UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE FARMACIA

TESIS DOCTORAL

Práctica de automedicación con antibióticos en pacientes adultos, con sospecha de infección del tracto urinario (ITU), de Addis Abeba (Etiopía)

Antibiotic self-medication practice among adult patients suspected of urinary tract infection (UTI) in Addis Ababa, Ethiopia

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Mebrahtu Eyasu Belete

DIRECTORES

Juana Benedí González
Sagrario Martín-Aragón Álvarez
José Antonio Romero Garrido

UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE FARMACIA

TESIS DOCTORAL

«Práctica de automedicación con antibióticos en pacientes adultos, con sospecha de infección del tracto urinario (ITU), de Addis Abeba (Etiopía)»

«Antibiotic self-medication practice among adult patients suspected of urinary tract infection (UTI) in Addis Ababa, Ethiopia»

Memoria para optar al grado de Doctor
Presentada por
Mebrahtu Eyasu Belete

Directores:
Juana Benedí González
Sagrario Martín-Aragón Álvarez
José Antonio Romero Garrido

Madrid, 2023

ACKNOWLEDGMENTS

First of all, I would like to express my heartfelt thanks to my supervisors Professor Juana Benedí González (PhD), Professor Sagrario Martín-Aragón (PhD) and Professor José Antonio Romero Garrido (PhD), all three from the Department of Pharmacology, Pharmacognosy and Botany, Faculty of Pharmacy, Complutense University of Madrid (Spain), for their indispensable contributions and strict follow-ups during my entire stay in the Doctorate PhD Program of «Farmacia».

Additionally, in the part-one study, next to my advisors, I wish to extend my acknowledgement to Saint Paul's Hospital Millennium Medical College, Research Directorate Director Office of Addis Ababa, Ethiopia for granting a financial support for this PhD research project part and definitely, this work would not have been possible without the support of the College. My appreciation is likewise addressed to the study participants who voluntarily provided the data, the different administrative offices at the Addis Ababa Health Bureau and the sub-cities, and the Woredas, including the health extension workers who greatly helped us in getting the sampled households.

In the part two study, I would like to extend my acknowledgement to the management body and the patients of the study settings for allowing me to do the research activities and for their participation respectively. Particularly, in part three studies, I would like to thank Mr. Debebe (from *The Center for Food Science and Nutrition* of Addis Ababa University), Mr. Kalkidan (from Arsho Diagnostic Center), Mr. Daniel (from Saint Paul's Hospital Millennium Medical College), and Mr. Biruk, Mr. Bihonegn, and Mr. Abiy (from Ethiopian Public Health Institute), all from Addis Ababa for their support during my stay in the study areas.

Last, but not least to Saint Paul's Hospital Millennium Medical College, provost office, Addis Ababa, Ethiopia for its support in giving adequate time to do my PhD study.

Lastly, I would like to thank my family for all their love and encouragement. Most importantly, I wish to thank my loving and supportive wife, Mrs. Samrawit Abebe Gangul and further for my daughter, Kidist Mebrahtu Eyasu and for my sons, Robel Mebrahtu Eyasu, and Yodahie Mebrahtu Eyasu Belete who provide unending inspiration.

Mebrahtu Eyasu Belete

Table of Contents

PREFACE

This final PhD dissertation report has three major interconnected parts.

PART ONE: «Prevalence and determinants of self-medication practice among selected households in Addis Ababa community, Ethiopia». This research work has been published at *PLoS ONE* (Shafie *et al.*, 2018). The following are the objectives:

General Objective

To assess the prevalence and determinant factors of self-medication practice in selected households of three sub-cities of Addis Ababa, Ethiopia.

Specific Objectives

- To determine the prevalence of self-medication practice (SMP).
- To assess the level of knowledge of the participants about SMP.
- To identify factors associated to SMP.

PART TWO: «Self-medication practice with Antibiotics by adult patients who are suspected of urinary tract infections in Addis Ababa Community». It includes the following general and specific objectives:

General objective

To assess the antibiotic self-medication practice among adult patients suspected of urinary tract infections (UTIs) in Addis Ababa, Ethiopia.

Specific objectives

- To characterize the socio-demographic characteristics of the study patients.
- To determine the prevalence of antibiotic self-medication practice (ASMP) among adult patients suspected with UTIs.
- To identify the associated risk factors of ASMP among adult patients suspected with UTIs.
- To determine the antibiotic susceptibility test towards patient isolated bacteria from urine sample.
- To assess the level of knowledge of the patients towards antibiotics.
- To identify common uropathogenic bacteria among adult patients confirmed with UTIs.

— To determine the prevalence of multi-drug resistant (MDR) bacteria against the tested antibiotics.
— To describe the number of antibiotics (from the 2 antibiotics until the maximum) resisted by the micro-organisms.
— To determine the presence of self-medicated patients among those with multi-drug resistant bacteria induced urinary tract infections.

PART THREE: «*In vitro* antioxidant, antibacterial activities and phytochemical screening of selected ethno-medicinal plants against patient isolated MDR uropathogenic bacteria». The general and specific objectives of this study were the following:

General Objective

To evaluate the in vitro antioxidant, antibacterial activities, qualitative and quantitative phytochemical composition, GC-MS characterization of active constituets and synergistic interactions of ciprofloxacin with the crude leave extracts of *Rhamnus prinoides, Thymus schimperi* and *Justicia shimperiana*.

Specific Objectives

Section 1) Phytochemical Profile and Antibacterial activities of the extracts

— To determine the qualitative phytochemical composition of the different crude extracts.
— To determine the antibacterial activity of the crude extracts.
— To determine the antibacterial activity of the extracts' combination.
— To identify the minimum inhibitory concentration (MIC) of the crude extracts.
— To determine the MIC of the extracts' combination.
— To identify the minimum bactericidal concentration (MBC) of the extracts.
— To identify the MBC of the extracts' combination.
— To assess synergistic activity of the extracts' combination with ciprofloxacin.

Section 2) Antioxidant activity of the extracts

— To determine the antioxidant and free radical scavenging activities of the three extracts.
— To estimate the quantity of the phenol, flavonoid and proanthocyanidin contents.

Section 3) GC-MS chemical profile

To identify the bioactive chemical constituets through GC-MS analyses on the extracts.

List of Tables

PART-ONE (TABLE 1-7)

PART TWO (TABLE 1-12)

PART-THREE: (TABLE 1-11)

List of Figures

PART-ONE (FIGURE 1-6)

PART-THREE (FIGURE 1-10)

Resumen

Antecedentes

La automedicación es una práctica recomendada por la Organización Mundial de la Salud (OMS) como parte de la atención primaria de salud. En los países en desarrollo, la práctica de la automedicación con antibióticos es la forma más común de autocuidado para tratar diferentes enfermedades/síntomas. Sin embargo, una práctica inadecuada puede entrañar peligros potenciales como el autodiagnóstico incorrecto y el desarrollo de bacterias multi-resistentes (*multi-drug resistance, MDR*). Además, se dispone de pocos informes de estudios sobre la asociación de la automedicación con antibióticos y el desarrollo de MDR entre los pacientes adultos con infección del tracto urinario (ITU). Actualmente, el aumento global de las infecciones bacterianas MDR supone un problema de salud pública. Para afrontarlo, las plantas medicinales podrían ser fuentes potenciales de fármacos antiinfecciosos en la lucha contra estas infecciones.

Objetivo

El objetivo principal de este estudio es evaluar la prevalencia de la práctica de la automedicación, de la práctica de automedicación con antibióticos, particularmente, y de los factores asociados a la misma entre participantes adultos de Addis Abeba, Etiopía. El objetivo secundario es evaluar tres especies vegetales de la medicina tradicional de Etiopía (*Thymus schimperi*, *Rhamnus prinoides* y *Justicia shimperiana*) como fuentes potenciales de principios activos con actividad antiinfecciosa frente a microorganismos patógenos del tracto urinario.

Metodología

Para evaluar la prevalencia de la práctica de automedicación se realizó un estudio transversal de una muestra de participantes procedentes de hogares seleccionados de una comunidad de Addis Abeba, desde abril hasta mayo de 2016, con un periodo de recuerdo de dos meses. Para evaluar la práctica de la automedicación con antibióticos se realizó un estudio transversal multicéntrico en Addis Abeba, desde noviembre de 2020 hasta agosto de 2021, en pacientes adultos con sospecha de ITU. La recolección de datos se llevó a cabo mediante encuestadores apropiadamente entrenados y formados que utilizaron cuestionarios previamente probados y validados. Los datos se analizaron con el paquete estadístico SPSS (SPSS Inc, Chicago, IL;

versión de software 21). Para identificar la asociación de las variables independientes con las variables dependientes (práctica de automedicación, práctica de automedicación con antibióticos) se realizaron análisis de regresión logística binaria simple y múltiple. La magnitud de las asociaciones se cuantificó mediante una proporción de probabilidades ajustadas con un intervalo de confianza (IC) del 95%. En todas las pruebas estadísticas, la asociación de las variables se consideraba significativa si el valor de p era inferior o igual a 0,05.

En el estudio de laboratorio clínico, sólo se estudiaron las muestras de orina con un crecimiento significativo (>105 ufc/mL). Tras obtener las cepas puras, éstas se sometieron a métodos convencionales de identificación bioquímica para identificar los diferentes uropatógenos Gram-negativos y Gram-positivos. Los datos de resistencia se interpretaron de acuerdo con el *Clinical Laboratory Standards Institute.*

En segundo lugar, se investigó la actividad antibacteriana y antioxidante *in vitro* de los extractos crudos hidrometanólicos de *Rhamnus prinoides, Thymus schimperi* y *Justicia shimperiana.* Además, se realizaron análisis fitoquímicos cualitativos y cuantitativos siguiendo protocolos estándar. Se estudió la actividad antioxidante de los extractos hidrometanólicos de las respectivas especies vegetales mediante los ensayos FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl) y ABTS (2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid). La actividad antibacteriana y las concentraciones bactericidas mínimas (CBM) de los extractos se determinaron por el método de difusión en pozos de agar. La Concentración Mínima Inhibitoria (CMI) se determinó mediante el método de microdilución en placas de 96 pocillos. En el estudio de la actividad antibacteriana, los resultados de CIM y CBM se expresaron como media ± desviaciones estándar. En el estudio de la actividad antioxidante, los datos se analizaron utilizando el software SPSS (versión 25) y Microsoft Office Excel 2017. Los resultados se expresaron como media ± desviación estándar. El coeficiente de correlación de Pearson se utilizó para analizar la asociación entre los contenidos fitoquímicos totales frente a los tres ensayos de actividad antioxidante. El valor $P \leq 0,05$ se consideró estadísticamente significativo. El resultado del cribado fitoquímico se clasificó como altamente (+++), moderadamente (++), ligeramente (+) y ausente (-), respectivamente. Finalmente, para identificar y caracterizar el perfil químico bioactivo de los extractos crudos, se utilizó el análisis GC-MS.

Resultados

En el estudio de la comunidad, de los 604 participantes, 422 (69,9%) eran mujeres y 182 (30,1%) eran hombres, siendo la edad media de 41,04 (± 13,45) años. La prevalencia de la

automedicación en este estudio fue del 75,5%. Las tres dolencias más frecuentes tratadas con automedicación fueron dolor de cabeza 117 (25,7%), dolor abdominal 59 (12,9%) y tos 54 (11,8%). Los dos motivos principales de la automedicación fueron la levedad de la enfermedad 216 (47,4%) y el conocimiento previo del medicamento 106 (23,2%). Los dos medicamentos más consumidos fueron el paracetamol 92 (20,2%) y los remedios tradicionales 73 (16,0%), mientras que los puntos de venta de medicamentos 319 (83,3%) fueron la principal fuente de medicamentos. Las dos fuentes de información sobre medicamentos más frecuentes fueron los profesionales sanitarios 174 (45,4%) y la experiencia de tratamientos anteriores 82 (21,4%). Además, se encontraron diferencias estadísticamente significativas entre los encuestados que declararon practicar la automedicación en función de los ingresos y de los conocimientos sobre la práctica de la automedicación adecuada.

En el estudio multicéntrico, para evaluar la práctica de automedicación con antibióticos, participaron un total de 531 pacientes adultos. La edad media (± DE) era de 43,74 (± 16,16) años. La mayoría de los pacientes, 368 (69,3%), eran mujeres. La prevalencia global de la práctica de automedicación con antibióticos fue del 10,4%. La infección del tracto urinario (ITU) 14 (25,5%) fue autodeclarada como la queja más común para la práctica de la automedicación con antibióticos. El antibiótico más frecuentemente declarado fue la amoxacilina 28 (50,9%). La razón principal para la automedicación con antibióticos fue la experiencia pasada/simplemente conocer el nombre del medicamento 29 (52,7%). Un número de 48 (87,3%) pacientes que practicaban automedicación declararon que la automedicación con antibióticos era una práctica inaceptable. Los pacientes con una edad igual o superior a 50 años, con ingresos mensuales inferiores o iguales a 4.000 birrs y sin antecedentes de ITU, eran más propensos a la automedicación. De las 129 bacterias aisladas, 106 (82,2%) eran resistentes a dos o más antibióticos. Las dos bacterias más aisladas fueron *Escherichia coli,* 88 (68,2%), y *Klebsiella pneumoniae*, 10 (7,8%). En cuanto a los antibióticos testados, casi el 91% de los aislados de *E. coli* eran resistentes a la ampicilina, mientras que para amoxicilina y doxiciclina lo eran al 100%. Los aislados de *K. pneumoniae* eran resistentes al 100% para los tres fármacos (ampicilina, amoxicilina y doxiciclina). Entre los aislados de bacterias MDR aisladas, 9 (8,5%) pacientes practicaban automedicación con antibióticos y casi un tercio de las bacterias eran resistentes a seis antibióticos (33,3%).

En cuanto al estudio de las especies vegetales de interés, los contenidos más altos de fenoles, flavonoides y proantocianidinas se detectaron en el extracto de *Thymus schimperi* en comparación con los otros extractos. En la prueba de actividad antioxidante, el extracto de *T. schimperi* tuvo el mayor porcentaje en la inhibición de DPPH (CI 50%), con mayor poder

antioxidante reductor FRAP actividad reductora, 2521,60 mMole sulfato ferroso Eq (mMole Fe2+); y la mayor actividad antioxidante/ensayo ABTS/ con una media de 27296,65 µMole trolox Eq (µMole trolox). El contenido total de fenoles mostró una correlación negativa casi perfecta con el valor de la actividad antioxidante del ensayo DPPH (CI 50%) (r=-0,999; p=0,023). Además, el contenido total de flavonoides de los extractos se correlacionó significativamente con el ensayo ABTS (media de la actividad de barrido; r=0,999; p= 0,032). En la actividad antibacteriana, *T. schimperi* mostró la mayor actividad antibacteriana de manera dependiente de la concentración en las dosis probadas en comparación con los otros extractos. *T. schimperi,* a dosis de 1000 mg/mL, mostró el diámetro máximo de la zona de inhibición (DZI) frente a los aislados de *E. coli* MDR (DZI: 20,00 ± 0,00 mm), *K. pneumoniae* ESBL (DZI: 14,50±0,55 mm), *E. coli* (ATCC25922) (DZI: 18,5±0,55 mm) y *K. pneumoniae* (ATCC700603) (DZI: 14,83±0,40 mm) en comparación con los controles negativos y los demás extractos. Al igual que *T. schimperi* y su combinación con *Rhamnus prinoides* en la prueba frente a los aislados de *E. coli* MDR, *T. schimperi* fue el único extracto con la CIM más baja (4 mg/mL) entre los extractos evaluados frente a *E. coli* (ATCC25922). El control positivo (ciprofloxacino) tuvo el valor de CIM más bajo en comparación con todos los extractos independientes y la combinación de dos extractos. La CIM más baja de *T. schimperi* en la evaluación de la actividad antibacteriana frente a los aislados de *K. pneumoniae* MDR fue de 8 mg/mL, sin embargo, fue de 4 mg/mL (CIM) para la actividad frente a *K. pneumoniae* (ATCC700603). El MBC más bajo de *T. schimperi* en la prueba frente a los aislados MDR *K. pneumoniae* ESBL fue de 32 mg/mL; en la actividad frente a *K. pneumoniae* (ATCC700603) fue de 64 mg/mL; en la evaluación frente a los aislados de *E. coli* MDR y frente a *E. coli* (ATCC25922) fue de 16 mg/mL. La relación entre la CBM y la CIM de *T. schimperi* sola y en combinación con *R. prinoides* en la prueba frente a *K. pneumoniae* (ATCC700603) fue bacteriostática. Salvo en el caso de *E. coli* MDR (aditivo), la interacción de las combinaciones entre el extracto de *T. schimperi* y ciprofloxacino se clasificó como indiferente frente al resto de las cepas. A diferencia de *E. coli* (ATCC25922) (antagonista), las combinaciones de *R. prinoides* y ciprofloxacino mostraron una interacción indiferente. En los análisis GC-MS, en el extracto de *T. schimperi* se identificaron 14 compuestos bioactivos. Los cuatro compuestos predominantes fueron el ácido hexanedioico, éster bis (2-etilhexilo) (73,88%); timol (11,68%); o-Cimen-5-ol (7,95%); y *p*-tert-butilcatecol (2,19%). En los extractos de *R. prinoides* se identificaron seis compuestos y los tres compuestos bioactivos abundantes identificados fueron el ácido hexanedioico, éster bis (2-etilhexilo) (79,36%); beta-D-glucopiranósido, metilo (10,03%); y desulfosinigrina (8,28%). En el extracto de *Justicia*

shimperiana se identificaron el ácido hexanedioico, éster de mono (2-etilhexilo) (59,6% de área de pico), la debrisoquina (12,18%) y el 8,11,14-heptadecatrienoato de metilo (10,79%).

Conclusión

En la comunidad, la automedicación se practicó con una serie de fármacos, desde el paracetamol hasta los antimicrobianos. Claramente, la prevalencia de la práctica de automedicación con antibióticos entre la población de pacientes adultos es significativa. La edad, los ingresos mensuales y la ausencia de antecedentes de ITU se asociaron significativamente con la práctica de automedicación antibiótica. Entre los pacientes automedicados, algunos presentaban bacterias MDR. La práctica de la automedicación es inevitable; por lo tanto, las autoridades sanitarias y los profesionales de la salud deberían aplicar enérgicamente las leyes existentes de regulación de medicamentos para evitar la venta libre de antibióticos y su uso inadecuado. Dado que la práctica de la automedicación es inevitable, las autoridades y los profesionales de la salud deberían educar al público no sólo sobre las ventajas y desventajas de la automedicación, sino también sobre su uso adecuado. Por lo que respecta específicamente a este estudio, también debería prestarse mayor atención a los pacientes de más edad y a los que poseen las rentas más bajas.

Entre los extractos vegetales estudiados, el de *Thymus schimperi* mostró la mayor actividad antioxidante en todos los ensayos. El contenido total de fenoles, flavonoides y proantocianidinas fue mayor en *T. schimperi* en comparación con los demás extractos. Estadísticamente, las actividades DPPH (CI 50%) y ABTS se correlacionaron con el contenido total de fenoles y flavonoides, respectivamente. *T. schimperi* tuvo una mejor actividad antibacteriana *in vitro* frente a los aislados uropatógenos MDR *E. coli* y *K. pneumoniae* ESBL. En los análisis GC-MS se identificaron compuestos de los cuales se han descrito aplicaciones en el tratamiento de diferentes enfermedades. Y así, el extracto de *T. schimperi* presentó 14 compuestos, el de *R. prinoides* presentó seis y el de *J. shimperiana* cinco.

Palabras clave: Prevalencia; Práctica de automedicación; Hogares; Comunidad; Resistencia a fármacos; Prueba de susceptibilidad a los antibióticos; Pacientes adultos; *In vitro; Rhamnus prinoides; Thymus schimperi; Justicia shimperiana;* Actividad antioxidante; Actividades antibacterianas; Cribado fitoquímico; Bacterias MDR; *Escherichia coli; Klebsiella pneumoniae;* Addis Abeba; Etiopía.

ABSTRACT

Introduction

Self-medication (SM) is a recommended practice by the World Health Organization (WHO) as part of the primary health care. In developing countries, antibiotic self-medication practice (ASMP) is the most common form of self-care to treat different diseases and symptoms. However, inappropriate practice can have potential dangers such as incorrect self-diagnosis, and development of multidrug resistant (MDR) bacteria. Urinary tract infections (UTIs) are among the most common bacterial infections that people self-treat. Even so, there are few study reports on the relationship between ASMP and MDR development in adult UTI patients. Currently, the global increase in MDR bacterial infections poses a public health problem. To tackle this, medicinal plants could be potential sources as anti-infective drugs to fight against these infections.

Objective

This study aimed to evaluate the overall community self-medication practice, antibiotic self-medication practice, and their associated factors among adult participants in Addis Ababa, Ethiopia. The secondary objective was to evaluate three plant species from traditional Ethiopian medicine (*Thymus schimperi, Rhamnus prinoides* and *Justicia shimperiana*) as potential sources of active ingredients with anti-infective activity against pathogenic microorganisms of the urinary tract.

Methodology

To assess the prevalence of self-medication practice, a cross-sectional study of a sample of participants from selected households in a community in Addis Ababa was conducted from April to May 2016, with a recall period of two months. To assess the practice of self-medication with antibiotics, a multicentre-institutional based cross-sectional study was conducted from November 2020 up to August 2021 in Addis Ababa, among adult patients suspected of UTI. Data collection was carried out by appropriately trained and educated enumerators using pre-tested and validated questionnaires. Data were analysed using the SPSS statistical package (SPSS Inc, Chicago, IL; software version 21). To identify the association of the independent variables with the dependent variables (self-medication practice, antibiotic self-medication practice), simple and multiple binary logistic regression analyses were performed. The magnitud of the associations was quantified using an adjusted odds ratio (AOR) with a 95%

confidence interval (CI), and in all statistical tests, a p-value ≤ 0.05 was considered statistically significant.

In the clinical laboratory study, only urine samples with significant growth (>10^5 cfu/mL) were further studied. After obtaining the pure strains, the strains were subjected to conventional biochemical identification methods to identify different Gram-negative and positive uropathogens. Resistance data was interpreted according to the Clinical Laboratory Standards Institute.

Second, the hydromethanolic crude extracts of *Rhamnus prinoides*, *Thymus schimperi*, and *Justicia shimperiana* were investigated for *in vitro* antibacterial and antioxidant activities. Furthermore, qualitative and quantitative phytochemical screenings were done following standard protocols. The hydro-methanolic extract of these plants was studied for antioxidant activities using FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) assays. Antibacterial activity and Minimum Bactericidal Concentrations (MBC) of the extracts were determined by agar well diffusion method. The Minimum Inhibitory Concentration (MIC) was determined by the microdilution method in 96-well plates. In the antibacterial study, results of zone of inhibitions, MIC and MBC were summarized as means (± standard deviations). In antioxidant activity study, data were analyzed using SPSS (Version 25) and Microsoft Office Excel 2017. Results were expressed as mean ± standard deviation. One-way anova was performed to compare mean differences of the phytochemical contents among the extracts. List Significant Difference test was used to identify where the mean differences were. The Pearson correlation coefficient was used to analyze the association among total phytochemical contents versus the three antioxidant activity assays. P-value ≤ 0.05 was considered as statistically significant. The presence of phytochemical screening result was classified as highly (+++), moderately (++), slightly (+) and absent (–), respectively. Finally, to identify and characterize the bioactive chemicals profile of the crude extracts, GC-MS analyses was used. The Minimum Inhibitory Concentration (MIC) was determined by the microdilution method in 96-well plates. In the antibacterial study, results of zone of inhibitions, MIC and MBC were summarized as means ± standard deviations. In the antioxidant activity study, data were analyzed using SPSS (Version 25) and Microsoft Office Excel 2017. Results were expressed as mean ± standard deviation. One-way ANOVA was performed to compare mean differences of the phytochemical contents among the extracts. List Significant Difference test was used to identify where the mean differences were. The Pearson correlation coefficient was used to analyze the association among total phytochemical contents versus the three antioxidant

activity assays. P-value ≤ 0.05 was considered as statistically significant. The presence of phytochemical screening result was classified as highly (+++), moderately (++), slightly (+) and absent (–), respectively. Finally, to identify and characterize the bioactive chemicals profile of the crude extracts, GC-MS analyses were used.

Result

In the community study, among the 604 participants, 422 (69.9%) were female and 182 (30.1%) were male and being the mean age of 41.04 (± 13.45) years. The prevalence of self-medication (SM) in this study was 75.5%. The three most frequently reported ailments were headache 117 (25.7%), abdominal pain 59 (12.9%) and cough 54 (11.8%). The two main reasons for self-medication (SM) were mildness of illness 216 (47.4%) and previous knowledge about the drug 106 (23.2%). The two most frequently consumed medications were paracetamol 92 (20.2%) and traditional remedies 73 (16.0%), while drug retail outlets 319 (83.3%) were the main source of drugs. The two most frequently reported source of drug information were health professionals 174 (45.4%) and experience from previous treatment 82 (21.4%). Moreover, there were statistically significant differences among respondents who reported practicing self-medication (SM) based on income and knowledge about appropriate self-medication practice (SMP).

Additionally, within the multicenter study to assess the practice of self-medication with antibiotics, a total of 531 adult patients were participated. The mean age (± SD) was 43.74 (± 16.16) years. Majority, 368 (69.3%), of the patients were female. The overall prevalence of ASMP was 10.4%. UTIs 14 (25.5%) was self-reported as the most common complaint for antibiotic self-medication practice. The most frequently self-reported antibiotic was amoxacillin 28 (50.9%). The primary reason for antibiotic self-medication was past experience/simply knowing the name of drug before 29 (52.7%). About 48 (87.3%) of the patients, who practiced antibiotic self-medication, self-reported that antibiotic self-medication was unacceptable practice. Patients whose age was 50 years and above, monthly income less than or equals 4000 birrs and those patients with no history of UTIs were significantly associated and more likely to have antibiotic self-medication. MDR was reported for 106 (82.2%) of the total 129 bacteria isolates, which were resistant to two or more of antibiotics. The two commonly isolated bacteria were *Escherichia coli*, 88 (68.2%) and *Klebsiella pneumoniae*, 10 (7.8%). Within the tested antibiotics, nearly 91% of the isolated *E. coli* was ampicillin resistant, while for amoxicillin and doxycycline, it was 100% resistant. The *K. pneumoniae* isolates were 100% resistant for all the three tested drugs (ampicillin, amoxicillin,

and doxycycline). Among those who had MDR bacteria isolates, 9 (8.5%) of them had ASMP, and almost one third of the bacteria were resistant to six antibiotics (33.3%).

In the phytochemical screening of the selected ethno-medicinal plants, the highest phenolic, flavonoid and proanthocyanidin contents were detected in *T. schimperi* extract compared to the other extracts. In the antioxidant activity test, *T. schimperi* extract had the highest percent in DPPH inhibition (IC 50%), with highest reducing antioxidant power FRAP reducing activity, 2521.60 mMole ferrous sulfate Eq (mMole Fe2+) and the highest antioxidant activity/ABTS assay/ with a mean of 27296.65 µMole trolox Eq (µMole trolox). The Total Phenol Contents showed almost a perfect negative correlation with the antioxidant activity value from DPPH (IC 50%) assay (r= -0.999; p=0.023). Additionally, the total flavonoid contents of the extracts correlated significantly with ABTS assay (mean of scavenging activity; r=0.999; p= 0.032). In the antibacterial activity, *T. schimperi* showed the highest antibacterial activity in a concentration dependent manner in the doses tested compared to the other extracts. *T. schimperi* among the extracts at 1000 mg/mL dose showed a maximum diameter of the zone of inhibition (DZI) against clinical isolate (CI) MDR *E. coli* (DZI: 20.00±0.00 mm), *K. pneumoniae ESBL* (DZI: 14.50±0.55 mm), *E. coli* (ATCC25922) (DZI: 18.5±0.55 mm) and *K. pneumoniae* (ATCC700603) (DZI: 14.83±0.40 mm) compared to the negative controls and the rest extracts. Like *T. schimperi* alone and its combination with *R. prinoides* in the test against the CI MDR *E. coli, T. schimperi* was the only extract with the lowest MIC (4 mg/mL) among the extracts evaluated against *E. coli* (ATCC25922). The positive control (ciprofloxacin) had the lowest MIC value compared to all independent extracts and the two extracts combination. The lowest MIC of *T. schimperi* in antibacterial activity evaluation against CI MDR *K. pneumoniae ESBL* was 8 mg/mL but 4 mg/mL (MIC) for activity against *K. pneumoniae* (ATCC700603). The lowest MBC of *T. schimperi* in the test against CI MDR *K. pneumoniae ESBL* was (32 mg/mL); in activity against *K. pneumoniae* (ATCC700603) (64 mg/mL); and in the evaluation against patient isolated MDR *E. coli* and *E. coli* (ATCC25922) (16 mg/mL). The ratio of MBC to MIC of *T. schimperi* alone and in combination with *R. prinoides* in the test against *K. pneumoniae* (ATCC700603) was bacteriostatic. Except for CI MDR *E. coli* (additive), the interaction of the combinations between *T. schimperi* extract and ciprofloxacin was categorized as indifference against the rest strains. Unlike for *E. coli* (ATCC25922) (antagonist), the *R. prinoides* and ciprofloxacin combinations showed an indifference interaction. In the GC-MS analyses, in *T. schimperi* extract, 14 bioactive compounds were identified. The four predominant compounds were hexanedioic acid, bis (2-ethylhexyl) ester (73.88%); thymol (11.68%); o-Cymen-5-ol (7.95%); and p-tert-Butylcatechol (2.19%). While in *R. prinoides* extracts, six compounds were identified and the three abundant bioactive

compounds identified were hexanedioic acid, bis (2-ethylhexyl) ester (79.36%); beta-D-glucopyranoside, methyl (10.03%); and desulphosinigrin (8.28%). *J. shimperiana* extract had five bioactive compounds. The predominant compound was hexanedioic acid, mono (2-ethylhexyl) ester (59.6%), debrisoquine (12.18%), and methyl 8, 11, 14-heptadecatrienoate (10.79%).

Conclusion

In the community, self-medication was practiced with a range of drugs from the conventional paracetamol to antimicrobials. Clearly, the prevalence of arbitrary antibiotic self-medication practice among the adult patient population is significant. Age, monthly income, and no history of UTIs were significantly associated with antibiotic self-medication practice. Among self-medicated patients, some patients had MDR bacteria. The practice of self-medication is inevitable; hence, the Ethiopian health authorities and professionals should strongly implement the existing drug regulation laws to prevent the Over the Counter selling of antibiotics and its inappropriate use. Since the practice of self-medication is unavoidable, authorities and health professionals should be required to educate the public not only about the advantages and disadvantages of self-medication but also about its proper use. Specific to this study, more attention should also be given to older patients and patients with lower income.

Among all vegetal extracts, *Thymus schimperi* exhibited the highest antioxidant activity in all assays. The total phenol, flavonoid and proanthocyanidin content were highest in *T. schimperi* compared to the other extracts. Statistically, DPPH (IC 50%) and ABTS scavenging activities were correlated with total phenol, and flavonoids, respectively. *T. schimperi* had a better *in vitro* antibacterial activity against uropathogenic MDR *E. coli* and *K. pneumoniae ESBL* isolates. The GC-MS analyses identified compounds for which applications in the treatment of different diseases have been described. And so, *T. schimperi* extract had 14 compounds, *R. prinoides* (six) compounds and *J. shimperiana* had five compounds.

Keywords: Prevalence; Self-medication Practice; Antibiotic; Households; Community; Drug resistant; Antibiotic susceptibility test; Adult patients; *In vitro*; *Rhamnus prinoides*; *Thymus schimperi*; *Justicia shimperiana*; Antioxidant activity; Antibacterial activities; Phytochemical screening; MDR bacteria; *Escherichia coli; Klebsiella pneumoniae;* Addis Ababa; Ethiopia.

PART-ONE:

Prevalence and determinants of self-medication practice among selected households in Addis Ababa community, Ethiopia

List of Abbreviations

AA	Addis Ababa
AOR	Adjusted Odd Ratio
Coef.	Coefficient
CI	Confidence Interval
GI	Gastrointestinal
IRB	Institutional Review Board
NSAIDs	Non-steroidal Anti-inflammatory Drugs
OTC	Over the Counter
SD	Standard Deviation
SM	Self-Medication
SMP	Self-Medication Practice
SPHMMC	Saint Paul's Hospital Millennium Medical College
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
WSMI	World Self-Medication Industry

CHAPTER 1. INTRODUCTION

1.1 Background

The World Health Organization (WHO) has defined self-medication (SM) as the selection and use of medicinal products by the consumer to treat self-recognized illnesses or its symptoms, or the intermittent or continued use of a medication prescribed by a physician for a chronic or recurring disease or symptom (WHO, 1998), or without periodic consultation with health care provider" (WHO, 2000).

According to target eight of the Millennium Development Goal (MDG), essential drugs should be accessible and affordable in developing countries with the appropriate information and communication. Self-medication products are those not requiring a medical prescription which are produced, distributed, and sold to consumers for use on their own initiative (UN, 2008). In line with this, self-medication (SM) is one of the contributors for rational drug use. SM is identified as one of the key aspects of primary health care by the WHO (WHO, 1998) and World Self-Medication Industry (WSMI, 2010). SM is one aspect of self-care which people practice for themselves in order to maintain health or prevent and deal with illnesses (Suleman *et al.*, 2009).

Appropriate SM is beneficial to individuals and health care systems because this approach is affordable and convenient, particularly when people have mild diseases or symptoms. Further, it allows patients to take responsibility, and build confidence to manage their own health. It has also a crucial role in the health care system in saving time spent in waiting for doctors, alleviating health care costs spending on settings with resources constrained (Abosede, 1984; Huges *et al.*, 2001; Bennadi, 2013), and transportation cost (Sherazi *et al.*, 2012).

Globally, it has been estimated that more than 50% of antibiotics are sold without prescription (Cars and Nordberg, 2005). Although dispensing antibiotics without prescription in the developed world is minimal (Hoxha *et al.*, 2005; Gastelurrutia *et al.*, 2013; Chang *et al.*, 2017; Horumpende *et al.*, 2018 (a); the problem is greater or worse in Africa and other low-income countries due to poor healthcare regulations and which is substantiated by reports briefing that the prevalence of self-medication in developing countries is in the range of 12.7% to 95% (Gupta *et al.*, 2011; Wijesinghe *et al.*, 2012; Divya *et al.*, 2016).

SM with antimicrobials has been found a common practice in low-income countries and it has been shown to be significantly related to many factors including availability and

accessibility, lack of access to health care facilities, high prevalence of infectious diseases, poor awareness, poor regulation, and lack of supervision by health professionals (WHO, 2016). Most of the public or state hospitals rendered the health service almost to 3/4th of the country's population are not well equipped and are unable to fully deliver services to the public. This is contributing highly to the practice of SM by consuming medications with consultation of paramedics (Pradhan, 2004; WHO, 2017). Moreover, the use of prescription-only medications including antimicrobials becomes a prevailing practice in Ethiopia. WHO reports that SM with antimicrobial agents is becoming widespread, with one of its greatest risks being antimicrobial resistance (Donmez *et al.*, 2018).

In developing countries, the prevalence of difficult-to-treat bacterial infections is escalating daily, and they have become a serious threat to public health, leading to an increased mortality rate and decreased quality of life (Garofalo *et al.*, 2015; Grigoryan *et al.*, 2008). Particularly, in sub-Saharan Africa, where the number of deaths attributed to antimicrobial resistance each year is higher than in other parts of the world (O'Neill, 2014), irrational use of antibiotics is more common (Morgan *et al.*, 2011; Afari-Asiedu *et al.*, 2020).

In Africa, including Ethiopia, people use not only non-prescription drugs but also prescription drugs without supervision (Ayalew, 2017). The possible reason for this is the low number of health care practitioners and their inaccessibility to all. In Ethiopia, as a general rule, those drugs legally classified as over-the-counter (OTC) drugs must be used primarily to treat a condition that does not require the direct supervision and prescription of/from a doctor. Then, OTC drugs are reasonably safe and well-tolerated. However, they still have some risks such as interaction with other drugs, supplements, foods or drinks, and further, they could cause problems for individuals with certain medical conditions. Therefore, they must be dispensed by pharmacy personnel providing sufficient counseling about the condition, the drugs and the determination on the maximum quantity dispensed at one time (Food, Medicine and Health Care Administration and Control Authority of Ethiopia, FMHACA, 2012). In spite of this, the regulation of non-prescription medicines is not clearly distinguished from that of prescription medicines (World Self-medication Industry, WSMI, 2010).

1.2 Statement of the problem

Globally, SM has become a public health problem due to its prevalence and harmful effects. It is being practiced in both developing and developed countries (Hanafy *et al.*, 2016) but widely practiced in developing countries as many drugs are prescribed over the counter

without the guidance of physician (Shah *et al*., 2018). Rahman *et al*. (2008) indicated that almost 80% of the world population depends on the use of unconventional medicines as its first source of health care. While the medical community tends to reject most self-medication practices (SMP) for the fear of health risks and the need for expert diagnosis, public authorities tend to be more tolerant, highlighting the economic advantages for managing minor ailments (Fainzang, 2017).

SM may facilitate the indiscriminate use of antibiotics, for example, in therapies for minor and self-limiting conditions (Grigoryan *et al*., 2006; Berzanskyte *et al*., 2006; Sapkota *et al*., 2010). From a public health perspective, it should also be stressed that non-responsible SMPs incur significant healthcare costs, particularly with the cost of adverse drug reactions and drug interactions indicating a real economic burden (Bennadi, 2013; Sultana *et al*., 2013).

Non-responsible SM is defined as the practice whereby people use at their own initiative drugs which are not allowed to use without a prescription (Galato *et al*., 2009), and furthermore, the misuse or abuse of OTC medicines (Mortazavi *et al*., 2017). However, SMP could potentially leads to some health-related complications, namely, incorrect drug selection, drug resistance, uncontrolled adverse effects or drug reactions or interactions (Ruiz, 2010; Panda *et al*., 2016), misdiagnosis and delay in medical care (Hughes *et al*., 2001; Bennadi, 2013; Patil, 2017), disruption in drug distribution systems (Purreza *et al*., 2013), delay in treatment of serious illnesses, disguising symptoms of acute illnesses, sudden death in certain cases (Nunes de Melo *et al*., 2006; Sanchez, 2014; Purreza *et al*., 2013; Mortazavi *et al*., 2017), continued hospitalization intervals, and an upsurge in morbidity rate in the community (WHO, 2000; Goossens *et al*., 2005).

Moreover, SM is a grave threat to health as it increases the risk of antibiotics resistance development (WHO, 2000; Rather *et al*., 2017). In 2014, according to a study commissioned by the UK government, approximately 700,000 people would die annually from drug-resistant bacteria, and by 2050, it could well soar to 10 million, which exceed the worldwide deaths from cancer (Piddock *et al*., 2016). Antibiotic-resistant bacteria have historically been hospital based, but they are now becoming more common in the community (Levy, 2002; Carlet and Pittet, 2013).

The increase in antibiotic resistance rates is mainly credited to inadequate prescriptions of antibiotics and their pressure/excessive use in the community, largely in primary care. Other associated factors are such as patient perceptions towards patient–physician interaction, including SM, knowledge regarding antibiotics, and the lack of adequate policies for the

restriction and control of prescribed antibiotics (Costelloe *et al*., 2010; Hulscher *et al*., 2010; Napolitano *et al*., 2013; Awad *et al*., 2015). In Malaysian Statistics on Medicine 2009–2010, the Ministry of Health reported a 16% increased use of both prescribed and purchased OTC antimicrobials annually, and this can contribute to antimicrobial resistance (Aslam *et al*., 2020).

Findings of different studies revealed that there are many factors associated with SMP. For example, a study conducted in China in four consecutive China National Health Surveys (CNHS) showed that having five or more family members, educational background with high and technical school, and socio-economic status with upper class were factors positively associated with SMP (Yuefeng *et al*., 2012).

As reported by Pagan *et al*. (2006), there are also other factors that contribute for SMP such as urge of self-care, feeling of sympathy toward family members in sickness, poverty, ignorance, misbeliefs, availability of drugs other than in pharmacy, and lack of easy access to professional health-care services. Further, other determinants of SMP were lack of government sponsored health insurance coverage, satisfaction with pharmacy counseling, adequate medication knowledge, and improvement of symptoms (James *et al*., 2006, Suleman *et al*., 2009, Gupta *et al*., 2011).

Similarly, studies performed in Uganda and territory of the city of Nisˇ substantiated these findings and explained that participants of male sex, having long distance travel to health facility, with a perception of hospital drugs do not work, advised by relatives/friends, their previous experiences/old prescriptions use, and media (television, magazines, and internet) were found as associated factors for respondent's choice towards SM (Ocan *et al*., 2014; Dimitrijević *et al*., 2014).

The temporal increment of SMP might be related to the advancement of information technology and high access of drugs from several sources which make the patients to be closer to the disease and drug related information (Bennadi, 2013; Patil, 2014). Moreover, several worldwide websites allow patients to access antibiotics without medical diagnosis and a prescription (El Zowalaty *et al*., 2016). People can google about their health issues and medications (Pradhan, 2004). Therefore, there should be guidelines to follow strictly for regulating patients as well as comedics to ensure optimum utilization of SMP (Sherazi *et al*., 2012).

In Ethiopia, and particularly in the selected households among the Addis Ababa community, the magnitude and pattern of SMP, factors affecting SM, whether the practice is

appropriate or not, whether the public is knowledgeable on the rational use and risk of the medicines, the common types of illnesses leading to SMP, and major types of drugs consumed have not yet been adequately studied. Therefore, the major aim of this study was to determine the prevalence and determinants of SM in selected households among the Addis Ababa community.

CHAPTER 2. LITERATURE REVIEW

In this literature review, there are various studies carried out in Africa and the other world about SM. Some of the published studies are discussed below on their prevalence, sociodemographic profile of study participants, reasons of SMP, types of ailments/disease cause SMP, types of drugs used, source of information and other related variables.

Worldwide, the prevalence of SMP is not similar across countries. As an example, SMP has been reported 12.7% in Spain (as a European country), 75% in Chile and 61% in Mexico (as South and Central American countries), and 40-60% in the Vietnamese, 32% in the Chinese, and 71% in the Indians (as Asian countries) (Shaghaghi *et al*., 2014). According to a systematic review, in developing countries, the overall prevalence of SM ranged from 8.3 to 87% (Parulekar *et al*., 2016).

In developed nations, such as those that make up Europe, a major concern of irrational SM with antibiotics is reported. For instance, in Greece, of the approximately 78% self-medicated individuals with antibiotics, 45% received the antibiotics without prescription at least one time (Skliros *et al.*, 2010). A survey, covered 19 European countries, on the prevalence of antimicrobial drug SM in the previous 12 months indicated that the prevalence of actual SM varied from 1 to 210 per 1,000, and intended SM from 73 to 449 per 1,000; both rates were high in Eastern and Southern Europe and low in Northern and Western Europe (Grigoryan *et al*., 2006).

In other countries such as USA, a very high degree of SM is reported among migrants (Horton and Stewart, 2012). A survey conducted in South Australia on SM with OTC drugs and complementary medications in elderly population similarly indicated increasing prevalence from 17.7% in 2000-2001 to 35.5% in 2003-2004 (LynnYeen *et al*., 2009).

A relatively higher proportion of SMP is reported in various Asian countries. In India, around 87% of professional students practiced SM (Verma *et al*., 2010). In another area of India, a community prevalence of 56% is reported (Gupta *et al*., 2011). On the contrary, a study conducted in five cities of the nearby Pakistan reported 15.7 and 8.3% prevalence in urban and rural areas, respectively (Hussain *et al*., 2011). In Sri Lanka, unlike the study in Pakistan, there was 33.9 and 35.3% prevalence of SM in urban and rural areas (Wijesinghe *et al*., 2012). In other areas such as Malaysia and Nepal, prevalence rates of 77.6% among health care professionals (Ali *et al*., 2012) and 59% among respondents (Shankar *et al*., 2002) are reported, respectively.

Drug category used for SM are analgesics (like paracetamol, dipyrone, and aspirin); antibiotics (amoxicillin, ampicillin, erythromycin, ciprofloxacin, chloramphenicol, cefaclor, and amoxicillin/clavulanic acid); cough remedies; laxatives and oral rehydration salts are the commonest drugs employed in SM (LynnYeen *et al*., 2009; Togoobaatar *et al*., 2010; Hussain *et al*., 2011). The primary diseases that led to SM with the above drugs were headache, fever, upper and lower respiratory tract diseases such as cough, cold and infection, GI diseases and skin problems (Grigoryan *et al*., 2006; James *et al*., 2006; Skliros *et al*., 2010; Verma *et al*., 2010).

A cross-sectional study on antibiotic consumption carried out in the general population of Mureș County (Central Region of Romania) has found that 62.65% of participants (624/996 participants) considered that antibiotics are used to treat a bacterial infection and 61.45% (612 participants) used an antibiotic at least once in the previous year, with distribution of 68.7% (rural) and 56.8% (urban) of the participants. About 10.34% of the participants used antibiotics following recommendations from family/friends, and 22.9% used antibiotic left over from their last prescription. Of those who consumed antibiotics (868 participants), 65.9% consulted their physician every time before taking the medication. Furthermore, 82.3% of the total of 996 participants considered that the use of non-prescription antibiotics has a negative effect, and 85.14% had heard about the antibiotic resistance of bacteria (Voidăzan *et al*., 2019).

In Vietnam, a cross-sectional study was performed in five highland provinces with 1000 residents, and 83.3% of them had SM in the last 12 months, with a mean of SM, 4.5 times (± SD=4.1). Female (OR=0.62, $p<0.01$), ethnic minorities, higher number of members having health insurance in family (OR=0.82, $p<0.01$) and higher annual household income (OR=0.78, $p<0.05$) were associated with the lower likelihood of "only buy medicines at pharmacy stores when having illness in the last 12 months". Moreover, females (OR=0.59, $p<0.05$), white-collar workers (OR=0.25, $p<0.01$) and individuals with higher number of children in the family (OR=0.68, $p<0.05$) were less likely to practice SM. People who belonging to ethnic minorities, white-collar workers (Coef.=−0.32, $p<0.01$) and individuals with higher number of members having health insurance in family practiced SM less often in the last 12 months compared to other groups. Meanwhile, individuals having higher number of members in the family (Coef.=0.07, $p<0.01$) and higher annual household income (Coef.=0.08, $p<0.01$) practiced SM more often in the last 12 months (Ha *et al*., 2019).

In 2017, a cross-sectional nationally representative household survey was carried out biannually by National Statistical Office (Thailand). It was reported by Chanvatik *et al*. (2019)

who indicated that the one-month prevalence of antibiotic use was 7.9% for three common conditions; flu (27.0%), fever (19.2%) and sore throat (16.8%). The majority of antibiotics (70.3%) were provided by public or private healthcare facilities, and 26.7% by pharmacies. Thai adults had low level of knowledge about antibiotics; only 2.6 gave correct answers to all six statements related to antibiotics, while 13.5% gave wrong answers to all six statements. People who had higher education level and belong to richer wealth quintiles and receive antibiotics and antimicrobial resistance information have significantly higher levels of knowledge about antibiotics. In the last 12 months, only 17.8% of participants had heard information about the proper use of antibiotics and antimicrobial resistance; mostly from doctors (36.1%), health workers (24.8%) and pharmacists (17.7%).

A sub-study of the Indonesia Basic Health Research Study 2010 focused on 12,226 participants aged 15 years and above residing in high-risk malaria-endemic provinces showed one in five respondents used traditional medicine for malaria symptoms. Most of the respondents had multiple episodes of malaria infection and used traditional medicine alongside free antimalarial drug treatments. Those participants consumed traditional medicine as SMP for the purpose of treating the general health/common illness on daily basis (OR: 3.75, 95% CI: 2.93 4.79); those without a hospital in local vicinity (OR: 1.31, 95% CI: 1.10 1.57), and those living in poorer quality housing, were more likely to use traditional medicine for malaria symptoms (Suswardany *et al*., 2017).

Among 315 residents in the United Arab Emirate population, 31.7% (n=100/315) of the participants reported the use of non-prescription antibiotics within a three-month study period. SM with antibiotics was significantly associated with ethnicity and employment status. About 21.9% (n=69/315) of the participants having SMP with antibiotics had a previous experience with the disease they treated, and this was their main reason for this behavior. The primary sources of antibiotics were those purchased from community pharmacies (70; 22.2%) and household (21; 6.7%) (Abduelkarem *et al*., 2019).

As to a cross sectional study report from Ras Al-Khaimah residents of United Arab Emirates, the prevalence of SMP among the respondents was 52.1%. A headache (155 [37.5%]) was the most common clinical condition treated through SMP. Familiarity with the treatment/medication (198 [48%]) was the most common cited reason, whereas the advertisement and friend's advice were the most (182 [44%]) cited sources of information for SM usage. The majority (265 [64.1%]) of the respondents were considered SMP as safe. However, 19 respondents reported side-effects or complications during the due course of SM.

It was observed that there was a statistically significant association ($p < 0.05$) between age and employment status of participants with SMP (Sridhar *et al.*, 2018).

In Tabriz (North-West of Iran), a descriptive-analytic cross-sectional study carried out in 2017 on the population covered by the health centers of Tabriz, indicated that the incidence of SM was 70.9% for participants who reported illness in the last month. The chance of SM was higher in young ($p=0.007$) and middle aged ($p=0.012$) groups, and housewives ($P=0.048$); and was lower among participants who were not literate ($p=0.047$). There was no significant relationship between gender and SM ($p=0.553$). The most frequently mentioned drugs used in SM were analgesics, cold medicines, and antibiotics, respectively. More frequent reasons for SM were the previous experience of the disease, the assumption that the ailment was not important, and the high cost of visits, respectively (Shaamekhi *et al.*, 2019).

In Jordan, using an internet-based questionnaire mainly spread through social media platforms, a descriptive cross-sectional study was conducted among 274 OTC product users and the results showed that analgesics were the most commonly used OTC products among the participants (50.4%). The majority used the OTC products only as needed rather than on a regular basis. Only 42.4% of the participants sought a pharmacist's help in determining the dose of the OTC medicine. Most of the participants were very interested in reading a patient information leaflet (80.3%) and the side effects and contraindications (89.5%). The majority of participants agreed that antibiotics have to be prescribed (68.5%), and anti-allergy medications should not be used as sleep aid medications (75.0%). About 53.4% thought that OTCs are sometimes enough to treat their health conditions without the need to follow-up with a physician (Taybeh *et al.*, 2020).

A community based cross-sectional study conducted in Asmara (Eritrea), in 16 selected sub-districts (response rate: 99.5%: ($N = 577$)), from September to November 2017, indicated that the prevalence of SM with antibiotics was 45.1%. The majority of participants practiced once or twice in a period of 12 months. The main reasons were previous successful experience (34.4%) and the illness being 'not serious enough to seek medical care' (25.7%). Of those who self-medicated, 84.1% of them used amoxicillin at least once. Wound infection (17.9%) and sore throat (13.9%) were the most self-recognized complaints that required SM. Antibiotics were supplied and recommended mostly by the community drug outlets. Sex ($p = 0.046$), knowledge ($p = 0.019$) and attitude ($p < 0.001$) of the participants were significantly associated with the practice of SM with antibiotics (Ateshim *et al.*, 2019).

According to Bahta *et al.* (2020), in Eritrea, the extent of dispensing antibiotics without prescription was 87.6% and the most common antibiotics were ciprofloxacin (47.8%) and co-trimoxazole (37.5%). Furthermore, 12.4% of the drug retail outlet attendants did not dispense antibiotics because they preferred a referral to health facilities (52.6%), following administrative restrictions not to sell antibiotics (42.1%) or did not have the necessary antibiotics (31.6%). Private community pharmacies (AOR = 7.68, 95% CI: 1.67, 35.37; p = 0.009) and private drug shops (AOR = 10.65, 95% CI: 1.96, 57.93; p = 0.006) were more likely to dispense antibiotics compared to the governmental community pharmacies. Dispensing antibiotics without prescription was more likely to occur in the Maekel region (AOR = 3.76, 95% CI: 1.19, 11.92; p = 0.024) compared to the remaining regions combined.

In Ghana, a community survey was conducted at two time points, 2017 and 2018, on 1,100 randomly selected households over one year with focus on antibiotic use episodes in the past month and showed that antibiotics were used in 585 (53.2%) households in the month prior to the surveys. A total of 676 (21.2%) participants out of 3,193 members from the 585 participants used antibiotics for 761 episodes of illness. Out of the 761 antibiotic use episodes, 659 (86.6%) were used inappropriately. Paying for healthcare without health insurance (Odds Ratio (OR): 2.10, 95% CI: 1.1–7.4, p = 0.026), not seeking healthcare from health centers (OR: 2.4, 95% CI: 1.2–5.0, p = 0.018), or pharmacies (OR: 4.6, 95% CI: 1.7–13.0, p = 0.003) were significantly associated with inappropriate antibiotic use (Afari-Asiedu *et al.*, 2020).

A cross-sectional study was conducted in 2016 on 1091 participants (age ≥18 years) living in five districts in peri-urban areas of Maputo City (Mozambique) and revealed that 20.9% (228/1091) were used non-prescribed antibiotics. Most of the non-prescribed antibiotics were purchased in pharmacies (199/228; 87.3%). The proportion of use of non-prescribed antibiotics was higher in those who purchased from informal markets (82.6%; 14/17) and home stores (66.7%; 12/18), compared to pharmacies (24.6%; 199/810) (p=0.000). Being male, living in the Central A, Aeroporto B or 25 de Junho neighborhoods, purchase of antibiotics in informal markets or obtaining from home stores, not completing the course and having poor knowledge on the use of antibiotics were significant (p<0.05). Main reasons for use of non-prescribed antibiotics were a perception that there was no need to attend a health facility (26.8%), followed by someone else's advice (7.7%), symptoms similar to a previous episode (6.2%) and poor quality of care in health facilities (6.7%) (Mate *et al.*, 2019).

In Lomé (Togo) a community based cross-sectional study was conducted from March to June 2017 among people aged 60 years and older and included a total of 370 participants with a median age of 65 years. Almost three elderlies in five (57.6%) were multimorbid (had two or more chronic diseases). Conventional drugs (78.4%), medicinal plants (14.3%) and other dietary supplements (9.5%) were used by participants. The prevalence of polypharmacy was 22.7% (95% CI:18.5–27.3%). Concurrent use of conventional drugs and medicinal plants or other dietary supplements was observed among 17.0% of participants and 67.3% reported SM. Multimorbidity (AOR = 4.55; 95% CI: [2.42–8.54]) and female sex (AOR = 1.86; 95% CI: [1.00–3.47]) were associated with polypharmacy (Gbeasor-Komlanvi *et al.*, 2020).

In a rural District of Kilimanjaro region (North-Eastern Tanzania), a study was done among 300 residents from April 1st to 28th 2017 and found that the overall SMP was 58%. Among the antibiotics, the most commonly used drug was amoxicillin (43%) and among the antiprotozoal drugs, metronidazole was the one used (10%). The three most common symptoms that led participants to have SMP were cough (51.17%), headache/fever/malaria (25.57%) and diarrhea (21.59%), respectively. The most common reasons for SMP were urgency illness (24%), health facility charges (20.33%), proximity of pharmacy to home (17%) and no given reason (16.66%). Almost all reported that SM is not better than seeking medical consultation, 98% can result into harmful effects and 96% can result to drug resistance (Horumpende *et al.*, 2018 (b)).

In Lilongwe (Malawi) a community based cross-sectional, mixed-methods design, study was conducted among 105 residents and found that SM with antimicrobials was a common practice. The sources of drugs were market vendors, pharmacies, drugs shared with friends and family, and those leftover from previous treatments. The lack of medical supplies, long distances to health facilities, poor attitudes of medical professionals towards patients, and past experience with the disease and treatment were the main factors that influence SM. About 74% of individuals were unable to differentiate antimicrobials from other categories of drugs, and 92.4% wrongly responded that antimicrobials could be used to stop a fever. Over 54% respondents wrongly believed that antimicrobials are effective in treating common colds. About 53% reported that they would use antimicrobials to treat upper respiratory infections (Sambakunsi *et al.*, 2019).

In Meket District (Northeast Ethiopia), a community based cross-sectional study was conducted among 722 adult household members from 5th April to 5th May 2017, and the overall prevalence of SM was found to be 35.9%. The independent variables showing an association with SM were being unmarried (AOR = 2.17, 95% CI = 1.18, 4.01), previous experience of

SM (AOR = 1.78, 95% CI = 1.22, 2.61), accessibility of pharmacies (AOR = 3.71, 95% CI = 1.31, 10.51), peer/family pressure (AOR = 2.88, 95% CI = 1.98, 4.18) and presence of medication at home (AOR = 1.80, 95% CI = 1.11, 2.92) (Kassie *et al*., 2018).

In Jigjiga town (Eastern Ethiopia), a community based cross-sectional study was conducted on 547 adult community members from 27th June to 12th July 2017 and the magnitude of SM was found to be 37.5% (95% CI: (33.6%–41.7%). Educational status of secondary school (AOR = 0.46; 95% CI: 0.22–0.98), high income (AOR =3.00; 95% CI: 1.77–5.06), advised by neighbors, friends or relatives to take drug for their complaint (AOR = 2.59; 95% CI: 1.62–4.14), used old prescription/past experience to bought drugs (AOR = 12.19; 95% CI: 6.65–22.35), follow advertisements of drugs by television (AOR = 0.21; 95% CI: 0.05–0.85), and perception about that hospital drugs (clinics, health centers and hospitals) do not work (AOR = 2.36; 95% CI: 1.39–3.99) were significantly associated with SM (Amha *et al*., 2019).

At Harar City (Ethiopia), a cross-sectional study was conducted through exit interview in selected drug outlets among 370 clients from March to April 2017. Many participants practiced SM to alleviate their headache (30.3%), to treat their respiratory disorders (29.5%), and to treat their gastrointestinal disorders (27%). The most common reasons for having SM were prior experience (57.8%) and seeking less expensive service (20.5%). About 40.3% of the participants reported pharmacy professionals were their source of information, 18.9% were advised by neighbors, friends, or relatives, but 31.9% had no source for SM. Among drug category, analgesic (42.2%) was the most frequently used. One-third (31.1%) of the participants were expected to obtain counseling from pharmacy professionals on the drug side effects and selection (Mamo *et al*., 2018).

A community based cross-sectional study was conducted among households at Gondar town (Northwest Ethiopia), from March to June 2018. The overall prevalence of SMP among households at Gondar town were 50.2%. The odds of SMP among unmarried participants (AOR=3.12; 95% CI: 2.35, 5.34), influenced by peer (AOR=3.58; 95% CI: 2.89, 7.28), poor perceived quality of health care services (AOR=4.67; 95% CI: 2.56, 7.96) and access to pharmacy (AOR=2.32; 95% CI: 1.65, 6.76) were higher compared with their counterparts. In the contrary, the lesser odd was observed among knowledgeable participants about medications (AOR=0.27; 95% CI: 0.16, 0.39) compared with non-knowledgeable (Jember *et al*., 2019).

A systematic review of SMP in Ethiopia was performed by Ayalew (2017) which included 450 cross-sectional studies. The prevalence of SM varied from 12.8 to 77.1%, with an average of 36.8%. The most common illnesses/symptoms of SM utilized were fever/headache, GIT diseases, and respiratory diseases. The major reasons for SMP were previous experience of treating a similar illness and feeling that the illness was mild. Analgesics/antipyretics, antimicrobials, gastrointestinal drugs, and respiratory drugs were the common drug classes used in SM. Mainly, these drugs were obtained from drug-retail outlets. The use of SM was commonly suggested by pharmacy professionals and friends/relatives.

A systematic review and meta-analysis of observational studies, 27 studies with 9586 participants, done in Ethiopia by Sisay *et al*. (2018), showed that the pooled prevalence of SM in Ethiopia was 44.0% (95% CI: 35.1, 52.8). Geographical-based subgroup analyses revealed that the highest prevalence was observed at the capital of Ethiopia, Addis Ababa, 62.8% (95% CI: 42.3, 83.2). Population based analyses indicated that healthcare professionals and students were the main practitioners of SM. Besides, the prevalence of SMP in pregnant women was approximately 22.9% (95% CI: 9.8, 36). The most common reasons to practice SM were previous experience of clients and/or familiarity of treatments, 31.3% (95% CI: 21.5, 41.1) and perceived mildness of the illness, 31.1% (95% CI: 26.0, 36.2). The pooled prevalence of analgesics, antimicrobial agents and GI drugs were 46.1% (95% CI: 36.2, 56.1), 28.2% (95% CI: 19.6, 36.8), and 14.9% (95% CI: 7.8, 21.9), respectively.

At Assendabo town (Southwest Ethiopia), the prevalence of SMP (modern and traditional medicines) was 39% (Suleman *et al*., 2009). Around 11.4% of the participants had at least one episode of illness within two weeks. Above 50% of the participants, improvement in their illness was reported. Mildness of illness was a major reason for SMP. Besides, 80.6% of self-medicating individuals had no information on drug adverse effects. In Jimma town, in a Southwest Ethiopia's study, the prevalence of SMP was 27.6%. Headache, and relative cheapness of SMP were the most common illness and reason, respectively (Worku and G/Mariam, 2003). Likewise, in Silte Zone (Ethiopia), the prevalence of SMP with antibiotics or antimalarials was 14.5% (Wabe *et al*., 2012). The income level and educational status of the participants primarily affected the SMP. Headache, fever, and cough were the commonest illnesses.

CHAPTER 3. OBJECTIVE

3.1 General Objective

To assess the prevalence and determinant factors of self-medication practice in selected households of three sub-cities of Addis Ababa, Ethiopia.

3.2 Specific Objectives

- To determine the prevalence of self-medication practice (SMP)
- To assess the level of knowledge of the participants about SMP
- To identify factors associated to SMP

CHAPTER 4. METHODOLOGY

4.1 Study Area and Period

The current study was conducted on selected households in Addis Ababa from April to May 2016. Addis Ababa is the capital city of the Federal Democratic Republic of Ethiopia and hub of political, economic, and cultural activities of the country. It is the headquarters of the African Union (AU), the United Nations Economic Commission for Africa (UN-ECA) and the host for multi-lateral funding organizations such as the World Bank, the European Commission, United Nations Development Program (UNDP) and others. In addition, the city also gathers over 103 embassies and heads of diplomatic missions, different international, regional and sub-regional organizations and several international Non-government Organizations (NGOs) (Addis Ababa City Administration, 2016).

As compared to other regions of Ethiopia, Addis Ababa holds the highest concentration of health care facilities and health professionals. Nurses share the highest number among the estimated 10,000 health professionals that needs to cater for health-related needs of the city's 3.3 million people (Addis Ababa City Administration, 2016).

The city is divided in 12 sub-cities and 117 woredas. There are 12 public hospitals in the city: Tikur Anbessa hospital, St. Paul's hospital, Yekatit 12 hospital, Gandhi Memorial hospital, Ras Desta hospital, Zewditu hospital, Menilik hospital, St. Peter's hospital, Tirunesh-Beijing hospital, Alert Center, Federal Police Referral and Army hospitals. In addition, there are 31 private hospitals as well as many other health facilities. The health system in the city which is also considered as the capital of Africa is a two-tier system: hospitals and health centers (Addis Ababa City Administration, 2016).

4.2 Study Design

A community based cross-sectional study design was conducted by using semi-structured questionnaires to assess the objectives of this study.

4.3 Source Population

All households of the Addis Ababa sub-cities were the source population for this study.

4.4 Study Population

The selected households of the three sub-cities and available during the data collection period were used as study population based on the sampling technique employed.

4.5 Inclusion and Exclusion Criteria

4.5.1 Inclusion Criteria

Being a resident, preferably adult household head of Addis Ababa.

4.5.2 Exclusion Criteria

Those who were under 18 years of age, mentally incompetent, unable to listen or speak well, and unwilling were not included in the study

4.6 Sample Size Determination and Sampling Technique

4.6.1 Sample Size Determination

The sample size was determined using the single population proportion formula (Cochran, 1963) with its components: d= 5%, margin of error; P = 0.39, the estimated proportion of self-medication practice in Assendabo town, Ethiopia (Suleman *et al.*, 2009) present in the sample; $\frac{Z\alpha}{2}$ = 1.96, the value found in the statistical table that contains the area under the normal curve at 95% confidence level; 5% non-response rate.; and design effect for using multi-stage sampling taken as 1.5.

The sample size was as follows: Where $no = \text{sample size of the study}$

$$no = \frac{(Z\alpha/2)^2 \mathrm{P}\,(1-\mathrm{P})}{d^2} * de$$

$$no = \frac{(1.96)^2 * 0.39(1-0.39)}{(0.05)^2} * 1.5$$

$$no = 549$$

When consider the 10 % contingency, the minimum and final sample size was 604 residents.

4.6.2 Sampling Technique

For this study, a multistage sampling technique was used. Three Sub-cities of Addis Ababa (Gullele, Arada and Ldeta) were selected by lottery method and the sample size was apportioned based on the number of the households in each Sub-city. Woredas were selected from the Sub-cities using simple random sampling. Then, the sample allocated for each woreda was apportioned to the Woredas in the Sub-city depending on the number of households in each woreda. The households in the woreda to be included in this study were selected by simple random sampling. The house number of the households was used as a

sampling frame. If one household were unwilling to participate, the next random sample would be taken.

Table 1. Allocation of the study participants from each of the selected woredas

Sr. No.	Sub-city	Woreda	Total number of households in the woreda	Sample obtained
1	Gulele	10	4366	46
		3	5256	55
		9	6470	68
		8	6349	66
2	Arada	6	5810	64
		7	5020	55
		10	3123	35
		1	2950	33
3	Ldeta	5	5874	56
		1	4500	43
		4	5328	51
		7	3314	32

4.7 Data Collection Procedure

Ten data collectors, who were health extension workers, highly familiarized with the households for other unrelated issues such as health education and health services in the sub-cities, and with culturally accepted and advanced health communication skills in local languages were recruited. Before data collection, adequate training was given for all data collectors on the objectives of the study, significance and how to make an effective interview with the study participants using the questionnaire. A structured questionnaire was employed to gather the necessary information on socio-demographic characteristics, prevalence, and determinants of self-medication practice.

4.8 Variables

4.8.1 Independent Variables

- The **sociodemographic variables** (such as: age, marital status, and ethnicity),
- The **self-medication practice assessing questions** (such as: Source of information, ways of request the drug, reason of self-medication, perceived outcome of your self-medication and symptom/disease that led to your self-medication).

- The **knowledge assessing questions** (such as: discontinue taking drugs before the date advised, check expiry date of drugs during purchasing or before use, do you know that some drugs could not be given with other drugs?) for the details on the variables, refer annex I (data collection tool: questionnaire).

4.8.2 Dependent Variable

- Self-medication practice status (yes or no).

4.9 Operational Definition

- **Traditional medicine.** The use of different types of traditional medicine practices such as using plant materials, animal products and/or religious practices (use of holy water for drinking and washing, use of soil or mud from a religious site, etc.).
- **Self-Medication (SM).** The self-reported treatment of common health problems by the study participants with modern and/or traditional medicines without direct medical or traditional healer supervision or intervention (further it might include consultation with pharmacists and extensive use of previous prescription drugs in the last two months).
- **Knowledge on appropriate Self-Medication Practice (SMP)**: Respondent was considered as knowledgeable if the respondent answered 50% or more of the knowledge questions correctly which were associated with appropriate self-medication practice.
- **Self-Medication Practice (SMP):** Action by which a study participant suspects his/her illness and takes a drug (modern or traditional) in the last two months without prescription from a physician.
- **Physician**: Any person who is medically qualified to prescribe medications.

4.10 Data Analyses

Before the actual data analyses, the data was explored for its completeness, outliers, and missing values. Data was coded, entered and analyzed using SPSS statistical package (SPSS Inc, Chicago, IL, software version 21). Descriptive statistical analyses (frequency, percentage, and mean) was computed to describe findings using tables, and graphs were plotted. To identify the independent variables association with the dependent variable (SMP), simple and multiple binary logistic regression analyses were done. In the simple binary logistic regression,

all factors with p-value <0.25 were considered as candidates for the multiple binary logistic regression. In all statistical tests, association of variables was significant if p was less than or equal to 0.05.

4.11 Data Quality Control

The pre-structured questionnaire initially formulated in English was translated to Amharic and pre-tested for its accuracy, completeness, and consistency two weeks prior to the actual data collection on 31 individuals (5% of the sample size) at Kolfe Keranio Sub-city, a non-study area. Furthermore, the coordinator and principal investigator observed and gave feedback to the data collectors on a daily basis. Completeness, accuracy, and clarity of the collected data were checked carefully by the principal investigators and any error was addressed on the following day before starting next day activities.

4.12 Ethical Clearance

The proposal was reviewed for any unethical issues by the Institutional Review Board (IRB) of Saint Paul's Hospital Millennium Medical College (SPHMMC). Data was collected after the sub-cities, and woredas administrators were officially communicated. During the data collection process, all participants' ethical issues, privacy, confidentiality, beneficence, and justice were adhered according to IRB guidelines. Participants' information like name and address were not recorded and coding was used in the questionnaires. All data was exclusively used for the purpose of the current research. Written consent was obtained from each one of the participants and all were clearly informed that they had the right not to participate if they were unwilling.

CHAPTER 5. RESULTS

5.1 Socio-demographic characteristics of study participants

A total of 604 participants were involved in this study. Of the total, 235 (38.9%) were from Gulele sub-city (68 from woreda nine, 66 from woreda eight, 55 from woreda three and 46 from woreda ten), 187 (31.0%) were from Arada sub-city (64 from woreda six, 55 from woreda seven, 35 from woreda ten and 33 from woreda one), and the rest, 182 (30.1%) were from Ldeta sub-city (56 from woreda five, 51 from woreda four, 43 from woreda one and 32 from woreda seven).

The different socio-demographic characteristics of study participants are presented in Table 2. Most of the study participants (69.9%) were female. One hundred and eighty-two (30.1%) of the study participants were in the age group of 25-34 and the mean (±SD) age of respondents was 41.04 (±13.45). The minimum and maximum ages of the participants were 18 and 84, respectively (the range was 66). Three hundred and fifty-eight (59.3%) of study participants were followers of Orthodox Christianity, (26.7%) were Muslim and (11.9%) were Protestant Christians. Two hundred and twenty-one (36.6%) of study participants were Amhara followed by Oromo (26.5%) and Gurage (15.7%) in their ethnicity. Most of the study participants (67.5%) were married. The mean (±SD) monthly income of study participants was 1654.36 (±1284.94) birr. Two hundred and forty-five (40.6%) of the study participants earned more than 1500 birr in a month. Two hundred and three (33.6%) of study participants had attended higher education, followed by secondary education (27.8%) and being able to read and write but having no formal education (24.7%). The occupation of two hundred and thirty-one (38.2%) of study participants was employment in private organization followed by government employee and being a house-wife (25.8 and 16.2%, respectively). The household responsibility of majority of study participants was being mother (56.8%) followed by being a father (23.7%).

Table 2. Socio-demographic characteristics of study participants at Addis Ababa from April to May 2016

Variable	Frequency	Percent
Sub-city		
Gulele	235	38.9
Arada	187	31.0
Ldeta	182	30.1
Sex		
Female	422	69.9
Male	182	30.1
Marital status		
Married	408	67.5
Single	109	18.0
Separated	44	7.3
Divorced	43	7.1
Age (years)		
18-24	32	5.3
25-34	182	30.1
35-44	157	26.0
45-54	131	21.7
≥ 55	102	16.9
Religion		
Orthodox Christian	358	59.3
Muslim	161	26.7
Protestant Christian	72	11.9
Others*	13	2.2
Ethnicity		
Amhara	221	36.6
Oromo	160	26.5
Gurage	95	15.7
Tigray	78	12.9
Silte	28	4.6
Others**	22	3.6
Academic status		
Illiterate	72	11.9
Read and write but no formal education	149	24.7
Primary education	12	2.0
Secondary education	168	27.8
Higher education	203	33.6
House hold responsibility		
Mother	343	56.8
Father	143	23.7
Child	82	13.6
Relative	36	6.0
Occupation		
Private employee	231	38.2
Government employee	156	25.8
House-wife	98	16.2

Private owned job	56	9.3
Student	25	4.1
Unemployed	25	4.1
Pension	13	2.2
Monthly income (birr)		
< 500	135	22.4
500-1000	139	23.0
1001-1500	85	14.1
> 1500	245	40.6

*Catholic, Johova's witness; **Hadya, Hadere, Kambata, Sidama, Wolayta

5.2 Self-medication practice

The finding of this study revealed that from the total 604 study participants, 456 (75.5%) practiced SM. The source of information for those study participants who are users of modern medicine for SM is presented in Table 3. Advice from a health professional but without prescription covered 45.4%. Other sources of information included experience of previous treatment (21.4%), a friend's advice (16.4%), self-decision (12.5%), and reading information in books or on the internet (4.2%).

This study has also assessed the kind of source of modern medicine in case of those respondents who used modern medicine for SM. The finding indicated that the source was drug retail outlets (pharmacy and drug store) in the majority (83.3%) of the cases. Other sources which constituted 6.8, 6.5 and 3.4%, respectively, included from a neighbor, remnants from previous treatment and other sources like a friend, supermarket, a shop, and from a health professional (Table 3).

Regarding the way of request of drug in case of those who obtained drugs from drug retail outlets, the majority (54.9%) pointed out mentioning the name of the drug, followed by disclosing any signs and symptom of their illness to the pharmacy professional (26.3%), providing a piece of paper with the name of the drug (11.3%) and taking a drug container to the drug retail outlet (7.5%) (Table 3).

When asked about the ailment that initiated the respondent to practice SM, the majority mentioned headache (25.7%). Abdominal pain and cough were the second and third most common causes of morbidity, with a frequency of 59 (12.9%) and 54 (11.89%), respectively. Other episodes of illness included diarrhea 43 (9.4%), toothache 39 (8.6), and stomach pain 29 (6.4%) (Table 3).

Table 3. Practices related to SM in Addis Ababa residents from April to May 2016.

Variable	Frequency	Percent
Self-Medication status		
Those Practicing SM (Yes)	456	75.5
Those did not practice SM (No)	148	24.5
Source of information about modern medicine		
Health professional but without prescription	174	45.4
Experience from previous treatment	82	21.4
Friend	63	16.4
Self-decision	48	12.5
Book/internet	16	4.2
Source of modern medicines		
Pharmacy/drug store	319	83.3
Neighbour	26	6.8
Remnant from previous treatment	25	6.5
Others*	13	3.4
Way of requesting medicines from pharmacy/drug store		
Mentioning the name of the drug	175	54.9
Telling symptoms of disease to a pharmacy professional	84	26.3
A piece of paper with the name of the drug	36	11.3
Taking the drug container	24	7.5
Type of illness that become reason for self- medication		
Headache	117	25.7
Abdominal pain	59	12.9
Cough	54	11.8
Diarrhea	43	9.4
Toothache	39	8.6
Stomachache (dyspepsia)	29	6.4
Hypertension	13	2.9
Fever	11	2.4
Eye disease	11	2.4
Constipation	9	2.0
Asthma	5	1.1
Combination of illnesses**	27	5.9
Others***	39	8.9

*Friend, shop, supermarket and health professional but by the request of respondent.

**Fever and cough, cough and headache, headache and abdominal pain, urinary tract infection and pain, cough and joint pain, toothache and abdominal pain, disease of thyroid gland and diarrhea, headache and dyspepsia, headache and eye disease, headache and hypertension, diarrhea and headache.

***Common cold, *diabetes mellitus*, urinary tract infection, sore throat, tonsillitis, joint disease, eye disease, malaria, sinusitis, dysmenorrhea, 'mich', skin disease, heart disease, typhoid fever.

5.3 Reasons for not practicing SM

Out of 604 participants, 148 (24.5%) participants did not practice SM because of the following reasons: fear of using a wrong drug (25.7%), fear of side effects of drugs (23%), fear of wrong diagnosis of their illness (18.2%), fear of wrong use of drugs (16.9%), and absence of any ailment in the past two months (16.2%) (Figure 1).

Figure 1. Study participant reasons for not practicing SM.

5.4 Drug groups /drug used for SM Practice

Out of those respondents who practiced SM, the majority (66.9%) had used modern medicine followed by both modern and traditional medicine (17.1%) and traditional medicine (16.0%) (Figure 2).

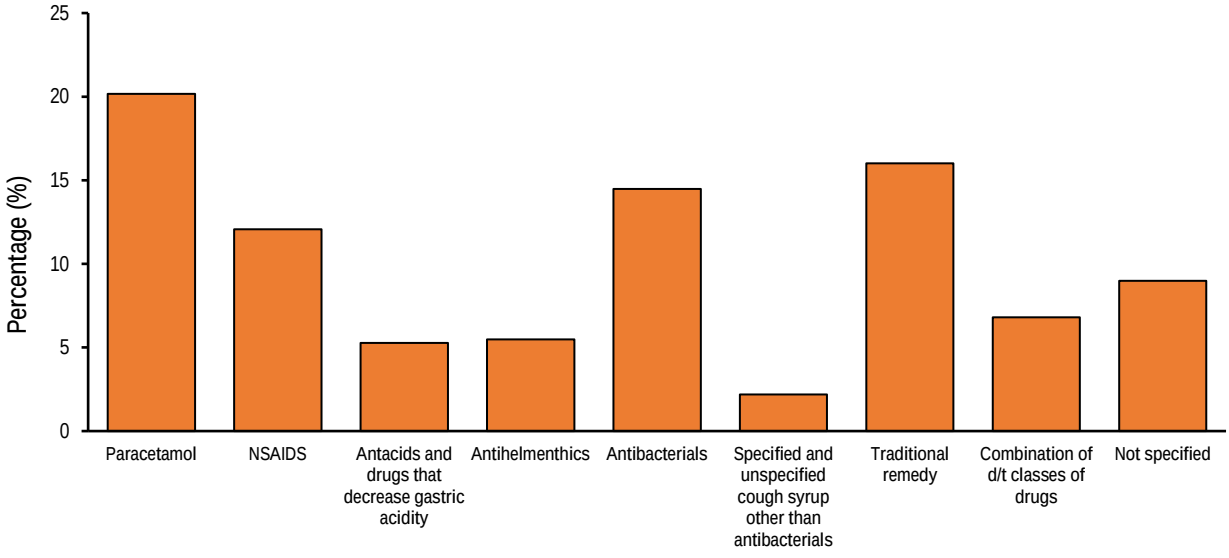


Figure 2. Frequency of drug/drug groups used among SM practiced study participants.

Ninety-two (20.2%) of those participants who practiced SM were known to use paracetamol. Other medications used were traditional remedies constituting 73 (16%), followed by antibacterials 66 (14.5%), NSAIDs 55 (12.1%), anti-helminthes 25 (5.5%), and anti-acids and drugs that decrease gastric acidity 24 (5.3%).

Analyses done to determine the most frequently used individual drugs indicated that paracetamol is used by a 20.2% of those who practiced SM. Other drugs that were commonly used for the practice included traditional remedies, diclofenac, amoxicillin, metronidazole, and albendazole, constituting 16.0, 9.9, 6.6, 4.4 and 2.4%, respectively. A significant proportion (31.1%) of drugs used for SM was in the category of prescription drugs (Table 4).

Table 4. Most commonly used individual drugs for self-medication and prescription category of the drugs used in Addis Ababa from April to May 2016.

Drug/Drug type	Frequency	Percent
Paracetamol	91	20.0
Traditional remedies	73	16.0
Diclofenac	45	9.9
Amoxicillin	30	6.6
Metronidazole	20	4.4
Anti-acids	12	2.6
Albendazole	11	2.4
Mebendazole	9	2.0
Omeprazole	9	2.0
Ciprofloxacin	7	1.5
Niclosamide	6	1.3
Others*	143	31.4
Prescription category of drugs used for SMP		
OTC drug	162	35.5
Prescription drug	142	31.1
Traditional remedies	73	16.0
The drug used not specified	50	11.0
Combination of both prescription and OTC	29	6.4

*Indomethacin, ibuprofen, ampicillin, insulin, chloroquine, tinidazole, vitamin B, ketoconazole, acetylsalicylic acid, hyoscine, chloramphenicol eye drop, tetracycline eye drop, salbutamol, dextromethorphan, metformin, amlodipine, ranitidine, hydrochlorothiazide, prednisolone, almetamine, oral rehydration salt, combination of drugs.

5.5 Reasons for SM Practice

Reasons for practicing SM were analyzed and shown in Table 5. Among the most common reasons for SMP were perception of mildness of illness 216 (47.4%), previous knowledge about the medication 106 (23.2%), and urgency use of the drug 48 (10.5%).

Table 5. Reasons of study participants for practicing SM in Addis Ababa from April to May 2016.

Reason for SMP	Frequency	Percent
Minor illness	216	47.4
I know the drug before	106	23.2
Urgency case	48	10.5
Time constraint	28	6.1
Self-medication is cheap	25	5.5
I believe visiting health facility has nothing to add	10	2.2
Long wait at health facilities	10	2.2
Previous health institution visit has not produced any benefit	10	2.2
Health facility too far	3	0.7

5.6 Treatment outcomes of SM Practice

Two hundred and seventy-five (45.5%) of those who practiced SM reported finding relief from the illness. Seventy-nine (13.1%) reported no improvement. Seventy-two (11.9%) said they were completely cure of their illness (Figure 3).

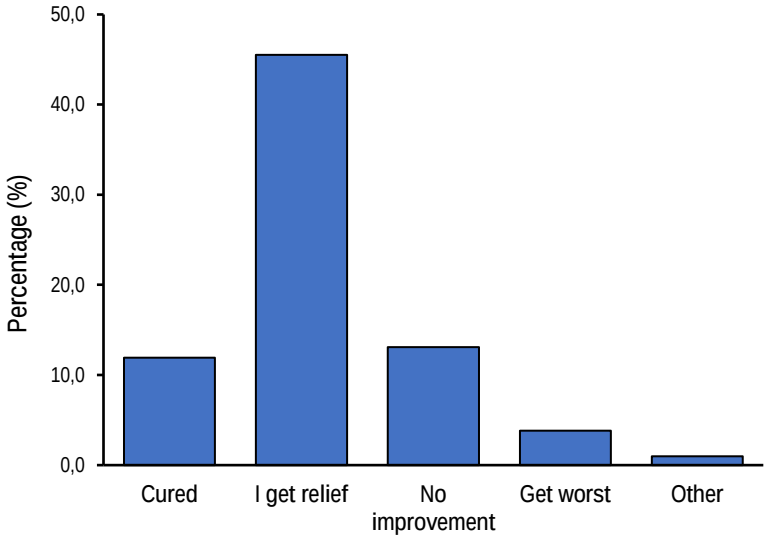


Figure 3. Treatment outcomes of SMP of study participants.

5.7 General Status of Participants during SM Practice

Those study participants who practiced SM were asked about presence of any special physiological and/or pathological condition during SM. The majority (64.7%) had no special condition. Fifty-four (11.8%) were nursing mothers. Twenty-nine (6.4%) were pregnant

during SM. The rest (17.1%) had reported presence of chronic disease like hypertension, diabetes mellitus, HIV, and kidney disease (Figure 4).

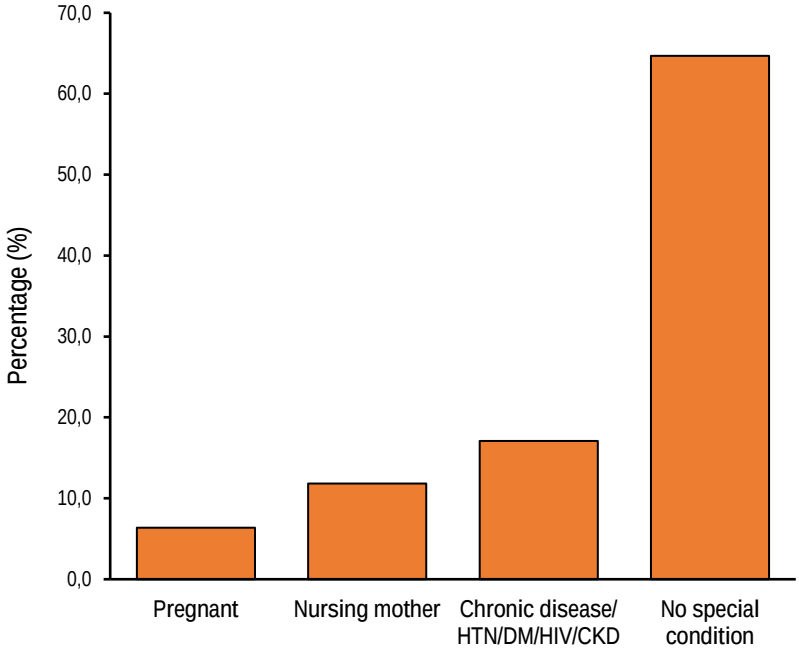


Figure 4. General status of SM practiced study participants.

5.8 Knowledge about Appropriate SM Practice

Thirteen questions were asked in order to assess the knowledge of the respondents about appropriate SM. Three hundred and seventy-nine (62.7%) respondents said they knew some drugs should not be taken with other drugs simultaneously. Four hundred and eighty-five (80.3%) said they knew the drugs that should not be taken with alcoholic drinks. Three hundred and seventy-four (61.9%) reported they had adequate knowledge on the fact that some drugs should not be taken with certain food items. When asked whether the study participant knew which drugs should or should not be given to children, four hundred and forty-eight (74.2%) said they knew which type of drugs they are dealing with (Table 6). Four hundred and forty-one (73%) and four hundred and thirty-two (71.6%) study participants reported they knew which drugs should not be taken by pregnant and nursing mothers, respectively.

Three hundred and thirty-three (55.1%) study participants said they were aware of availability of drugs that should not be taken by patients having chronic diseases. Four hundred and twelve (68.2%) study participants reported they had no habit of discontinuing

drug intake before the date advised by the health care provider. The majority of study participants (81%) said they had no habit of taking drugs with alcoholic drinks. When asked to participants whether or not they had a habit of sharing drugs with family members, friends and/or neighbors, the majority (74.7%) answered they were not in the habit of sharing (Table 6). Study participants were asked about their belief regarding whether a same drug can be a remedy and a poison. The majority (60.81%) said they believe a drug can be a remedy and a poison. Three hundred and eighty-five (63.7%) of study participants said they had habit of checking the expiry date of a drug before purchase and consumption (Table 6).

Table 6. Knowledge of study participants about appropriate SMP in Addis Ababa from April to May 2016.

Knowledge questions	Yes		No	
	N	%	N	%
Do you know availability of drugs that should not be simultaneously taken with other drugs?	379	62.7	225	37.3
Do you know availability of drugs that should not be taken with alcoholic drinks?	485	80.3	119	19.7
Do you know availability of drugs that should not be taken with certain types of food items?	374	61.9	230	38.1
Do you know availability of drugs that should not be given to children?	448	74.2	156	25.8
Do you know availability of drugs that should not be given to pregnant women?	441	73.0	163	27.0
Do you know availability of drugs that should not be taken by patients having chronic disease?	333	55.1	271	44.9
Do you know availability of drugs that should be taken by nursing mothers?	432	71.6	171	28.4
Do you know any drug that can be available in different dosage forms?	483	80	121	20
Have you the habit of discontinuing drug intake before the date advised by the health care provider?	132	31.8	412	68.2
Have you the habit of drinking alcohol while taking drugs?	115	19.0	489	81.0
Do you share drugs with family members, friends and neighbours?	153	25.3	451	74.7
Do you believe that a same drug can be a remedy and a poison?	367	60.8	237	39.2
Do you have the habit of checking expiry date of drugs during purchase or before use?	385	63.7	219	36.3

5.9 Level of Knowledge towards SM Practice

The level knowledge of respondents about appropriate SMP was computed from the thirteen questions. The minimum and maximum scores to the questions were 1 and 13, respectively. The mean knowledge score was 9.07 with a standard deviation of 2.6. Finally, the overall knowledge category of study participants indicated that the majority of study participants (83.4%) had good knowledge about appropriate SM (Figure 5).

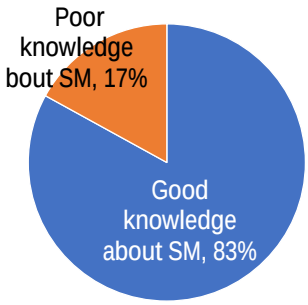


Figure 5. Knowledge of study participants about appropriate SMP.

5.10 Attitude towards SM Practice

Study participants were asked to reveal their attitude towards SMP at the time of illness. The majority (51%) said they could agree or disagree depending on the condition and/or disease of the patient. One hundred and ninety-seven (32.6%) agreed on the practice of SM without any condition. A very small proportion (2%) said they had no comment (Figure 6).

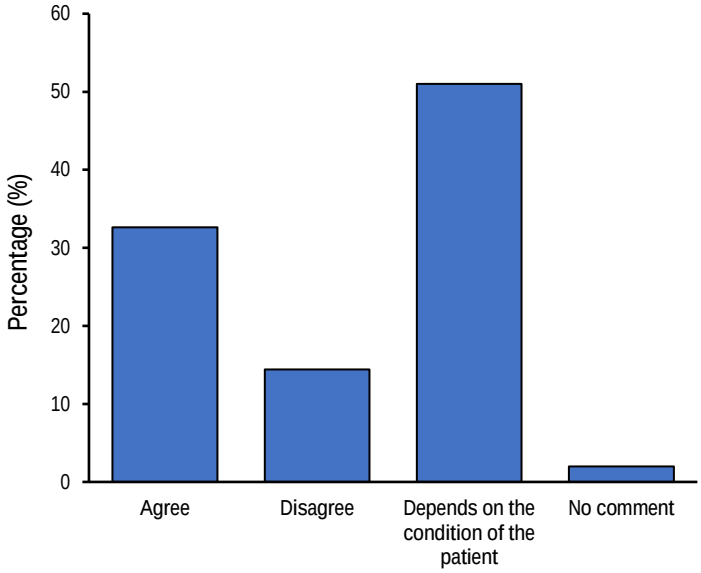


Figure 6. Attitude of study participants towards SMP.

5.11 Factors affecting self-medication practice

To identify factors affecting SMP, model chi-squares were utilized. According to the predicted probabilities, logistic regression was fitted. On binary logistic regression analyses, the study participant's income, age, and knowledge about appropriate SMP were associated with SMP. Those study participants at the age group of 25-34 were 0.52 times less likely to practice SM than those who were at the age group of ≥ 55 years (COR = 0.52[95% C.I:0.29-0.92]). Those study participants having a monthly income of 500-1000 birr were 1.67 times more likely to practice SM than those who had a monthly income of >1500 birr (COR = 1.67(95% C.I:1.05-2.65]). On the contrary, those study participants whose monthly income was 1001-1500 birr were 0.40 times less likely to practice SM than those whose monthly income was >1500 birr (COR = 0.40[95% C.I:0.19-0.84]) (Table 7).

This study also indicated that those study participants who had poor knowledge about appropriate SM were 1.97 times more likely to practice SM than those who had good knowledge (COR = 1.97[95% C.I: 1.24-3.12]) (Table 7). Finally, after obtaining statistically significant variables at $p \leq 0.05$ in binary logistic regression analyses, multivariable logistic regressions were carried out to see the independent predictors of SMP of respondents. After adjusting for potential confounders, the monthly income of respondent and knowledge about appropriate SMP were found to be independent predictors of SMP. Accordingly, those study participants whose monthly income was 1001-1500 birr were less likely to practice SM than those whose income was >1500 birr (AOR =0.34[95% C.I:0.16-0.74]). Those study participants who had poor knowledge about appropriateness of SM were 2.04 times more likely to practice SM than those who had good knowledge (AOR = 2.04[95% C.I:1.24-3.03]) (Table 7).

Table 7. Factors affecting SMP in Addis Ababa, from April to May 2016.

Variables	SMP				Odds ratio	
	Yes		No		Crude	Adjusted
	N	%	N	%		
Age (years)						
18-24	20	3.31	12	1.99	1.51(0.66-3.48)	1.55(0.65-3.71)
25-34	151	25.00	31	5.13	0.52(0.29-0.92)*	0.55(0.30-1.03)
35-44	125	20.70	32	5.30	0.64(0.36-1.15)	0.69 (0.37-1.26)
45-54	87	14.41	44	7.29	1.27(0.72-2.24)	1.34(0.74-2.41)
≥55	73	12.09	29	4.80	1	1
Monthly income (birr)						
<500	98	16.22	37	6.13	1.27(0.79-2.06)	1.00(0.59-1.69)
500-1000	93	15.39	76	12.58	1.67(1.05-2.65)*	1.4(0.85-2.29)
1001-1500	76	12.58	9	1.49	0.40(0.19-0.84)*	0.34(0.16-0.74)*
>1500	189	31.29	56	9.27	1	1
Sex						
Female	325	53.81	97	16.06	1.30(0.88-1.94)	0.75(0.49-1.17)
Male	131	21.69	51	8.44	1	1
Knowledge status of respondent						
Good knowledge	392	64.90	112	18.54	1	1
Poor knowledge	64	10.59	36	5.96	1.97(1.24-3.12)*	2.04(1.24-3.33)*

*There is statistically significant association ($p \leq 0.05$).

CHAPTER 6. DISCUSSION

This study attempted to assess SMP among Addis Ababa community residents and further analyze different associated factors that affect the practice such as, illness for which drug was used, the pharmacologic category of drug utilized, the reason to practice SM, and perceived outcome of the SM.

The prevalence of SM in this study was found to be 75.5%. The three most frequently reported ailments were headache 117 (25.7%), abdominal pain 59 (12.9%), and cough 54 (11.8%), respectively. The two main reasons for SM were mildness of illness 216 (47.4%) and previous knowledge about the drug 106 (23.2%). Paracetamol 92 (20.2%) and traditional remedies 73 (16%) were the two most frequently consumed medications.

According to the findings of this study, 75.5% of the study participants had practiced SM. This is by far greater than those findings reported from other Ethiopian parts, Gondar town (Northwest Ethiopia) (50.2%) (Jember *et al*., 2019), Jigjiga town (37.5%) (Amaha *et al*., 2019), Harar City (Eastern Ethiopia) (57.8%) (Mamo *et al*., 2018), Meket district (Northeast Ethiopia) (35.9%) (Kassie *et al*., 2018), three towns of North West Ethiopia, (Teferra and Alemayehu, 2001), Sire town (27.26%) (Jaleta *et al*., 2016), Nekemte town (36.7%) (Sado and Gedi, 2014), Jimma town (27.6%) (Worku and G/Mariam, 2003), Assendabo town (39%) (Suleman *et al*., 2009), and Kolladiba town (62.8%) (Abraha *et al*., 2014).

SMP is also more prevalent in this study in comparison to reports from students of Mekelle University (42.67%) and students from the University of Gonder (38.5%) (Abay and Amelo, 2010; Girma *et al*., 2011). However, in-line with SM practice of Pharmacy students from Rift Valley University, Abichu campus (Addis Ababa, Ethiopia) (72.8%) (Beyene *et al*., 2017), Private University Malaysia (77.6%) (Ali *et al*., 2012), Egypt (62.9%) (Helal and Abou-ElWafa, 2017), and Karachi (Pakistan) (76%) (Zafar *et al*., 2008).

Still, it is higher than some community studies done in Pokhara valley of Nepal (38.2%) (Paudel and Aryal, 2020), Ras Al-Khaimah, United Arab Emirate (52.1%) (Sridhar *et al*., 2018), Kilimanjaro region, North-Eastern Tanzania, (58%) (Horumpende *et al*., 2018 (b)), Assam (57.6%) (Hiyeswar *et al*., 2016), Barabanki (69.6%) (Keshari *et al*., 2014), and Iraq (53.4%) (Nadia *et al*., 2016). In a systematic review and meta-analysis of observational studies in Ethiopia by Sisay *et al*. (2018), geographical-based subgroup analyses revealed that the highest prevalence was observed at the capital of Ethiopia, Addis Ababa, 62.8%.

This study prevalence finding is more or less in line with studies conducted in Limmu Genet (Jimma Zone, Southwest, Ethiopia) (78.1%) (Bekele *et al*., 2018), Vietnam (83.3%) (Ha *et al*., 2019), Tabriz (North-West of Iran) 70.9% (Shaamekhi *et al*., 2019), Khartoum (Sudan) (81.8%) (Awad *et al*., 2006) and Sudan (73.9%) (Awad *et al*., 2005). Further, a systematic review on SMP in Ethiopia, all studies with cross-sectional design, done by Ayalew (2017) revealed that the prevalence of SM varied from 12.8 to 77.1%. Therefore, the present study finding on prevalence of SM is also in agreement with the maximum practice of SM in the country, Ethiopia.

This variation might be due to the difference in the study population, the fact that those study participants who used traditional remedies including herbs for SM were included as practitioners of SM in the current study and availability of a plethora of information and drug retail outlets in the capital city of Addis Ababa. Various studies conducted in different locations of the world have shown a range of SM practices between 8.3 to 87% (James *et al*., 2006; Pagan *et al*., 2006; Verma *et al*., 2010; Hussain *et al*., 2011; Gupta *et al*., 2011; Ali *et al*., 2012; Correa Da Silva *et al*., 2012; Wijesinghe *et al*., 2012; Wabe *et al*., 2012; Jalilian *et al*., 2013).

Higher period prevalence has been reported in most of the developing countries. This has been associated with several factors, particularly the lack of access to healthcare, availability of antibiotics as OTC drugs, and the relatively higher prevalence of infectious diseases (Begashaw *et al*., 2016). Prevalence of SM varies from region to region, with its rate of use being driven by societal norms, personal traits, pharmacy staff, the regulatory policies of the country, social determinants of health, beliefs, culture of the population, and variation on recall periods used in each study (Jember *et al*., 2019; Eticha and Mesfin, 2014; Jha *et al*., 2013; Hughes *et al*., 2001; Geissler *et al*., 2000).

The reasons for this wide variation might be the differences in socio-demographic characteristics of the study participants including academic status, non-availability of medical facilities in some areas, easy availability of drugs, and types of drugs that the study intends to identify.

The findings of this study indicated that there is a statistically significant association between monthly income and SMP. Those study participants whose monthly income was 1001-1500 birr were less likely to practice SM than those whose monthly income was > 1500 birr (AOR = 0.34 (95% C.I:0.16±0.74). This finding is contrary to a community study carried out in Jigjiga town, Eastern Ethiopia which showed that high income was associated with SMP

(Amaha *et al*., 2019), among Vietnamese residents in highland provinces (Ha *et al*., 2019) and also similar with the study conducted in China in four consecutive China National Health Surveys (CNHS) (Yuefeng *et al*., 2012). Further, the current study differs from a study from Kolladiba in which female respondents (70.1%), married respondents (65.2%), respondents with the level of secondary education (35.4%), respondents who were merchants (34.7%) and respondents who had very low income (49.4%) have been found to practice SM more than their counterparts from their respective categories (Abraha *et al*., 2014).

The findings of the current study are hardly close to those from a study from Sudan where the middle-income group has found to be more self-medicating and from a study in Sire town where no association was obtained between SMP with sex, educational status, occupation, and income (Jaleta *et al*., 2016; Awad *et al*., 2005). Unlike to the current work, a study done in Jordan by Gogazeh (2020) revealed that financial problem was the major reason behind SMP (4.72 out of 5). The reason might have been that the low-income group in this current study could not afford the cost of medication, that the city administration would provide free medical service for those who could not afford the cost of health care, and because of differences in categorization of income categories.

The current study indicated that three hundred and five (50.5%) study participants had SMP with modern medicines. The rest used either traditional medicine alone or in combination with modern medicine for SM purpose. This finding is almost comparable to the finding from Kolladiba town where 51.8% of study participants used modern drugs for SM (Abraha *et al*., 2014) and the SM practice of traditional remedies (16%) of this study is also comparable to a study done among community dwelling elderly people in Lomé (Togo) (14.3%) (Gbearsor-Komlanvi *et al*., 2020). However, in a study conducted in Western Nepal, only 8.7% of participants practiced SM with herbal remedies (Shankar *et al*., 2002) and also only 1% of participants practiced SM using herbal remedies at Limmu Genet (Jimma Zone, Southwest, Ethiopia) (Bekele *et al*., 2018). The very large magnitude of involvement of traditional medicine for SM in our study and the Kolladiba study indicate a great deal of attention needed to be given to this area of medicine in the country.

The kind of sources of information on modern drug used for SM in this study was health professionals advice without prescription (45.4%) and it is similar to that mentioned in studies conducted among communities in Addis Ababa residents (Bogale *et al*., 2019), Gondar town (Northwest Ethiopia) (Jember *et al*., 2019), Limmu Genet residents (Jimma Zone, Southwest Ethiopia) (Bekele *et al*., 2018), Pokhara Valley of Nepal (Paudel and Aryal, 2020), in all Jordan regions (North, Middle, and South) (Gogazeh, 2020), United Arab Emirates

residents (Abduelkarem *et al*., 2019), and also in Mekelle town, health science students from University of Gonder, students from Mekelle University, on the urban slum community of India, residents from Assendabo town, and in many others studies (although it differs in magnitude of the source) (Suleman *et al*., 2009; Abay and Amelo, 2010; Girma *et al*., 2011; Gupta *et al*., 2011; Eticha and Mesfin, 2014).

This study finding is different from studies done at Punjab (Pakistan) (Aziz *et al*., 2018) and Ras Al-Khaimah (United Arab Emirates) (Sridhar *et al*., 2018) in which media advertisements were the most common source of information for SM practice for 46.7 and 44% of the participants, respectively. Differently, in Jigjiga Town (Eastern Ethiopia) the main source of information for practicing SM was some advice from neighbors, friends or relatives to take drug for their complaint 138 (67.3) (Amaha *et al*., 2019).

Drug retail outlets were identified as the main source of modern medicine used for SM in this study. This study finding is similar to previous studies done in Ethiopian towns such as Sire, Mekelle and Kolladiba (Abraha *et al*., 2014; Eticha and Mesfin, 2014; Jaleta *et al*., 2016). Drug retail outlets were also the main source of modern medicine used for SMP in Asmara (Eritrea) (Ateshim *et al*., 2019), and Maputo City (Mozambique) (Mate *et al*., 2019).

Moreover, significant proportions (31.1%) of modern drugs used for SM in this study were prescription only drugs among the total self-medicated drugs. The reasons for this might be a weak enforcement of regulations regarding drug handling and dispensing (Suleman *et al*., 2009). The main reasons for SM of antibiotics in developing countries include OTC sale of antibiotics, high cost of medical consultation, low satisfaction with medical practitioners, and misconceptions regarding the efficacy of antibiotics (Abay and Amelo, 2010) and poor perceived quality of health care (Jember *et al*., 2019).

This study also indicated the possibility of obtaining drugs from neighbors and through remnants from previous treatment which implies the use of low-quality drugs, development of resistance to antimicrobials, and treatment failures. Delivering health education on the appropriate use of drugs to the community may alleviate these problems.

In this study, the most common types of ailments that led to SM were headache followed by abdominal pain, cough, diarrhea, and toothache. These study results are similar to the findings from community studies performed at different Ethiopian parts as with a study done at Gondar town (Northwest Ethiopia) which found the common ailments that led to SMP were headache (63.1%), respiratory tract infections (cough, cold/flu) (30.9%), fever (18.9%), GI infections (diarrhea, vomiting) (38.9%) and dysmenorrhea (7.6%) (Jember *et al*., 2019);

in Jigjiga town (Eastern Ethiopia): GI disease, fever, eye and skin infection, urinary tracts infection, cough/cold and other respiratory tract infection, and headache (Amaha *et al.*, 2019); in Limmu Genet town (Jimma Zone, Southwest Ethiopia): cough 54 (22.4%); fever 52 (21.7%); diarrhea 51 (20.7%); headache 36 (14.8%) (Bekele *et al.*, 2018); in Meket District (Northeast Ethiopia): headache/fever 217 (30.1%), respiratory tract infection 122 (16.8%), joint pain 78 (10.8%) and eye pain 73 (10.1%) (Kassie *et al.*, 2018).

Additionally, a study done among the general population of Ras Al-Khaimah (United Arab Emirates), the common symptoms/illnesses treated by SMP were headache (37.5%); fever (28.8%); cold/cough (21.5%); gastric problem (15.7%) and constipation (13.1%) (Sridhar *et al.*, 2018).

In a study conducted a few years ago among residents of Addis Ababa, the three most commonly reported illnesses for which study participants sought SM were GI disease, headache/fever and respiratory problems (Andualem and Gebre-Mariam, 2004). A similar study conducted in Sire town reported headache, cough and cold, and diarrhea as the three most commonly occurring illnesses that led to SM (Jaleta *et al.*, 2016). Headache and fever, respiratory tract infections and gastrointestinal disorders were also the three most common ailments that became reasons for SM in the Kolladiba study (Abraha *et al.*, 2014). More or less similar ailments with different magnitude were mentioned as reasons for practicing SM in students from Mekelle University, residents of Assendabo town, University of Gonder students, residents of Jimma town, urban slum areas of India, university students of Brazil, and in many communities (Suleman *et al.*, 2009; Gupta *et al.*, 2011; Worku and G/Mariam, 2003; Abay and Amelo, 2010; Girma *et al.*, 2011; Hussain *et al.*, 2011; Shankar *et al.*, 2002). Thus, as reported by the different findings, headache and cough were the most common ailments for which study participants would get treatment via SM.

The most commonly employed drug/drug classes for SM in this study were paracetamol, followed by traditional remedies, NSAIDs, antibacterials, anthelmintics and antiacids and other drugs that decrease gastric acidity. These findings are in agreement with studies done at Limmu Genet town (Jimma Zone, Southwest Ethiopia), the common drugs used for practicing SM were analgesics, antimicrobials, antihelmintics, antimalarial and traditional medicines (Bekele *et al.*, 2018). At Jigjiga town (Eastern Ethiopia), the drugs used for SM were antibiotics 84 (41.0%), analgesics 56 (27.3%), antihelmintics 28 (13.7%), and antacids 25 (12.2%) were frequently used drugs (Amha *et al.*, 2019). In Meket District (Northeast Ethiopia) (Kassie *et al.*, 2018), at Punjab, Pakistan (Aziz *et al.*, 2018) and in all

Jordanian regions (North, Middle and South) (Gogazeh, 2020), the analgesic/antipyretic drugs were the most common drugs used for SM practice.

That analgesics are the most commonly employed drugs for SM in this study is in line with study findings from Addis Ababa (where analgesics/antipyretics, antimicrobials and gastrointestinal (GI) drugs were the three most commonly employed drug categories for SM), Kolladiba town (where analgesics, antibiotics and GI drugs were reported as the three most commonly employed drugs categories for SM), Sire town (where the three most commonly employed drug categories for SM were analgesics, antibiotics and traditional remedies) and Mekelle town (where analgesics, GI drugs and respiratory drugs were the three most commonly employed drug categories for SM) (Andualem and Gebre-Mariam, 2004; Abraha *et al*., 2014; Eticha and Mesfin, 2014; Jaleta *et al*., 2016). Similar drug/drug classes with different magnitude were mentioned in studies conducted in Nepal, and among students at University of Gonder and Mekelle Universities. In the Nepal study, paracetamol, NSAIDs, cold remedies, antiacids, and herbs were the most commonly employed drug/drug classes for SMP (Shankar *et al*., 2002). A study conducted among students of University of Gonder pointed out paracetamol, NSAIDs, antacids, antihelmintics, antibiotics and antimalarial drugs as the most commonly used drugs for SM (Abay and Amelo, 2010). The most commonly used drugs for SM reported in the study conducted on students from Mekelle University were paracetamol, NSAIDs, antibiotics, cough syrup, and antiacids (Girma *et al*., 2011).

Our study indicated that the most commonly employed individual drugs were paracetamol, diclofenac, amoxicillin, metronidazole and albendazole. It is worth noting that a significant proportion of study participants (20%) employed antimicrobials for SM. Use of antimicrobials for SM was also seen in different studies conducted in different parts of the country. For example, 24.10%, 24.70% and 33.00% of study participants used antimicrobials for SM in Sire, Kolladiba, and Mekelle studies, respectively (Jaleta *et al*., 2016; Abraha *et al*., 2014; Eticha and Mesfin, 2014). One major problem with SM with antimicrobials is the emergence of drug resistant pathogenic microbes (Awad *et al*., 2005). The use of antimicrobial agents for SM was also reported and mentioned as one public health problem in other studies conducted in Mekelle University, Gonder University, Jimma town, Iran, Bahrain, and Sudan, among others (Worku and G/Mariam, 2003; Abay and Amelo, 2010; Girma *et al*., 2011; James *et al*., 2006; Wabe *et al*., 2012; Awad *et al*., 2005; Skliros *et al*., 2010).

Thus, use of prescription drugs without prescription should be discouraged and then, appropriate health education should be provided by all concerned bodies in order to raise the

awareness of the society on appropriate utilization of drugs, in general and antimicrobials in particular.

The way of requesting drugs by the participants who obtained drugs from drug retail outlets in this study was by mentioning the name of the drug in majority of the cases followed by telling symptoms of illness to the pharmacy professional and by showing a piece of paper with the name of drug. The majority of self-medicating individuals (57.4%) in the Addis Ababa study indicated a similar way of drug request for SM as the current study (Andualem and Gebre-Mariam, 2004). Similar ways of requesting drugs were obtained in a study conducted at Mekelle town where the majority (20.8%) requested drugs by telling illness symptoms (Eticha and Mesfin, 2014).

A study conducted in India indicated that the majority of respondents were requested by explaining symptoms of illness (Gupta *et al.*, 2011). Whereas the majority of participants practicing SM from Assendabo town study reported worsening of their condition, in our study the majority (60.5%) reported relief from their illness. In contrast to the finding of our study, 82.3% of study participants who practiced SM in the Mekelle study showed improvement in their disease symptoms due to the use of the drug (Eticha and Mesfin, 2014).

Regarding reasons of respondents for choosing SM, the majority of study participants in the current study pointed perception of mildness of illness (47.4%) followed by previous knowledge about the medication (23.2%) and urgency use of the drug (10.5%). This study reasons' of SMP are in agreement with a study performed at Gondar town (Northwest Ethiopia) whose participants reported that the common three reasons for their households SMPs were severity of illnesses (44.8%), emergency cases (35.3%), and reducing medical cost (17%) (Jember *et al.*, 2019). In a study on Meket district (Northeast Ethiopia) (Kassie *et al.*, 2018), the main reasons were perceptions of illness as mild 130 (50.19%), similarity of symptoms with previous illnesses 36 (13.9%) and inability to afford health care fee 33 (12.74%). Moreover, our findings are in line with those from a study performed at Asmara community (Eritrea) by Ateshim *et al.* (2019) who reported that previous successful experience, mildness of illness, and urgency/to get quick relief were the three common reasons for SM with antibiotics.

This finding is more or less similar to the finding from Sire town where study participants mentioned reduced cost of SM, mildness of illness, the urgent nature of the case, and previous experience with drug used as the top four reasons for SM (Jaleta *et al.*, 2016). Further, it is similar to a study done in all Jordanian regions (North, Middle and South), where

the three common reasons of SMP were financial problems, previous similar complaints, and trusting pharmacist and pharmaceutical services (Gogazeh, 2020).

Our study indicates that those participants who had poor knowledge about appropriate SM were 2.04 times more likely to practice SM than those who had good knowledge (AOR = 2.04[95% C.I:1.24-3.03]). Our study finding is similar to that of the study done among selected households at Gondar town (Northwest Ethiopia) whose participants non-knowledgeable on medicines were observed to have SMP compared to their counterparts (Jember *et al.*, 2019). A study carried out on selected households living in five districts in peri-urban areas of Maputo City (Mozambique) showed that having poor knowledge on the use of antibiotics ($p<0.001$; OR =2.60) was associated to SMP with antibiotics (Mate *et al.*, 2019). Likewise, the finding of our study is also in line with a community based cross sectional study carried out at Asmara (Eritrea) which indicated that non-knowledgeable participants on antibiotics were 2.13 times at odds of practicing SM (Ateshim *et al.*, 2019). The reason for this might lie in the fact that those community members who had good knowledge about appropriate SMP preferred to consult a physician before practicing SM or/and might fear the bad adverse drug reactions during SM.

The findings of the current study indicate that a significant proportion of self-medicating individuals were pregnant (6.4%), nursing mothers (11.8%) or had a chronic disease(s) (17.1%). This is in line with the finding from Addis Ababa where a significant percent of SM individuals was pregnant (6.1%), breast-feeding (6.7%), or had a chronic disease(s) (87.2%) (Andualem and Gebre-Mariam, 2004). This might be due to reduced awareness or lack of knowledge of participants about teratogenic potential of drugs, effect of drugs on nursing an infant and the effect of drugs on other drugs that are taken for treatment of other diseases.

In this study, the majority of participants (51%) said they might agree or disagree to practice SM depending on the illness and the drug used for treatment followed by agreement (32.6%) and disagreement (14.4%) with SMP. Similar to this study finding on the participants agreement towards SMP, at Jigjiga town (Eastern Ethiopia), among the community members of the selected households, about 29.3% of them agreed practicing SM while the rest, 70.7%, did not (Amha *et al.*, 2019). This study finding differs from that of a study done in a rural District of Kilimanjaro region (Northeastern Tanzania), in which almost all 300 residents thought SM was not better than seeking medical consultation (Horumpende *et al.*, 2018 (b)). Even though this current finding is contrary to the finding from Gonder University students, where 55.5% agreed with SMP, it is in agreement with the finding from Mekelle University

students where only 36.7% agreed to the practice of SM (Abay and Amelo, 2010; Girma *et al*., 2011).

The finding from a study in Bahrain indicated that the majority (76.9%) of respondents had a positive attitude favoring SM (James *et al*., 2006). This difference in attitude towards SM from place-to-place might be due to differences in access to information about medicines, different types of study participants and access to medicines.

CHAPTER 7. STRENGTH and LIMITATIONS of THE STUDY

7.1 Strength of the study

The strengths of this study are: a) an adequate sample size that represents the Addis Ababa community by using appropriate sampling techniques, and b) utilization of appropriate statistical methods and tests to minimize biases and analyze the data.

7.2 Limitations of the study

Possible limitations of this study are: a) Recall bias and provision of socially acceptable responses by the study participants, and b) potential variation in interviewer techniques that lead to unique responses. To minimize the recall bias, proper definition and articulation of the research question, administering the interview properly and consistently, and shortening the recall period to two months were done. To minimize the possibility of providing socially acceptable responses, the study participants were assured about the confidentiality of the information they provide and declared in the research consent form every answer they provide was valuable. Moreover, potential variation in the interviewer technique that might have elicited unique responses was minimized by assigning supervisors to follow the data collection process and make corrections for any errors. Questions were also made possibly neutral in tone and placed in logical order, and interviewers were advised to continually practice and refine their interviewing techniques.

CHAPTER 8. CONCLUSION

The majority of the study participants had practiced SM. Their common reasons for this practice were mildness of illness, previous knowledge about the drug, and urgencies of the illness. The most typical sources of drug information about the available modern drugs for SMP were consultation by a health professional, drug experience from previous treatment, and friends. Drug retail outlets were the main sources for obtaining drugs. Headache, abdominal pain, cough, diarrhea, and toothache were the main ailments of the participants that took to SM and their respective frequently used drugs were paracetamol, traditional remedies, NSAIDs, antibacterials, and antihelmintics. Monthly income and knowledge about appropriate SM were both significantly associated with SMP.

CHAPTER 9. RECOMMENDATIONS

Although appropriate SMP is one of the components of self-care adopted by the WHO, its irrational use is very likely to bring serious health consequences as observed in this current study. Therefore, based on the findings of this study, we recommend to the drug regulatory and health authorities to allocate some resources to provide health information on SMP (such as proper time, type of drugs, and advantages and disadvantages of SM), enforce rules determining drug prescription and dispensing, and to increase awareness among community members on antimicrobial drug resistance and its public health impact. In the future, this kind of study should be performed in various seasons and for longer periods. In any educational programs on drugs, the involvement and active participation of pharmacy professionals to fight against the negative consequences of SMP and educate people looking for SM should be considered.

PART-TWO:

Self-medication practice with antibiotics by adult patients who are suspected of Urinary Tract Infections in Addis Ababa community

List of abbreviations

ASMP	Antibiotic self-medication practice
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
MDR	Multiple Drug Resistance
SPHMMC	Saint Paul's Hospital Millennium Medical College
UPEC	Uropathogenic *Escherichia coli*
UTIs	Urinary Tract Infections

CHAPTER 1. INTRODUCTION

1.1Background

Urinary tract infection (UTI) is a spectrum of diseases caused by microbial invasion of the genitourinary tract (Khadka *et al.*, 2012). It may involve the lower urinary tract or both the lower and upper urinary tracts (Sobel and Kaye, 2010). UTIs are classified as either symptomatic or asymptomatic. The definition of a symptomatic UTI generally requires the presence of urinary tract-specific symptoms in the setting of significant bacteriuria with a quantitative count of $\geq 10^5$ colony forming units of bacteria per milliliter (cfu/mL) in one urine specimen (Nicolle *et al.*, 2005; 2001).

Symptomatic UTIs are divided into lower tract (acute cystitis) or upper tract (acute pyelonephritis) infections. Cystitis is defined as significant bacteriuria with associated bladder mucosal invasion, whereas pyelonephritis is defined as significant bacteriuria with associated inflammation of the renal parenchyma, calices and pelvis (Connolly and Thorp, 1999). Asymptomatic bacteriuria is defined as the presence of bacteria in the urine, without clinical signs or symptoms suggestive of a UTI (Nicolle *et al.*, 2005).

Worldwide, UTIs are some of the most commonly occurring infections and affecting approximately 150 million people each year (Flores-Mireles *et al.*, 2015). UTIs are among clinical practice's most common and recurrent bacterial infections and account for one-third of all community-acquired or nosocomial infections (Foxman, 2010). The associated cost of healthcare is enormous, accounting for EUR 661 million in direct costs for treating and EUR 939 million in indirect costs, totaling EUR 1.6 billion annually (Foxman, 2002). However, UTIs are significantly understudied (Losada *et al.*, 2016). It is worth highlighting some facts such as an estimated 50% of women have reported having had a UTI at some point in their lives and 8.3 million office visits and more than 1 million hospitalizations have come at an overall annual cost of more than $1 billion (Michno *et al.*, 2018). The factors identified and showing an association with high prevalence of UTI within the population include age, gender, sexual activity, contraceptive use, previous history of UTI, indwelling catheter equipment and hygiene problems (Shatalov, 2015; Seifu and Gebissa, 2018; Kashef *et al.*, 2010). Other risk factors for UTI include low educational level, low immunity, employment status, incomplete bladder emptying, bladder dysfunction, and prostate syndrome (Kebamo *et al.*, 2017).

The source of UTI pathogens is generally considered to be the patient's own flora. Urinary tract infections are preceded by colonization of the vagina and periurethral area by uropathogens from the GI tract (Hilbert, 2011). In several studies, Gram-negative bacteria

are responsible for 80–85% of UTIs and major causative organisms are *E. coli* (75–87%) followed by *Klebsiella* spp, *Citrobacter* spp*, Enterobacter* spp*, Pseudomonas* and *Proteus* spp and also caused by Gram-positive pathogens, such as *Enterococcus faecalis* and *Staphylococcus saprophyticus* (Flores-Mireles *et al*., 2015; Rampure *et al*., 2013; Thakur *et al*., 2013; Gupta *et al*., 2011). Women are much more susceptible than men to community-acquired UTIs, in part, due to the female anatomy in that a much shorter urethra allows pathogens easier access to the bladder (Ronald, 2003). Moreover, UTIs would be more frequent in diabetic patients (Kamoun *et al*., 2013).

Escherichia coli is reported as one of the most common organisms found in the genital tract of non-pregnant (9–28%) and pregnant women (24–31%). The possible reason why *Escherichia coli* is the most prevalent in most of the studies is its commonness as a urinary pathogen (Behzadi *et al*., 2010). Vaginal *E. coli* strains are considered to be a reservoir for vaginal and/or endocervical colonization in pregnant women, and an important step in the development of urinary tract, intra-amniotic and puerperal infections through 'fecal-vaginal-urinary/neonatal' transmission (Haider *et al*., 2010; Hamdan *et al*., 2011).

UTI is an extremely common condition that occurs in both males and females of all ages. The prevalence and incidence of infection are higher in women than in men, which are likely the result of several clinical factors including anatomical differences, hormonal effects, and behavior patterns (Griebling, 2009). UTI are not only common, but also highly recurrent. In particular, sexually active women, the elderly and pre-pubertal children are highly susceptible to chronically recurrent UTI, resulting in increased use of antibiotics and negatively affecting quality of life (Foxman, 2002).

1.2 Statement of the problem

The most influential factors for antibiotic resistance crisis are inappropriate antibiotics use, over dosage and self-medication with antibiotics (Rather *et al*., 2017; Piddock *et al*., 2016). About 80% of antibiotics are used in the community and the rest are used in hospitals (Kotwani and Holloway, 2011; Cars *et al*., 2001; Wise *et al*., 1998). It is estimated that 20-50% of all antibiotics use is inappropriate (Cizman, 2003)**.** The increased and inappropriate use of antibiotics has serious implications since it contributes to selective pressure, favoring the development antibiotic resistance (Vila, 2010; Dougherty and Pucci, 2014). Several study reports and reviews indicated the existence of correlation between antibiotic use and bacterial resistance (Stein *et al*., 2018; Anyanechi and Saheeb, 2014; Chon et al., 2012; Davies and Davies, 2010).

Antibiotic resistance involves a significant economic burden worldwide (Ahmed *et al.*, 2019). Annually, there is an estimation of 700,000 lives lost due to antimicrobial resistance. This is further projected to the mortality of at least of 10 million lives by the coming 2050 (Williams, 2016) and by 2030, antimicrobial resistance may lead to poverty among 24 million people in the world (WHO, 2019). It is also important to note that there have not been many new discoveries of antibiotics to combat the antibiotic resistant pathogens (Piddock, 2012; Bartlett *et al.*, 2013; WHO, 2014).

SM with antibiotics is prevalent in developing countries, including Africa (Ethiopia) and is considered as a global health problem (Morgan *et al.*, 2011; Mahmoud *et al.*, 2020). The causes of non-prescription sales of antibiotics for the purpose of SM possibly could vary from country to country due to different underlying contexts (Gebretekle and Serbessa, 2016). The purchase of antimicrobial drugs without prescription is estimated to be 58% in Asia, 47% in Southern Europe, 30% in Eastern Europe, 25% in South America, 39% in Middle East (Morgan *et al.*, 2011). However, appropriateness of SM is not much known. Therefore, a certain level of knowledge and health orientation is needy to make it responsible SM (Afolabi *et al.*, 2008).

The use of antibiotics to treat malaria is one of the worst scenarios of SM with antibiotics (Ajibola *et al.*, 2018). Additionally, one of the misuses of antibiotics is treating patients following rise in body temperature/fever (≥ 37°C) symptom developed which is common to many clinical diseases (Salgado *et al.,* 2016).

Males were more exposed to SM with antibacterial drugs compared to their female counterpart (Sunny *et al.,* 2019). Predictors of SM of antimicrobials included age, female gender, previous experience with antimicrobials, severity of illness, patient education, advice from friends, and socioeconomic status (Ocan *et al.*, 2015; Al Rasheed *et al.*, 2016). Further, factors such as health-care utilization, the proximity of pharmacies, and increased waiting time in health-care facilities, also appear to be significantly associated with SM of antimicrobials (Gebrekirstos *et al.*, 2017; Al Rasheed *et al.*, 2016).

However, little was known about the association of previous SM with antibiotics and the drug resistance bacteria development among UTIs suspected patients in the study settings in Addis Ababa, Ethiopia. Therefore, this study was conducted to describe the experience of ASMP of the study patients, the causative bacteria identified from the urine sample, the antibiogram of the culture sensitivity test, and other associated factors from the clinical data and sociodemographic profiles of the study patients regarding SM with antibiotics.

1.3 Significance of the study

In this study, the findings are crucial to raise the awareness on the dangers of ASMP in the population and enforce the current law of antibiotics to be used under prescription order from physician doctors. These study findings could generate information on timely status of the drug resistance bacteria induced UTIs and identify the impact of ASM on the development of drug resistance.

National and international organizations that work on antibiotic drug resistance could utilize this study finding to set cost effective interventional strategies such as educational training (small group, peer run, use of opinion leaders, and different materials).

CHAPTER 2. LITERATURE REVIEW

2.1 Antibiotic self-medication Practice

A study conducted in India showed that SM with antibiotics was reported by 42.3% of the participants prescribed by pharmacists at some point in their lives. The majority of participants (61.8 and 75.5%) of the sample thought antibiotics have antiviral and analgesic effect, respectively. About 67.3, 38 and 54.7% of participants thought antibiotics should be prescribed post-extraction, should be taken before any dental work and knew that antibiotics have side effects, respectively. The majority of participants (74.5%) knew that unnecessary use of antibiotics makes them ineffective leading to antibiotic resistance. In the sample, a 90.7% believed it was important to complete the course of antibiotics prescribed by dentist but 43% used to discontinue antibiotics on feeling better (Sohail *et al.*, 2020).

A study report from Sir Salimullah Medical College Mitford Hospital in Dhaka revealed that a total 607 (17.56%) microorganism were isolated from 3455 urine samples. Among them, the majority of cases 189 (31.14%) were female and 19-45 was the most common age group. The most predominant organism was *Escherichia coli* (86.32%) followed by *Pseudomonas* (4.45%), *Klebsiella* (3.62%), *Proteus* (2.31%) and as Gram-positive organisms *Staphylococcus* (0.82) and *Enterococcus* (0.49%) stand out. *Escherichia coli* was highly resistant to amoxyclav (82.63%) followed by cephradine (75.19%), ciprofloxacin (73.28%) and highly sensitive to imipenem (3.05%). *Pseudomonas* showed high degree resistance to cefixime (92.59%), ceftriaxone (88.89%) and sensitivity to imipenem (25.62%), meropenem (25.93%) (Akter *et al.*, 2020).

Of 2160 dental patients, over a third of the study population (39.7%) admitted to the practice of ASM. Most of the respondents (58.4%) who indulged in SM were females, and it was prevalent in the older adults between 32–42 years old (36%). There was a negative correlation between age and SMP ($p < 0.001$) observed with point biserial correlation test. Binary logistic regression analyses found an odds ratio of 0.97 ($p < 0.001$). The most consumed antibiotics were amoxicillin/clavulanic acid (52%), amoxicillin (31.1%), and azithromycin (10.1%). It is imperative to educate patients about the harmful effects of SM and to stress the need for governments to implement stricter laws on non-prescription drug availability (Aragoneses *et al.*, 2021).

In patients attending the El-Mahsama family practice center, which serves a rural community in Ismailia governorate in Egypt, frequency of SM among the study sample has reached 96%. More than half of the participants (53.6%) reported that the first reason behind

using SM was cost saving. Meanwhile, the most prevalent conditions that make them use these medications by themselves were headaches (17%), aches and pain (other than headache) (39.2%), and fevers (11.8%). The most frequent self-administered drugs were analgesics (59.5%) and antibiotics (23.5%) (Zeid *et al.*, 2020).

In Hawassa (Ethiopia), among patients attending adult outpatient department at Hawassa University Comprehensive Specialized Hospital, the overall prevalence of symptomatic UTI was 32.8% (95% CI: 28.3–37.6). The predominant isolated bacteria were *E. coli* 46 (36.2%) followed by *S. aureus* 21 (16.5%). Gram-negative bacteria were highly resistant to ampicillin (71.4%), and tetracycline (68.2%). Gram-positive bacteria were highly resistant to norfloxacin (77.7%). The overall prevalence of multi-drug resistant isolates was 102 (80.3%). Being female, being not formally educated, and possessing SM history were more likely associated with UTI (Mechal *et al.*, 2021).

Regarding a study conducted among 279 patients at Kiruddu National Referral Hospital Kampala (Uganda), in which the majority were females and from the outpatient department, overall, 212 (76%) participants had taken an antibiotic in the past 6 months, and some 22.2% (n=47) of the participants had practiced SM. Male participants and Muslims were more likely to self-medicate. Employees and patients with tertiary education were less likely to practice SM. About 33% (n=70) of the participants had not completed treatment dosage during their last course of antibiotic treatment because of feeling better (60%, n=42), lack of money to purchase the medication (15.7%, n=11) and side effects (10%, n=7). Whereas 169 participants (79.7%) believed that not completing treatment would have an impact on their personal health, only 96 participants (45.3%) believed that this behaviour could affect the health of others (Nabaweesi *et al.*, 2021).

In a study done at Soba (Khartoum, Khartoum state, Khartoum, Sudan), it was found that 340 (100%) respondents practiced SM. The principal for seeking self-medication included Malaria as reported by 165 people (17.4%) followed by diarrhea (162 people) (17.1%). Drugs commonly used for SM included antibiotics (35.9%) followed by analgesics (31.8%). Among reasons for seeking SM, about 237 persons (50%) cited cost-effectiveness as the primary reason, as well as flexibility of pharmacies in place and time, 251 (37.9%) found pharmacists their sources of medicine information, followed by doctors 176 (26.5%). For these reasons 214 (62.9%) used repeated prescriptions, some people go to herbal remedy, included cough as reported by 137 (20.12%), followed by cold (17.9%) (Isameldin *et al.*, 2020).

In a study report including 504 patients and their relatives visiting the General Hospital, Karaikal, 33.9% of them reported SM with antimicrobials in the past 6 months with prevalence significantly affected by male gender (adjusted odds ratio [OR] = 1.83; 95% confidence interval [CI]: 1.2–2.79), a preference for SM with antimicrobials during minor illnesses (adjusted OR = 3.05; 95% CI: 1.77–5.25) and a history of SM with antimicrobials among family members (adjusted OR = 3.43; 95% CI: 2.16–5.43). The most commonly self-prescribed drug was amoxicillin (55.2%) and the antimicrobials were mostly obtained from pharmacies (91.8%). Self-prescribed antimicrobials were commonly used to treat cold, cough, and fever. The reasons for SM with antimicrobials were easiness to do so (32.2%), to save their time (35.7%) and money (32.2%) (Moktan and Shehnaz, 2020).

In Nigeria's study, about 374 (46.8%) of 800 outpatient clinic visiting patients had self-medicated with antibiotics 6 months preceding the study with commonly used drugs such as ampicillin/cloxacillin (31.3%) and amoxycillin (24.8%). Respiratory tract conditions were the most common reasons for taking antibiotics, 100 (27.2%). The primary reason for SM was the availability of the drug at the local drug store. Being divorced or widowed (AOR 0.302, 95% CI 0.117-0.781, p = 0.01) and age > 70 years (AOR 0.18, 95% CI 0.04-0.84, p = 0.03) were significantly and negatively associated with ASM (Tobin and Atulomah, 2020).

Approximately 34.9% of the 384 selected visitors of Central Polyclinic of Kabul (Afghanistan), self-medicated with antibiotics in the past 12 months. Amoxicillin was the most common antibiotic used for SM. The sore throat was a common health problem, and previous experience was the source of recommendation for SM. The most common reasons for SM with antibiotics were cost-saving, previous experience, and lack of time (Negarandeh *et al.*, 2021).

In Malaysia's study, about 50% of 270 patients had moderate knowledge on antibiotics. The knowledge and attitude on antibiotics usage exhibited a significant difference (p=0.001) amongst these patients in association with literacy. The results showed that the literate respondents had significant knowledge on the identification of antibiotics (p=0.021), their roles, and adverse effects (p=0.004). The attitude of the respondents towards following the prescription was significantly associated with their literacy (p=0.004) (Hanish *et al.*, 2021).

According to Mazzariol *et al.* review (2017), the increased prevalence of multi-drug resistant (MDR) *Enterobacteriaceae*, the limiting available treatment options for infections caused by these organisms and the lack of new antibiotics provide a good rationale for using older antibiotics, such as fosfomycin, that have been shown to retain some activity against MDR bacteria.

In the Middle East's systematic review (Alhomouda *et al*., 2017), the prevalence of SMA was from 19 to 82%. The main determinants of SMA were age, sex, and educational and income levels of the individuals. Broadly, the most cited reasons for SMA were sociocultural, economic, and regulatory factors. Penicillins were the antibiotics most commonly used. The antibiotics were obtained mainly via stored leftover drugs, pharmacies without prescriptions, and friends/relatives. SMA was mainly for upper respiratory tract problems. The primary sources of drug information were relatives/friends and previous successful experience. Inappropriate drug use such as wrong indication, short and long duration of treatment, sharing of antibiotics, and storing antibiotics at home for use at a later time were reported.

SMA is a common practice among Haitian patients. Among 200 outpatients in Haiti, 45.5% of them practiced SMA. ASMP was less prevalent among patients with the highest education level (23.1%; OR: 0.89 (0.5-1.75), p = 0.001). Mild symptoms (28.6%) and vaginal itching (44.4%) were the main reasons for SMA. SM using amoxicillin was reported by 67%. Amoxicillin for UTIs is the most commonly used medication. In Haiti, marketing OTC antibiotics is illegal, but most patients buy non-prescribed antibiotics in pharmacies (Moise *et al*., 2017).

In Ghana, a study performed at two health facilities showed that of the total 261 participants enrolled, 19.9% of them acknowledged using antibiotics prior to their visit to the laboratory. However, about 31% of the participants' urine samples were positive for antimicrobial activity. Age intervals (20–30, 31–40 and 41–50 years) of the participants had significant association and lower odds of urine antimicrobial activity. Participants who had urine antimicrobial activity were more likely to have no growth on their culture plates than participants who had no urine antimicrobial activity [OR 2.39(1.37–4.18), p = 0.002]. The most commonly used antibiotics were the penicillins, fluoroquinolones and metronidazole. Although, majority of the participants (54.8%) had knowledge towards antibiotics, most of them had inadequate information on their proper use. The commonest indications for antibiotic use were aches and pains (30.3%), diarrhea (43.3%) and urinary tract infections (28.0%). Prior antibiotic use was found to increase the likelihood of obtaining a culture negative result and could affect the outcome of bacteriological tests (Donkor *et al*., 2019).

Among 1,007 episodes of complicated UTIs, 97 (9.6%) were due to *P. aeruginosa*. Resistance rates of *P. aeruginosa* were: antipseudomonal cephalosporins 35 of 97 (36.1%), aminoglycosides 30 of 97 (30.9%), piperacillin–tazobactam 21 of 97 (21.6%), fluoroquinolones 43 of 97 (44.3%), and carbapenems 28 of 97 (28.8%). The multidrug resistance rate was 28 of 97 (28.8%). Independent risk factors for *P. aeruginosa* complicated UTI were male sex (OR 2.61, 95% CI 1.60–4.27), steroid therapy (OR 2.40, 95% CI 1.10–

5.27), bedridden functional status (OR 1.79, 95% CI 0.99–3.25), antibiotic treatment within the previous 30 days (OR 2.34, 95% CI 1.38–3.94), indwelling urinary catheter (OR 2.41, 95% CI 1.43–4.08), and procedures that anatomically modified the urinary tract (OR 2.01, 95% CI 1.04–3.87). Independent risk factors for multidrug resistance rate *P. aeruginosa* cUTI were age (OR 0.96, 95% CI 0.93–0.99) and anatomical urinary tract modification (OR 4.75, 95% CI 1.06–21.26). Readmission was higher in *P. aeruginosa* complicated UTI *(*cUTI) patients than in other etiologies (23 of 97 [23.7%] vs 144 of 910 [15.8%], p = 0.04), while 30-day mortality was not significantly different (7 of 97 [7.2%] vs 77 of 910 [8.5%], p = 0.6) (Gomila *et al.,* 2018).

In Pakistan, self-medication with antibiotics was self-reported by 135(527) participants (26%), with a higher prevalence of men than women (48% vs 38%, respectively). The main reason for SM was previous experience with the same antibiotic (68%). The most commonly used antibiotics were amoxicillin-clavulanic acid (40%) and major indications for self-medication were sore throat (29%) and flu (24%). Of the 527 respondents, only 104 (20%) were aware of antimicrobial resistance (Shabnam and Azim, 2015).

Of 300 patients attended Dalhatu Araf Specialist Hospital (Lafia Nasarawa State, Nigeria), 180 (60%) of them were positive for UTI. The most common organisms were *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Proteus mirabilis*. *In vitro* antibiotic susceptibility tests revealed that the Gram-negatives bacteria were sensitive to quinolones (ofloxacin, ciprofloxacin, pefloxacin) and erythromycin, while the Gram-positive isolates were sensitive to lincomycin, erythromycin and quinolones (ofloxacin, ciprofloxacin, pefloxacin) (Kolawole *et al*., 2009). In another study done in Nepal, the reported positive rate of UTI among Nepalese patients attending general hospitals ranged from 23.1 to 37.4% (Rai *et al*., 2008).

A total of 347 *E. coli* isolates were collected from urine samples of community-acquired uncomplicated UTIs patients. The susceptibility rates of antibiotics were as follows: amikacin 100% (347), imipenem 100% (347), ciprofloxacin 57.1% (198), cefotaxime 74.9% (260), ampicillin 30% (104), trimethoprim/sulfamethoxazole 66.9% (232), and fosfomycin 98% (340). All fosfomycin-resistant *E. coli* isolates were extended-spectrum beta-lactamase (ESBL)-producing. In 85 cases of ESBL-producing *E. coli*, the fosfomycin susceptibility rate was 91.8% (78/85) (Choe *et al*., 2017).

In a meta-analysis study done on developing countries by Ocan *et al*., (2015), a total of 34 studies involving 31,340 participants were included, and the overall prevalence of

antimicrobial self-medication was 38.8% (95% CI: 29.5-48.1). Most studies assessed non-prescription use of antibacterial (17/34: 50%) and antimalarial (5/34: 14.7%) agents. The common disease symptoms managed were, respiratory (50%), fever (47%) and GI (45%). The major sources of antimicrobials included, pharmacies (65.5%), leftover drugs (50%) and drug shops (37.5%). Twelve (12) studies reported inappropriate drug use; not completing dose (6/12) and sharing of medicines (4/12). The main determinants of antimicrobial self-medication include, level of education, age, gender, past successful use, severity of illness and income. Reported negative outcomes of antimicrobial self-medication included allergies (2/34: 5.9%), lack of cure (4/34: 11.8%) and causing death (2/34: 5.9%). The commonly reported positive outcome was recovery from illness (4/34: 11.8%).

Uncomplicated UTI is one of the most common indications for antibiotic use in the community. However, the Gram-negative organisms that can cause the infection are becoming more resistant to antibiotics. Many multidrug resistant organisms retain susceptibility to two old antibiotics, nitrofurantoin and fosfomycin. Advantages over newer drugs include their high urinary concentrations and minimal toxicity. Fosfomycin is a potential treatment option for patients with uncomplicated UTI due to resistant organisms. Nitrofurantoin may be more effective and can be used for urinary infections in pregnant women (Gardiner *et al.*, 2019).

In a Nigerian study (Akingbade *et al.*, 2014), *Escherichia coli* isolates obtained shows high resistance to cloxacillin (92.5%), amoxicillin (90.8%), ampicillin (90.8%), erythromycin (75.8%), cotrimoxazole (70%), streptomycin (70.0%) and tetracycline (68.3%) while 85.8 and 84.2% were susceptible to gentamycin and ceftazidime, respectively. Sixteen *Escherichia coli* strains were observed to be resistant to more than two classes of antibiotics. The resistant plasmid DNA was detectable in 6 (37.5%) of the 16 MDR *Escherichia coli* having single sized plasmids of the same weight 854 bp and were all resistant to erythromycin, cefuroxime, cloxacillin, amoxicillin, ampicillin and cotrimoxazole.

About 137 of 469 patients who met the criteria of UTI had a positive urine culture. A MDR pathogen was found in 36.5% of these. Overall susceptibility was less than 85% for standard antimicrobial agents. Logistic regression identified residence in nursing homes, male gender, hospitalization within the last 30 days, renal transplantation, antibiotic treatment within the last 30 days, indwelling urinary catheter and recurrent UTI as risk factors for MDR or any of these resistances. For patients with no risk factors, ciprofloxacin had 90%, pip/taz 88%, gentamicin 95%, cefuroxime 98%, cefpodoxime 98% and ceftazidime 100% susceptibility. For patients with one risk factor ciprofloxacin had 80%, Pip/taz 80%, gentamicin 88%, cefuroxime 78%, cefpodoxime 78% and ceftazidime 83% susceptibility. For two or more

risk factors, ciprofloxacin drops its susceptibility to 52%, cefuroxime to 54% and cefpodoxime to 61%. Pip/taz, gentamicin and ceftazidime remain at 75% and 77%, respectively (Bischoff *et al.*, 2018).

2.2 Treatment of UTIs

Urine culture is the gold standard for detection of UTI. However, asymptomatic bacteriuria is common, particularly in older women, and should not be treated with antibiotics. Conversely, in symptomatic women, even growth as low as 10^2 colony-forming unit/mL could reflect infection. Resistance is increasing to fluoroquinolones, β-lactams, and trimethoprim-sulfamethoxazole. Most uropathogens still display good sensitivity to nitrofurantoin. First-line treatments for UTI include nitrofurantoin, fosfomycin, and trimethoprim-sulfamethoxazole (when resistance levels are <20%). These antibiotics have shown minimal collateral damage and resistance. In pregnancy, β-lactams, nitrofurantoin, fosfomycin, and trimethoprim-sulfamethoxazole can be appropriate treatments. Interpreting the probability of UTI based on symptoms and testing allows for greater accuracy in diagnosis of UTI, decreasing overtreatment and encouraging antimicrobial stewardship (Chu and Lowder, 2019).

Antimicrobial susceptibility testing was available for 5246 *E. coli* urine isolates from 4870 patients. *E. coli* was most commonly resistant to amoxicillin (43.1%), cotrimoxazole (24.5%) and ciprofloxacin (17.4%). Resistance rates were low for meropenem (0.0%), fosfomycin (0.9%) and nitrofurantoin (1.5%). Significantly higher rates of resistance to ciprofloxacin (32.8 vs 15.8%) and cotrimoxazole (30.6 vs 23.9%) were found in urological patients compared with patients on other wards (p <0.01). In multivariable analyses, predictors for *E. coli* resistance against ciprofloxacin and cotrimoxazole were: treatment in the urological unit, male sex, and only to a lesser extent, urine sample obtained from indwelling catheters. Age ≥65 years was associated with higher resistance to ciprofloxacin (OR 1.42, 95% CI 1.21–1.67; p <0.001), but lower resistance to cotrimoxazole (OR 0.76, 95% CI 0.67-0.86; p <0.001) (Erb *et al.*, 2018).

In a Shashemene's study (Ethiopia), the overall prevalence of UTI in the area was 90.1%. Most frequently isolated uropathogen in our study was *Escherichia coli* (39.3%). While, *Staphylococcus* species (20.2%), *Leuconostoc* species (11.4%), *Raoultella terrigenal Klebsiella* spp./ (8.4%), *Salmonella typhimurium* (6.3%), *Dermacoccus nishinomiyaensis* (6.3%), *Citerobacter freundii* (5.2%) and *Issatchenkia orientalisl Candida kruseil* (2.7%) were the other isolates. Some of UTI risk factors was statistically significant (p < 0.05). Gentamicin was the most effective drug against most of the isolates followed by

chloramphenicol and nitrofurantoin. In contrast, amoxicillin, vancomycin and cephalexin were the antibiotics to which most of the isolates developed resistance (Seifu and Gebissa, 2018).

In a study report which had a total of 607 outpatients, approximately 68% were treated inappropriately. Inappropriate regimens consisted of 50.9% (n = 309) incorrect durations, 35.1% (n = 213) incorrect choice of antibiotic, and 12.4% (n = 75) incorrect doses. Ten percent of patients developed a reinfection within 30 days. Recurrence of UTI with the same pathogen within 30 days occurred in 5.1%. Catheter use and advanced age are both risk factors for recurrence UTIs and inappropriate treatment (Wattengel *et al.*, 2020). Uncomplicated urinary tract infections (UTIs) are common in women and are usually managed in primary care (Butler *et al.*, 2015; Foxman, 2014).

A German study showed there was no strong association between the urine culture results, as later reported by the laboratory, and the treatment strategy (antibiotic or symptomatic) women decided to follow. Of those women with a confirmed UTI (positive urine culture), 63% took an antibiotic, while 55% of those with a negative result also took an antibiotic. The respective figures for non-steroid anti-inflammatory drugs were 42% (40/96) in the case of a positive urine culture and 45% (10/22) in the case of a negative result (Gágyor *et al.*, 2020).

Community-acquired symptomatic UTIs are treated with empirical antimicrobial therapy upon diagnosis based on patient symptoms. Urine cultures are recommended for complicated and recurrent cases, and may be performed for uncomplicated cases, although physician guidelines vary. Since treatment will precede identification of the pathogen, local trends for antibiotic resistance must be accounted for. The recommended first-line antibiotic therapy for cystitis is either nitrofurantoin (100 mg daily for 5 days) or trimethoprim-sulfamethoxazole (SXT) (160 mg-800 mg daily for 3 days). Nitrofurantoin should be avoided if pyelonephritis is suspected, as this drug only reaches an effective concentration in the bladder. SXT should be avoided if resistance in the area is >20% or if the patient has been treated with this antibiotic in the last three months. Another option for treatment is pivmecillinam (400 mg daily for 3-7 days), but this drug is not approved for use in North America and some European countries. Fosfomycin (3 g single dose) can also be used, but some studies suggest it is less effective than nitrofurantoin or SXT. Although amoxicillin and ampicillin should be avoided due to endemic resistance, 3-7 days courses of amoxicillin-clavulanic acid, as well as cephalosporins such as cefaclor, cefdinir and cefpodoxime proxetil, may be used. However, they exhibit less effectiveness and are associated with more adverse effects than the recommended front-line therapies (nitrofurantoin and SXT). Fluoroquinolones

are highly effective in 3-day courses, resistance is minimal, and they are well-tolerated, but are only recommended as second-line therapies as they are highly useful for more serious infections and their judicious use will delay the rise of resistance. Pyelonephritis is a much more serious condition, often requiring hospitalization and parenteral administration of antibiotics—either ceftriaxone (400 mg) or a consolidated 24 h dose (7 mg drug/kg body weight) of an aminoglycoside (gentamicin or tobramycin), in addition to oral ciprofloxacin (Gupta *et al.*, 2011; Hilbert, 2011).

As to Hilbert and his colleagues (2011) conclusion, the rising antibiotic resistance among uropathogens, and especially the emergence of MDR clonal groups, has provided urgency to the development of novel preventative and therapeutic strategies. Some older drugs, such as fosfomycin, may prove to be very useful in treating antimicrobial-refractory UTIs, especially those due to ESBL producers. Newer drugs, such as the recently approved doripenem, have proven highly effective in the clinic to treat complicated UTIs. Research into novel anti-virulence therapies, such as inhibiting the production of, or adherence by, Uropathogenic *Escherichia coli* (UPEC) fimbriae is still an early stage but holds promise for future development. The use of probiotics to prevent vaginal UPEC colonization and the use of an immuno-stimulatory uropathogen extract (Vaginal vaccine, SolcoUrovac®) are currently in clinical trials to determine efficacy in preventing recurrent UTIs. Another preventative strategy is vaccination, and experimental vaccines have been developed that are effective in preventing UTIs in primates. In summary, a combination of traditional and innovative prevention and treatment strategies is being deployed to combat the threat of emerging antibiotic resistance among uropathogens.

CHAPTER 3. OBJECTIVES

3.1 General objective

To assess the antibiotic self-medication practice among adult patients suspected of urinary tract infections (UTIs) in Addis Ababa, Ethiopia.

3.2 Specific objectives

- To characterize the sociodemographic characteristics of the study patients.
- To determine the prevalence of antibiotic self-medication practice among adult patients suspected with UTIs.
- To identify the associated risk factors of antibiotic self-medication practice among adult patients suspected with UTIs.
- To determine the antibiotic susceptibility test towards patient isolated *bacteria* from urine sample.
- To assess the level of knowledge of the patients towards antibiotics.
- Identify common uropathogenic bacteria among adult patients confirmed with UTIs.
- To determine the prevalence of multi-drug resistant bacteria against the tested antibiotics.
- To describe the number of antibiotics (from the 2 antibiotics until the maximum) resisted by the micro-organisms.
- To determine the presence of self-medicated patients among those with multi-drug resistant bacteria induced urinary tract infections.

CHAPTER 4. METHODOLOGY

4.1 Study area

This study was conducted at three (3) institutions. These study settings were Saint Paul's Hospital Millennium Medical College (SPHMMC), Arsho Diagnostic Laboratories (two branches; the Piazza branch and Meskel flower branch) and Ethiopian Public Health Institute (EPHI). St. Paul's Hospital was built in 1969 (was named St. Paul General Specialized Hospital until 2008) by Emperor Haile Selassie in collaboration with the German Evangelical Church, as a source of medical care for underserved populations. Since 2008, it has been a teaching hospital (Saint Paul's Hospital Millennium Medical College). It currently has 392 beds, with an annual average of 200,000 patients and a catchment population of more than 5 million. Approximately 75% of the patients receive medical services free of charge. There is a clinical and non-clinical staff of over 1,300 persons in over 13 departments. Most recently it has been launched its new Hemodialysis unit. The department of Microbiology (culture Unit) provides a drug susceptibility test service to adult patients suspected of UTI coming from different departments.

Arsho was the first clinical testing laboratory to be established in Ethiopia. The original site illustrated in Piazza is still the best known of the company's laboratories. Arsho is generally accepted to be the leading laboratory and the most widely respected. It was set up in 1972 by Dr. Arshavir Terzian and run by him until his sudden death in 2002. The Piazza branch is the registered Head office of the company. It is where the first private medical laboratory in Ethiopia was established in 1972. It is still the biggest branch in terms of foot traffic and income. By now, the total number of Arsho branches in Addis Ababa are nine (Africa Business Directory, 2021). The Ethiopian Public Health Institute (EPHI) is the result of the merger in April 1995 of the former National Research Institute of Health (NRIH), the Ethiopian Nutrition Institute (ENI) and the Department of Traditional medicine (DTM) of the Ministry of Health. The merger was affirmed by the council of ministers' regulation No 4/1996, which recognized the Institute as an autonomous public authority having its own legal personality (EPHI, 2021a). EPHI provides a laboratory service for patients who brought the laboratory order sheets from different clinics, health centers and hospitals. It provides about 82 types of diagnostic tests for patients (EPHI, 2021b).

4.2 Study period

The study was conducted from 5th November 2020 up to 7th August 2021 to assess the ASMP among adult patients suspected of UTI in Addis Ababa, Ethiopia.

4.3 Study design

Institutional based cross-sectional study design was used to assess the ASMP of adult patients suspected of UTI in Addis Ababa, Ethiopia.

4.4 Source population

All adult patients suspected of UTI and visited the Department of Bacteriology, culture unit of the study settings in Addis Ababa, Ethiopia.

4.5 Study population

All adult patients suspected of UTI and visited the Department of Bacteriology, culture unit of the study settings presented during data collection and volunteer to participate in Addis Ababa, Ethiopia.

4.5.1 Inclusion and exclusion

All adult patients (≥ 18 years of age) and were volunteer to participate in this study were included. However, adult patients who were seriously ill and/or with serious psychiatric problems were excluded from the study.

4.6 Sample size determination and sampling technique

4.6.1 Sample size determination

The sample size was determined using the single population proportion formula (Cochran, 1963) with its components: d= 5%, margin of error; P = 0.50, the estimated proportion of antibiotic self-medication among adult patients suspected with urinary tract infection present in the population; $\frac{Z\alpha}{2}$ = 1.96, the value found in the statistical table that contains the area under the normal curve at 95% confidence level; and 10% non-response rate.

The sample size was determined as follows: Where $no = \text{sample size of the study}$

$$no = \frac{(Z\alpha/2)^2 \mathrm{P}\,(1-\mathrm{P})}{d^2}$$

$$no = \frac{(1.96)^2 * 0.50(1-0.50)}{(0.05)^2}$$

$$no = 384$$

The minimum sample size of this study was 423 adult patients including (10% non-response rate, 39). However, 531 patients were participated.

4.6.2 Sampling technique

Taking all adult patients consecutively as sampling technique was employed to recruit adult patients suspected with UTIs until the required sample size was achieved to assess their ASMP in the study settings in Addis Ababa, Ethiopia. To recruit the patients, a team of data collectors was in the Department of Microbiology, culture unit of the study settings and in discussion with the laboratory technologists (working in culture unit), patients were communicated to participate while came to give urine sample and those fulfilled the inclusion criteria of the study were included. The diagnosis procedure was a normal daily activity of the culture Units of the study settings in Addis Ababa, Ethiopia.

For urine culture test, clean catch mid-stream urine samples were collected using sterile wide mouth container from enrolled patients. The minimum acceptable volume of urine sample was 10 ml. All the samples were analyzed immediately after arrival to the culture unit to ensure that a bacterium presented in the urine was isolated and to avoid over population.

4.7 Study Variables

4.7.1 Dependent variable

Antibiotic self-medication practice status (yes or no)

4.7.2 Independent variables

- Sociodemographic profiles (e.g., age, gender, and marital status)
- Antibiotic Self-medication practice statements (e.g., Have you ever treated yourself (self-medicated with antibiotics?; reason(s) of self-medication practice).
- Antibiotic Self-medication Knowledge statements (e.g., Do you know what antibiotics are?; What are antibiotics used for?)
- Clinical variables (e.g., comorbidity; history of catheterization)

4.8 Operational definition

ASM is an action by which an adult patient suspects her/his illness and took an antibiotic drug in the last twelve (12) months without prescription from a prescriber. Drug resistant stated that bacteria strains were resistant two or more antibiotics (Magiorakos *et al.*, 2012). In this study, the total number of knowledge related questions was twenty-one. Each correct response to a statement on knowledge of antibiotics was given a score of 1, whereas incorrect responses were given a score of 0. The minimum and maximum possible score in the

knowledge domain were 0 and 21, respectively. Some of the questions were with incorrect responses, and the scores were inverted. The total score less than 80 and ≥ 80% were categorized as poor and good knowledge respectively.

Total Score (%) = $\frac{\text{Obtained score - least possible score}}{\text{Maximum possible score - least possible score}} \times 100$ (Karuniawati *et al*., 2021).

4.9 Data collection technique

4.9.1 Interviewer administered questionnaire

Data was collected using interviewer administered structured questionnaire to assess the antibiotic self-medication practice of suspected UTI patients after provided their verbal and written consent to participate and recorded all the relevant information required. The tool was adopted from published articles such as from Shafie *et al*. (2018) and Moise *et al*. (2017) with some modifications. The questionnaire had five components: sociodemographic profile (8 questions), knowledge assessing statements (21 questions), for ASMP assessment (25 questions), clinical data (4 questions), and antimicrobial susceptibility test (2 questions). The overall data collection was done by trained laboratory and pharmacy professionals.

4.9.2 Strategic follow up

Once the recruited adult patients gave a urine culture sample, the patient sample specimen identity number was registered on the date of data collection. Further, the follow up was important to obtain the isolated organism and its antimicrobial drug susceptibility test result among the patients with bacterial induced UTIs.

4.9.3 Laboratory protocols

4.9.3.1 Urine culture and identification

Urine specimens were directly inoculated onto blood agar (Oxoid, England) and MacConkey agar (BD, USA) using a sterile standard calibrated wire loop (0.001), and streaked culture plates were incubated at 37ºC aerobically for 24 hours. Number and type of colony count were done on blood agar plate, and then significant bacteriuria was determined. Cultures with colony counts greater than 10^5 cfu/mL, for single isolated bacteria, were considered significant. Identification of bacterial isolates was done using colony characteristics on blood

agar, MacConkey agar and gram reaction of the bacteria and biochemical tests in accordance with standard procedures. Organisms isolated from urine specimens of the patients were identified and tested for antimicrobial susceptibilities (Cheesbourgh, 2006).

4.9.3.2 Antimicrobial Susceptibility Tests

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, England) using Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). Briefly, using a sterile wire loop, 3-5 pure colonies were picked from blood agar plate or MacConkey agar and emulsified in nutrient broth (Oxoid, England) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was adjusted to the optical density of McFarland 0.5 tubes to standardize the inoculums size. A sterile cotton swab was dipped into the suspension and distributed the bacteria suspension evenly over the entire surface of Mueller-Hinton agar (Oxoid, England). The antimicrobial agents tested were such as Amoxicillin (10 µg), Chloramphenicol (30 µg), Nitrofurantoin (300 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Cephalothin (30 µg), Ceftriaxone (30 µg), Norfloxacin (NOR), Doxycycline (30 µg), Trimethoprim-Sulfamethoxazole (25 µg), Tetracycline (30 µg) and other antibiotics (CLSI, 2012).

4.10 Data quality management

Before the actual data collection, the prepared English questionnaire was translated to Amharic version and again back to English to check its consistency to assess the socio-demographic and ASMP, whereas some clinical data were extracted from the patient chart. Then, the data extraction questionnaire and laboratory findings assessing tools were pretested two weeks prior to actual data collection at International Clinical Laboratory (ICL), Addis Ababa, Ethiopia. Based on the pretested questionnaire feedback from adult patients and the diagnosis procedure, some modifications on the questionnaire were done.

During data collection, this research project included four data collectors. Three of them were laboratory technologists and one pharmacist. Before data collection, data collectors and local supervisor (one) were trained on the data collection tool and sampling techniques by the principal investigator. Supervision was carried out regularly during data collection period both by the principal investigator and local supervisor. The collected data was checked on daily basis for completeness and consistence.

4.11 Data analyses

Data was entered and analyzed using SPSS version 25 software. All patient data were explored for its outliers and missing values. Descriptive statistics such as proportions, frequencies, means and standard deviations were used to describe the characteristics of the study patients. A binary logistic regression was employed to identify factors associated with ASP. All factors with p-value ≤ 0.20 in the bivariable analysis were considered as candidates for the multivariable regression model. In all statistical tests, $p < 0.05$ was considered statistically significant. In clinical laboratory study, only urine samples with significant growth were further studied (significant growth was defined as the presence of $> 10^5$ colony-forming units per milliliter (cfu/mL) of urine (Cheesbourgh, 2006). After obtaining the pure strains, the strains were subjected to conventional biochemical identification methods to identify different Gram-negative and positive uropathogens. Resistance data were interpreted according to the Clinical Laboratory Standards Institute. Reference strains of *E. coli (ATCC 25922*) were used for quality control for antimicrobial susceptibility (CLSI, 2012).

4.12 Ethical Clearance

Ethical clearance was obtained from St. Paul's Hospital Millennium Medical College Institutional Review Board (IRB), Addis Ababa, Ethiopia. A supporting letter and the ethical clearance were taken to all study settings. Written and informed verbal consent was taken from the adult patients after clear explanations about the purpose and aims of the study. To maintain their privacy and keep the medical case issue confidential, unique identifiers were removed and the data collected was in a locked cabinet in order to ensure confidentiality.

CHAPTER 5. RESULT

5.1 Sociodemographic characteristics of study patients

In this study, a total of 531 patients were included. The mean score of age (± SD) was 43.74 (± 16.16) years. The minimum and maximum ages of the patients were 18 and 90 years, respectively. About one-third of the patients' age was 50 years and above, 188 (35.4%). Majority, 368 (69.3%) patients were female, 254 (47.8%) patients were with college and above educational background, and 347 (65.3%) patients were married. Most of the patients were government employed, 131 (24.7%), and 518 (97.6%) of the patients came from urban areas. The main two religions of this study patients were Orthodox (316 (59.5%)) and Muslim (119 (22.4%)). Majority of the patient's monthly income was 2000 birr and below. Most of the study patients were from the Arsho Diagnostic Center (352 (66.3%) (Table 1).

Table 1. Sociodemographic characteristics of adult patients suspected of UTIs in Addis Ababa, Ethiopia, 2020/21 (n=531).

Variable	Category	Frequency, N (%)
Study settings	Arsho	352 (66.3)
	EPHI	108 (20.3)
	SPHMMC	71 (13.4)
Sex	Male	163 (30.7)
	Female	368 (69.3)
Age (years)	Mean ± SD	43.74 (± 16.16)
	18-24	36 (6.8)
	25-29	74 (13.9)
	30-34	89 (16.8)
	35-39	50 (9.4)
	40-44	57 (10.7)
	45-49	37 (7)
	≥ 50	188 (35.4)
Religion	Orthodox	316 (59.5)
	Muslim	119 (22.4)
	Protestant	93 (17.5)
	Jehovah's witness	2 (0.4)
	Catholic	1 (0.2)
Marital status	Married	347 (65.3)
	Single	134 (25.2)
	Widowed	31 (5.8)
	Divorced	14 (2.6)
	Separated	5 (5.8)
Education	Illiterate	16 (3)
	Write and read only	42 (7.9)
	Primary school	94 (17.7)
	Secondary school	125 (23.5)
	College and above	254 (47.8)
Occupation	Government	131 (24.7)
	Private (owner)	108 (20.3)
	House wife	104 (19.6)
	Private (employed)	99 (18.6)
	Retired	40 (7.5)
	Jobless	21 (4)
	Student	14 (2.6)
	Others*	14 (2.6)
Residence	Urban	518 (97.6)
	Rural	13 (2.4)
Monthly income (birr)	≤ 2000	204 (38.4)
	2001-3000	79 (14.9)
	3001-4000	44 (8.3)
	4001-5000	69 (13)
	5001-6000	19 (3.6)
	≥ 6001	116 (21.8)

Others *: farmers, house servants, and church servants.

5.2 Knowledge Towards Antibiotics

In this study, 367 (70.8%) of the patients heard the term antibiotics. About 382 (71.9%) of the total patients self-reported that antibiotics are drugs which treat bacterial infections. However, some patients, 39 (7.3%), self-reported that the clear indications of antibiotics were for the purpose of treating viral infections.

As it can be seen in Table 2, the self-reported response to the statements posed; 370 (71.9%) of the patients self-reported that broad-spectrum antibiotics are better than narrow-spectrum ones; 279 (52.5%) reported, higher doses result in faster recovery; 379 (71.4%), lower doses result in less adverse reactions; 404 (76.1%), switching antibiotics enhances drug effects; 418 (78.7%), switching antibiotics reduces adverse reactions; 422 (79.5%), intravenous is better than oral medication; 322 (60.6%), ASM practice could cause drug resistance; 486 (91.5%), ASM practice could result into disease complication; 506 (95.3%) ASM practice could result into harmful effects; and 469 (88.3%) of the patients self-reported that ASM could not be practiced for all drugs.

According to most of the patients' self-report, some drugs are not taken with other drugs, 480 (90.4%); not taken with other alcoholic beverages, 510 (96%); and not taken with some kinds of food, 514 (96.8%), respectively. About 482 (90.8%) and 386 (72.7%) of the patients self-reported that the same drug could be given by oral, injection, topical, or other routes and had a practice of checking the expiry date of the drugs before purchasing or before using them, respectively. Most of the patients had a good/poor knowledge. About 107 (20.2%) of the total patients had a good knowledge towards antibiotics (Table 2).

Table 2. Knowledge towards antibiotics of the adult patients suspected of urinary tract infection in Addis Ababa, Ethiopia.

SN.	Knowledge Statements	Count, N (%)	
		Yes	No
K1	I heard about the word antibiotics	376 (70.8)	155 (29.2)
K2	Antibiotics treat		
K2.1	Bacterial infection	382 (71.9)	-
K2.2	Viral infection	39 (7.3)	-
K2.3	I do not know	110 (20.7)	-
K3	Broad-spectrum antibiotics are better than narrow-spectrum ones	370 (69.7)	161 (30.3)
K4	Higher doses result in faster recovery	252 (47.5)	279 (52.5)
K5	Lower doses result in less adverse reactions	152 (28.6)	379 (71.4)
K6	Switching antibiotics enhances drug effects	127 (23.9)	404 (76.1)
K7	Switching antibiotics reduces adverse reactions	113 (21.3)	418 (78.7)
K8	Intravenous is better than oral medication	422 (79.5)	109 (20.5)
K9	Antibiotic self-medication could cause drug resistance	322 (60.6)	209 (39.4)
K10	Antibiotic self-medication can be practiced for all drugs	62 (11.7)	469 (88.3)
K11	Antibiotic self-medication can result into disease complication	486 (91.5)	45 (8.5)
K12	Antibiotic self-medication can result into harmful effects	506 (95.3)	25 (4.7)
K13	Some drugs cannot be taken with other drugs	480 (90.4)	51 (9.6)
K14	Some drugs cannot be taken with alcoholic drinks	510 (96)	21 (4)
K15	Some drugs cannot be taken with some kinds of foods	514 (96.8)	17 (3.2)
K16	Some drugs are contraindicated or cannot be given to children	514 (96.8)	17 (3.2)
K17	Some drugs are contraindicated or cannot be given to pregnant	515 (97)	16 (3)
K18	Some drugs cannot be given to breast feeding mothers	504 (94.9)	27 (5.1)
K19	Some drugs Cannot be taken by people with chronic diseases	431 (81.2)	100 (18.8)
K20	Same drug can be given by oral, injection, topical or other routes	482 (90.8)	49 (9.2)
K21	Checking expiry date of the drugs you purchase or before use	386 (72.7)	145 (27.3)
	Level of knowledge		
	Poor Knowledge	424 (79.8)	
	Good Knowledge	107 (20.2)	

K: Knowledge question number; Some drugs: some antibiotics.

5.3 Common Self-Reported Reasons for avoiding ASM Practice

Generally, there were 476 (89.6%) patients who did not practice self-medication with antibiotics. Ninety-nine (20.8%) of the patients mentioned multiple reasons such as fearing not to use wrong drug, fear of side effects, fear of wrong diagnosis, and wrong use of a drug why they did not self-medicate with antibiotics.

However, among the self-reported single reasons mentioned by the patients for avoiding the practice of self-medication with antibiotics, the most common two reasons were fearing the

side effects of drugs (93 (19.5%)) and having the chance of wrong diagnosis (79 (16.6%)). About 23 (4.8%) of the patients who self-reported why they refused the practice of antibiotic self-medication with antibiotics was due to their interest to visit their doctors for the sake of consultation and prescription (Table 3).

Table 3. Common self-reported reasons of the adult patients for avoiding the self-medication with antibiotics during the past 12 months in Addis Ababa, Ethiopia.

Reasons for Avoiding ASM Practice (N=476 patients)	Frequency, N (%)
Not to use a wrong drug (≠ 1)	51 (10.7)
Fear of side effects (≠ 2)	93 (19.5)
Fear of wrong diagnosis (≠ 3)	79 (16.6)
Fear of wrong use of a drug (≠ 4)	57 (12)
All above mentioned (≠ 1, ≠ 2, ≠ 3, ≠ 4)	99 (20.8)
Lack of Drug knowledge to have ASMP	28 (5.9)
Want doctor visit (for consultation and prescription)	23 (4.8)
Due to My chronic illness	12 (2.5)
No illness in the past 12 months	8 (1.7)
Easy access for health facility	7 (1.5)
Others*	19 (4.1)
Total	476 (100)

*Others: those mentioned both (≠ 1, and ≠ 3), (≠ 2 and ≠ 3), and (≠ 3 and ≠ 4); ASMP; Antibiotic self-medication Practice.

5.4 Antibiotic Self-Medication Practice

In this study, the overall prevalence of antibiotic self-medication practice was 55/531 (10.4%). The two common ways of requesting the antibiotics from different drug sources were by mentioning the name of the drug 30 (54.5%), and by mentioning the sign and symptom of illness 13 (23.6%). The prominent sources of drugs for the self-medication with antibiotics in this study were community pharmacies 50 (90.9%). Most of the patients' antibiotic selection basis for the purpose of self-medication was indications of the drugs 27 (49.1%), and type of antibiotics 20 (36.4%). The sources of drug related information for self-medication with antibiotics in majority of the self-reported patients were their own experience 14 (25.5%) followed by community pharmacists, previous doctors' prescription, and opinions of friends 11 (20%) each.

As to patients' self-reports, the two most common diseases/conditions treated by self-medication with antibiotics were urinary tract infections 14 (25.5%), and peptic ulcer disease 9 (16.4%). Other symptoms or diseases such as fever 6 (10.9%), tonsilitis 5 (9.1%), and

unexplained infections 6 (10.9%) were also reported and treated by self-medication with antibiotics (Table 4).

Table 4. Self-Reported Information on Antibiotics Self-medication among Adult Patients Attending the Study Settings, Addis Ababa, Ethiopia.

Variables	Frequency, (%)
Antibiotic self-medication Practice (ASMP)	
Yes	55 (10.4)
No	476 (89.6)
Source of information	
Community pharmacists	11 (20)
Family members	3 (5.5)
Opinion of friends	11 (20)
My own experience	14 (25.5)
Previous doctor's prescription	11 (20)
Internet searching	2 (3.6)
Pharmacist and friends	1 (1.8)
Others*	2 (3.6)
Selection basis	
Type of antibiotics	20 (36.4)
Brand of antibiotics	6 (10.9)
Price of antibiotics	1 (1.8)
Indications (use)	27 (49.1)
type and side effects of antibiotics	1 (1.8)
Source of Drug	
Community Pharmacies	50 (90.9)
Leftover from previous prescription	2 (3.6)
From neighbor	1 (1.8)
Open Market/shop	2 (3.6)
Ways of requesting the drug	
By mentioning the name of the drug	30 (54.5)
By mentioning the sign and symptom of illness	13 (23.6)
By showing drug container	9 (16.4)
By showing a piece of paper (With Drug name)	3 (5.5)
Diseases/symptoms ≠	
Urinary tract infection	14 (25.5)
Peptic Ulcer Disease	9 (16.4)
Abdominal pain	7 (12.7)
Fever	6 (10.9)
Unexplained Infections	6 (10.9)
Tonsilitis	5 (9.1)
Cough	4 (7.3)
Wound infection	1 (1.8)
Typhoid disease	2 (3.6)
Toothache	2 (3.6)
Others**	5 (9.1)

Others*: Neighbor and Open Market; Others**, such as diarrhea, tape worm infection; ≠; the total percentage for the diseases/symptoms treated is not equivalent to 100% as patients might have more than one response.

5.5 Self-medicated antibiotics and Reasons for ASMP

According to the self-report of the patients, the three commonly self-medicated antibiotics were amoxicillin 28 (50.9%), ciprofloxacin 13 (23.6%), and sulfamethoxazole-trimethoprim combination 5 (9.1%). Although some patients self-reported that they self-medicated with antibiotics, they were unable to mention the names of the 8 (14.5%) antibiotics. In this study, the three most common self-reported reasons of self-medication with antibiotics were knowing the drug before self-medication 29 (52.7%), minor illness 14 (25.5%), and urgency case 7 (12.7%) (Table 5).

Table 5. Self-Medicated Antibiotics and Reasons for Self-Medication by Adult Patients Suspected of UTIs attending at the Study Settings, Addis Ababa, Ethiopia.

Variables	Frequency, (%)
Self-medicated Antibiotics ≠	
Amoxicillin	28 (50.9)
Ciprofloxacin	13 (23.6)
Sulfamethoxazole-trimethoprim	5 (9.1)
Ampicillin	4 (7.3)
Tetracycline	3 (5.5)
Ceftriaxone	4 (7.3)
Norfloxacillin	2 (3.6)
Amoxacillin-Clavulanic acid	1 (1.8)
Cefixime	1 (1.8)
Cloxacillin	1 (1.8)
Unable To Mention the Drug Name	8 (14.5)
Reasons for Antibiotic self-medication	
I know the drug before	29 (52.7)
Minor illness	14 (25.5)
Urgency case	7 (12.7)
Time constraint	3 (5.5)
Self-medication is cheap	1 (1.8)
Health institutions are far, and I know the drug	1 (1.8)

≠; the total percentage for the antibiotics used is not equivalent to 100% as patients might have used more than one antibiotic type.

5.6 Antibiotic Self-Medication Information

The self-report of most of the self-medicated patients were; 20 (36.4%) of them never checked drug leaflet instructions; 20 (57.1%) reported, partially understood the drug leaflet instructions; 48 (87.3%), antibiotics self-medication is unacceptable practice; 34 (61.8%), the outcomes of their self-medication with antibiotics were finding relief from their illness; 31 (56.4%), they could not treat common infections successfully in case they contracted them; 36 (65.5%) had no concern at all for using counterfeit antibiotics; and 25 (45.5%), they stopped taking antibiotics after finishing/ran out their drugs.

Further, only 2 (3.6%) of the patients with antibiotic self-medication practice used the same drugs having different names. The maximum number of drugs used for self-medication with antibiotics was only one drug, 46 (83.6%). Majority of the patients determined the dose of the antibiotics used was by consulting pharmacists 36 (65.5%). About 44 (80%) of the patients self-reported that they never changed the dose of the antibiotics during the course of therapy. However, the main reason for those patients who changed the dose during the course of therapy was health improvement at some time before finishing the expected length of days of therapy.

Additionally, most of the patients self-reported that they never switched (47 (85.5%)) the antibiotics used by other antibiotics during the self-medication period. But, among those who switched their antibiotics during the self-medication time, their main reason was the failure of the former antibiotics in treating their diseases/symptoms, 8 (88.9%) (Table 6).

Table 6. Antibiotic Self-medication information

Variables	**Frequency, (%)**
Checking Drug leaflet instructions	
Yes, always	19 (34.5)
Yes, sometimes	16 (29.1)
Never	20 (36.4)
Understanding Drug leaflet instruction	
Fully understood	15 (42.9)
Partly understood	20 (57.1)
Attitude towards Antibiotic Self-medication Practice	
Better Practice	2 (3.6)
Acceptable Practice	5 (9.1)
Unacceptable practice	48 (87.3)
Outcomes of ASMP	
Cured from the illness	17 (30.9)
Get relief from the illness	34 (61.8)
No improvement	4 (7.3)

Successful treatment status during infection Using ASMP	
Yes, I can	15 (27.3)
I am not sure	9 (16.4)
No, I can not	31 (56.4)
Concern for Using Counterfeit Antibiotics	
Yes, very strongly	6 (10.9)
Yes, partially	13 (23.6)
No concern at all	36 (65.5)
Stop Taking Antibiotics	
After symptoms disappeared	20 (36.4)
After Antibiotics ran out	25 (45.5)
After completion of the treatment course	8 (14.5)
After consulting a pharmacist	2 (3.6)
Using the same drug with different names	
Yes	2 (3.6)
No	53 (96.4)
Maximum Number of drugs used in a single illness	
One	46 (83.6)
Two	6 (10.9)
Three	2 (3.6)
Four	1 (1.8)
I know the dosage of the antibiotics	
By checking the package insert	5 (9.1)
By consulting a pharmacist	36 (65.5)
By consulting my families/friends	3 (5.5)
From the internet	1 (1.8)
From my previous experience	6 (10.9)
By checking the drug leaflet and consulting a pharmacist	2 (3.6)
Others	2 (3.6)
Changing the dose during Self-medication	
Yes, always	3 (5.5)
Yes, sometimes	8 (14.5)
Never	44 (80)
Reasons for changing the dose during ASMP	
Due to improvement	5 (41.7)
Worse thing happened	4 (33.3)
To decrease the side effects	2 (16.7)
Other	1 (8.3)
Switching Antibiotics During SMP	
Yes, always	3 (5.5)
Yes, sometimes	5 (9.1)
Never	47 (85.5)
Reason of Switching Antibiotics During SMP	
The former antibiotics did not work	8 (88.9)
Due to improvement	1 (11.1)

5.7 Clinical data of the patients

This study revealed that 211 (39.7%) of the patients had co-morbidities. According to the patients' self-report, 365 (68.7%), 279 (52.5%) and 105 (19.8%) of them had clinical presentations of UTIs, history of UTIs and catheterization, respectively. The urine culture test findings showed 129 (24.3%) of the patients had urine test identification with bacterial isolates (Table 7).

Table 7. Clinical data of the patients

Variable	**Frequency (%)**
Co-morbidity	
Yes	211 (39.7)
No	320 (60.3)
UTI symptoms	
Yes	365 (68.7)
No	166 (31.3)
History of Catheterization	
Yes	105 (19.8)
No	426 (80.2)
History of UTI	
Yes	279 (52.5)
No	252 (47.5)
Urine culture result	
Yes (organism isolate)	129 (24.3)
No organism isolates	402 (75.7)

5.8 Urine Culture Identification Test and Isolation

In this study, there were 531 (100%) patients who submitted a urine sample for culture test. Of these patients, 402 (75.7%) of the patients had no bacteria isolate. However, 129 (24.3%) of the patients were diagnosed with UTI and had bacteria isolates. The prevalence of urinary tract infection among the UTI suspected adult patients was 24.3%. The two common bacteria identified and isolated were *E. coli*, 88 (68.2%); and *K. pneumoniae*, 10 (7.8%) (Table 8).

Table 8. Urine Culture Result of Adult Patients Suspected of Urinary Tract Infections in study settings, Addis Ababa, Ethiopia.

Isolation status		Frequency, N (%)
Culture result	*E. coli*	88 (68.2)
	K. pneumoniae	10 (7.8)
	Providencia Species	6 (4.7)
	Enterococcus faecalis	5 (3.9)
	Klebsiella Oxytoca	4 (3.1)
	Pseudomonas Species	3 (2.3)
	Staphylococcus aureus	3 (2.3)
	Enterococcus faecium	3 (2.3)
	Morganella Morganni	2 (1.6)
	Others*	5 (3.9)
	Total isolates	129 (100)

Others*; *Citrobacter freundi* (1), *Citrobacter diversus* (1), *Enterobacter spp.* (1), *Enterococcus spp.* (1), *and Staphylococcus aureus* (1).

5.9 Antibiotic Susceptibility Test

Totally, 39 antibiotics were checked for antibiotic susceptibility tests by the order of disease specialists from different health institutions to the study settings of culture unit. Accordingly, the following antibiotic susceptibility test findings were reported: among 55 *E. coli* isolates, 50 (90.9%) of them were ampicillin resistant, 32 (100%) of the isolates were amoxicillin resistant, 22 (100%) of the isolates were doxycycline resistant. However, low levels of resistances were reported to meropenem and cefepime which were 1/29 (3.4%) and 12/49 (24.5%) respectively. From fluoroquinolones class of antibiotics, 22 (100%) of the *E. coli* isolates and 24 (96%) of 25 *E. coli* isolates were moxifloxacin and levofloxacin sensitive respectively. Unlike to moxifloxacin, 23 (42.6%) of 54 *E. coli* isolates were ciprofloxacin resistant, 9 (31%) of 29 *E. coli* isolates were norfloxacin resistant and 2 (50%) of the 4 *E. coli* isolates were nalidixic acid resistant. Twenty-eight (65.1%) of the 43 *E. coli* isolates were cephalothin resistant. Among 38 *E. coli* isolates tested with amikacin, 35 (92.1%) of them was sensitive to amikacin.

Additionally, of 56 *E. coli* isolates, 29 (51.8%) of them were ceftriaxone sensitive, 18 (78.3%) of the 23 *E. coli* isolates tested were piperacillin-tazobactam sensitive and 15 (78.9%) of the 19 *E. coli* isolates were tobramycin sensitive.

Within the tested antibiotics on *K. pneumoniae* isolates, 6 (100%), for ampicillin, 5 (100%) for amoxicillin, and 4 (100) for doxycycline were totally resistant. *Providencia species* were totally resistant for 6 (100%) isolates carried on ampicillin and amoxicillin each. For *K. oxytoca*, cotrimoxazole was resistant to 3 (100%) of the isolates (Table 9).

Table 9. Antibiotic Susceptibility Test Results of Bacteria Isolated from Symptomatic UTI Adult Patients at the study settings in Addis Ababa, Ethiopia.

Drug		*E. coli*	*K. pneumoniae*	*E. faecalis*	*Prov.* Species	*K. Oxytoca*	*Pseud. Species*	*M. Morganni*	*S. aureus*	Others	*E. faecium*
AMP	S	5 (9.1)	-	4 (80)	-	-	-	-	1 (33.3)	-	-
	R	50 (90.9)	6 (100)	1 (20)	6 (100)	4 (100)	-	1 (100)	2 (66.7)	2 (100)	3 (100)
AMPS	S	1 (16.7)	-	4 (100)	-	-	-	-	1 (50)	1 (100)	-
	R	5 (83.3)	1 (100)	-	-	2 (100)	-	-	1 (50)	-	-
AUG	S	14 (43.75)	2 (66.7)	3 (100)	-	-	-	-	1 (50)	1 (50)	-
	R	14 (43.75)	-	-	-	2 (100)	-	-	1 (50)	1 (50)	-
	I	4 (12.5)	1 (33.3)	-	-	-	-	-	-	-	-
AMK	S	35 (92.1)	5 (100)	1 (100)	3 (100)	4 (100)	1 (50)	-	-	-	-
	R	2 (5.3)	-	-	-	-	1 (50)	-	-	-	-
	I	1 (2.6)	-	-	-	-	-	-	-	-	-
AMX	S	-	-	4 (100)	-	-	-	-	-	-	-
	R	32 (100)	5 (100)	-	6 (100)	4 (100)	-	1 (100)	2 (100)	1 (100)	3 (100)
AZS	S	-	-	-	-	-	-	-	-	-	-
	R	-	1 (100)	-	-	-	-	-	-	-	-
BEP	S	-	-	2 (100)	-	-	-	-	-	-	-
	R	-	-	-	-	-	-	-	2 (100)	1 (100)	2 (100)
CFP	S	21 (67.8)	4 (100)	-	3 (100)	1 (100)	-	-	1 (50)	1 (100)	-
	R	10 (32.2)	-	-	-	-	2 (100)	-	1 (50)	-	-
CFT	S	29 (56.9)	5 (71.4)	-	3 (50)	1 (50)	-	-	1 (50)	1 (50)	-
	R	22 (43.1)	2 (28.6)	-	3 (50)	1 (50)	2 (100)	1 (100)	1 (50)	-	-
CFRO	S	22 (40)	3 (42.9)	-	2 (40)	1 (25)	-	1 (100)	1 (50)	1 (33.3)	-
	R	32 (58.2)	4 (57.1)	-	3 (60)	3 (75)	2 (100)	-	1 (50)	1 (33.3)	-
	I	1 (1.8)	-	-	-	-	-	-	-	1 (33.4)	-
NTF	S	48 (70.6)	3 (30)	2 (66.7)	4 (80)	-	-	-	1 (100)	2 (66.7)	-
	R	19 (27.9)	6 (60)	1 (33.3)	1 (20)	2 (100)	-	1 (100)	-	1 (33.3)	1 (100)
	I	1 (1.5)	1 (10)	-	-	-	-	-	-	-	-
GEN	S	57 (77)	7 (77.8)	-	4 (66.7)	4 (100)	-	1 (100)	2 (66.7)	1 (50)	-
	R	15 (20.3)	2 (22.2)	-	2 (33.3)	-	2 (100)	-	1 (33.3)	1 (50)	-
	I	2 (2.7)	-	-	-	-	-	-	-	-	-
IMPN	S	3 (75)	-	-	-	-	-	-	-	-	-
	R	1 (25)	-	-	-	-	-	-	-	-	-
LVF	S	24 (96)	1 (50)	-	2 (100)	-	-	-	-	2 (100)	2 (100)
	R	1 (4)	1 (50)	-	-	-	-	-	-	-	-
LNZD	S	-	-	1 (100)	-	-	-	-	-	-	-
MTC	S	-	-	-	-	-	-	-	1 (50)	1 (100)	-
	R	-	-	-	-	-	-	-	1 (50)	-	-
MXF	S	22 (100)	-	-	2 (100)	-	-	-	-	1 (100)	1 (100)
CZD	S	19 (59.4)	4 (80)	-	3 (100)	1 (100)	1 (33.3)	-	-	1 (100)	-
	R	11 (34.4)	1 (20)	-	-	-	2 (66.7)	-	-	-	-
	I	2 (6.2)	-	-	-	-	-	-	-	-	-
CIP	S	30 (55.6)	2 (50)	-	2 (66.7)	-	-	-	1 (100)	2 (66.7)	1 (50)
	R	23 (42.6)	2 (50)	-	1 (33.3)	1 (100)	1 (100)	-	-	1 (33.3)	1 (50)
	I	1 (1.8)	-	1 (100)	-	-	-	-	-	-	-
CPTN	S	14 (32.6)	3 (60)	-	2 (33.3)	1 (25)	-	-	1 (50)	1 (50)	-
	R	28 (65.1)	2 (40)	-	4 (66.7)	3 (75)	2 (100)	-	1 (50)	-	-
	I	1 (2.3)	-	-	-	-	-	-	-	-	-
CFPM	S	34 (69.4)	5 (83.4)	-	4 (100)	1 (100)	1 (50)	-	1 (50)	2 (66.7)	-
	R	12 (24.5)	1 (16.6)	-	-	-	1 (50)	1 (100)	1 (50)	1 (33.3)	-
	I	3 (6.1)	-	-	-	-	-	-	-	-	-
CEF	S	29 (51.8)	5 (55.6)	-	3 (60)	1 (100)	-	-	1 (50)	2 (100)	-
	R	26 (46.4)	4 (44.4)	-	2 (40)	-	1 (100)	-	1 (50)	-	-
	I	1 (1.8)	-	-	-	-	-	-	-	-	-
NOR	S	20 (69)	-	-	1 (50)	-	-	-	-	-	1 (33.3)
	R	9 (31)	2 (100)	2 (100)	1 (50)	2 (100)	-	-	-	-	2 (66.7)
DOX	S	-	-	-	1 (16.7)	-	-	-	1 (50)	-	-
	R	22 (100)	4 (100)	4 (100)	5 (83.3)	2 (100)	-	1 (100)	1 (50)	-	1 (100)
PCN	S	-	-	2 (100)	-	-	-	-	-	1 (100)	-
PTDB	S	18 (78.3)	2 (40)	-	1 (100)	-	-	1 (100)	-	2 (100)	-
	R	3 (13)	-	-	-	-	-	-	-	-	-
	I	2 (8.7)	3 (60)	-	-	-	-	-	-	-	-
SMX	R	2 (100)	-	-	-	-	-	-	-	-	-

TTC	S	9 (25.7)	1 (20)	-	1 (16.7)	-	-	1	1 (50)	1 (50)	-
	R	26 (74.3)	4 (40)	4 (100)	5 (83.3)	2 (100)	1 (100)	1	1 (50)	1 (50)	2 (100)
TOBR	S	15 (78.9)	2 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)	-	-	-
	R	4 (21.1)	-	-	-	-	-	-	-	1 (50)	-
	I	-	-	-	-	-	-	-	-	1 (50)	-
VAN	S	-	-	3 (100)	-	-	-	-	-	1 (100)	2 (100)
NA	S	2 (50)	-	-	-	-	-	-	-	-	-
	R	2 (50)	1 (100)	-	-	-	-	-	-	1 (100)	1 (100)
MER	S	28 (96.6)	4 (100)	-	-	-	1 (50)	2 (100)	-	2 (100)	-
	R	1 (3.4)	-	-	-	-	1 (50)	-	-	-	-
CFLX N	S	-	-	-	-	-	-	-	-	1 (100)	-
	R	1 (100)	-	-	-	-	-	-	-	-	-
CFOX	S	3 (75)	2 (100)	-	-	-	-	-	-	-	-
	R	1 (25)	-	-	-	-	-	-	-	-	-
COT	S	9 (24.3)	2 (40)	-	-	-	-	1 (100)	-	-	-
	R	28 (75.7)	3 (60)	-	-	3 (100)	-	-	-	2 (100)	-
CFXM	S	17 (43.6)	3 (75)	-	-	1 (50)	-	1 (100)	-	1 (100)	-
	R	22 (56.4)	1 (25)	-	1 (100)	1 (50)	1 (100)	-	-	-	-
CFZO	S	25 (33.3)	3 (37.5)	-	2 (40)	2 (50)	-	1 (100)	1 (50)	2 (100)	-
	R	49 (65.3)	5 (62.5)	-	3 (60)	2 (50)	2 (100)	-	1 (50)	-	-
	I	1 (1.4)	-	-	-	-	-	-	-	-	-
CLDA	S	-	-	-	-	-	-	-	1 (100)	-	-
ERY	S	-	-	-	-	-	-	-	1 (50)	-	-
	R	-	-	-	-	-	-	-	1 (50)	-	-

AMP, ampicillin; AMPS, ampicillin-sulbactam; AUG, augmentin; AMK, amikacin; AMX, amoxacillin; AZS; azithromycin; BEP, benzathine penicillin; CFP, cefpodoxime; CFT, cefotaxime; CFRO, cefuroxime; NTF, nitrofurantoin; GEN, gentamycin; IMPN, imipenem; LVF, levofloxacin; LNZD, linezolid; MTC, methicillin; MXF, moxifloxacin; CZD, ceftazidime; CIP, ciprofloxacin; CPTN, cephalothin; CFPM, cefepime; CEF, ceftriaxone; NOR, Norfloxacin; DOX, doxycycline; PCN, penicillin; PTDB, piperacillin-tazobactam; SMX, sulphamethoxazole; TTC, tetracycline; TOBR, tobramycin; VAN, vancomycin; NA; nalidixic acid; MER, meropenem; CFLXN, cefalexin; CFOX, cefoxitin; COT, cotrimoxazole; CFXM, cefixime; CFZO, cefazolin; CLDA, clindamycin; ERY, erythromycin.

5.10 Multi-Drug Resistance

Among the total bacterial isolates (n=129), the overall prevalence of multi-drug resistance bacteria was reported for 106 (82.2%) bacterial isolates; which were resistant to two or more of antibiotics. Within the overall MDR findings, the three most common multi-drug resistances were reported for *E. coli*, 69 (65.5%), *K. pneumoniae*, 9 (8.5%), and *Providencia species,* 6 (5.7%), respectively. Regarding to the number of drugs resisted, the three groups of antibiotics commonly resisted were R2, 14 (13.2%), R3, R6, and R8, 13 (12.3%) each group and R4, 11 (10.4%), respectively. One (1.4%) of the *E. coli* isolates and 1 (50%) of *S. aureus* isolates were resistant to eighteen and sixteen antibiotics, respectively. In this study, more than 80% of the findings showed that the common uropathogenic organisms which developed multi-drug resistance were Gram-negative bacteria (Table 10).

Table 10. Multi-Drug Resistance Pattern of Bacterial Isolates from Symptomatic UTI Patients Attending at the study settings, Addis Ababa, Ethiopia.

Bacteria Isolates		R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R18	MDR, N (%)
E. coli	Count, (%)	8 (11.6)	8 (11.6)	7 (10.1)	7 (10.1)	10 (14.5)	5 (7.2)	7 (10.1)	2 (2.9)	2 (2.9)	4 (5.8)	3 (4.3)	1 (1.4)	1 (1.4)	3 (4.3)	-	1 (1.4)	69 (100)
	% Within NDR	57.1	61.5	63.6	70	76.9	83.3	53.8	33.3	50	66.7	100	100	100	100	-	100	65.1
K. pneumoniae	Count, (%)	1 (11.1)	-	2 (22.2)	1 (11.1)	1 (11.1)	1 (11.1)	1 (11.1)	-	1 (11.1)	1 (11.1)	-	-	-	-	-	-	9 (100)
	% Within NDR	7.1	-	18.2	10	7.7	16.7	7.7	-	25	16.7	-	-	-	-	-	-	8.5
E. faecalis	Count, (%)	2 (50)	1 (25)	1 (25)	-	-	-	-	-	-	-	-	-	-	-	-	-	4 (100)
	% Within NDR	14.3	7.7	9.1	-	-	-	-	-	-	-	-	-	-	-	-	-	3.8
Prov. Species	Count, (%)	1 (16.7)	-	1 (16.7)	-	-	-	1 (16.7)	2 (33.3)	-	1 (16.7)	-	-	-	-	-	-	6 (100)
	% Within NDR	7.1	-	9.1	-	-	-	7.7	33.3	-	16.7	-	-	-	-	-	-	5.7
K. Oxytoca	Count, (%)	-	-	-	-	-	-	2 (50)	2 (50)	-	-	-	-	-	-	-	-	4 (100)
	% Within NDR	-	-	-	-	-	-	15.4	33.3	-	-	-	-	-	-	-	-	3.8
Pseudo. Species	Count, (%)	-	-	-	-	1 (33.3)	-	1 (33.3)	-	1 (33.3)	-	-	-	-	-	-	-	3 (100)
	% Within NDR	-	-	-	-	7.7	-	7.7	-	25	-	-	-	-	-	-	-	2.8
M. Morganni	Count, (%)	1 (50)	-	-	1 (50)	-	-	-	-	-	-	-	-	-	-	-	-	2 (100)
	% Within NDR	7.1	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	1.9
S. aureus	Count, (%)	-	1 (50)	-	-	-	-	-	-	-	-	-	-	-	-	1 (50)	-	2 (100)
	% Within NDR	-	7.7	-	-	-	-	-	-	-	-	-	-	-	-	100	-	1.9
Others	Count, (%)	-	3 (75)	-	-	1 (25)	-	-	-	-	-	-	-	-	-	-	-	4 (100)
	% Within NDR	-	23.1	-	-	7.7	-	-	-	-	-	-	-	-	-	-	-	3.8
E. faecium	Count, (%)	1 (33.3)	-	-	1 (33.3)	-	-	1 (33.3)	-	-	-	-	-	-	-	-	-	3 (100)
	% Within NDR	7.1	-	-	10	-	-	7.7	-	-	-	-	-	-	-	-	-	2.8
Total	Count, (%)	14 (13.2)	13 (12.3)	11 (10.4)	10 (9.4)	13 (12.3)	6 (5.7)	13 (12.3)	6 (5.7)	4 (3.8)	6 (5.7)	3 (2.8)	1 (0.9)	1 (0.9)	3 (2.8)	1 (0.9)	1 (0.9)	106 (100)
	% Within NDR	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Notes: NDR, Number of Drug Resistance; **N**, number; **MDR**, Multi-Drug Resistance (for two or more antibiotics); **R2**, resistance to two antibiotics; **R3**, resistance to three antibiotics; **R4**, resistance to four antibiotics; **R5**, resistance to five antibiotics; **R6**, resistance to six antibiotics; **R7**, resistance to seven antibiotics; **R8,** resistance to eight antibiotics; **R9**, resistance to nine antibiotics; **R10**, resistance to ten antibiotics; **R11**, resistance to eleven antibiotics; **R12,** resistance to twelve antibiotics; **R13**, resistance to thirteen antibiotics; **R14**, resistance to fourteen antibiotics; **R15**, resistance to fifteen antibiotics; **R16**, resistance to sixteen antibiotics; **R18**, resistance to eighteen antibiotics. *Prov. Species, Proventcia Species.*

5.11 Multi-Drug Resistant Report versus the Antibiotic self-medication

As it can be seen in Table 11, among those patients who had MDR bacteria, 9/106 (8.5%) of them had antibiotics self-medication practice. Three (33.3%) of the patients who had MDR and ASMP were reported of having resistance to six antibiotics. Among the patients with no self-medication practice history but with MDR, most of them developed resistance to thirteen antibiotics (13.4%).

Table 11. The cross tabulation of Multi-Drug Resistant Report versus the Antibiotic self-medication Practice in the study settings, Addis Ababa, Ethiopia.

Variable			Antibiotic SM Practice N (%)		Total
			Yes	No	
MDR	R2	Count (%)	1 (7.1)	13 (92.9)	14 (100)
		% Within ASMP	11.1%	13.4%	13.2%
	R3	Count (%)	0	13 (100)	13 (100)
		% Within ASMP	0.0%	13.4%	12.3%
	R4	Count (%)	1 (9.1)	10 (90.9)	11 (100)
		% Within ASMP	11.1%	10.3%	10.4%
	R5	Count (%)	1 (10)	9 (90)	10 (100)
		% Within ASMP	11.1%	9.3%	9.4%
	R6	Count (%)	3 (23.1)	10 (76.9)	13 (100)
		% Within ASMP	33.3%	10.3%	12.3%
	R7	Count (%)	0	6 (100)	6 (100)
		% Within ASMP	0.0%	6.2%	5.7%
	R8	Count (%)	1 (7.7)	12 (92.3)	13 (100)
		% Within ASMP	11.1%	12.4%	12.3%
	R9	Count (%)	0	6 (100)	6 (100)
		% Within ASMP	0.0%	6.2%	5.7%
	R10	Count (%)	0	4 (100)	4 (100)
		% Within ASMP	0.0%	4.1%	3.8%
	R11	Count (%)	1 (16.7)	5 (83.3)	6 (100)
		% Within ASMP	11.1%	5.2%	5.7%
	R12	Count (%)	1 (33.3)	2 (66.7)	3 (100)
		% Within ASMP	11.1%	2.1%	2.8%
	R13	Count (%)	0	1 (100)	1 (100)
		% Within ASMP	0.0%	1.0%	0.9%
	R14	Count (%)	0	1 (100)	1 (100)
		% Within ASMP	0.0%	1.0%	0.9%
	R15	Count (%)	0	3 (100)	3 (100)
		% Within ASMP	0.0%	3.1%	2.8%
	R16	Count (%)	0	1 (100)	1 (100)
		% Within ASMP	0.0%	1.0%	0.9%
	R18	Count (%)	0	1 (100)	1 (100)
		% Within ASMP	0.0%	1.0%	0.9%
Total		Count (%)	9 (8.5%)	97 (91.5)	106 (100)
		% Within ASMP	100.0%	100.0%	100.0%

5.12 Factors Associated with Antibiotics Self-medication Practice

As stated in Table 12 below, the current study revealed that patients who were 50 year-old and above were about 4 times (AOR: 4.338 (CI: 1.407-13.380); P-Value: 0.011) more likely to have antibiotic self-medication practice compared to patients who were 18-24 year-old. Additionally, most of the patients whose monthly income was less than 4000 birrs (Adjusted Odds Ratio (AOR): 2.610 (Confidence interval (CI): 1.331-5.117); p: 0.005) and those patients with no history of urinary tract infections (UTI) (AOR: 1.952 (CI: 1.062-3.588); p: 0.031) were significantly associated with ASMP compared to patients with high-incomes and UTI histories, respectively.

Table 12. Multiple Logistic regression analysis of the study variables towards Antibiotic Self-medication Practice in the study settings, Addis Ababa, Ethiopia.

Variable	ASMP		Crude Odds Ratio (95%, CI; P< 0.05)	Adjusted Odds Ratio (95%, CI; P< 0.05)
	Yes	No		
Age				
18-24	7 (12.7)	29 (6.1)	1	1
25-29	13 (23.6)	61 (12.8)	1.133 (0.409-3.140)	1.237 (0.428-3.576)
30-34	11 (20)	78 (16.4)	1.712 (0.606-4.838)	2.170 (0.725-6.496)
35-39	7 (12.7)	43 (9)	1.483 (0.470-4.676)	1.888 (0.566-6.305)
40-44	4 (7.3)	53 (11.1)	3.198 (0.864-11.844)	3.914 (0.979-15.639)
45-49	4 (7.3)	33 (6.9)	1.991 (0.529-7.499)	1.997 (0.493-8.092)
≥ 50	9 (16.4)	179 (37.6)	4.801 (1.659-13.896); 0.004*	4.338 (1.407-13.38); 0.011*
Married status				
Single	22 (40)	112 (23.5)	1	1
Married in lifetime	33 (60)	364 (76.5)	2.167 (1.214-3.868); 0.009*	1.659 (0.881-3.125)
Educational level				
College and above	34 (61.8)	220 (46.2)	1	1
Secondary and below	21 (38.2)	256 (53.8)	1.884 (1.062-3.342); 0.03*	0.945 (0.475-1.878)
History of UTI				
Yes	36 (65.5)	243 (51.1)	1	1
No	19 (34.5)	233 (48.9)	1.817 (1.013-3.258); 0.045*	1.952 (1.062-3.588); 0.031*
Monthly income (Birr)				
≥ 4001	32 (58.2)	172 (36.1)	1	1
≤ 4000	23 (41.8)	304 (63.9)	2.459 (1.394-4.337); 0.002*	2.610 (1.331-5.117); 0.005*

Married in lifetime; include currently married, divorced, and separated patients.

CHAPTER 6. DISCUSSION

This study involved a total of 531 patients collated from three studies in Addis Ababa, Ethiopia. The overall prevalence of self-medication with antibiotics among adult patients suspected of urinary tract infections was 10.4%. This proportion is similar to a previous study (Nepal and Bhatta, 2018). However, this study's proportion is lower and inconsistent with the findings of studies conducted in Kampala (Uganda) (Nabaweesi *et al.*, 2021), Khartoum (Sudan) (Isameldin *et al.*, 2020), Eritrea (Ateshim *et al.,* 2019), Egypt (Zeid *et al.*, 2020), Dominican Republic (Aragoneses *et al.,* 2021), Saudi Arabia (Al Rasheed *et al.*, 2016), Pakistan (Gillani *et al.*, 2021) and Haiti (Moise *et al.*, 2017). The discrepancies in the prevalence results across the studies may be attributed to uncontrolled distribution of drugs, presence of pharmacies which dispense antibiotics without prescriptions, sociodemographic characteristics, sample size, the presence/absence of co-morbidities, and the level of knowledge regarding antibiotics.

According to patient self-reports, the most frequently self-medicated antibiotic in this present work was amoxicillin. Similar to the current study's result, it has been reported and substantiated in some earlier studies conducted in India (Moktan and Shehnaz, 2020), Kabul (Afghanistan) (Negarandeh *et al.*, 2021), Yemen (Afadly *et al.*, 2017), Haiti (Moise *et al.*, 2017), Myanmar (Win *et al.*, 2021) and Saudi Arabia (Al Rasheed *et al.*, 2016). This might be explained by the fact that amoxicillin is more "familiar" than other antibiotics and the ease of purchase. Penicillins, which are a class of drugs that includes amoxicillin, were reported to be the most common antibiotics for self-medication (Roien *et al.*, 2021; Donkor *et al.*, 2019; Alhomouda *et al.*, 2017).

The most common self-medicated condition treated with antibiotics in this study were urinary tract infections. This is similar to previously studies (Donkor *et al.*, 2019; Gardiner *et al.*, 2019). However, this result differs from those of Shabnam and Azim (2015) which found that flu symptoms, or more specifically, a sore throat prompted most episodes of antibiotic self-medication (Negarandeh *et al.*, 2021).

The primary reason for antibiotic self-medication amongst this study group was previous experience and/or simple familiarity with the drug name. This is similar to previous studies (Shabnam and Azim, 2015; Alghadeer *et al.*, 2018; Abduelkarem *et al.*, 2019; Ateshim *et al.,* 2019). Unlike to this study, other studies reported that other reasons such as cost-saving, previous experience and lack of time (Negarandeh *et al.*, 2021), availability of the drug at the local drug store (Tobin and Atulomah, 2020), cost saving (Zeid *et al.*, 2020), and socio-

cultural, economic, and regulatory factors (Alhomouda *et al.*, 2017) of seeking self-medication with antibiotics.

In this study, symptom resolution was most cited as a positive result of self-medication. This result agrees with an earlier study (Ocan *et al.*, 2015). The primary sources of antibiotics procurement were local pharmacies. Earlier studies found similar results (Al Rasheed *et al.*, 2016; Albawani *et al.*, 2017; Abduelkarem *et al.*, 2019; Moktan and Shehnaz, 2020; Yeika *et al.*, 2021). Lax prescribing protocols or loose pharmacy practices are not uncommon in many low and middle-income countries contributing to misuse (Ngu *et al.*, 2018). Additionally, this study showed that the main source of information for the antibiotics self-medication practice in most of the patients was the past/own experience. This result is in line to a previous study (Nusair *et al.*, 2021). But other studies reported different findings from this study (Sattar *et al.*, 2018; Anaba *et al.*, 2021).

Patients with a monthly income under or equal to 4000 birr were statistically more likely to practice self-medication with antibiotics compared to those with higher incomes. This result is similar to studies conducted in Wolaita Soddo, Addis Ababa, and Kolladiba, Ethiopia (Abrha *et al.*, 2014; Chang *et al.*, 2017; Shafie *et al.*, 2018; Mathewos *et al.*, 2021).

In low-income countries, self-medication predominates over clinic visits due to its lower costs. The WHO claims that self-medication provides a cheap alternative to people who are unable to afford the healthcare costs, and thus, self-medication is often the first response to illness among people with low income (Suleman *et al.*, 2009; Abdulraheem *et al.*, 2016). Usually, most people prefer self-medication rather than suffer the high cost of laboratory tests and other services often ordered at clinics.

The patients of this study who had no history of urinary tract infections were more likely to self-medicate compared to patients with infection histories. This practice is similar to that of pulmonary tuberculosis patients who, too, were less likely to self-medicate with antibiotics (Ngu *et al.*, 2018). As reported by Pons *et al.* (2017), similar statement to this current result, patients with more chronic diseases, are consequently more accustomed to a set of signs and symptoms related to their health conditions and may therefore opt to seek a prescription.

The result in this study showed that majority of the patients, 50 years of age and older, were statistically more likely to self-medicate. This is similar to a previous study (Al Rasheed *et al.*, 2016). Unlike to the current findings, other study reported a negative

association between increasing age and self-medication practice with antibiotics (Pons *et al*., 2017; Tobin and Atulomah, 2020).

Some patients of this study who presented MDR bacteria isolates practiced antibiotic self-medication. Earlier studies conducted at Hawassa (Ethiopia) (Mechal *et al*., 2021), Karachi (Pakistan) (Abdullah *et al*., 2013), and Lahore (Aftab *et al*., 2018) support this finding. Additionally, several earlier reports and reviews indicated that the inappropriate use of antibiotics contributed to the development of antibiotic resistance (Davies and Davies, 2010; Vila, 2010; Chon *et al*., 2012; Anyanechi and Saheeb, 2014; Dougherty and Pucci, 2014; Stein *et al*., 2018). Usually, most of the experts have advised the establishment of antibiotic stewardship index to gauge the proportion of a country's gross domestic product utilization that is spent in publicly funded health programs (Walsh and Toleman, 2012).

The present study indicated that, among the adult patients suspected of UTIs, the most common uropathogenic bacteria identified and isolated was *Escherichia coli*. This result is in line with previously conducted studies (Aftab *et al*., 2018; Akter *et al*., 2020; Nazir *et al*., 2021; Mechal *et al*., 2021; Obakiro *et al*., 2021). The possible reason why *Escherichia coli* presents the highest prevalence in most of the studies is its commonness as a urinary pathogen (Behzadi *et al*., 2010) especially in women due to puerperal infections through fecal-vaginal-urinary transmission (Haider *et al*., 2010; Hamdan *et al*., 2011).

In this study, *E. coli* was found to be highly resistant to amoxicillin 32 (100%), doxycycline 22 (100%), and least resistant to meropenem 1 (3.4%). Other studies bear out these same results in terms of high resistance towards amoxicillin (Bhatt *et al*., 2012; Mahdi *et al*., 2016; Parajuli *et al*., 2017; Somily *et al*., 2017). Meropenem was reported to be the most effective drug. However, it should not be administered empirically unless infections are life threatening. Carbapenems are considered the drugs of last resort (Shakya *et al*., 2017). Despite of this, use of "last-resort" antibiotics, such as carbapenems has increased. Between 2000 and 2010, the global consumption of carbapenems increased by 45% (Van Boeckel *et al*., 2014).

In the current work, among the antibiotics tested on *E. coli* isolates, the fourth-generation fluroquinolone, moxifloxacin, showed maximum efficacy and was 100% sensitive against *E. coli*. This finding is supported by an earlier report (Lemmen *et al*., 2003). In this study, more than 80% of self-medicating patients self-reported that the practice was unacceptable. This result is dissimilar to a study performed in Kabul (Afghanistan) (Roien *et al*., 2021).

CHAPTER 7. CONCLUSION

This study revealed that SM with antibiotics for urinary tract infections is a common practice in Addis Ababa, Ethiopia. Worryingly, some of these patients have developed multi-drug resistance urinary tract infections. The two most common uropathogenic organisms detected were *E. coli* and *K. pneumoniae.* In most of the cases, these uropathogenic bacteria were resistant to the commonly prescribed antibiotics.

CHAPTER 8. STRENGTHS and LIMITATIONS

Some possible limitations to the study are the nature of the study design, which was a cross-sectional study, or possible recall bias for some of the questions. The strengths of this study were its urban setting and hospital/diagnostic centers whose laboratories were accredited by national and international organizations. The study focused on the presence of antibiotic self-medication practice among the adult patients with MDR bacteria-induced UTIs.

CHAPTER 9. RECOMMENDATION

This study makes the following recommendations targeting the reduction of antibiotic self-medication:

- Ethiopian public health authorities must enforce existing laws on antibiotics and develop a collaborative pharmacy approach to stem the dispensing of antibiotics without a prescription.
- Develop a public health campaign to enlighten the general public regarding inappropriate antibiotic use.
- Promote antibiotic according to microorganism sensitivity.
- Develop hospital protocols which target evaluation and treatment of patients with suspected urinary tract infections.
- Educate medical students regarding appropriate antibiotic use and its misuse.
- Pursue research into sensitive and specific point-of-care tests for rapid diagnosis of UTIs.
- Promote antibiotic stewardship indexes to gauge the proportion of a country's gross domestic product spent for publicly funded health programs (Walsh and Toleman, 2012).

PART-THREE:

In Vitro Antioxidant, Antibacterial Activities and Phytochemical Screening of Selected Ethno-Medicinal Plants against Patient Isolated MDR-Uropathogenic Bacteria, Addis Ababa, Ethiopia

List of Abbreviations

ABTS	2, 2′-azino-di-(3-ethylbenzthiazoline sulfonic acid
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standard Institute
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
E. coli	*Escherichia coli*
EPHI	Ethiopian Public Health Institute
FIC	Fractional Inhibitory Concentration
FRAP	Ferric Reducing Antioxidant Power
GC-MS	Gas-chromatographic coupled with Mass Spectrometry
IC50%	Inhibitory Concentration 50%
J. shimperiana	*Justicia shimperiana*
K. pneumoniae **ESBL**	*Klebsiella pneumoniae Extended Spectrum Beta-lactamase*
MBC	Minimum Bactericidal Concentration
MDR	Multiple Drug Resistance
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton Broth
MIC	Minimum inhibitory Concentration
OD	Optical Density
QE	Quercetin Equivalence
R. prinoides	*Rhamnus prinoides*
T. schimperi	*Thymus schimperi*
TFC	Total Flavonoid Content
TPC	Total Phenol Content
TPROC	Total Proanthocyanidin Content

CHAPTER 1. INTRODUCTION

1.1 Background

Since primeval times, human beings have used natural products to relieve and treat diseases for themselves and their livestock. Traditional medicine has been in existence even before the advent of modern medicine. It continues to remain as an alternative care available for the majority of the developing countries due to its intrinsic qualities, unique, and holistic approaches as well as its accessibility and affordability (Nigussie, 2021; Nigussie *et al*., 2021).

Plants have been used for the treatment of various disorders regardless of their safety and efficacy profiles. Previous studies indicated that approximately 80% of the global population relies on traditional medicine for their primary healthcare (Joos *et al*., 2012; Pathak and Das, 2013). According to the WHO, medicinal plants are believed to be the best source for getting a diversity of drugs (Yuan *et al*., 2016; WHO, 2021). Nowadays, there is a widespread interest in medicines derived from plants, and it is reported that green medicine is safe and reliable because it contains components and secondary metabolites such as polyphenols, alkaloids, volatile oils, among others, which have therapeutic properties. To this end, formulation of plants for standardization and regulation of phytomedicinal products is the most alternative way (Oluduro, 2012; Abew *et al*., 2014). At present, at least 25 % of the active substances of produced pharmacological drugs are obtained from plants (Sekar and Kandavel, 2010). Ethnobotanical study and knowledge on medicinal plants would contribute to improve human health on a local and/or a global level (Yineger *et al*., 2008).

The genus *Rhamnus* consists of 137 species and 19 synonyms (Anonymous, 2021). *Rhamnus prinoides* is a dicotyledonous angiosperm, which belongs to the genus *Rhamnus* under the family of *Rhamnaceae*. It is commonly known as dark blinkblaar, dogwood, and shiny leaf (Dlamini and Turner, 2002). *Rhamnus prinoides* L'Herit (*Rhamnus prinoides*) is an endemic plant to Ethiopia which grows to a height of about six meters, ecologically widespread, and locally cultivated from medium to high altitudes (1000-3200 m) (Chen *et al*., 2020) and it is widely planted in gardens (Campbell *et al*., 2019; Chen *et al*., 2020). Although, *R. prinoides* is a slow growing plant in low rainfall areas, it can grow 1 m per annum in wet areas (Ferede *et al*., 2018).

In Ethiopia, *Rhamnus prinoides* is traditionally used to prepare alcoholic beverages, 'tella' and 'tej' (Amharic) and treat different kinds of bacterial infections. Intensive studies on *Rhamnus prinoides* revealed that it exhibits strong antioxidant properties due to presence of polyphenols in sufficient amounts (Chen *et al*., 2020). Moreover, the antimicrobial activities of

crude extracts from leaves, bark, and root of Rhamnus prinoides had been studied extensively (Molla *et al.*, 2016; Campbell *et al.*, 2019).

In Ethiopia, *Rhamnus prinoides* (also known as “Gesho” in Amharic) is used as laxative, diuretic, preventive for syphilis, depurative and cholagogue. For children with tonsillitis or with tonsils removed (a common practice because the tonsils are considered responsible for 'sickness' in general), some macerated leaves of gesho are put in the mouth to relieve the pain (Muzila *et al.*, 2010; Kiringe, 2006). In Ethiopia, *R. prinoides* (Gesho) is used to add flavour to the local drinks, tella and tej brewed from fermented barley, sorghum or finger millet (d’Avigdor *et al.*, 2014).

The search for new antibacterial agents, particularly, has increased in the last decade mainly because of the increase in bacterial infections especially in countries with poor populations and more so because of bacterial resistance to current antibiotics (Ahameethunisa and Hopper, 2010). Chemical and biological investigations of folk medicinal plants with the reputation of being curative have provided the world with many clinical drugs (Izzo and Ernst, 2009).

Justicia schimperiana (Hochst. ex Nees) is very common in villages and towns as well as in cities growing on waste places or grown as a hedge. They are used as a boundary marker around native compounds. The plant is relatively fast growing and prefers altitudes of 2400 m or above (Getahun, 1976).

Justicia schimperiana is a plant belonging to the family of *Acanthaceae*. *J. schimperiana* and is locally known as “sensel or smiza” in Amharic, and “umuga” in Oromifa. In the local communities, this plant is commonly claimed to be used for treatment of many diseases such as constipation (Murthy *et al.*, 1993), Scabies (Gedif and Hahn, 2003), skin lesion (Giday *et al.*, 2007), tooth ache (Wondimu *et al.*, 2007) and stomach-ache and burning (Teklehaymanot, 2009). Moreover, the crude extract of *J. schimperiana* has exhibited antibacterial activity against *Neisseria gonorrhea* and *Shigella flexineri* (Geyid *et al.*, 2005).

Supposedly, *Justicia shimperiana* has different local names in Ethiopia including “dhumuugaa” in Afaan Oromoo, “Sensel” or “Simiza” in Amharic, and “Surpa,” “Kasha,” or “Keteso” in Sidama. This plant has several medicinal uses in different areas of Ethiopia. Accordingly, the ethnobotanical study reports showed that the plant is used in the treatment of various ailments such as evil eye, hepatitis B (jaundice), rabies, asthma, common cold, stomachache, diarrhea, tapeworm infestation, anthrax, wound, external parasite, ascariasis,

and skin irritation (Teklehaymanot and Giday, 2007; Yineger *et al*., 2008; Abera, 2014; Chekole *et al*., 2015).

The genus *Thymus* is one of the most taxonomically complex genera in the *Lamiaceae* family. It includes 250–350 species and varieties of wild growing evergreen species of herbaceous perennials, subshrubs and aromatic (Kindl *et al*., 2015). *Thymus*, an aromatic plant belonging to the *Lamiaceae* family (Derbie and Chandravanshi, 2011; Hailemariam and Emire, 2013) has been reported to be found in different parts of Ethiopia. The two species, *Thymus schimperi* Ronniger and *T. serrulatus* are locally known as Tosign in Amharic, and both are endemic to the Ethiopian highlands. They grow on edges of roads, in open grassland, on bare rocks and on slopes, between 2,200-4,000 m altitudes. Both species are perennial herbs, woody at the base and are 5-40 cm high (Derbie and Chandravanshi, 2011). In west highlands of Ethiopia *T. schimperi* is one of herbal spices grown in home garden and used as ingredients of mixed spice used in cooking and for medicinal purposes (Alemayehu *et al*., 2015). *T. schimperi* was used not only to cure human diseases, but also for cattle, while its flower for honey around Odo Bulu, Bale and Demaro region of Ethiopia (Yineger *et al*., 2008). *T. schimperi* is also used for treatment of circulatory disorder in Ada'a District, East Shewa Zone of Oromia Regional State, Ethiopia (Meresa, 2017).

1.2 Statement of the problem

The use of herbal knowledge in the development of pharmaceutical industries and primary healthcare in many nations of the world is rapidly growing amidst rising clinical health and unstable therapeutic concerns associated with synthetic drugs. Pharmacopeias derived from natural herbs is now rife in the global pharmaceutical market and has become a huge investment (Mahomoodally, 2013). In recent decades alone, there have been growing global concerns on the rising cost of buying synthetic drugs, assessing their toxicological profile, and redressing their periodic side-effects and unstable efficacy (Gupta *et al*., 2016). Thus, plant-derived medicine accounts for more than a quarter of today's pharmacopoeia and over EUR 3.5 billion in annual export value of pharmaceutical (Eddouks and Ghanimi, 2013). Herbs can be very effective in programs for resolving urinary tract infections and typhoid fever (Tambekar and Dahikar, 2010).

The WHO estimated that about 65% of the world population use medicinal plants for their primary health care. In addition, approximately 39% of the drugs developed since 1980 have been derived from plants and their derivatives (Nwonuma *et al*., 2020). Biomedical science has exploited plants as the potential sources of drugs to prevent and cure human

diseases. The WHO has recognized antimicrobial resistance as a global health security threat that requires action across government sectors and society as a whole (Van Duin and Doi, 2017).

Antibiotics are commonly used to treat bacterial infections. However, the uncontrolled use of antibiotics certainly contributes to the emergence of multidrug resistance (MDR) against many bacterial strains (Bologa *et al.*, 2013). Patients infected with MDR bacteria may suffer from a prolonged disease that is difficult to treat and requires higher costs of treatment. The emergence of MDR bacteria has further compromised the accessibility and affordability of many currently prescribed antibiotics worldwide (Falcone and Paterson, 2016; Van Duin and Doi, 2017). To this effect, the search for an innovative antibiotic from natural sources is ultimately an important segment of modern medicine could overcome the socio-economic and health impacts caused by MDR microbes (Bakal *et al.*, 2017).

Almost in every territory of Ethiopia, the traditional medicine is involved in the delivery of healthcare as it is integrated within the local culture, and this makes it accessible to the majority of the population (Bekele and Reddy, 2015). However, regulation of the traditional medicine practice is difficult under the nationwide framework on its safety and effectiveness using the common medicine categorizations and descriptions (Adams *et al.*, 2013; WHO, 2013). In Ethiopia, there has not yet been any herbal remedy officially confirmed by the Ethiopian Food and Drug Authority (EFDA) to ensure the overall quality of herbal medicines. Therefore, the experience of the European Union countries could be used as springboard to Ethiopia in establishing a regulatory platform to ensure the safety, efficacy and quality of traditional herbal medicines. As it is known, in the European Union, the European Medicine Agency (EMA) has a well-organized legislative framework for registration of herbal medicines depending on traditional use of medicine and available scientific evidence (European Medicine Agency, 2019; Demeke *et al.*, 2022).

Over the past several decades, UTI is one of the most common infections. It is mostly caused by Gram-negative bacteria namely *E. coli*, *P. aeruginosa* and *P. pneumoniae* (Fair and Tor, 2014). In this regard, medicinal plants can be used to overcome socioeconomic and health impact caused by MDR bacteria, including methicillin resistance *staphylococcus aureus* and MDR Gram-negative bacteria such as *E. coli* and *K. pneumoniae* (Rossiter *et al.*, 2017; Gadisa *et al.*, 2019; Khameneh *et al.*, 2019). The majorities of emerging infectious events are caused by bacteria which can be associated with evolution of drug resistant strains and overwhelming of the natural host defenses (Malka *et al.*, 2010).

For years, plants have been evaluated for antimicrobial and their resistance-modifying activities (Gibbons, 2004). Due to the synergistic effects of combining extracts with antibiotics, multi-target effects have been achieved (Wagner and Ulrich-Merzenich, 2009). The combinations of drug-herbals have been reported to improve the efficacy of chemotherapeutic agents with low side-effect profiles to the living tissues (Mgbeahuruike *et al*., 2017) and antibiotics having no intrinsic antibacterial activity as well as susceptibility of bacteria to previously ineffective antibiotics (Aiyegoro *et al*., 2010).

Importantly, the method to tackle these infectious diseases might be establishing the therapeutic potential of medicinal plants. To this end, this study investigated the *in vitro* antibacterial and antioxidant activities of the hydromethanolic leaf extracts of *Thymus schimperi*, *Rhamnus prinoides*, and *Justicia shimperiana* in the test against MDR uropathogenic *E. coli* and *K. pneumoniae ESBL* and reference strains. Furthermore, it was performed a qualitative/quantitative phytochemical screening, the identification and characterization of the bioactive phytochemical constitutes using gas chromatography coupled with mass spectrometry (GC-MS), and the study of synergistic activities between ciprofloxacin and plant extracts.

1.3 Significance of the study

The present work has revealed that the studied plants are potential sources of antimicrobial agents and demonstrated the importance of such plant extracts as an alternative medicine for MDR bacteria induced UTIs regardless of safety and efficacy profiles as well as providing basic nutrition requirements. In future, verifying the safety and efficacy of lead compound obtained from plant sources will allow and favor its combination with standard antibacterial drugs (drug-herb combination).

CHAPTER 2. LITERATURE REVIEW

Living cells are highly susceptible to injury induced by oxygen and the more reactive products of its metabolism. Organisms, therefore, have the ability to produce or acquire antioxidant compounds that help to mitigate the deleterious effects of living in Earth's highly oxidizing environments. But Earth was an oxygen-free (anaerobic) planet until microbes evolved the ability to produce molecular oxygen (O_2) through photosynthesis (Ruszczycky and Liu, 2017).

Plant antioxidants are a natural reservoir of bioactive compounds. They play important roles in plant acclimation and adaptation to environmental challenges but are also beneficial for human health. As sedentary organisms, plants cannot escape from environmental challenges, originating from natural origin (e.g., temperature, water availability, soil composition, pests) or from anthropogenic practices (e.g., destruction of habitats, pollution). Diverse abiotic factors, like pollution as well as nutrient deficiency, temperature regimes (heat/cold), water supply (drought/flooding), light intensity, day/night rhythms, and radiation, modify the balance between production and scavenging of reactive oxygen species (ROS) and induce a phenomenon well known as oxidative stress (Meena *et al.*, 2017; García-Sánchez *et al.*, 2020; Gou *et al.*, 2020; Nadarajah, 2020).

Natural products have gained significant appreciation as an alternative and/or complementary healthcare approach with extensive pharmaceutical and biological properties (Aryal *et al.*, 2021). Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radicals are any species capable of independent existence that contains one or more unpaired electrons which react with other molecule by taking or giving electrons, and involved in many pathological conditions (Madhavi *et al.*, 1996), and they play very important roles in human health and beneficial in combating against several diseases like cardiovascular disorders, lung damage, and inflammation. These free radicals are highly unstable and when the amount of them exceed in the body, they can damage the cells and tissues and may involve in several diseases. Thus, there is a need of antioxidants of natural origin because they can protect the human body from the diseases caused by free radicals (Mishra *et al.*, 2007; Upadhye *et al.*, 2009).

The generation of highly reactive oxygen species (ROS) with a lone unpaired electron induce oxidative stress and plays a key role in the pathogenesis of numerous physiological conditions, including cellular injury, aging, cancer, and hepatic, neurodegenerative, cardiovascular and renal disorders (Madamanchi *et al.*, 2005; Losada-Barreiro and Bravo-Díaz, 2017). Many plants synthesize secondary metabolites naturally, including flavonoids and

polyphenols which act as antioxidants and play a critical role in different biological activities (Carvalho *et al*., 2010; Nichols and Katiyar, 2010). Therefore, plants and natural products could be a major source of antioxidants that can scavenge free radicals and protect from excess oxidative stress-induced ailments.

Medicinal plants are effective in the treatment of infectious diseases occurring on both human and animals of different species (Adetutu *et al*., 2011). Additionally, several studies have reported that medicinal plants contain different bioactive ingredients/secondary metabolites which provide health benefits (Saeidi *et al*., 2015). Alkaloids, flavonoids, tannins, phenols, saponin, steroids, glycosides, and terpenes are some of the plant's major secondary metabolites (De Silva *et al*., 2017) that have antioxidant, anti- inflammatory, anti-cancer, and anti-microbial properties (Majekodunmi, 2015).

The antibacterial activities of plants can be related to phytochemical compounds which can protect the human body against microbial infection. The most important phytochemicals are flavonoids, alkaloids, and terpenoids (Kumar *et al*., 2013). Flavonoids (Khalid *et al*., 2019) and terpenoids (Broniatowski and Mastalerz, 2015) have been recognized to show strong antibacterial activities. In a related study, it has been reported that the essential oils have antibacterial, antifungal, antioxidant and anti-diabetic activties (Sut *et al*., 2020).

Recently, there is an increase in significancy of plants and plant extracts due to their therapeutic properties. Further, plants have some advantages such as efficacy, safety, cultural acceptability, better compatibility with human body and lesser side effects (Khomarlou *et al*., 2017). Antibacterial activity of methanol and chloroform leaves extracts of *R. prinoides* against *Staphylococcus aureus*, *Streptococcus pyogenes* Rosenbach, and *Streptococcus pneumoniae* revealed minimum inhibitory concentration ranging from 8.13-32.5 mg/mL and 8.13-16.25 mg/mL, respectively, compared to a positive control cefotaxin (0.03 mg/mL) and ampicillin (0.01 mg/mL) ranged from 23.67- 28.00 mg/mL in both fractions, respectively (Molla *et al*., 2016).

The acetone leaf extracts of *R. prinoides* showed antimycobacterial activity with minimum inhibitory concentration (MIC) values ranging from 0.625 to >2.5 mg/mL aganist three fast-growing mycobacteria species *i.e. Mycobacterium smegmatis* Trevisan*, Mycobacterium aurum* Tsukamura and *M. fortuitum* Da Costa Cruz and one pathogenic *M. tuberculosis* strain (Dzoyem *et al*., 2016). Further, semi-purified ethanolic stem and stem bark extracts of *R. prinoides* with higher contents of polyphenols and flavonoids displayed anti-inflammatory activity through reducing the Nitric Oxide production at the dosage of 11.11-

100 µg/mL and the COX-2 inhibitory activity with an IC50 value at 20.61 ±0.13 µg/mL (*Chen et al.*, 2020).

Aqueous extract of *Justicia schimperiana* (200 mg/kg and 400 mg/kg, p.o.) showed significant tolerance ($p < 0.05$) to oral glucose load at 1 and 2 hours after glucose load. The extract also produced significant ($p < 0.05$) blood glucose reduction at 4 hours after its administration in normoglycemic mice. The extract at 400 mg/kg dose level produced significant ($p < 0.05$) reduction in blood glucose level at 2, 3 and 4 hours of treatment in streptozotocin (45 mg/kg) induced diabetic mice. Acute oral toxicity studies of aqueous extract of *Justicia schimperiana leaves* in rats showed no death or signs of toxicity at the dose of 2000 mg/kg indicating the safety nature of the extract (Tesfaye *et al.*, 2016). Furthermore, the phytochemical analysis on the *n*-hexane extract of *Justicia schimperiana* showed the presence of alkaloids, polyphenols, flavonoids, glycosides, saponins, triterpenes, and quinones (Abebe *et al.*, 2014). Additionally, another experimental study also confirmed that chloroform, methanol, and aqueous fractions of the leaves of *Justicia schimperiana* had an antimalarial activity (Abdela *et al.*, 2014).

In a study of evaluation, the *in vivo* hepatoprotective activity of *Justicia schimperiana* (Hochst. ex Nees) used in Ethiopian traditional medical practices for the treatment of liver diseases, pretreated mice with the hydro-alcoholic extracts of *J. schimperiana* significantly suppressed the plasma aspartate aminotransferase activity (AST) ($p < 0.01$) and ALT activity ($P < 0.05$) when compared with the carbon tetrachloride (CCl4) intoxicated control. Within the Soxhlet extract of the plant, the methanol extract of *J. schimperiana* showed significant hepatoprotective activity. Further fractionation of this extract using solid phase extraction and testing them for bioactivity indicated that the fractions did not significantly reverse liver toxicity caused by CCl4. However, the percentage hepatoprotection of the distilled water fraction was comparable with that of the standard drug silymarin at the same dose (50 mg/kg) as evidenced by biochemical parameters (Umer *et al.*, 2010).

The 80% methanolic leaf extract of *J. schimperiana* was evaluated for its activity against castor oil induced diarrhea, enteropooling, and gastrointestinal (GI) motility in mice. A significant reduction ($p < 0.001$) in the total defecation and diarrheal drops was produced by all the test doses of the extract. Percentage inhibition of wet feces was 42.58, 65.07, and 74.96% at 100, 200, and 400 mg/kg doses of the extract, respectively. The extract also significantly inhibited castor oil-induced enteropooling at all test doses. The percent reduction in mean weight of intestinal contents was 66.96, 67.83, and 76.52% at 100, 200, and 400 mg/kg doses of the extract, respectively. The extract significantly reduced GI movement of

charcoal meal as well at 200 ($p < 0.01$) and 400 mg/kg ($p < 0.001$) doses (Mekonnen *et al*., 2018).

The methanol extract of *J. schimperiana* showed strong inhibition activity against *S. dysentery* and *E. coli* with a zone size of 14.5 ± 0.5 mm and 16 ± 0.2 mm and MIC values of 3.12 mg/mL against *E. coli* and *S. dysentry*. Highly prominent activity was produced by the ethanol extract of *R. chalepensis* with the highest zone of 15 ± 0.5 mm diameter observed in *S. typhi*, followed by *S. aureus* 13 ± 0.11 mm with the MIC value of 1.56 mg/mL against *S. typhi.* Four antibiotics were used as standard for the testing of antibacterial activity against six different human pathogens. Among the antibiotics Ciprofloxin showed maximum zone of inhibition ranging from 20-35 mm followed by kanamycin, tetracycline and chloramphenicol (Tedila *et al*., 2019). Essential oils are complex mixtures of volatile substances. Usually, they are generally present at low concentrations and used for their flavor and fragrances in food, pharmaceutical and perfumery industries (Maffei *et al*., 2011).

The anti-rabies activities of three plant extracts were tested in three different doses: 300, 2000 and 5000 mg/kg in mice and compared with positive control based on the difference in mean survival time of group of mice challenged with rabies virus (CVS-11). The result showed that *P. dodecandra*, *J. schimperiana* and combination of all the three plant extracts at 300 and 2000 mg/kg dose levels and *C. macrostachyus* at 300 and 5000 mg/kg doses didn't significantly ($p > 0.05$) increase the survival period of mice. However, at 5000 mg/kg dose level for *P. dodecandra* ($p = 0.002$), *J. schimperiana* ($p = 0.038$) and combination of all the three extracts ($p = 0.021$) and at 2000 mg/kg dose level for root bark of *C. macrostachyus* ($p = 0.011$)*;* the plant extracts were significantly ($p < 0.05$) increasing the survival time of mice (Zewde *et al*., 2019). Partly, in a chemical composition identification test, from the essential oil of *Justicia schimperiana*, 28 compounds were identified making up 75.18%, the essential oil of *Justicia schimperiana* has not showed obvious activity against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (Abebe *et al*., 2018).

In Ethiopia *T. schimperi* possesses antibacterial, anthelmintic, and antifungal activity (Melka *et al*., 2016; Awraris and Bhagwan, 2011; Bekalo *et al*., 2009). Aqueous and methanol 80% extract of *T. schimperi* showed a reduction in blood glucose levels in a dose- and time-dependent manner. *T. schimperi* aqueous and methanol extracts at 250 and 500 mg/kg doses showed reduction of (22.65% and 33.15%) and (30.06% and 38.35%) in plasma glucose levels, respectively after 4 h of extract administration (Guesmi *et al*., 2014). The oral administration of aqueous extract of *T. schimperi* leaves and its essential oil distillate at doses of (250, 500, 750 and 1000 mg/kg) and (1 and 1.5 mL/kg) was respectively evaluated for

their diuretic and anti-hypertensive activity against salt-sucrose induced hypertensive rats. The aqueous extract of *T. schimperi* leaves for all mentioned doses showed positive diuretic activity at 5 hour and the two higher doses significantly increased Na^+, K^+ and Cl^- content of urine (Dires *et al*., 2018).

T. schimperi oil had moderate antibacterial effect on tested *Enterobactericeae*; 17 mm and 24 mm inhibition zone in diameter against *E. coli* and *K. pneumoniae,* respectively. It had also considerable inhibition zone in diameter; methicillin-susceptible staph (*MSSA*) and methicillin-resistant staph (*MRSA*) (24 mm) (Cutillas *et al*., 2018). *T. schimperi, which is found in* Bale, is composed of carvacrol (63%) as the major constituent whereas *T. schimperi* from Gonder, Shewa and Wello is composed of thymol (36%-38%) as a dominant phytoconstituent. The essential oil of *T. serrulatus* collected from the Tigray region had thymol (49%) as the major constituent, similar to *T. schimperi* which is found around Dinsho village (South-Central Ethiopia) (Dagne *et al*., 1998; Debelo *et al*., 2015). The essential oil of *T. schimperi* indicated strong effect on the fungal pathogen (*Aspergillus niger*) after applying 10 µL/disc of the oil, which completely inhibited up to a diameter of 36 mm and its minimum inhibitory concentration (MIC) was estimated to be lower than 5 µL/disc, which was related with other finding (Esubalew *et al*., 2017).

In an evaluation study, the antibacterial activities of the essential oils (EOs) of *Thymus serrulatus* and *Thymus schimperi* collected from Ofla (Ofl), Alamata (Ala), Yilmana Densa (Yil), Tarmaer (Tar), Butajira (Buta), and Bale (Bal) in Ethiopia against cariogenic bacteria (*Streptococcus mutans* and *Lactobacillus*) isolated from human teeth. Inhibition zones (IZs), minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) were measures of the antibacterial activity. Significant bacterial inhibitions resulted in concentration and EO-dependent manner. At 128 µL/mL, IZs against *S. mutans* were 37.33 mm (Tar), 36.00 mm (Bal), 33.67 mm (Yil), 33.33 mm (Ofl), 30.00 (Ala), and 29.67 mm (Buta) and IZs against *Lactobacillus* were 31.00 mm (Tar), 30.67 mm (Yil), 27.67 (Bal), 27.00 (Buta), 26.67 (Ofl), and 21.33 (Ala). The respective inhibition zones due to 3% DMSO (negative control) and 3% H_2O_2 (positive control) were 0.00 mm/30.00 mm against *S. mutans* and 0.00 mm/29.00 mm against *Lactobacillus*. At 128 µL/mL concentration, all the EOs resulted in significantly higher inhibition zones than that of 3% H_2O_2 against *S. mutans* and *Lactobacillus* (Damtie and Mekonnen, 2020).

The antibacterial potential of mixed oil depends on the concentrationss and type of the EOs and bacteria species. The combined EOs of *Blepharis cuspidata* and *T. schimperi* had inhibition zone (39 mm), its MIC and MBC value was 0.39 µL/mL against MRSA. It had inhibition zone (28-35 mm), MIC value 0.39-6.25 µL/mL and MBC (0.78-12.50 µL/mL) against MDR *E. coli* and *K. pneumoniae*. Whereas combined effects of *Blepharis cuspidata* and *Boswellia ogadensis* had MIC values ranges from 0.78-6.25 µL/mL for *E. coli* and *K. pneumoniae* and 1.56 µL/mL for MRSA. There was strong synergistic effect between the combination of *Blepharis cuspidata* and *T. schimperi*. This study revealed that Gram-negative bacteria were slightly less susceptible than gram positive (Gadisa *et al*., 2019).

CHAPTER 3. OBJECTIVES

3.1 General Objective

To evaluate the *in vitro* antioxidant, antibacterial activities, qualitative and quantitative phytochemical composition, GC-MS characterization, and synergistic interactions of ciprofloxacin with the crude leave extracts of *Rhamnus prinoides, Thymus schimperi,* and *Justicia shimperiana.*

3.2 Specific Objectives

3.2.1 Phytochemical Profile and Antibacterial activities of the extracts

- To determine the qualitative phytochemical screening of the different crude extracts.
- To determine the antibacterial activity of the crude extracts.
- To determine the antibacterial activity of the extracts combination.
- To identify the minimum inhibitory concentration (MIC) of the crude extracts.
- To determine the MIC of the extracts combination.
- To identify the minimum bactericidal concentration (MBC) of the extracts.
- To identify the MBC of the extracts combination.
- To assess synergistic activity of the extracts combination with ciprofloxacin.

3.2.2 Antioxidant activity of the extracts

- To determine the antioxidant and free radical scavenging activities of the three extracts.
- To estimate the quantity of the phenol, flavonoid and proanthrocyanidins contents.

3. 2. 3 GC-MS chemical profile

To identify the bioactive chemical constitutes through GC-MS analysis on the extracts.

CHAPTER 4. MATERIALS AND METHODS

4.1 Plant Collection and Study Areas

Fresh leaves of *Rhamnus prinoides* was collected from the cultivated garden of a family on 15th May 2021 in Addis Ababa, Ethiopia. Then after, it was identified by taxonomist at National Herbarium, Department of Biology, College of Natural and Computational Sciences, Addis Ababa University and the specimen (number ME 001) and deposited for future reference. The second plant, *Thymus schimperi* leaves were collected on 30th July 2021 from Debre Birhan town near villages, Amhara Region, Ethiopia which is 120 Km away from Addis Ababa to Northwest direction. The third plant, *Justicia shimperiana* leaves were collected from Debre Markos, East Gojjam Zone, Amhara Region, 300 Km away from Addis Ababa, Northwest, Ethiopia. The *in vitro* antioxidant study was carried out in Addis Ababa University, College of Natural and Computational Sciences, in the Center of Food Science and Nutrition, Addis Ababa, Ethiopia. However, the antibacterial study was done in Ethiopian Public Health Institute (EPHI), Traditional and Modern Drugs Studies Directorate, Pharmacology laboratory, Addis Ababa, Ethiopia. The GC-MS analysis of the extracts was done in *JIJE LaboGlass Private Limited Company*, Addis Ababa, Ethiopia.

4.2 Chemicals and Reagents Used

In this study, the chemicals used were 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich-America), ascorbic acid (vitamin C), 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid (ABTS) solution (Roche Diagnostic GmbH, Germany), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®) (Sigma-Aldrich, America), ferrous sulfate, potassium persulfate (Carl Roth GmbH+ Co.KG, Germany), ciprofloxacin powder, catechin, gallic acid, Dimethyl sulfoxide (DMSO), and methanol (Alpha Chemika, India). The solvent methanol was of analytical grade.

4.3 Study Design

A randomized experimental study design was used to carry out the phytochemical screening test and *in vitro* antibacterial activity evaluation of the selected medicinal plants against the patient isolated multi-drug resistance uropathogenic bacteria. All experimental tests were done in triplicates alongside the positive and negative controls.

4.3 Plant Extraction

Although a standardized extraction protocol has not been developed for herbal extracts, 20–95% of the solvents substances are frequently used by the herbal medicine industry to prepare plant crude extracts (Zhang *et al*., 2018). All three medicinal plants: *Rhamnus prinoides, Thymus schimperi, and Justicia shimperiana* were extracted with 80% methanol (and 20% distilled water). Two hundred grams of air-dried powdered plant (*Thymus schimperi),* 150 grams *(Rhamnus prinoides),* and 250 grams (*Justicia shimperiana*) materials were placed in a flat-bottom flask filled with 800 mL of methanol and 200 mL (distilled water) extracting solvents and macerated for 72 hours over a rotary shaker at 121 rpm. The suspension was filtered every 24 hours with Whatman Number 1 paper. The residue was re-macerated for the second and third times with fresh solvent. The resulting filtrate was then concentrated under reduced pressure in a rotary evaporator (R-300). The filtrate residue was further dried, followed by a water bath at 45°C, until the solvent was removed. After the solvent was evaporated, the remaining crude extracts were diluted with 10 mL of sterile distilled water and kept in an airtight bottle in the refrigerator until the experiment was carried out (Mulatu, 2020).

4.4 Phytochemical screening

The qualitative phytochemical tests for the identification in the hydromethanolic (80% methanol) crude extract of leaves of *Rhamnus prinoides, Thymus schimperi,* and *Justicia shimperiana* were carried out by the methods described in (Harborne, 1973; Sofowra, 1993; Evans, 2002; Ayoola *et al*., 2008; Aiyelaagbe and Osamudiamen, 2009; Farhan *et al*., 2012; *Godghate et al*., 2012; Pandey and Tripathi, 2014; Jaradat *et al.,* 2015; Prabhavathi *et al*., 2016). All the extracts (0.05 g/mL) were subjected to phytochemical screening and performed following the standard protocols using different reagents and chemicals for the detection of the following constituents.

Test for Alkaloids: To the filtrate in test tube, 1 mL of Mayer's reagent was added drop by drop. The formation of a greenish coloured or cream precipitate indicated the presence of alkaloids.

Test for steroid: Crude extract was mixed with 2mL of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Test for Flavonoids: 2 mL of each extract was added with 2 mL of 2.0% sodium hydroxide, formation of intense yellow colour is observed. To this, 2 drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for saponins: Crude extract was mixed with 5mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Anthocyanin: Approximately 2 mL of the prepared plant extracts were added to 2 mL of 2N HCl and ammonia. The appearance of a pink red coloration that turned blue violet indicated the presence of anthocyanin.

Test for Coumarin: About 3 mL of 10% NaOH were added to 2 mL of plant extracts. The formation of a yellow colour was an indication for the presence of coumarins.

Test for phenols and tannins: Two milliliters of 5% solution of $FeCl_3$ were added to 1 mL crude extracts. A black or blue-green colour indicated the presence of tannins and phenols.

4.5 Inoculum preparations and standardization

The patient isolated MDR bacterial strains from urine sample came for urine culture, *E. coli*, and *K. pneumoniae ESBL* and reference strains such as *E. coli (ATCC25922)*, and *K. pneumoniae (ATCC700603)* were utilized to evaluate antibacterial activities of crude plant extracts. The clinical isolates were obtained from Arsho Medical Laboratory, Addis Ababa, Ethiopia. Each bacterial strain was inoculated and incubated (Incubator memmert ├) for 24 hours at 37°C. To prepare the final inoculum, the cultures were diluted with fresh Mueller-Hinton broth to achieve the required standardized turbidity of bacterial suspension (Optical Densities, OD) by measuring using UV-Visible Spectrophotometer at 625 nm, (OD values range from 0.08 to 0.1). It was equivalently match with the turbidity of 0.5 McFarland barium sulfate standard corresponding to 1.0×10^8 colony forming units (cfu/mL) following the guideline of Clinical and Laboratory Standard Institute (CLSI, 2015). The turbidity of the inoculum tube was adjusted visually by either adding bacterial colonies or by adding sterile normal saline solution to that of the already prepared 0.5 McFarland standard. Finally, the inoculum amount of the bacteria was 5.0×10^5.

4.6 Antibacterial assay of plant extracts

Antibacterial activity of hydromethanolic leaf extracts was determined by agar well diffusion method (Andleeb *et al.*, 2020; Andualem *et al.*, 2014). Multidrug resistant (MDR) bacterial colonies in a subculture on blood agar plate media were incubated for 24 hours at 35 ± 2°C. The MDR bacterial colonies were dissolved in a normal saline solution with a turbidity equivalent to the 0.5 McFarland standard. One hundred µL of each MDR bacterium was inoculated in Muller Hilton agar (MHA) by spreading the bacterium on the surface of the agar using a sterilized glass spreader. After thirty minutes of inoculation, the wells were prepared using a sterilized steel *cork borer* (1 cm in diameter). The labeled four wells were with 100 µL of 250 mg/mL, 500 mg/mL, and 1000 mg/mL of the crude extracts and making the final concentration of 25, 50, and 100 mg/well (Marami *et al.*, 2021) respectively. All plates were then incubated aerobically at 35 ± 2°C for 24 hours, then, the zone of inhibition was measured using a ruler. The experiment was done in three independent tests for each bacterial strains and the mean of zones of inhibitions was calculated for each extract (Tambekar and Dahikar, 2011). A 4% DMSO was used as a negative control. Ciprofloxacin (5 µg) was applied as a positive control.

4.7 Minimum Inhibitory Concentration

The minimum inhibitory concentrations (MICs) for all the crude extracts were evaluated against *E. coli* and *K. pneumoniae* ESBL and their reference strains were determined in triplicate using the 96-wells method in Mueller-Hinton broth according to CLSI (Clinical Laboratory Standardization Institute) (CLSI, 2013). A 4% DMSO was used to dilute crude plant extracts. To determine the MICs of each of the extracts, the concentrations prepared for each of the extracts, ranged between 0.03125 mg/mL and 64 mg/mL, while ciprofloxacin concentration ranged between 0.0024 and 5 µg/mL. For all the tested extracts and ciprofloxacin concentrations were prepared by serial dilution in double-strength MHB. A 100 µL of each of the bacterial strains was inoculated to each well. Blank Mueller-Hinton broth was used as a negative control. The wells were inoculated with the standardized (0.5 McFarland standard) bacterial inoculum and incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration that showed no growth in the Mueller Hinton broth. The result of bacterial inhibition was judged by comparison with growth in positive and negative controls (Wayne, 2012).

4.8 Minimum Bactericidal Concentration (MBC)

MBC was determined by a method described in different studies (Rouis *et al.*, 2013). In this method, the contents of all wells containing a concentration of the crude extracts above the minimum inhibitory concentration (MIC) value from each triplicate, in the MIC determination test, was streaked on Mueller Hinton agar with wire loop aseptically cleaned and incubated at 37°C for 24 hours. The lowest concentration of the extract which showed no bacterial growth after incubation was observed for each triplicate and noted as the MBC. The average value was taken for the MBC of test material against each bacterium. Further, the ratio of MBC/MIC of the extracts indicated the exact definition of the antibacterial effect. If the ratio MBC/MIC was ≤ 4, the effect was considered as bactericidal but if the ratio MBC/MIC was > 4, the effect was defined as bacteriostatic (Levison, 2004; Benjamin *et al.*, 2012).

4.9 Checkerboard Assay

Checkerboard analysis was used to determine the impact on potency of the combination of the extract and the antibiotic in comparison to their individual activities. This comparison is then represented as the Fractional Inhibitory Concentration (FIC) index value. The FIC index value takes into account the combinations that produce the greatest change from the individual antibiotic's MIC. To quantify the interactions between the extracts and the antibiotic being tested (the FIC index), the following equation is used: FIC index = (MIC of extract in combination/MIC of extract alone) + (MIC of antibiotics in combination/MIC of antibiotics alone). In antimicrobial combination, the interaction definition of synergy was as Σ FIC$\leq$ 0.5, additivity as 5 $<$ Σ FIC$\leq$ 1, indifference as 1 $<$ Σ FIC$\leq$ 4, and antagonism as Σ FIC$>$ 4 (Petersen *et al.*, 2006; Lorian, 2005).

4.10 *In vitro* Antioxidant Studies

In these *in vitro* antioxidant assays, 1g of each of the extracts was dissolved in 20 mL of 99% methanol to make a concentration of 50 mg/mL and then diluted to prepare the series concentrations for antioxidant assays. For comparison in all the assays, reference chemicals were used.

4.11 DPPH Radical Scavenging Assay

Free radical scavenging ability of the extracts was tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as described by (Ceylan *et al.*, 2015; Etim *et al.*, 2015; Ralte

et al., 2021). The hydrogen atom donating ability of the plant extracts was determined by the de-colorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.004% of DPPH (4 mg/100 mL) in methanol was prepared, and 3 mL of this solution was mixed with 1mL of extract in methanol at different concentrations (0.02-0.2 mg/mL). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid (3 mg/10 mL) was used as reference standard while methanol solution of DPPH was used as control. The optical density of the mixture was measured at 517 nm using PerkinElmer (Lambda 950 UV/VIS NIR spectrometer). Percentage of DPPH radical scavenging activity was calculated by the following equation:

Percentage of DPPH radical scavenging activity = {(A0− A1)/A0} × 100; where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract. The IC50 value was determined by using linear regression equation Y = Mx + C; where, Y = 50, M and C values were derived from the linear graph trendline (for both the standard and samples). The experiment was repeated three times at each concentration.

4.12 ABTS Radical Scavenging Assay

For ABTS assay, the procedure followed the method of Arnao *et al.* (2001) with some modifications. The stock solutions included 7.4 mM $ABTS^+$ solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 24 hours at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^+$ solution with 60 mL methanol to obtain an absorbance of 1.1±0.02 units at 734 nm using PerkinElmer (Lambda 950 UV/VIS NIR spectrometer). Fresh $ABTS^+$ solution was prepared for each assay. The extracts (40 µL) were allowed to react with 2850 µL of the ABTS+ solution for 2 hours in a dark condition. Then, the optical density was taken at 734 nm using the spectrophotometer. The standard curve was linear at 300 µM Trolox. Results were expressed in mM Trolox equivalents (TE)/g of the extract. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve. The ABTS scavenging capacity of the extract was compared with that of trolox standard. All determinations were performed in triplicate (n = 3).

4.13 Ferric Reducing Antioxidant Power (FRAP) assay

The total antioxidant potential of the crude extracts was determined according to a method described by Benzie and Strain (1996) as a measure of antioxidant power. The assay was

based on the reducing power of a compound (antioxidant). A potential antioxidant reduced the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/ tripyridyltriazine (TPTZ)). The FRAP reagent was consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$. The FRAP reagent was prepared by mixing the acetate buffer, TPTZ solution, and $FeCl_3 \cdot 6H2O$ solution in proportion of 10:1:1 (v/v/v). Briefly, an aliquot of appropriately diluted sample (1 mL) was mixed with 3 mL of freshly prepared FRAP reagent and mixed thoroughly. The reaction mixture was incubated at 37°C for 30 min. Optical density of the mixture was measured at 593 nm versus blank using PerkinElmer (Lambda 950 UV/VIS NIR spectrometer). Ferrous sulphate (0–1000 mM) was used to plot a calibration curve for quantification, and the results were expressed as mmol Fe^{2+} per 100 g of the extract. All samples were analyzed in triplicate.

4.14 Determination of Total Phenols

The total phenolic content of the three extracts was determined using the modified Folin–Ciocalteu method (Apak *et al*., 2006). A 0.0625 g of gallic acid/standard chemical was dissolved in 50 mL of methanol. Here, the extracts (50 µL of the 50 mg/mL (stock solution) of each) were mixed with 5 mL of Folin–Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 mL (75 g/L or 7.5%) of sodium carbonate (initially, it was kept in refrigerator for the last 24 hours). The mixture was vortexed for 15 s and allowed to stand for 30 min at 37^{o}C for colour to develop. The optical density was measured in triplicate at 765 nm using an AJI-C03 UV-VIS spectrophotometer.

4.15 Determination of Total Flavonoids

Total flavonoids were estimated using the method of Marinova *et al*. (2005). In this study, the standard chemical quercetin reagent (0.0125 g was dissolved in 50 mL of methanol) and run at a dose of 25-225 µL. One mL of 2% $AlCl_3 6H_2O$ which was dissolved in 100 mL of volumetric flask in water and added to each of the extracts and to the standard. Then, it was added to 50 µL of *R. prinoides and J. shimperiana*, and to 150 µL of *T. schimperi* and finally allowed to stand for 60 min at room temperature before the optical density was measured at 415 nm using an AJI-C03 UV-VIS spectrophotometer.

4.16 Quantitation of Total Proanthocyanidins

The total proanthocyanidin was measured by the Vanillin/HCl method according to Sun *et al*. (1998). Briefly, 500 µL (0.5 mL) of *T.* schimperi and *J. shimperiana*, and 100 µL (0.1 mL) of

R. prinoide from 50 mg/mL stock solution and were added to 3 mL of 4% Vanillin-methanol solution and hydrochloric acid (1.5 mL). The mixture was left for 15 min at room temperature, and then, the optical density was read at 500 nm. Total proanthocyanidins content was calculated from the standard curve for catechin.

4.17 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To identify and characterize the bioactive chemicals profile of the hydromethanolic leaf crude extracts, 1 µL amount of concentration from each of the extracts of *T. schimperi, R. prinoides,* and *J. schimperiana* extract (of 50 mg/mL stock solution) was injected into a gas chromatography system (Agilent Technologies 7890B) coupled with an inert mass spectrometer (Agilent 5977B) with Single Quadrupole. The separation of the crude extract was achieved using a DB-5ms, capillary column (30 m x 0.25 mm x 0.25 µm) via an inlet split/splitless mode. Helium was used as the carrier gas with a linear velocity/column flow of 1 mL/min. The injector temperature was set at 250°C and oven temperature was kept 110°C for 2 min, and then increase by 10°C/min rate to 200°C and then by 5°C /min rate to 280°C for 9 min. Further, the Mass spectrometer experimental condition was explained as follows: Ionization mode: EI; EMV mode: Gain Factor; Gain Factor: 1; Transfer line temperature: 280°C; Ion Source temp: 230°C; Quad temp: 150°C; Solvent delay: 3 min; and Acquisition mode: SCAN. Finally, The NIST 14 mass spectrometry database library was used to identify the bioactive compounds.

4.18 Ethical consideration

The study was carried out after obtaining ethical clearance from St. Paul's Hospital Millennium Medical College, Institutional Review Board (IRB), Addis Ababa, Ethiopia. Then, to proceed with the data collection, the study areas were communicated with an official letter together with the ethical clearance. In all the data, the study was done under the legal framework of the IRB guidelines.

4.19 Data Analysis

In antioxidant study, data were entered and statistically analyzed using SPSS Version 25 (SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 2017 (Microsoft, Redmond, WA, USA). All measurements were performed in triplicate, and results were expressed as mean ± standard deviation (SD). One-way ANOVA was performed to compare mean differences of the phytochemical contents (Total flavonoids, phenol, and proanthocyanidin contents) among the

three extracts. If any mean difference exists in the phytochemical contents, List Significant Difference (LSD) test was used to identify where the differences were. The Pearson correlation coefficient (r) was used to analyze the association among total flavonoids, phenol, and proanthocyanidin contents versus the antioxidant activity (DPPH [IC 50%], ABTS [mean] and FRAP [mean]). In all the statistical tests, $p \leq 0.05$ was considered as statistically significant. The findings of the antibacterial effects of the crude extracts in terms of zone of inhibitions, MIC and MBC were summarized in the form of means (± standard deviations). The presence of phytochemical screening results was classified as highly present (+++), moderately (++), slightly (+) and absent (–), respectively. Finally, to identify and characterize the bioactive chemicals profile of the crude extracts, GC-MS analysis was used.

CHAPTER 5. RESULT

5.1 Qualitative Phytochemical Analysis

The findings of the preliminary phytochemical analysis (in qualitative form) showed the presence or absence of the secondary metabolites in the three hydromethanolic leave crude extracts are described in Table 1. Accordingly, the results of the tests on *T. schimperi* included a maximum of six types of phytochemical groups, such as alkaloids, coumarins, flavonoids, phenols, saponins, and terpenoids. The next, the *R. prinoides* contains phenol, tannin, flavonoids, coumarins, anthocyanin, alkaloids, and saponins. Finally, the third plant *J. shimperiana* extract contained five types of phytochemical groups, such as saponin, coumarins, flavonoids, phenol, and tannin.

Table 1. Preliminary phytochemical evaluation of the three hydromethanolic crude leaf extracts.

SN.	Phytochemical constituents	*T. schimperi*	*R. prinoides*	*J. shimperiana*
1	Saponins	++	+	+++
2	Steroids	-	-	-
3	Alkaloids	++	++	-
4	Anthocyanin	-	+	-
5	Coumarins	+	+	++
6	Flavonoids	++	++	++
7	Terpinoids	-	-	-
8	Phenol	+++	+++	+++
9	Tannin	+++	+++	+++

+: Presence; -: Absence; (+): low; (++): Moderate; (+++): highest.

5.2 Quantitative Phytochemical Analysis

In this study, the quantitative phytochemical analysis of the three hydromethanolic crude leaf extracts of *T. schimperi, R. prinoides, and J. shimperiana* was carried out to determine the total phenolic content, flavonoids, and proanthocyanidins and the findings have been presented in Table 2.

The results demonstrated that the total flavonoid content was 90.39±0.35 Quercetin Equivalence (QE)/100 g of dry *R. prinoides* material; 82.43±2.53 QE/100 g of dry *J. shimperiana* material; and 227.96±3.69 QE/100 g of dry *T. schimperi* material. Among the three crude extracts, *T. schimperi* had the highest average (±SD) in phytochemical contents of flavonoids (227.96±3.69), phenolic (274.50±5.29), and proanthocyanidins (1618.06±1.32) (Table 2). The concentration-response (calibration) curve of the standard chemical quercetin in the determination of flavonoids contents was plotted in Figure 1A.

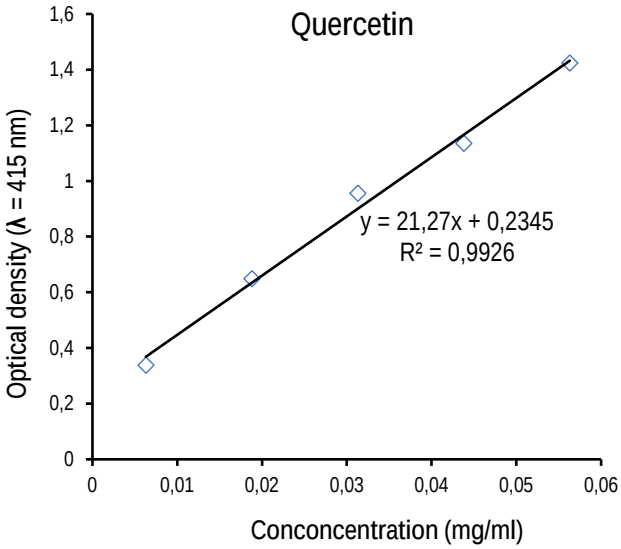


Figure 1A. Linear regression calibration curve of the standard quercetin for the determination of flavonoids.

As shown in Table 2, the total phenolic contents of the three hydromethanolic of crude extracts were determined using the standard gallic acid. The standard gallic acid linear regression calibration curve is presented in Figure 1B. Total phenolic content of the extract was calculated as gallic acid Equivalent/100 g of the dry plant materials. And, accordingly, the highest phenolic quantity was detected in *T. schimperi* (274.50±5.29) followed by *R. prinoides* (229.21±2.82) (Table 2).

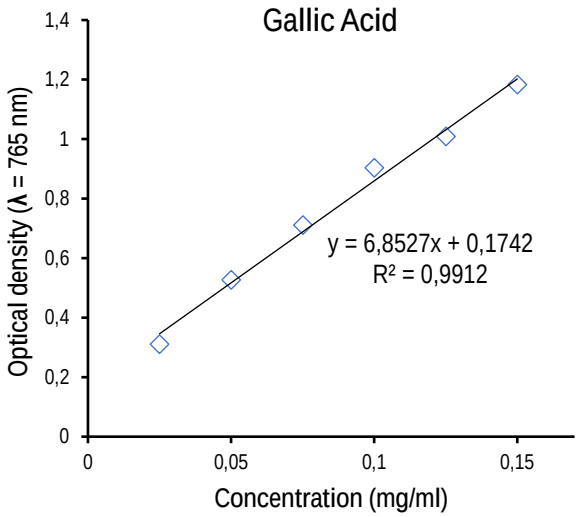


Figure 1B. Linear regression calibration curve of the standard gallic acid for the determination of the total phenol content.

Table 2. Quantity (Mean±SD) of phytochemicals of the three hydromethanolic crude extracts.

Sample type	Flavonoids (mg Q$^{\times}$ E/100 g)	Phenol (mg GA† E/100 g)	Proanthocyanidins (mg C‡E/100 g)
T. schimperi	227.96±3.69	274.50±5.29	1618.06±1.32
R. prinoides	90.39±0.35	229.21±2.82	406.24±7.58
J. shimperiana	82.43±2.53	83.59±2.19	1037.18±4.06

The standard chemicals: **Q$^{\times}$**: Quercetin; **GA†:** Gallic acid; and **C‡:** catechin.

Among the three extracts, the highest proanthocyanidin content (catechin equivalence per 100 g of the dry plant material) was found in *T. schimperi* (1618.06±1.32) followed by *J. shimperiana* (1037.18±4.06) (Table 2). The linear regression curve of the catechin standard concentration (mg/mL) versus the optical density was shown in Figure 1C.

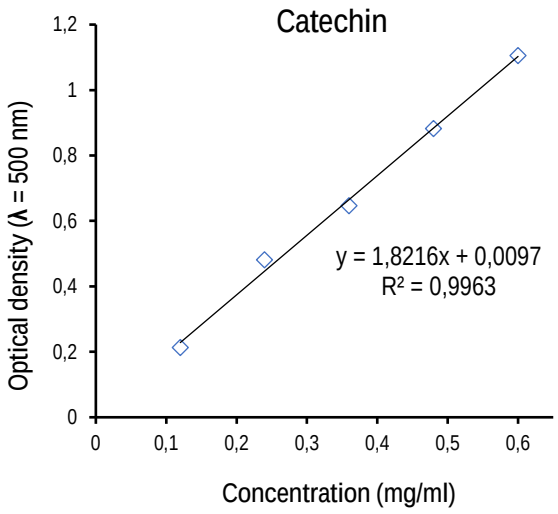


Figure1C. Linear regression calibration curve of the standard catechin for the determination of proanthocyanidins.

5.3 DPPH Radical Scavenging Assay

The result of various concentrations versus the DPPH scavenging activity in terms of percentage inhibition of the three hydromethanolic extracts with the positive control ascorbic acid is shown in Table 3. The synthetic antioxidant ascorbic acid was used as a positive control at the same concentrations of the tested extracts. The percentage inhibition of DPPH scavenging activities of the three hydromethanolic crude extracts was evaluated at concentrations of 0.02-0.2 mg/mL. There was an increase in DPPH radical scavenging activity with increasing concentrations of the extracts. At 0.2 mg/mL concentration, the percentage inhibition of all the extracts was relatively higher than the rest of the concentration. The

scavenging effect of the standard drug ascorbic acid was higher than all the extracts. However, in all the extracts (*T. schimperi, R. prinoides* and *J. shimperiana*) tested, *T. schimperi* exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentrations tested (0.02- 0.2 mg/mL) (Table 3).

Table 3. DPPH inhibition (%) by the three hydromethanolic leave crude extracts.

Tested samples	**% Inhibition of DPPH**						
	0.02 mg/mL	0.04 mg/mL	0.08 mg/mL	0.12 mg/mL	0.16 mg/mL	0.2 mg/mL	IC50%
T. schimperi	27.86	44.28	78.87	88.64	88.64	89.29	4.49
R. prinoides	14.95	37.28	41.87	57.71	75.78	84.03	9.79
J. shimperiana	5.12	6.01	16.46	17.93	24.55	35.03	30.68
Ascorbic acid (standard)	34.20	47.14	78.34	90.32	94.94	96.78	3.63

5.4 Ferric Reducing Antioxidant Power Assay (FRAP assay)

The FRAP assay results of the three hydromethanolic leave crude extracts were expressed as mmole equivalent of ferrous sulfate and are shown in Figure 2. Similar to DPPH assay, all the extracts showed an antioxidant activity in FRAP assay. The *T. schimperi* extract showed the highest reducing antioxidant power among the extracts with 2521.60 mmole ferrous sulfate Eq (mmole Fe^{2+}), followed by *R. prinoides* (1810.55 mmole Fe^{2+}). The linear regression curve of the standard compound (ferrous sulfate) used in FRAP assay at different concentrations is presented in Figure 1D.

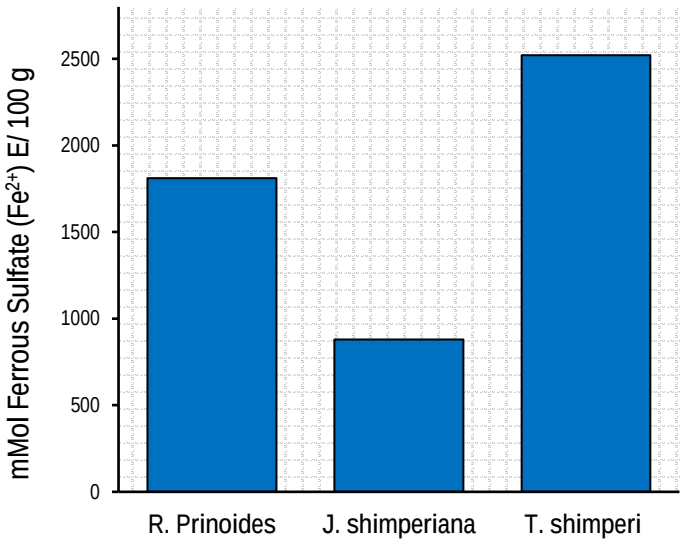


Figure 2. Ferric reducing antioxidant power assay (FRAP assay) of *R. prinoides*, *J. shimperiana* and *T. schimperi* of hydromethanolic leaf crude extracts. The results are expressed as mmol ferrous sulfate (Fe^{2+}) equivalence/ 100 g. The bars represent the mean ± standard deviation (SD) (n = 3).

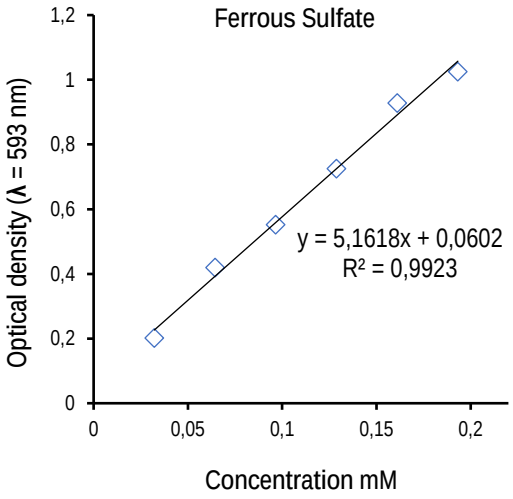


Figure 1D. Linear regression calibration curve of the standard ferrous sulfate in the FRAP assay.

5.5 ABTS Assay

The ABTS radical scavenging activity of the three hydromethanolic leaf extracts with reference compound, 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox®) is shown in Figure 3. The ABTS assay results were expressed as µmole equivalent of trolox®. The ABTS radical scavenging activity of the extracts was evaluated at 40 µL (n=3; triplicate). Therefore, the findings revealed that *T. schimperi* was the highest antioxidant activity with a mean of 27296.65 µmole trolox Eq (µmole trolox®), followed by *R. prinoides* (18857.62 µmole trolox®). The linear regression curve of the standard compound (Trolox®) in ABTS assay used at different concentrations is presented in Figure 1E.

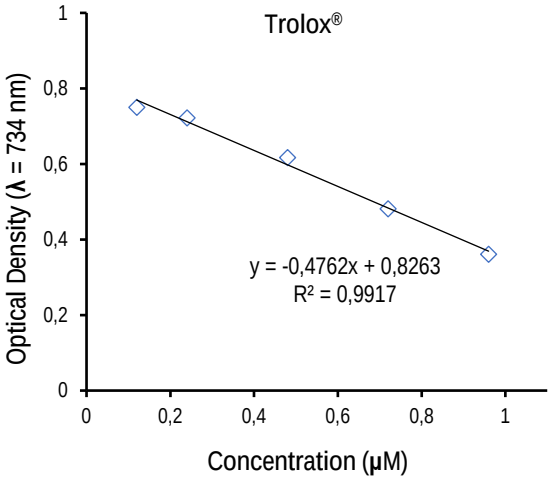


Figure 1E. The linear regression curve of the standard compound (Trolox®) used in ABTS assay.

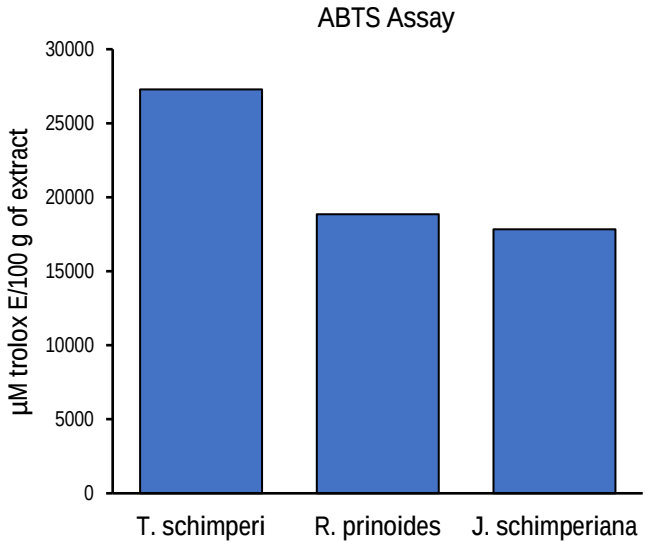


Figure 3. ABTS assay of *R. prinoides, J. shimperiana and T. Schimperi* of hydromethanolic extracts. Results are expressed as µmole *trolox*® equivalence/100 g of dried extract. Data was represented as the mean±SD (n = 3).

5.6 Analyses

According to the one-way ANOVA analyses, there was a statistical significance ($p < 0.05$) in mean differences of each measurement across the column among the three extracts (Table 4).

Table 4. One-Way Analysis of Variance (ANOVA)

Extracts	TFC (EC1)	TPC (EC1)	TPROC (EC1)	DPPH (IC50%)	ABTS (EC1)	FRAP (EC1)
T. schimperi	227.96±3.69¶^{A}	274.50±5.29¶^{A}	1618.06±1.32¶^{A}	4.49 ^{A}	27296.65¶^{A}	2521.60¶^{A}
R. prinoides	90.39±0.35¶^{A}	229.21±2.82¶^{A}	406.24±7.58¶^{A}	9.79 ^{A}	1887.62¶^{A}	1810.55¶^{A}
J. shimperiana	82.43±2.53¶^{A}	83.59±2.19¶^{A}	1037.18±4.06¶^{A}	30.68^{A}	17825¶^{A}	879.28¶^{A}
ANOVA	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

¶ Mean ±SD (n=3); measurements in each column having similar letters (A) had statistical difference among the extracts at $p \leq 0.05$ by Least Significant Difference (LSD) test; IC50%: Inhibitory Concentration; EC1: Effective Concentration.

5.7 Correlation between Antioxidant Assays and Phytochemical Contents

The Pearson correlation coefficients between the antioxidant assays and the Total Flavonoids Content (TFC), Total Phenol Content (TPC), and Total Proanthocyanidins Content (TPROC) were analyzed and presented in Table 5. The Total Phenol Contents showed almost a perfect negative correlation with the antioxidant activity value from DPPH (IC 50%) assay ($r = -0.999$) at a level of statistical significance ($p=0.023$). Further, the lowest IC50% means, the highest antioxidant activity. Additionally, these analyses revealed that the total flavonoid contents of

the extracts correlated significantly with ABTS assay (mean of scavenging activity; r=0.999; p = 0.032). However, the total proanthocyanidins content did not correlate with the antioxidant activities of the extracts (Table 5).

Table 5. Correlation Matrix Showing Relationship between Antioxidant Indices *Versus* TFC, TPC and TPROC of the three extracts.

Correlations							
		TFC (mg Q[x] E/100 g)	TPC (mg GA[†]E/100 g)	TPROC (mg C[‡]E/100 g)	DPPH (IC50%)	ABTS (mean)	FRAP (Mean)
TFC	Pearson Correlation	1	0.718	0.828	-0.692	0.999*	0.851
	Sig. (2-tailed)		0.490	0.379	0.513	0.032	0.352
	N	3	3	3	3	3	3
TPC	Pearson Correlation	0.718	1	0.204	-0.999*	0.753	0.976
	Sig. (2-tailed)	0.490		0.869	0.023	0.458	0.138
	N	3	3	3	3	3	3
TPROC	Pearson Correlation	0.828	0.204	1	-0.168	0.798	0.410
	Sig. (2-tailed)	0.379	0.869		0.893	0.412	0.731
	N	3	3	3	3	3	3
DPPH (IC50%)	Pearson Correlation	-0.692	-0.999*	-0.168	1	-0.728	-0.968
	Sig. (2-tailed)	0.513	0.023	0.893		0.481	0.162
	N	3	3	3	3	3	3
ABTS (mean)	Pearson Correlation	0.999*	0.753	0.798	-0.728	1	0.877
	Sig. (2-tailed)	0.032	0.458	0.412	0.481		0.319
	N	3	3	3	3	3	3
FRAP (Mean)	Pearson Correlation	0.851	0.976	0.410	-0.968	0.877	1
	Sig. (2-tailed)	0.352	0.138	0.731	0.162	0.319	
	N	3	3	3	3	3	3

*Correlation is significant at the 0.05 level (2-tailed); colors show repetitions of correlation.

5.8 *In Vitro* Evaluation of Antibacterial Activity

Except *J. shimperiana*, the rest of the results of this study showed the hydromethanolic crude leave extracts and their combination had an antibacterial activity against the patient isolated multi drug resistant (MDR) *E. coli* compared to the negative control. According to the records, *T. Schimperi* earned the highest diameter of zone of inhibition (DZI: 20.00±0.00 mm) at 1000 mg/mL concentration compared to all the uncombined tested extracts. However, the extract-extract combination of *T. Schimperi* and *R. Prinoides* had the same antibacterial activity as uncombined *T. Schimperi* extract at the maximum tested concentration (1000 mg/mL) (Table 6).

Largely, all the tested extracts except *J. shimperiana* showed a concentration dependent increase in antibacterial activity in the evaluation against *E. coli* (ATCC25922). *T. Schimperi* had the highest antibacterial activity at 1000 mg/mL and with this it had (DZI: 18.5±0.55 mm) diameter of the zone of inhibition compared to the other tested extracts. Following this, the extract-extract combination of *T. Schimperi and R. prinoides* had the second highest antibacterial activity (DZI: 14.5±0.55 mm) (Table 6).

In all the *in vitro* antibacterial activity evaluation against the *E. coli* (both clinical isolate and reference strain), the antibacterial activities of the extracts were totally lower than ciprofloxacin, the positive control. However, the antibacterial activity of ciprofloxacin against the *E. coli* (ATCC25922) was higher than its activity against to the MDR *E. coli clinical isolate*. This finding showed that the standard *E. coli* (ATCC25922) is a different strain and also, the clinically isolated *E. coli* had acquired a multi-drug resistance (Table 5).

In the *in vitro* antibacterial activity evaluation of the three extracts and the combination against the patient isolated MDR *K. pneumoniae* extended-spectrum β-lactamases *(ESBL)*, *T. schimperi* had a concentration-dependent maximum inhibition compared to the tested extracts at corresponding similar concentrations. However, the antibacterial activity of *T. schimperi* was relatively lower compared to ciprofloxacin (DZI: 17.83±0.41 mm). In the *T. schimperi and R. prinoides* combination, the antibacterial activity was the second with the highest zone of inhibition (DZI: 11.90±0.10 mm) (Table 6).

Activities against *K. pneumoniae* (ATCC700603), *T. schimperi* showed the highest zone of inhibition (DZI: 14.83±0.40 mm) compared to the extracts and the combination. Antibacterial activity of *R. prinoides* extract had also a concentration-dependent increase across the concentrations tested and had a higher antibacterial activity than the activity against the patient isolated MDR *K. pneumoniae ESBL* (Table 6). In both MDR patient isolated

K. pneumoniae extended-spectrum β-lactamases *(ESBL)* and *K. pneumoniae* (ATCC700603), the antibacterial activity of *J. shimperiana* was similar to the negative control (Table 6).

Table 6. The *in vitro* antibacterial activity of the hydromethanolic crude leave extracts against the MDR Clinical Isolate and standard bacteria strain.

Plant extracts (Against B_1)	***E. coli clinical isolate (MDR)***			
	Zone of Inhibition (mm): Mean ± SD			
	250 mg/mL (Extract)	500 mg/mL	750 mg/mL	1000 mg/mL
T. schimperi	17.17 ± 0.41	19.1 ± 0.98	19.70 ± 0.29	20.00 ± 0.00
R. prinoides	8.83 ± 0.26	9.5 ± 0.55	10.83 ± 0.41	11.00 ± 0.00
J. shimperiana	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
T. schimperi and R. prinoides	17.17 ± 0.41	19.17 ± 0.98	19.50 ± 0.49	20.00 ± 0.00
Ciprofloxacin (5µL/mL)	33.00± 0.00			
Negative control	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
Plant extracts (Against B_2)	***E. coli (ATCC25922)***			
	Zone of Inhibition (mm): Mean ± SD			
	250 mg/mL (Extract)	500 mg/mL	750 mg/mL	1000 mg/mL
T. schimperi	15.67 ± 0.52	17.67 ± 0.52	18.33 ± 0.52	18.5 ± 0.55
R. prinoides	8.83 ± 0.26	10.5 ± 0.55	10.66 ± 0.52	11.5 ± 0.55
J. shimperiana	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
T. schimperi and R. prinoides	10.17 ± 0.40	11.65 ± 0.35	12.00 ± 0.00	14.5 ± 0.55
Ciprofloxacin (5µL/mL)	37.00± 0.00			
Negative control	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
Plant extracts (Against B_3)	***MDR K. Pneumoniae CI***			
	Zone of Inhibition (mm): Mean ± SD			
	250 mg/mL (Extract)	500 mg/mL	750 mg/mL	1000 mg/mL
T. schimperi	12.5 ± 0.55	13.5 ± 0.55	14.00 ± 0.00	14.50 ± 0.55
R. prinoides	8.50 ± 0.50	8.75 ± 0.24	9.00 ± 0.00	10.00 ± 0.00
J. shimperiana	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
T. schimperi and R. prinoides	11.00 ± 0.00	11.58 ± 0.52	11.82 ± 0.12	11.90 ± 0.10
Ciprofloxacin (5µL/mL)	17.83 ± 0.41			
Negative control	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
Plant extracts (Against B_4)	***K. Pneumoniae (ATCC700603)***			
	Zone of Inhibition (mm): Mean ± SD			
	250 mg/mL (Extract)	500 mg/mL	750 mg/mL	1000 mg/mL
T. schimperi	12.5 ± 0.55	14.00 ± 0.00	14.00 ± 0.00	14.83 ± 0.40
R. prinoides	9.00 ± 0.00	10.00 ± 0.00	10.50 ± 0.55	11.00 ± 0.55
J. shimperiana	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
T. schimperi and R. prinoides	10.33 ± 0.52	11.5 ± 0.55	11.5 ± 0.55	11.5 ± 0.55
Ciprofloxacin (5µL/mL)	24.00 ± 0.00			
Negative control	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00

B1: *E. coli (CI) MDR; B2: E. coli (ATCC25922); B3: K. pneumoniae CI*; and B4: *K. Pneumoniae (ATCC700603).*

5.9 Minimum Inhibitory Concentration (MIC)

The findings of the MIC of the three hydromethanolic leave crude extracts are displayed in Table 7. In all the extracts, the concentration ranges for MIC determination were evaluated from 64 mg/mL up to 0.03125 mg/mL. within the tests, the MIC activity of the extracts against patient isolated MDR *E. coli* ranges from 4 mg/mL up to 8 mg/mL whereas against standard *E. coli* (ATCC25922) ranges 4 mg/mL up to 32 mg/mL. However, the concentration of the standard drug ciprofloxacin for the MIC tested against all *E. coli* strains was ranged from 5-0.0024 µg/mL.

Both *T. schimperi* alone and its combination with *R. prinoides* had the lowest MIC value (4 mg/mL) in the test against the clinical isolate MDR *E. coli*. However, *T. schimperi* was the only extract with the lowest MIC (4 mg/mL) among the extracts evaluated against *E. coli* (ATCC25922). The positive control (ciprofloxacin) had the lowest MIC value compared to all independent extracts and the two extracts combination. In the effect against the patient isolated MDR *E. coli*, ciprofloxacin MIC was identified at 0.156 µg/mL but MIC value was lowest enough in *E. coli* (ATCC25922) (0.0048 µg/mL). The lowest MIC of *T. schimperi* in antibacterial activity evaluation against patient isolated MDR *K. pneumoniae* was 8 mg/mL but 4 mg/mL (MIC) for activity against *K. pneumoniae* (ATCC700603) (Table 7).

5.10 Minimum Bactericidal Concentration (MBC)

Among the extracts tested for MBC determination, *T. schimperi* had the lowest MBC (16 mg/mL) in the evaluation against patient isolated MDR *E. coli* which were similar to the same concentration to the MBC against the reference strain *E. coli* (ATCC25922). The standard drug ciprofloxacin had the lowest MBC compared to the extracts. In the test against *E. coli* (ATCC25922), the MBC of ciprofloxacin was 0.0006 µg/mL. The lowest MBC of *T. schimperi* in antibacterial activity evaluation against patient isolated MDR *K. pneumoniae* was 32 mg/mL but 64 mg/mL (MBC) for activity against *K. pneumoniae* (ATCC700603) (Table 7). The ratio of MBC/MIC of the extracts revealed the exact definition of the antibacterial effect. Accordingly, for both *E. coli* strains, *T. schimperi* had a bactericidal effect. *R. prinoides* had a bacteriostatic in effect against patient isolated MDR *E. coli*. Whereas *T. schimperi* and *R. prinoides* combination showed bacteriostatic effect in the test against MDR *E. coli* clinical isolate (Table 7). In this study, the ratio of MBC to MIC of *T. schimperi* alone and in combination *R. prinoides* in the test against *K. pneumoniae* (ATCC700603) was bacteriostatic. However, in the test against patient isolated MDR *K. pneumoniae ESBL*, *T. schimperi* showed a bactericidal (Table 7).

Table 7. The MIC and MBC of the extracts and ciprofloxacin

Extract and ciprofloxacin	*MDR E. coli (CI)*			*E. coli* (ATCC25922)		
	MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
T. schimperi	4	16	4	4	16	4
R. prinoides	8	64	8	16	>64	ND
J. shimperiana	8	>64	ND	32	>64	ND
T. schimperi and R. prinoides	4	32	8	16	64	4
Ciprofloxacillin	0.156 µg/ml	0.156 µg/ml	1	0.0048 µg/ml	0.0006 µg/ml	0.125
Extract and ciprofloxacin	*MDR K. pneumoniae CI (ESBL)*			*K. pneumoniae* (ATCC700603)		
	MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
T. schimperi	8	32	4	4	64	16
R. prinoides	16	> 64	ND	16	> 64	ND
J. shimperiana	16	> 64	ND	16	> 64	ND
T. schimperi and R. prinoides	8	> 64	ND	8	64	8
Ciprofloxacin (µg/mL)	0.156	0.3125	2	0.156	0.156	1

ND: Not determined.

5. 11 Checkerboard Assay

To quantify the interactions, the Fractional Inhibitory Concentration (FIC) index of the checkerboard analysis in this study showed that the interaction of the combinations between *T. schimperi* extract and *ciprofloxacin* (positive control) was categorized as indifference in the activity against the three bacterial strains except for MDR *E. coli clinical isolate* (additive). Similarly, according to the FIC index value the *R. prinoides* and *ciprofloxacin* combinations showed an indifference interaction in the test against all the three bacterial strains except for *E. coli* (ATCC25922) (antagonist) (Table 8).

Table 8. Results of the Checkerboard analysis for the interactions between extracts and ciprofloxacin combination in the activities against the four bacteria strains.

Item	**Types of Bacterial Isolates**			
	MDR E. coli CI	*E. coli ATCC*	*MDR K. pneumoniae CI (ESBL)*	*K. pneumoniae ATCC*
TS_A	2	2	8	8
TS_C	1	2	16	8
CPR_A	0.156	0.0192	1.248	0.312
CPR_C	0.078	0.0096	0.312	0.624
FIC_{TS}	0.5	1	2	1
FIC_{CPR}	0.5	0.5	0.25	2
FIC_{index1}	1	1.5	2.25	3
Category	Additive	Indifference	Indifference	Indifference
RP_A	8	16	16	16
RP_C	8	32	16	32
CPR_{A2}	0.156	0.0096	0.624	0.312
CPR_{C2}	0.156	0.312	0.312	0.312
FIC_{RP}	1	2	1	2
FIC_{CPR2}	1	32.5	0.5	1
FIC_{index2}	2	34.5	1.5	3
Interaction category	Indifference	Antagonist	Indifference	Indifference

TS_A: *Thymus schimperi* alone; TS_C: *Thymus schimperi* combination; CPR_A: Ciprofloxacin alone; CPR_C: Ciprofloxacin combination; FIC_{TS}: Fractional Inhibitory Concentration of *Thymus schimperi;* FIC_{CPR}: Fractional Inhibitory Concentration index of Ciprofloxacin; FIC_{index1}: Fractional Inhibitory Concentration index of *Thymus schimperi* combined with ciprofloxacin; RP: *Rhamnus prinoides.*

5. 12 GC-MS Analyses

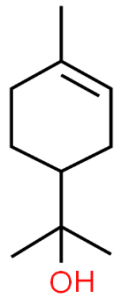
As shown in Table 9, the results obtained from GC-MS analyses of the hydromethanolic extract of *T. schimperi* clearly revealed the identification and presence of 14 bioactive compounds. The identified and matched compounds were represented with the name of the compound, molecular formula, molecular weight, retention time, percentage composition of the area, nature of the compounds, and chemical structure.

During the analyses, the first compound identified (peak 1) was observed at retention time (4.666 minutes) and the last compound (peak 14) was identified at longest retention time of (19.835 min). Among the compounds eluted, the major compounds identified in this *T. schimperi* crude extract were hexanedioic acid, bis (2-ethylhexyl) ester (RT: 19.835; 73.88%); thymol (RT: 5.799; 11.68%); O-Cymen-5-ol (RT: 5.931; 7.95%); and p-tert-Butylcatechol (RT: 7.853; 2.19%) respectively along with the other minor constituents. In the GC-MS analyses, chromatogram shows the peak area separation of the components. The mass spectrums of the three major bioactive compounds in *T. schimperi* crude extract confirmed are presented in Figure 4A-4C. The GC-MS chromatogram of hydromethanolic crude extract of *T. schimperi* is presented in Figure 6.

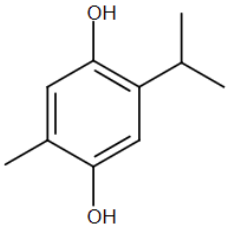
Table 9. The Bioactive Compounds Identified through GC-MS Analyses from the Hydromethanolic *T. schimperi* crude extract

Peak #	Name of the Compound (CPD)	Formula	MW	RT	Nature (CPD)	%Area
1	α-Terpineol	$C_{10}H_{18}O$	154.25	4.67	Menthane monoterpenoids	0.208649
2	Thymoquinone	$C_{10}H_{12}O_2$	164.201	5.31	Monoterpene	0.152454
3	Thymol	$C_{10}H_{14}O$	150.22	5.80	Monoterpenoid phenol	11.67551
4	o-Cymen-5-ol	$C_{10}H_{14}O$	150.221	5.93	Phenolic compound	7.944895
5	Rosefuran	$C_{10}H_{14}O$	150.22	6.29	Aromatic	0.313204
6	p-tert-Butylcatechol	$C_{10}H_{14}O_2$	166.217	7.85	Catechol derivative organic cpd	2.194801
7	p-Cymene-2,5-diol	$C_{10}H_{14}O_2$	166.2170	9.60	Essential oil	0.192658
8	4H-1,3,2-Dioxaborin, 6-ethenyl-2-ethyl-4-methyl-4-(2-methylpropyl)-	$C_{12}H_{21}BO_2$	208.11	10.41	-	0.167239
9	Phthalic acid, 2-cyclohexylethyl butyl ester	$C_{20}H_{28}O_4$	332.4	11.71	Ester	0.14886
10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	13.39	Ester	0.545681
11	E-10-Methyl-11-tetradecen-1-ol propionate	$C_{18}H_{34}O_2$	268.24	15.65	Fatty alcohol esters	0.254021
12	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	292.5	15.73	Esterified form of linoleic acid	1.434284
13	Phytol, acetate	$C_{22}H_{42}O_2$	338.5677	15.87	Diterpenoid	0.883479
14	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.5665	19.84	Diester of 2-ethylhexanol and adipic acid	73.88426

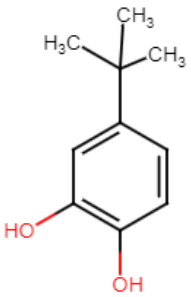
MWT: Molecular Weight; RT: Retention Time.



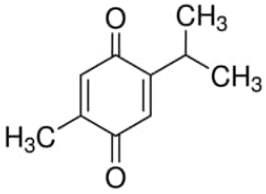
α-Terpineol



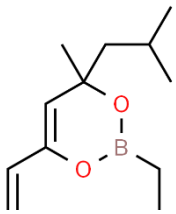
p-Cymene-2,5-diol



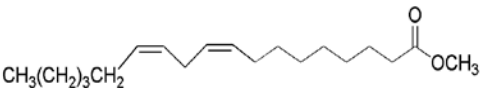
p-tert-Butylcatechol



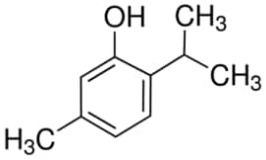
Thymoquinone



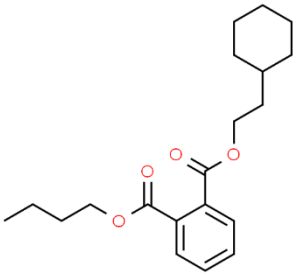
4H-1,3,2-Dioxaborin, 6-ethenyl-2-ethyl-4-methyl-4-(2-methylpropyl)-



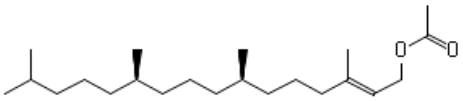
Linolenic acid, methyl ester



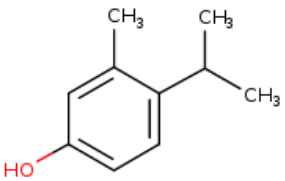
Thymol



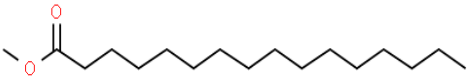
Phthalic acid, 2-cyclohexylethyl butyl ester



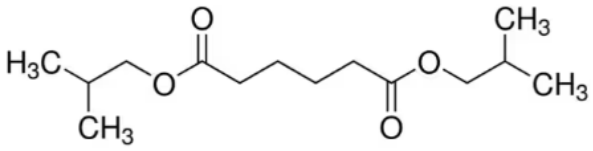
Phytol, acetate



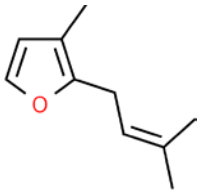
o-Cymen-5-ol



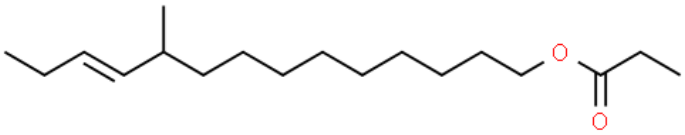
Hexadecanoic acid, methyl ester



Hexanedioic acid, bis(2-ethylhexyl) ester



Rosefuran



E-10-Methyl-11-tetradecen-1-ol propionate

Figure 5. Chemical Structure of the Bioactive Compounds Identified through GC-MS Analyses from the Hydromethanolic *T. schimperi* crude extract

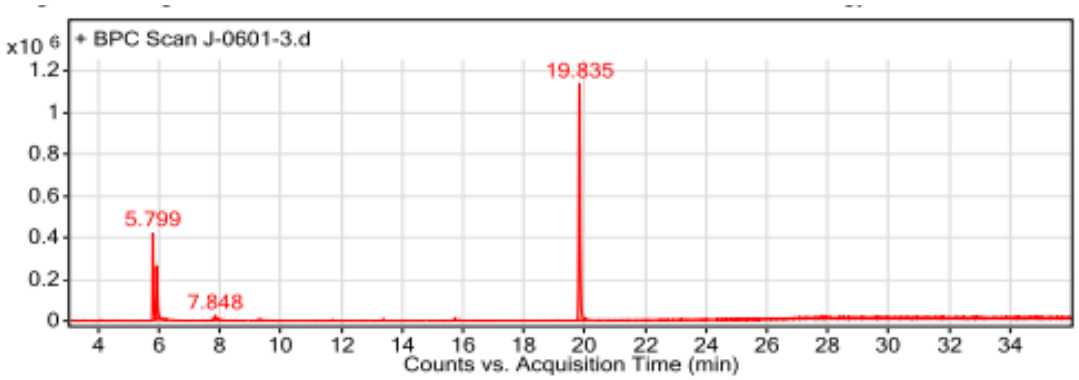


Figure 6. The GC-MS chromatogram of hydromethanolic crude extract of *T. schimperi.*

In the GC-MS analyses of the hydromethanolic crude extract of *R. prinoides*, six compounds were identified. In these analyses, the three abundant bioactive compounds identified among the eluted components were the following: hexanedioic acid, bis (2-ethylhexyl) ester (RT: 19.755; 79.36%); beta-D-Glucopyranoside, methyl (RT: 9.719; 10.03%); and Desulphosinigrin (RT: 11.132; 8.28%) (Table 10). The GC-MS chromatogram of hydromethanolic crude extract of *R. prinoides* is presented in Figure 8.

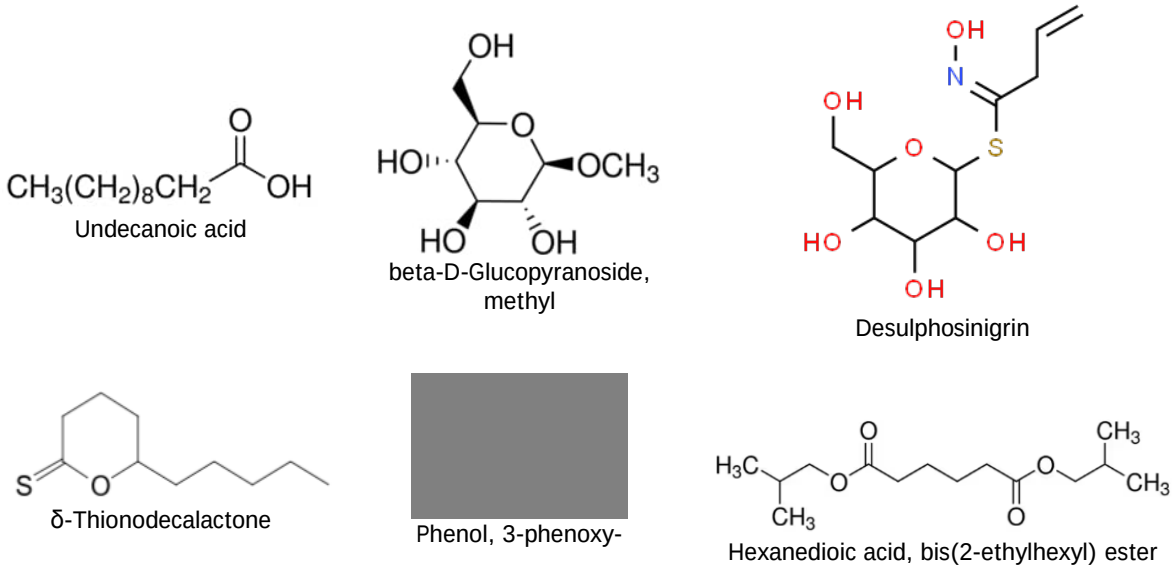


Figure 7. Chemical Structure of the Bioactive Compounds Identified through GC-MS Analyses from the Hydromethanolic *R. prinoides* crude extract.

Table 10. The Bioactive Compounds Identified through GC-MS Analysis from the Hydromethanolic Crude *R. prinoides* Extract.

Peak #	Name of the Compound (CPD)	Formula	MW	RT	% Area	Nature (CPD)
1	Undecanoic acid	$C_{11}H_{22}O_2$	186.29	8.008	1.42	Fatty acid
2	beta-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194.1825	9.719	10.03	O-glycosyl
3	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279.31	11.132	8.28	Carbohydrate
4	δ-Thionodecalactone	$C_{10}H_{18}OS$	186.314	15.859	0.51	Lactone
5	Phenol, 3-phenoxy-	$C_{12}H_{10}O_2$	186.21	19.172	0.41	Phenol
6	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.6	19.755	79.36	Diester of 2-ethylhexanol and adipic acid

MWT: Molecular Weight; RT: Retention Time.

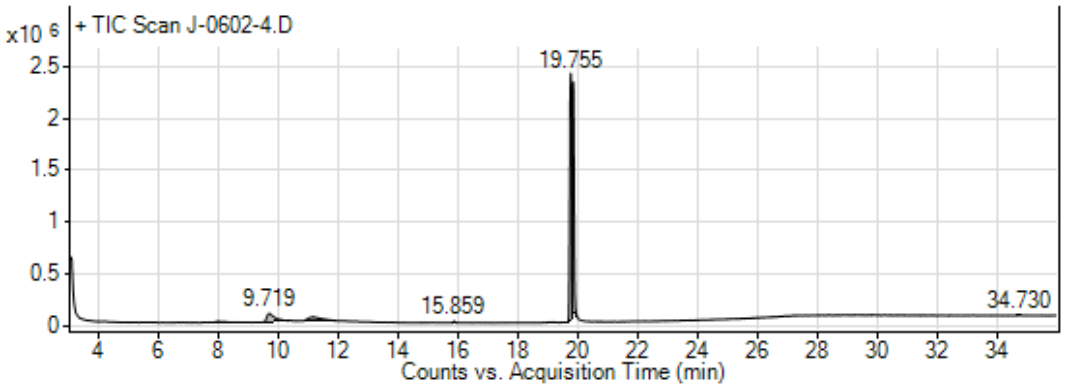
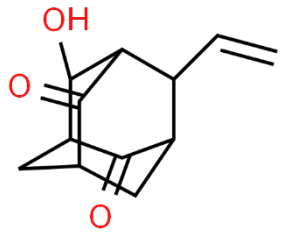


Figure 8. The GC-MS chromatogram of hydromethanolic crude extract of *R. prinoides.*

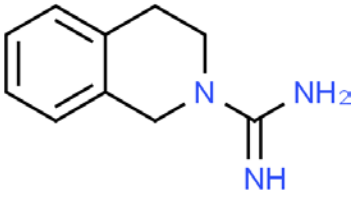
In this study, the GC-MS analyses of the hydromethanolic crude extract of *J. schimperiana* confirmed the presence of five bioactive compounds. The predominant compound was hexanedioic acid, mono (2-ethylhexyl) ester (RT: 19.841; 59.6% peak area). The second abundantly occurring bioactive compound was debrisoquine (RT: 13.364; 12.18%) followed by methyl 8, 11, 14-heptadecatrienoate (RT: 15.727; 10.79%) (Table 11). The GC-MS chromatogram of hydromethanolic crude extract of *J. schimperiana* is presented in Figure 10.

Table 11. The Bioactive Compounds Identified through GC-MS Analysis from Hydromethanolic Crude *J. schimperiana* Extract.

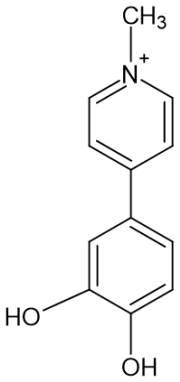
Peak #	Name of compounds	Formula	MWT	RT	% Area	Nature (Cpd)
1	4-Hydroxy-9-vinyladamantane-2,6-dione	$C_{12}H_{14}O_3$	206.24	12.288	6.80	-
2	Debrisoquine	$C_{10}H_{13}N_3$	175.23	13.364	12.18	Member of isoquinolines and a carboxamidine
3	1-Methyl-4-[4,5-dihydroxyphenyl]pyridinium bromide	$C_{12}H_{12}BrNO_2$	282.13	14.239	10.62	-
4	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	278.43	15.727	10.79	-
5	Hexanedioic acid, mono(2-ethylhexyl)ester	$C_{14}H_{26}O_4$	258.35	19.841	59.60	-



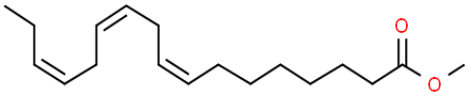
4-Hydroxy-9-vinyladamantane-2,6-dione



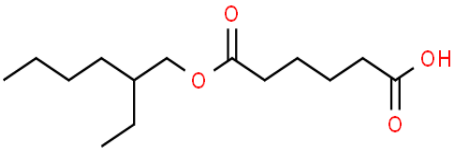
Debrisoquine



1-Methyl-4-[4,5-dihydroxyphenyl]pyridinium bromide



Methyl 8,11,14-heptadecatrienoate



Hexanedioic acid, mono(2-ethylhexyl)ester

Figure 9. Chemical structure of the Bioactive Compounds Identified through GC-MS Analysis from Hydromethanolic Crude *J. shimperiana* Extract.

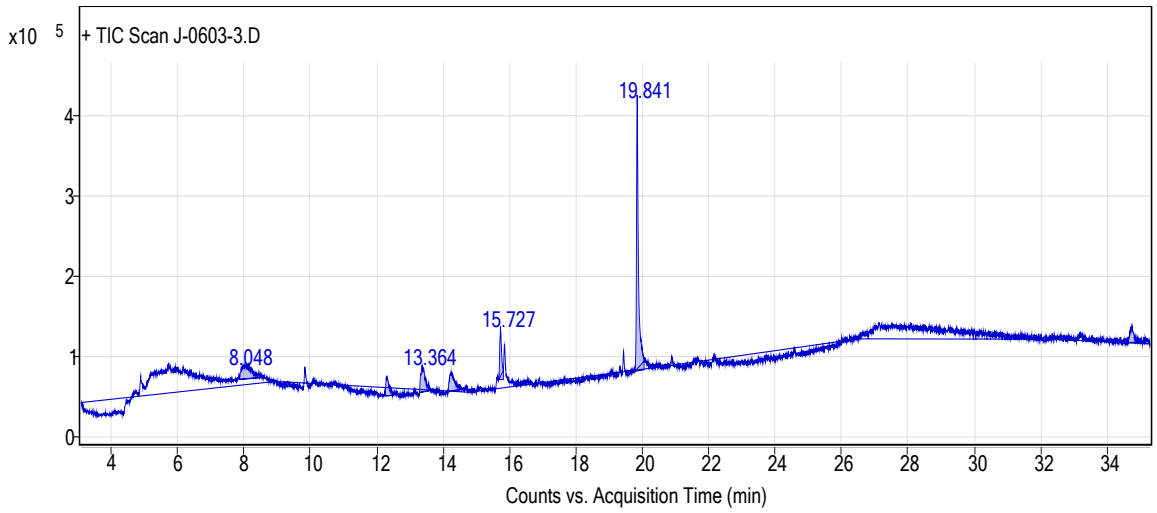
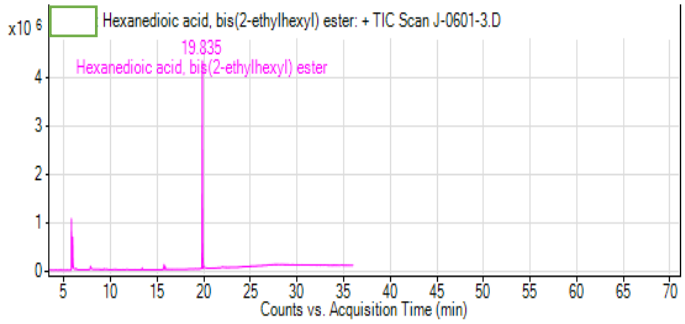
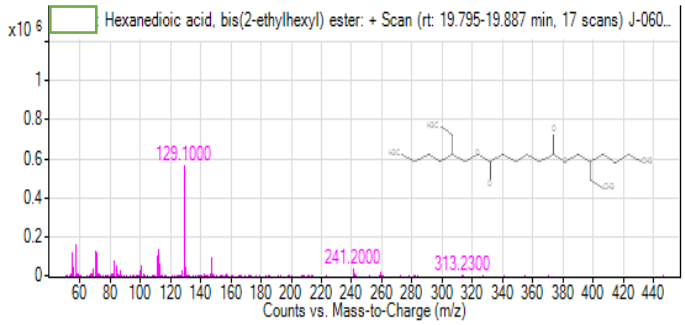
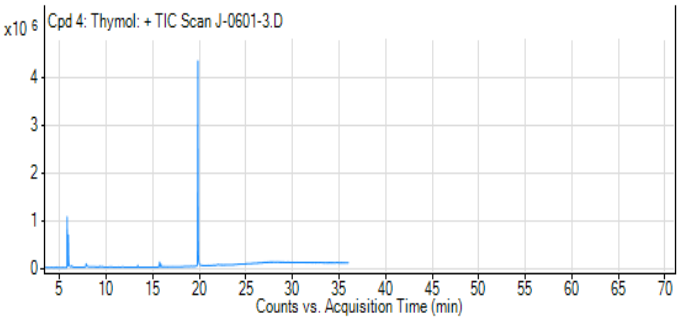
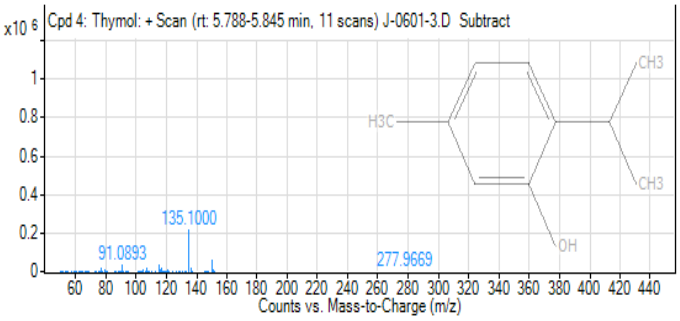


Figure 10. The GC-MS chromatogram of hydromethanolic crude extract of *J. schimperiana.*


Hexanedioic acid, bis(2-ethylhexyl) ester: + TIC Scan J-0601-3.D
x10 6
19.835
Hexanedioic acid, bis(2-ethylhexyl) ester
Counts vs. Acquisition Time (min)


Hexanedioic acid, bis(2-ethylhexyl) ester: + Scan (rt: 19.795-19.887 min, 17 scans) J-060...
x10 6
129.1000
241.2000
313.2300
Counts vs. Mass-to-Charge (m/z)


Cpd 4: Thymol: + TIC Scan J-0601-3.D
x10 6
Counts vs. Acquisition Time (min)


Cpd 4: Thymol: + Scan (rt: 5.788-5.845 min, 11 scans) J-0601-3.D Subtract
x10 6
135.1000
91.0893
277.9669
H3C
CH3
CH3
OH
Counts vs. Mass-to-Charge (m/z)

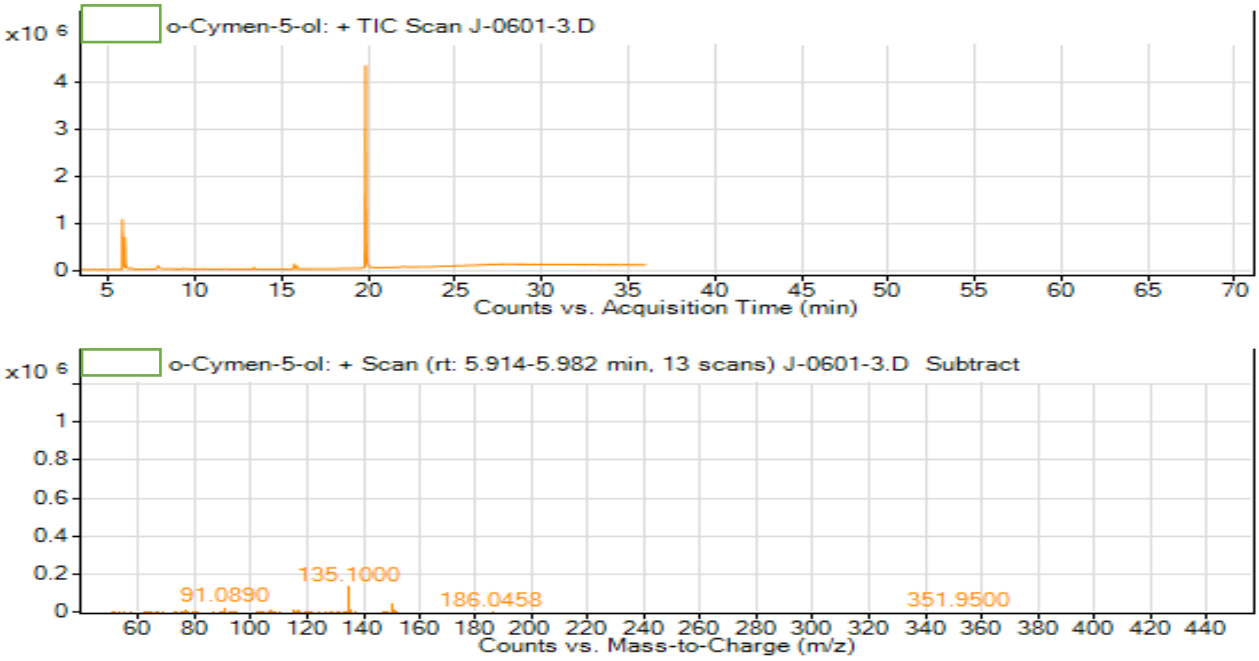


Figure 4A-4C. Peak fragmentation of the Chromatography Mass Spectrum of the three Major compounds from *T. schimperi*.

CHAPTER 5. DISCUSSION

In recent years, the traditional remedies exhibit peaking popularity due to their possible commercial application for medicinal or healthcare purposes (Strzepek-Gomółka *et al*., 2021). Generally, in the evaluation of *in vitro* antioxidant activities of the three hydromethanolic leave crude extracts using the three standard assays (DPPH, ABTS and FRAP Assays), *T. schimperi* has clearly shown the highest antioxidant activities among the extracts followed by *R. prinoides* and *J. shimperiana,* respectively. In the current study, all the tested plant extracts showed DPPH free radical scavenging activity. The greatest radical scavenging activity was shown by *T. schimperi* (IC50 = 4.49) and *R. prinoides* extract (IC50 = 9.79). The closer the IC50 value of the plant extract to that of the IC50 (IC50 = 3.63) of ascorbic acid, the greater the inhibitory effectiveness (Chand *et al*., 2018). Therefore, *T. schimperi* extract was more effective in scavenging free radicals than *R. prinoides* and *J. shimperiana*. A lower IC50 value indicates a higher antioxidant activity (Li *et al*., 2009; Boulfia *et al*., 2021). This study also revealed that all the three hydromethanolic crude extracts showed a concentration-dependent increase in the DPPH scavenging activity. This result agreed with a report of Motalleb *et al*., (2005) which showed the scavenging effects on the DPPH radical increased sharply with increasing concentration of the samples.

The phytochemical analyses of this study indicated that the Total Phenol Content (TPC) among the three extracts was highest in *T. schimperi* followed by *R. prinoides and J. shimperiana.* In the Pearson coefficient analyses, the TPC showed a nearly perfect negative correlation with the antioxidant activity value from DPPH (IC 50%) assay (r= -0.999) at a level of statistical significance (p=0.023). This finding was supported by earlier reports (Heim *et al*., 2002; Soongand and Barlow, 2004; Balasundram *et al*., 2006; Loizzo *et al*., 2012) and stated that phenolic compounds are major antioxidant constituents in selected medicinal plants and there are direct relationships between their antioxidant activity and total phenolic content with a relative difference in their antioxidant potencies. Furthermore, Narangerel *et al*., (2021) mentioned that a strong antioxidant activity is associated with a high amount of total phenolic content. According to Mediani *et al*., (2012), phenolic compounds of herbs have the ability to scavenge free radicals, and factors such as genetic and environmental conditions (growth season and plant maturity) can cause variations in their values. Moreover, there are numerous reports supporting that antioxidant potential of plants is due to their phenolic compounds (Chinnici *et al*., 2004; Burčul *et al*., 2018). In general, plants are a diverse source of phenolic compounds with antioxidant, anti-bacterial, antiviral, and anti-cancer potentials (Manach *et al*., 2004).

Additionally, the Pearson coefficient analyses of this study showed that the total flavonoid contents of the extracts had significant correlation with ABTS assay (mean of scavenging activity; r = 0.999; p= 0.032). This study finding is in agreement with a previous study report (Zhang *et al*., 2016). Further, the role of high levels of flavonoid contents in most of the antioxidant activity studies of plant materials as reported by (Du Toit *et al*., 2018; Milevskaya *et al*., 2018; Calado *et al*., 2015; Abdel-Hameed *et al*., 2014; Montoro *et al*., 2005) were implicated to their ability to scavenge reactive oxygen species (ROS) consisting of free radicals. Other effects of flavonoid include anticancer (Milevskaya *et al*., 2018), antimicrobial, and anti-inflammatory properties (Shahidi and Ambigaipalan, 2015).

Unlike the earlier study report by Li *et al*. (2021), total proanthocyanidin contents of this study did not show correlation with the antioxidant activity (in DPPH, ABTS, and FRAP assays). However, proanthocyanidins, a subclass of the most complex flavonoids, are the non-polar condensed tannins and polymer of flavan-3-ols and constitute an important group of polyphenols because of their bioactivities such as anti-inflammatory, antioxidant and anticancer activities (Jose *et al*., 2012; Zhou and Raffoul, 2012).

The antioxidant metabolites were held responsible for the reduction of ferric (Fe^{3+}) to form it as ferrous (Fe^{2+}) ion, this addition of $FeCl_3$ to form it as ferrous tripyridyltriazine by blue colored complex formation and can be determined by measuring of reduction of the colored complex at 593 nm (Rice-Evans *et al*., 1997). In the present study, the Pearson correlation analysis revealed that the FRAP scavenging activities of the extracts was not correlated with any of the determined phytochemical contents. However, a previous study report concluded that redox properties can be present in phenolic compounds and by them specific activity such as reducing agents, hydrogen donators and singlet oxygen quenchers (Labiad *et al*., 2017).

In Ethiopia, the medicinal plants exhibited by local healers for the community health have promising antibacterial activities on multidrug-resistant bacteria. This is due to biodiversity coupled with the chemical diversity found within each species (Yitbarek *et al*., 2010; Sasidharan *et al*., 2011; Chuah *et al*., 2014). The result of this study directs that, methanol crude extracts showed on antibacterial activities. The same result with *Kinsalin et al.* (2014) states that methanol extract possesses potent antimicrobial activity which in turn may be due to the presence of biologically active ingredients with antimicrobial activity in the medicinal plants. Among the reported mechanisms, the antimicrobial activity of flavonoids is due to their ability to bind with bacterial cell wall and to interact with extracellular and soluble

protein while that of tannins may be related to their ability to inhibit the microbial adhesions, cell envelop proteins and enzymes (Sharma *et al*., 2010; Mogana *et al*., 2020).

In this evaluation study of the hydromethanolic crude extracts, the findings clearly showed that, *T. schimperi* had the highest antibacterial activities against the patient isolated MDR uropathogenic bacteria (*E. coli and K. pneumoniae ESBL*) compared to the combined and uncombined extracts. This finding is similar to a previously reported study on *T. schimperi oil* that caused a moderate antibacterial activity on these specific organisms (Cutillas *et al*., 2018).

The extract-extract combination of *T. schimperi and R. prinoides* had a similar antibacterial activity in the test against patient isolated *MDR E. coli* with uncombined *T. schimperi.* However, the antibacterial activity evaluation revealed that the effect against the rest of these three organisms was lower than uncombined *T. schimperi* extract*.* This result clearly indicates that the combined extracts *of T. schimperi and R. prinoides* did not show synergistic activities. This study is different from Gadisa *et al*. (2019) report whose study on *T. schimperi* and *Blepharis cuspidata* combinations showed a synergistic activity. This could be due to the difference in the type of the combined plant material with *T. schimperi*, or a difference in the extraction method or other undisclosed factors.

Comparatively, the MIC of the *T. schimperi* extract had the lowest MIC than the other independent extracts during the antibacterial activity test, except with the MIC (equivalent) of its combination with *R. prinoides,* in the test against the clinical isolated MDR *E. coli* and *K. pneumoniae* EBSL. This result further confirmed that *T. schimperi* had the highest antibacterial activities for the entire patient isolated MDR bacteria and reference strains compared to the tested extracts. This current study finding is in line with Ibrahim and Kebede (2020) which stated that the lower MIC indicates that the plant extracts were stronger of activities of killing or inhibiting the test pathogens and vice versa. Additionally, this study clearly indicated that the ratio of MBC to MIC of the *T. schimperi*, except in the test against *K. pneumoniae (ATCC 700603)*, showed a bactericidal activity.

According to the checkerboard analyses of the present study, the interaction of the combinations between the *T. schimperi* and ciprofloxacin was additive. This is interpreted as an overall antibacterial activity of the extract and positive control combination equals the sum of the individuals' effect. Thirdly, following the extract-extract combinations' activity, the *R. prinoides* extract showed an antibacterial activity against the patient-isolated, MDR *E. coli* and

K. pneumoniae ESBL and their reference strains in a concentration-dependent manner, with a slight increased effect in both reference strains.

Potentially, the gas-chromatography coupled with mass spectrometry (GC-MS) could provide more precise information in qualitative analysis of different samples including plant extracts (Cong *et al.*, 2007). According to this work, the GC-MS analysis of the hydromethanolic crude leaves extract of *T. schimperi* showed the presence of fourteen (14) compounds. These identified compounds possess many biological properties. For instance, with interest of this study evaluation, the most abundant compound was hexanedioic acid, bis (2-ethylhexyl) ester (RT: 19.835; 73.88%) which was reported to have an antioxidant and antimicrobial activities (Tayade *et al.*, 2013). Thus, hexanedioic acid, bis (2-ethylhexyl) ester among the eluted compounds might be contributing to the highest activity of the crude extract of *T. schimperi* in the *evaluation of in vitro* antioxidant and antibacterial activities.

Additionally, the second highest compound identified from GC-MS analysis of the hydromethanolic crude extract of *T. schimperi* was thymol. In the evaluation against multidrug resistant *E. coli* and *K. pneumoniae*, the antibacterial activity of *T. schimperi* might be associated with thymol effect. Previously reported multiple studies confirmed so far that thymol exhibited an antibacterial activity when testing against Gram-negative strains (Trombetta *et al.*, 2005; Zarrini *et al.*, 2010; Khoury *et al.*, 2016; Marchese *et al.*, 2016; Rúa *et al.*, 2019; Kachur and Suntres, 2020).

Multiple study reports demonstrated that thymol alone, or as a phytochemical constituent in plant extracts along with other metabolites, exhibits potent antibacterial, antimicrobial, antifungal, and antiparasitic properties (Marchese *et al.*, 2016; Nagoor Meeran *et al.*, 2017). In this study, the hydromethanolic crude extract of *T. schimperi* showed antioxidant activities. These antioxidant activities might be associated with thymol. As reported before by Nagoor Meeran *et al.* (2015, 2016), thymol was shown to exhibit potent superoxide anion, hydroxyl and DPPH radical scavenging and reducing capacity in a concentration-dependent manner. Further, in the GC-MS analyes of the crude *T. schimperi*, the third abundant compound found was o-cymen-5-ol. Also, the antibacterial activities of the *T. schimperi* possibly could be related to the presence of o-cymen-5-ol. Similar to this study, earlier studies (Pizzey *et al.*, 2011; Lesielle Adaptive Skincare, 2022) reported that o-cymen-5-ol has an antibacterial activity.

Similar to the finding of the GC-MS analyses of *T. schimperi* extract, the major constituent of the hydromethanolic crude extract of *R. prinoides* was hexanedioic acid, bis (2-

ethylhexyl) ester. As previously mentioned, this compound has reported to have antioxidant and antibacterial activities (Tayade *et al*., 2013; Ferdosi *et al*., 2021). Further, the GC-MS analyses of the hydromethanolic crude extract of *R. prinoides* identified the second abundant bioactive compound known as β-D-Glucopyranoside, methyl among the total eluted. According to the existing literature body of evidence, β-D-Glucopyranoside, methyl was reported to have antibiotic, antibacterial, and antimycotics activities (Shaheed *et al*., 2018; National Center for Biotechnology Information, 2022). Additionally, a smaller number of reports described that the β-D-Glucopyranoside, methyl, which is a chemical component found in phenols and flavonoids, could potentialy have an antimicrobial, antibacterial effect, as well as it is used in the medication of different diseases (Salem, 2005; Panghal *et al*., 2011). Therefore, the antibacterial effects of the *R. prinoides* in this study might be mediated from the biological activities of these identified compounds in adequate amount in this GC-MS analyses.

In a PubChem Compound Summary (NCBI b, 2022), debrisoquine which is also the second highest compound identified from GC-MS analyses of hydromethanolic crude extract of *J. shimperiana* has a role as an antihypertensive agent, an adrenergic agent, a sympatholytic agent, and a human metabolite. Multiple reports (NCBI b, 2022) indicated that debrisoquine is involved in the development of different diseases and disorders. Also, DRUGBANK online stated that debrisoquine is indicated for the treatment of moderate and severe hypertension, either alone or as an adjunct, and for the treatment of renal hypertension involved in many drug-drug interactions (DRUGBANK, 2022).

CHAPTER 6. CONCLUSION

The findings have shown that among all the tested hydromethanolic extracts, *Thymus schimperi* exhibited the highest antioxidant activity in all the assays. *Thymus schimperi* had the highest in total phenol, flavonoid and proanthocyanidin contents compared to the other two extracts. Statistically, the total phenol, and flavonoids of the extracts were correlated with DPPH (IC 50%) and ABTS scavenging activities. Comparatively, due to the highest antioxidant activities and higher phytochemicals content, *T. schimperi* had a better *in vitro* antibacterial activity against uropathogenic MDR *E. coli* and *K. pneumoniae ESBL* isolates. These hydromethanolic extracts were subjected to GC-MS analysis. Accordingly, 14 compounds were identified in *T. schimperi*, 6 compounds from *R. prinoides* extract and 5 compounds from *J. shimperiana.* Literature survey showed that the three compounds with highest percent from *T. schimperi* were hexanedioic acid, bis(2-ethylhexyl) ester (RT: 19.835; 73.88%); thymol (RT: 5.799; 11.68%); and o-Cymen-5-ol (RT: 5.931; 7.95%) possess antibacterial activities.

And, in *R. prinoides* extract, hexanedioic acid, bis (2-ethylhexyl) ester (RT: 19.755; 79.36%); β-D-Glucopyranoside, methyl (RT: 9.719; 10.03%); and desulphosinigrin (RT: 11.132; 8.28%) had antioxidant and/or antibacterial activities.

CHAPTER 7. RECOMMENDATION

This study should be expanded using *in vivo* assays against the targeted MDR uropathogenic induced infections. Similar to the current study and based on the multiple study reports, the identified bioactive compounds which are found in the hydromethanolic crude plant extracts with antibacterial activity against MDR uropathogenic bacteria should be tested in separate compound or in combination with each other or combined with other known compounds with antibacterial activity using both *in vitro* and *in vivo* models for antibacterial evaluation.

FINAL DISCUSSION

This PhD thesis deeply studied the Self-medication Practice (SMP) among adult Ethiopian inhabitants in Addis Ababa community through visiting their residence to evaluate their overall SMP and interviewing them during their visit to a hospital/diagnostic centers/ as patients suspected of UTIs in Addis Ababa, in which SMP can endanger the inhabitants to several inherent risks. This kind of tendency could be obviously real within adult patients who are more likely to be vulnerable to inappropriate drug-pressure related clinical complications (Mok *et al*., 2021). In developing countries, like Ethiopia, in which universal health care accessibility is not yet achieved, the practice of self-medication is of concern (Dutta *et al*., 2017). To address this concern, particularly, a study was performed in Harar City (Eastern Ethiopia) in 2018 showing that about ten percent of participants had experienced adverse effects associated with SMP (Mamo *et al*., 2018).

In the selected households, this study showed that 75.5% of the participants had taken medication without a prescription. Comparatively, this study result was higher than most of the studies conducted on SMP such as in Dharwad (India) (46%) (Arpitha and Patil, 2022), Chittagong city (Bangladesh) (41%) (Hossain *et al*., 2018), Brazil (18.3%) (Pons *et al*., 2017), Srilanka (12.2%) (Wijesinghe *et al*., 2012), Portugal (26.2%) (Martins *et al*., 2002), Saudi Arabia (35.4%) (Alghanim, 2011), and Iran (35.4%) (Jalilian *et al*., 2013). However, this study result is lower than that of India (87.4%) (Garud *et al*., 2012), North Maharashtra (92.1%) (Durgawale *et al*., 2014), Vietnam (83.3%) (Van Ha *et al*., 2019). Particularly, at present, the overall prevalence of ASMP among the patients suspected of UTIs was found to be 10.4%. In comparison, in a systematic review authored by Do *et al*. (2021) and conducted in six low-income and middle-income countries, ASMP was found higher than this current study in Vietnam (55.2%), Bangladesh (45.7%), and Ghana (36.1%). But, the current study is similar to a finding in Mozambique (8%) but higher than ASMP from South Africa (1·2%), and Thailand (3·9%). Across different studies, the prevalence of SMP reported is with a wide range of differences. This variation could be due to differences in methodology adopted, the study population selected, or the operational definition of SMP. The extent of practices is influenced by social-economic characteristics, knowledge about specific diseases, and cultural variations could be associated with the difference among these results (Kumar *et al*., 2015).

The most self-medicated drug in the community residents was paracetamol. This study finding is supported by studies performed in Serbia (Lukovic *et al*., 2014), Brazil (Gadelha *et al*., 2016), Pakistan (Aziz *et al*., 2018) and coastal South India (Kumar *et al*., 2018) which reported analgesics and antipyretics as the most self-prescribed medications. Those residents

in the selected households of this study and having a poor knowledge towards drugs were significantly associated with SMP. This finding is in line with a previous report in Indonesia (Kurniawan *et al*., 2017) which revealed that poor knowledge had higher chances of practicing self-medication. In contrast, those with a good knowledge toward drugs have a chance of SMP (Khalid *et al*., 2021; Zawahir *et al*., 2021; Simegn and Moges, 2022).

In the present work, about 82.2% of patients diagnosed with UTIs had MDR bacterial infections. Within this MDR data, 8.5% of them had ASMP. Similarly, according to previous reports (Rather *et al*., 2017; Piddock *et al*., 2016), the most influential factors for antibiotic resistance crisis are inappropriate antibiotics use, over dosage and ASMP. This finding of the present study imposes an urgency of careful monitoring and regulation of the drug consumption, drug delivery, drug dispensing in developing countries to prevent SM (Saha *et al*., 2017).

In this study, MDR UTIs were common among the suspected patients including those with history of ASMP. In a harmonized report to this finding by Bhargava *et al*. (2022), it was reported that the prevalence of MDR pathogens in the community is higher. This finding implies that there are bacteria strains circulating in the community with a potential of causing severe and MDR infections. As a consequence, in the health care services, there is a decrease in antibiotic treatment options. This statement is also supported by Jernigan *et al*. (2020) and Bader *et al*. (2017) which stated that MDR bacteria are also causing healthcare-associated and community-acquired infections such as UTIs which cause a treatment failure. Therefore, for a proper management of UTI in a particular region, it is necessary an extensive investigation and implementation of antibiotic policy based on antimicrobial susceptibility tests.

The most common causes of MDR UTIs among the suspected patients of this study were *E. coli*, *K. pneumoniae ESBL* and *Providencia species*, respectively. This most common prevalent bacteria finding is supported by multiple studies performed previously (Ahmed *et al*., 2019; Patel *et al*., 2019; Mukherjee *et al*., 2020). There are different types of bacteria in the gastrointestinal tract. In drug exposure, the development of bacterial resistance is easier in the digestive microbiota due to a large number of bacteria (greater than 10^9 bacteria per gram of stool), promoting contact and the emergence of resistant mutants. Additionally, UTIs are most often of ascending origin by contamination from the perineal flora, reflecting the digestive flora. Therefore, this selective pressure due to ASMP of the same antibiotic has a definite clinical impact (Mouanga *et al*., 2021).

According to the patient self-reports, the most frequently self-medicated antibiotic in this study was amoxicillin. This finding is in agreement with several study reports (Widayati *et al*., 2011; Shah *et al*., 2014; Gelayee, 2017; Núñez *et al*., 2017; Horumpende *et al*., 2018; Ateshim *et al*., 2019; Torres *et al*., 2020; Chuwa *et al*., 2021; Muhammed *et al*., 2021; Mabilika *et al*., 2022; Simegn and Moges, 2022). Similarly, penicillins, which are a class of antibiotics including amoxicillin, were reported to be the antibiotics most commonly used in SM (Alhomoud *et al*., 2017). Usually, amoxicillin is used as a first-line antibiotic for different diseases such as UTIs, acute otitis media, and pneumonia (Alhomoud *et al*., 2017). This could be due to the safety profile of amoxicillin, offering a wide usage in a variety of population and age groups, and the accessibility of amoxicillin (Muhammed *et al*., 2021). However, other studies mentioned that ciprofloxacin (Aslam *et al*., 2022) and cephalosporin (Goossens *et al*., 2005) are the common antibiotics in SM.

Results of this study showed that both the patients and household residents, having low monthly income, were more likely to practice the antibiotic and/or the overall SM, respectively. This current study result is in agreement with studies conducted in Ethiopia (Jember *et al*., 2019) and China (Lie *et al*., 2018), in which both studies revealed that SM is associated with lower income. However, the present finding contradicts the finding reported by Abdelwahed (2022).

At present, 50-year-old and above patients were more likely to practice SM. In general, this study result is similar to multiple studies previously reported (Klemenc-Ketis and Mitrovic, 2017; Bogale *et al*., 2019; Amaha *et al*., 2019) which stated that there is a significant positive association between old age and SMP across countries. In the current study, patients with no history of UTIs were more likely to practice ASM. This report is opposite to the finding by (Klemenc-Ketiš and Kersnik, 2011) which stated that older individuals are prone to self-medication if they have chronic diseases. Among the patients with ASMP, the most common disease treated in this study was UTIs. This finding is different from reports by Alhomoud *et al*. (2017) and by Elmahi *et al*. (2022), respectively, which mentioned ASM as mainly used for upper respiratory tract problems. The primary reason for antibiotic self-medication amongst this patient group was previous experience and/or simple familiarity with the drug name. A similar study by Ateshim *et al*. (2019) also mentioned that the main reason for self-medication with antibiotics was previous successful experience.

In this study, the hydromethanolic extracts of the three plant extracts have shown antioxidant activities. And the highest DPPH free radical scavenging activity was seen in *T. schimperi* (IC50 = 4.49) followed by *R. prinoides* extract (IC50 = 9.79). Several study reports

(Li *et al*., 2009; Jadid *et al*., 2017; Boulfia *et al*., 2021) explained that the lower IC50 value of a sample indicates the higher antioxidant activity. Accordingly, the observed IC50 value showed that *T. schimperi* exhibited the highest antioxidant activity. Though *T. schimperi* extract showed the highest IC50 value, it was closer to that of the IC50 of ascorbic acid (IC50 = 3.63), the greater the inhibitory effectiveness (Chand *et al*., 2018). In the DPPH assay of this study, the percent inhibition of DPPH free radicals was observed in a concentration dependent manner by all extracts. This finding is in accordance with the reports by Motalleb *et al*. (2005) ans Jadid *et al*. (2017).

The quantitative phytochemical analyses performed in the three hydromethanolic extracts of this study showed that the Total Phenol Content (TPC), Total Flavonoids Content (TFC), and Total Proanthocyanidin Content (TPROC) were found in highest concentration in *T. schimperi* extract compared to the other extracts. In the Pearson coefficient analyses, TPC showed a significant negative correlation with DPPH ($r = -0.999$, $P = 0.023$), which showed that TPCs of the extracts had both hydrogen and electron-donating abilities of antioxidant capacity. In earlier studies, reports indicated that there is a direct correlation between antioxidant activity and TPC found in extracts, as phenolic compounds have an antioxidant activity (Deetae *et al*., 2012; Rahman *et al*., 2015; Jin *et al*., 2016; Aryal *et al*., 2019).

Also, the Pearson coefficient analyses of this study showed that there was a highest positive correlation between total flavonoid content (TFC) and ABTS radical scavenging activity. This study result is in line with previously reported studies (Fidrianny *et al*., 2013; Kainama *et al*., 2020). These results suggest that this study extracts can be used as a natural source of antioxidants. Multiple earlier reports (Floyd *et al*., 1983; Michiels, 2004; Montoro *et al*., 2005; Abdel-Hameed *et al*., 2014; Calado *et al*., 2015; Zhang *et al*., 2016; Du Toit *et al*., 2018; Milevskaya *et al*., 2018) stated that flavonoids, which are plant secondary metabolites, demonstrated to scavenge oxygen species (ROS) consisting of free (OH) radicals.

Zhang *et al*. (2022) reported that phenolic compounds are the most abundant secondary metabolites in plants, showing a wide range of distinct biological activities, and have received an increasing attention in recent years. Currently, evidence suggests phenolic compounds of plants were reported to have antioxidant activity (Martins *et al*., 2016), antimicrobial (Karunakaran *et al*., 2018) and prevention of diseases associated with oxidative stress (Yasir *et al*., 2016). The antimicrobial activity of flavonoids is due to their ability to bind with bacterial cell wall and to interact with extracellular and soluble protein while that of tannins may be related to their ability to inhibit the microbial adhesions, cell envelope proteins and enzymes (Sharma *et al*., 2010; Mogana *et al*., 2020). With all these phytochemical

constituents, the Ethiopian medicinal plants tested were shown the antibacterial activities against the clinical isolate MDR *E. coli* and *K. pneumoniae ESBL* and further the standard strains.

In this evaluation study of the hydromethanolic crude extracts, *T. schimperi* had the highest antibacterial activity against the clinical isolates MDR *E. coli and K. pneumoniae ESBL* compared to the other extracts. Previously, Nasir *et al*. (2015) and Cutillas *et al*. (2018) were reported, similar to the current finding, *T. schimperi* oil showed a moderate antibacterial activity on these specific organisms. The finding of this study is similar to several earlier study reports by Yitbarek *et al*. (2010), Sasidharan *et al*. (2011) and Chuah *et al*. (2014) which stated that the Ethiopian medicinal plants presented by local healers in the community were reported to have antibacterial activity against MDR bacterial infections.

The MIC of *T. schimperi* extract was the lowest MIC compared to independent extracts but similar to the MIC (equivalent) of its combination with *R. prinoides,* in the test against the clinical isolates MDR *E. coli* and *K. pneumoniae* EBSL. This result further confirmed that *T. schimperi* had the highest antibacterial activity for the entire patient isolated MDR bacteria and the standard reference strains compared to the tested extracts. This current study finding is in agreement with Ibrahim and Kebede (2020) which stated that the lower MIC indicates that the plant extracts were stronger of activities in killing or inhibiting the test pathogens and vice versa. Additionally, this study indicated that the ratio of MBC to MIC of the *T. schimperi*, except in the test against *K. pneumoniae (ATCC 700603)*, showed a bactericidal activity.

Additionally, the checkerboard analyses of the present study revealed that the interaction between the *T. schimperi* and ciprofloxacin combination was additive. This finding is interpreted as the overall antibacterial activity of the combination of the extract and the positive control equals to the sum of the individual antibacterial activity. Thirdly, following the extract-extract combinations' activity, the *R. prinoides* extract showed an antibacterial activity against the patient-isolated, MDR *E. coli* and *K. pneumoniae* ESBL and their reference strains in a concentration-dependent manner, with a slight increased effect in both standard reference strains.

In the GC-MS analyses of this study, a total of 14 constituents were identified in the hydromethanolic leaf extract of *T. schimperi*. Hexanedioic acid, bis (2-ethylhexyl) ester (RT: 19.835; 73.88%) was the main component among the total compounds eluted. Several study reports showed that hexanedioic acid, bis (2-ethylhexyl) ester has numerous biological activities such as antibacterial activity (Kamaruding *et al*., 2020; Daniel *et al*., 2021) and

antioxidant and antibacterial activities (Tayade *et al*., 2013; Ferdosi *et al*., 2021). The antioxidant and antibacterial activity of the *T. schimperi* extract in this study is the highest among the tested extracts. Therefore, together with other compounds eluted, hexanedioic acid, bis (2-ethylhexyl) ester offers this important antioxidant and antibacterial activity to have from *T. schimperi* extract.

Thymol, which is the second major component of *T. schimperi extract* during the GC-MS analyses, was reported as possessing different biological activities. Accordingly, *thymol* was stated to have antifungal activity (Shcherbakova *et al*., 2021), antibacterial activity including against Gram-negative bacteria strains (Trombetta *et al*., 2005; Zarrini *et al*., 2010; Khoury *et al*., 2016; Marchese *et al*., 2016; Rúa *et al*., 2019; Kachur and Suntres, 2020), antimicrobial, antibacterial, antifungal and antiparasitic properties (Marchese *et al*., 2016; Nagoor Meeran *et al*., 2017) and antioxidant activities (Nagoor Meeran *et al*., 2015; 2016). Therefore, the presence of thymol in higher concentration in the hydromethanolic extract of *T. schimperi* in this study could be the possible explanation for its higher antibacterial and antioxidant activities compared to other extracts.

Obtained results showed that the third compound in the GC-MS analyses of *T. schimperi* extract was o-cymen-5-ol. According to Pizzey *et al*. (2011), o-cymen-5-ol alone or together with zinc has shown direct antimicrobial effects, inhibiting oral disease-related processes, and showing synergistic effects against anaerobes. Further, o-cymen-5-ol was reported by ANECO (2022) that it has antibacterial activity, absorb specific wavelengths of ultraviolet light, inhibits oxidation, and in cosmetics preparations has antiseptic, anti-acne, and extends the shelf life of products. A report from Lesielle Adaptive Skincare (2022) reported that o-cymen-5-ol has an antibacterial activity. The *T. schimperi's* antibacterial and antioxidant activities possibly could be due to the presence of o-cymen-5-ol alone or together with other phytochemical constituents.

Similar to the finding of the GC-MS analyses of *T. schimperi* extract, the major constituent of the hydromethanolic crude extract of *R. prinoides* was hexanedioic acid, bis (2-ethylhexyl) ester. As previously mentioned, this compound has reported to have antioxidant and antibacterial activities (Tayade *et al*., 2013; Ferdosi *et al*., 2021).

The second abundant bioactive compound known as β-D-Glucopyranoside (methyl) among the total eluted compounds in the GC-MS analyses of *R. prinoides*, was reported to have antibiotic, antibacterial, and antimycotics activities (Shaheed *et al*., 2018; National Center for Biotechnology Information, 2022). A smaller number of reports (Salem, 2005;

Panghal *et al.*, 2011) also described that the β-D-Glucopyranoside (methyl), which is a chemical component found in phenols and flavonoids, could potentially have an antimicrobial, antibacterial effect, as well as it is used in the medication of different diseases. Therefore, the antibacterial effects of the *R. prinoides* in this study might be mediated from the biological activities of these identified compounds in adequate amounts in this GC-MS analyses.

In a PubChem Compound Summary (NCBI b, 2022), debrisoquine which is also the second highest compound identified from GC-MS analyses of hydromethanolic crude extract of *J. shimperiana* has a role as an antihypertensive agent, an adrenergic agent, a sympatholytic agent and a human metabolite. Multiple reports (NCBI b, 2022) indicated that debrisoquine is involved in the development of different diseases and disorders. Also, DRUGBANK online stated that debrisoquine is indicated for the treatment of moderate and severe hypertension, either alone or as an adjunct, and for the treatment of renal hypertension involved in many drug-drug interactions (DRUGBANK, 2022).

The community study has some limitations. These include; the study design was a cross-sectional study, by its nature, its conclusions' are a point-time findings; as the data was collected using interviewer administered questionnaires, the truthfulness of the self-reported answers of the participants were difficult to prove. Furthermore, this study was conducted in the capital city of the country (Addis Ababa, Ethiopia); therefore, these findings cannot be generalized to the whole country.

FINAL CONCLUSIONS

1. The overall SMP of the study participants from the selected households was a rampant practice. Commonly, this practice included the use of the OTC, traditional remedy, and the antibacterial drugs, respectively. Community members with lower monthly income and poor knowledge were more likely to have self-medication practice (SMP) compared to their counterparts.

2. Community members within the catchment area of the diagnostic centers/study settings, among the adult patients suspected of UTIs, showed that about 10% of patients had antibiotic self-medication practice (ASMP). Worryingly, in this study, the inappropriate use of ASMP is a potential threat for MDR development by bacteria.

3. A significant number of patients with a history of ASMP had MDR bacteria induced UTIs. The two bacterial isolates identified as the most common causes of UTIs in the community were *Escherichia coli* and *Klebsiella pneumoniae ESBL*. These bacteria were resistant to most of the tested antibiotics.

4. Currently, there are MDR bacteria strains spreading in the community, which are difficult to treat by the currently available antibiotics, causing MDR UTIs.

5. Patients who had a monthly income less than or equal to 4000 birr, were 50 year-old and above and had no previous history of UTIs were statistically associated with ASMP.

6. As for the three vegetal species, the *Thymus schimperi* extract showed both the highest antioxidant activity and the highest antibacterial activity against patient isolated MDR *E. coli* and *K. pneumoniae ESBL* and reference strains.

7. The interaction of the combination of *T. schimperi* extract with ciprofloxacin was classified as additive in the case of *E. coli* MDR.

8. The DPPH and ABTS scavenging activities were correlated with total phenol and flavonoid contents, respectively. Comparatively, *Thymus schimperi* extract had the highest total phenol, flavonoid and proanthocyanidin contents followed by *Rhamnus prinoides* extract.

9. In the GC-MS analyses for identifying bioactive compounds, *Thymus schimperi* extract had 14 compounds, *Rhamnus prinoides* had 6 compounds and *Justicia shimperiana* had 5

compounds. The compounds identified have been reported to posess applications in different disease treatments.

10. The presence of the different bioactive compounds and the highest antioxidant activity reported with *Thymus schimperi* may explain, at least in part, the best antibacterial activity against these patient isolated MDR *E. coli* and *K. pneumoniae ESBL* isolates.

REFERENCES

Abay SM, Amelo W. Assessment of self-medication practices among medical, pharmacy, and health science students in Gondar University, Ethiopia. *J. Young Pharm*. 2010; 2:306-10.

Abdela J, Engidawork E, Shibeshi W. *In vivo* antimalarial activity of solvent fractions of the leaves of *Justicia schimperiana* hochst. Ex Nees Against *Plasmodium berghei* in Mice. *Ethiop. Pharm. J.* 2014; 30:95-108.

Abdel-Hameed ESS, Nagaty MA, Salman MS, Bazaid SA. Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (Opuntia ficus indica Mill.) growing in Taif, KSA. *Food Chem*. 2014; 160: 31-38.

Abdelwahed RNK, Jassem M, Alyousbashi A. Self-Medication Practices, Prevalence, and Associated Factors among Syrian Adult Patients: A Cross-Sectional Study. *J. Environ. Public Health.* 2022; 9274610:7.

Abduelkarem AR, Othman AM, Abuelkhair ZM, Ghazal MM, Alzouobi SB, *et al*. Prevalence of Self-Medication with Antibiotics Among Residents in United Arab Emirates. *Infect. Drug Resist.* 2019:12 3445-3453.

Abdullah FE, Mushtaq A, Irshad M, Rauf H, Afzal N, Rasheed A. Current efficacy of antibiotics against Klebsiella isolates from urine samples - A multi-centric experience in Karachi. *Pak. J. Pharm. Sci*. 2013; 26 (1):11-15.

Abdulraheem IS, Adegboye A, Fatiregun AA. Self-medication with Antibiotics: Empirical Evidence from a Nigerian Rural Population. *J. Pharm. Res. Int*. 2016; 11(5): 1-13.

Abebe H, Kibret B, Haile A. Phytochemical investigation on leaf extract of *Adhatoda schimperiana*, Ethiopia. *J. Med. Plants Stud.* 2014; 2(2): 23-31.

Abebe W, Zhang W, Zhang S, Xie G. Chemical Composition and Antimicrobial Activity of Essential Oil from *Justicia schimperiana. J. Pharmacogn. Nat. Prod*. 2018; 4: 154.

Abera B. Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia. *J. Ethnobiol. Ethnomedicine.* 2014; 10 (1):40.

Abew B, Sahile S Moges F. *In vitro* antibacterial activity of leaf extracts of *Zehneria scabra* and *Ricinus communis* against *Escherichia coli* and methicillin resistance *Staphylococcus aureus*. *Asian Pac. J. Trop. Biomed.* 2014; 4: 816-820.

Abosede OA. Self-medication: An important aspect of primary health care. *Soc. Sci. Med.* 1984; 19(7):699-703.

Abraha S, Molla F, Melkam W. Self-medication practice: the case of Kolladiba Town, North west Ethiopia. *IJPSR***.** 2014; 5:670-677.

Acar A, Aydın M, Arslan D. Development of infusion tea formulations with food wastes: Evaluation of temperature and time effects on quality parameters. *Applied Food Research*. 2022; 2(1):100087.

Adams D, Dagenais S, Clifford T, *et al*. Complementary and alternative medicine use by pediatric specialty outpatients. *Pediatrics* 2013; 131(2): 225-232.

Addis Ababa City Administration. Addis Ababa health bureau fact sheet. 2016.

Adetutu A, Morgan WA, Corcoran O. Ethnopharmacological survey and *in vitro* evaluation of wound-healing plants used in South-western Nigeria. *J. Ethnopharmacol.* 2011; 137: 50-56.

Afadly S, Ballaswad M, Amra A, *et al*. Self-medication with antibiotic amongst adults attending community pharmacies in Mukalla district, Yemen. *Lat. Am. J. Pharm.* 2017; 36(2): 224-228.

Afari-Asiedu S, Oppong FB, Tostmann A, Ali Abdulai M, Boamah-Kaali E, Gyaase S, *et al*. Determinants of Inappropriate Antibiotics Use in Rural Central Ghana Using a Mixed Methods Approach. *Front. Public Health.* 2020; 8:90.

Afolabi AO. Factors influencing the pattern of self-medication in an adult Nigerian population. *Ann. Afr. Med.* 2008; 7(3); 120-127.

Africa Business Directory. Arsho Medical Laboratories PLC. Available at: https://directory.africa-business.com/business/80ca4e2db5/ARSHO-MEDICAL-LABORATORIES-PLC. Accessed on August 05, 2021.

Aftab L, Islam A, Raza M. Urinary Tract Infection Causative Bacterias and Antibiotic Sensitivity Pattern in Emergency Department of Fatima Memorial Hospital, Lahore. *PJMHS*. 2018; 12(1):579.

Ahameethunisa AR, Hopper W. Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. *BMC Compl. Alter. Med*. 2010; 10:6.

Ahmed I, Rabbi MB, Sultana S. Antibiotic resistance in Bangladesh: A systematic review. *Int. J. Infect. Dis.* 2019; 80:54-61.

Ahmed SS, Shariq A, Alsalloom AA, Babikir IH, Alhomoud BN. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *Int. J. Health Sci.* 2019; 13: 48-55.

Aiyegoro OA, Afolayan AJ, Okoh AI. Interactions of antibiotics and extracts of *Helichrysum pedunculatum* against bacteria implicated in wound infections. *Folia Microbiologica* 2010; 55 (2):176-180.

Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in Mangifera indica leaf from Ibadan, Oyo State. *Plant Sci. Res*. 2009; 2:11-13.

Ajibola O, Omisakin OA, Eze AA, Omoleke SA. Self-Medication with Antibiotics, Attitude and Knowledge of Antibiotic Resistance among Community Residents and Undergraduate Students in Northwest Nigeria. *Diseases* 2018; 6(2):32.

Akingbade O, Balogun S, Ojo D, Akinduti P, Okerentugba PO, Nwanze JC, Okonko IO. Resistant plasmid profile analysis of multidrug resistant Escherichia coli isolated from urinary tract infections in Abeokuta, Nigeria. *Afr. Health Sci*. 2014; 14 (4):821-828.

Akter R, Hossain E, Afroz S, Khan TM, Rahman M, Paul S. Detection of Bacterial Pathogen in Urinary Tract Infection with Their Antimicrobial Resistance Pattern in A Tertiary Care Hospital. *Sir. Salimullah Med. Coll. J.* 2020; 28: 33-36.

Al Rasheed A, Yagoub U, Alkhashan H, Abdelhay O, Alawwad A, Al Aboud A, *et al*. Prevalence and predictors of self-medication with antibiotics in Al Wazarat Health Center, Riyadh City, KSA. *Biomed. Res. Int*. 2016; 2016: 1-8.

Albawani SM, Hassan YB, Abd-Aziz N, Gnanasan S. Self-medication with antibiotics in Sana'a City, Yemen. *Trop. J. Pharm. Res.* 2017; 16 (5): 1195-1199.

Alemayehu G, Asfaw Z, Kelbessa E. Ethnobotanical study of medicinal plants used by local communities of Minjar-Shenkora District, North Shewa Zone of Amhara Region, Ethiopia. *J. Med. Plants Stud*. 2015; 3(6):1-11.

Alghadeer S, Aljuaydi K, Babelghaith S, Alhammad A, Alarifi MN. Self-medication with antibiotics in Saudi Arabia. *Saudi Pharm. J*. 2018; 26: 719-724.

Alghanim SA. Self-medication practice among patients in a public health care system. *East Mediterr. Heal. J*. 2011; 17: 409-416.

Alhomoud F, Aljamea Z, Almahasnah R, Alkhalifah K, Basalelah L, Alhomoud FK. Self-medication and self-prescription with antibiotics in the Middle East-do they really happen? A systematic review of the prevalence, possible reasons, and outcomes. *Int. J. Infect. Dis*. 2017; 57: 3-12.

Ali AN, Kai JTK, Keat CC, Dhanaraj SA. Self-medication practices among health care professionals in a private University, Malaysia. *Int Curr Pharm J.* 2012; 1:302-10.

Amaha MH, Alemu BM, Atomsa GE. Self-medication practice and associated factors among adult community members of Jigjiga town, Eastern Ethiopia. *PLoS One* 2019; 14(6): e0218772.

Anaba EL, Cole-Adeife MO, Oaku RI. Prevalence, pattern, source of drug information, and reasons for self-medication among dermatology patients. *Dermatol Ther.* 2021; 34(2): e14756.

Andleeb S, Alsalme A, Al-zaqri N, Warad I, Alkahtani J. *In-vitro* antibacterial and antifungal properties of the organic solvent extract of *Argemone mexicana* L. *J. King Saud. Univ. Sci*. 2020; 32(3): 2053-2058.

Andualem G, Umar S, Getnet F, Tekewe A, Alemayehu H, Kebede N. Antimicrobial and phytochemical screening of methanol extracts of three medicinal plants in Ethiopia. *Advan. Biol. Res.* 2014; 8: 101–106.

Andualem T, Gebre-Mariam T. Self-medication practices in Addis Ababa: a prospective study. *Ethiop. J. Health Sci*. 2004; 14(1):1-11.

ANECO. O-cymen-5-ol. *Available at: https://www.anecochem.com/sale-15370896-o-cymen-5-ol.html.* 2022. Accessed on September 14, 2022.

Anonymous. Plants of the World online. 2021.

Anyanechi C, Saheeb B. Toothache and self-medication practices: a study of patients attending a Niger Delta tertiary hospital in Nigeria. *Ann. Med. Health Sci. Res*. 2014; 4(6): 884-888.

Apak R, G¨uçl¨u K, Ozy¨urek M, Karademir SE, Erça˘g E. The Cupric Ion Reducing Antioxidant Capacity (CUPRAC) and polyphenolic content of some herbal teas. *Int. J. Food Sci. Nutr.* 2006; 57(5-6): 292–304.

Aragoneses JM, Aragoneses J, Rodríguez C, Algar J, Suárez A. Trends in Antibiotic Self-Medication for Dental Pathologies among Patients in the Dominican Republic: A Cross-Sectional Study. *J. Clin. Med.* 2021; 10(14):3092.

Arnao M, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* 2001; 73:239-44.

Arpitha VS, Patil PS. Prevalence and Patterns of Self-Medication Practices in Urban Field Practice Area, Dharwad. *Natl. J. Community Med*. 2022; 13(7):419-423.

Aryal B, Niraula P, Khadayat K, *et al*. Antidiabetic, Antimicrobial, and Molecular Profiling of Selected Medicinal Plants. *Evid. Based Complement. Alternat. Med*. 2021; 2021:5510099.

Aslam A, Gajdács M, Zin CS, Abd Rahman NSB, Ahmed SI, Jamshed SQ. Public Awareness and Practices towards Self-Medication with Antibiotics among the Malaysian Population. A Development of Questionnaire and Pilot-Testing. *Antibiotics* 2020; 9:97.

Aslam A, Zin CS, Jamshed S, Rahman NSA, Ahmed SI, Pallós P, Gajdács M. Self-Medication with Antibiotics: Prevalence, Practices and Related Factors among the Pakistani Public. *Antibiotics* 2022; 11: 795.

Ateshim Y, Bereket B, Major F, Emun Y, Woldai B, Pasha I, *et al*. Prevalence of self-medication with antibiotics and associated factors in the community of Asmara, Eritrea: a descriptive cross sectional survey. *BMC Public Health*. 2019; 19:726.

Awad A, Eltayeb I, Matowe L, Thalib L. Self-medication with antibiotics and antimalarials in the community of Khartoum State, Sudan. *J. Pharm. Pharm. Sci*. 2005; 8: 326-331.

Awad AI, Aboud EA, Singer AC. Knowledge, attitude and practice towards antibiotic use among the public in Kuwait. *PLoS One* 2015; 10(2): e0117910.

Awad IA, Eltayeb BI, Capps AP. Self-medication practices in Khartoum State, Sudan. *Eur. J. Clin. Pharmacol*. 2006; 62: 317-324.

Awraris D, Bhagwan SC. Investigation of the levels of selected metals in the leaves of Thyme (*T. schimperi* and *T. vulgaris*) Grown in Ethiopia. *Springer* 2011; 141:317-328.

Ayalew MB. Self-medication practice in Ethiopia: a systematic review. *Patient Prefer. Adherence*. 2017; 11: 401-413.

Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, *et al*. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop. J. Pharm. Res*. 2008; 7:1019-1024.

Aziz MM, Masood I, Yousaf M, Saleem H, Ye D, Fang Y. Pattern of medication selling and self-medication practices: A study from Punjab, Pakistan. *PLoS One* 2018; 13(3): e0194240.

Bader MS, Loeb M, Brooks AA. An update on the management of urinary tract infections in the era of antimicrobial resistance. *Postgrad. Med*. 2017; 129(2):242-258.

Bahta M, Tesfamariam S, Weldemariam DG, Yemane H, Tesfamariam EH, Alem T, *et al*. Dispensing of antibiotics without prescription and associated factors in drug retail outlets of Eritrea: A simulated client method. *PLoS One* 2020; 15(1): e0228013.

Bakal SN, Bereswill S, Heimesaat MM. Finding novel antibiotic substances from medicinal plants - Antimicrobial properties of Nigella sativa directed against multidrug-resistant bacteria. *Eur. J. Microbiol. Immunol*. 2017; 7(1):92-98.

Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem.* 2006; 99(1): 191-203.

Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. *Clin. Infect. Dis.* 2013; 56(10):1445-1450.

Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. 1966; 45(4):493-496.

Begashaw B, Tessema F, Gesesew HA. Health care seeking behavior in Southwest Ethiopia. *PLoS One* 2016; 11(9): e0161014.

Behzadi P, Elham B, Hodjjat Y, Aghapour R. A survey on urinary tract infections associated with the three most common uropathogenic bacteria. Maedica – *A. J. Cinical Med.* 2010; 5(67):111-115.

Bekalo TH, Woodmatas SD, Woldemariam ZA. An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia. *J. Ethnobiol. Ethnomed*. 2009; 5:26.

Bekele BB, Berkesa ST, Tefera E, Kumalo A. Self-Medication Practice in Limmu Genet, Jimma Zone, Southwest Ethiopia: Does Community Based Health Insurance Scheme Have an Influence? *Journal of Pharmaceutics.* 2018; 1749137: 8.

Bekele G, Reddy PR. Ethnobotanical study of medicinal plants used to treat human ailments by Guji Oromo tribes in Abaya District, Borana, Oromia, Ethiopia. *Univers. j. plant sci.* 2015; 3(1):1-8.
Benjamin TT, Adebare JA, Remi Ramota R, Rachael K. Efficiency of some disinfectants on bacterial wound pathogens. *Life Sci. J.* 2012; 9:2012.
Bennadi D. Self-medication: A current challenge. *J Basic Clin Pharm*. 2013; 5(1):19-23.
Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 1996; 239:70-76.
Berzanskyte A, Valinteliene R, Haaijer-Ruskamp FM, Gurevicius R, Grigoryan L. Self-medication with antibiotics in Lithuania. *Int. J. Occup. Med. Environ. Health*. 2006; 19(4): 246-253.
Beyene A, Getachew E, Doboch A, Poulos E, Abdurahman K. Knowledge, attitude and practice of self-medication among pharmacy students of rift Valley University, Abichu campus, Addis Ababa, Ethiopia. *J. Health Med. Informat*. 2017; 8(269):2.
Bhargava K, Nath G, Bhargava A, Kumari R, Aseri GK and Jain N. Bacterial profile and antibiotic susceptibility pattern of uropathogens causing urinary tract infection in the eastern part of Northern India. *Front. Microbiol*. 2022; 13:965053.
Bhatt C, Shrestha B, Khadka S, Swar S, Shah B, Pun K. Etiology of urinary tract infection and drug resistance cases of uropathoges. *J. Kathmandu Med. Coll*. 2012; 1(2): 114-120.
Bischoff S, Walter T, Gerigk M, Ebert M, Vogelmann R. Empiric antibiotic therapy in urinary tract infection in patients with risk factors for antibiotic resistance in a German emergency department. *BMC Infect. Dis.* 2018; 18(1):56.
Bogale AA, Amhare AF, Chang J, *et al*. Knowledge, attitude, and practice of self-medication with antibiotics among community residents in Addis Ababa, Ethiopia. *Expert Rev. Anti. Infect. Ther*. 2019;1-8.
Bologa CG, Ursu O, Oprea T, Melançon CE, Tegos G. Emerging trends in the discovery of natural product antibacterials. *Curr. Opin. Pharmacol*. 2013; 13: 678-687.
Boulfia M, Lamchouri F, Senhaji S, Lachkar N, Bouabid K, Toufik H. Mineral Content, Chemical Analysis, *In Vitro* Antidiabetic and Antioxidant Activities, and Antibacterial Power of Aqueous and Organic Extracts of *Moroccan Leopoldia comosa* (L.) Parl. Bulbs. *Evid. Based Complementary Altern. Med.* 2021:9932291:17.
Broniatowski M, Mastalerz P. Biochimica et Biophysica Acta Studies of the interactions of ursane-type bioactive terpenes with the model of *Escherichia coli* inner membrane - Langmuir monolayer approach. *Biochim. Biophys. Acta - Biomembr*. 2015; 1848(2): 469-476.
Burčul F, Generalić Mekinić I, Radan M, Rollin P, Blažević I. Isothiocyanates: cholinesterase inhibiting, antioxidant, and anti-inflammatory activity. *J. Enzyme Inhib. Med. Chem*. 2018; 33(1):577-582.
Butler CC, Hawking MKD, Quigley A, McNulty CAM. Incidence, severity, help seeking, and management of uncomplicated urinary tract infection: a population-based survey. *Br. J. Gen. Pract.* 2015; 65: e702-707.
Calado JCP, Albertao PA, de Oliveira EA, Sisto MH, Frankland ACH, Marcucci MC. Flavonoid contents and antioxidant activity in fruit, vegetables and other types of food. *Agric. Sci.* 2015; 6: 426-435.
Campbell M, Zhao W, Fathi R, Mihreteab M, Gilbert ES. *Rhamnus prinoides* (gesho): A source of diverse anti-biofilm activity. *J. Ethnopharmacol.* 2019; 241:111955.
Carlet J, Pittet D. Access to antibiotics: a safety and equity challenge for the next decade. *Antimicrob. Resist. Infect. Control*. 2013; 2:1.
Cars O, Mosltad S, Melander A. Variation in antibiotic use in the European Union. *Lancet* 2001; 357:1851-1853.
Cars O, Nordberg P. Antibiotic resistance–The faceless threat. *Int. J. Risk Saf. Med.* 2005; 17:103–10.
Carvalho M, Ferreira PJ, Mendes VS *et al.* Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. *Food Chem. Toxicol.* 2010; 48: 441-447.
Ceylan Y, Usta K, Usta A, Maltasc AE, Yildizc S. Evaluation of antioxidant activity, phytochemicals and ESR analysis of Lavandula Stoechas. *Acta Phys. Pol. A*. 2015; 128:483-487.
Chand RN, Gopalan RD, Christi K, Chand RN, Gopalan RD, Christi K. Evaluation of antioxidant properties in thirteen Fijian medicinal plants used in Alzheimer's. *Dis. Relat. Illn*. 2018; 8: 11-17.
Chang J, Ye D, Lv B, Jiang M, Zhu S, Yan K, *et al*. Sale of antibiotics without a prescription at community pharmacies in urban China: a multi-center cross-sectional survey. *J. Antimicrob. Chemother*. 2017; 72:1235-1242.

Chang J, Wang Q, Fang Y. Socioeconomic differences in self-medication among middle-aged and older people: data from the China health and retirement longitudinal study. *BMJ Open.* 2017; 7: e017306.

Chanvatik S, Kosiyaporn H, Lekagul A, Kaewkhankhaeng W, Vongmongkol V, hunyahan A, *et al*. Knowledge and use of antibiotics in Thailand: A 2017 national household survey. *PLoS One* 2019; 14(8): e0220990.

Chaudhary H, Shah K. Self-medication practices in dental patients in rural Gujarat, India. *Int. J. Oral Care Res*. 2015; 3:15-20.

Cheesbrough M. *District Laboratory Practice in Tropical Countries*. 2nd ed. United States of America, New York: Cambridge University Press; 2006.

Chekole G, Asfaw Z, Kelbessa E. Ethnobotanical study of medicinal plants in the environs of Tara-gedam and Amba remnant forests of Libo Kemkem District, Northwest Ethiopia. *J. Ethnobiol. Ethnomedicine*. 2015; 11:4.

Chen GL, Munyao Mutie F, Xu YB, Saleri FD, Hu GW, Guo MQ. Antioxidant, anti-inflammatory activities and polyphenol profile of *Rhamnus prinoides*. *Pharmaceuticals* 2020; 13(4): 55.

Chinnici F, Bendini A, Gaiani A, Riponi C. Radical scavenging activities of peels and pulps from cv. Golden delicious apples as related to their phenolic composition. *J Agric Food Chem.* 2004; 52 (15):4684-4689.

Choe HS, Lee SJ, Chang IH, Kim TH, Chung H, Chung JM, *et al.* The Antibiotic Susceptibility of Escherichia coli from Community-Acquired Uncomplicated Urinary Tract Infection: A Focused on Fosfomycin. *Urogenital Tract Infection.* 2017; 12(2): 77.

Chon SY, Doan HQ, Mays RM, Singh SM, Gordon RA, Tyring SK. Antibiotic overuse and resistance in dermatology. *Dermatol. Ther.* 2012; 25(1):55-69.

Chu CM, Lowder JL. Diagnosis and treatment of urinary tract infections across age groups. *Am. J. Obstet. Gynecol*. 2018; 219(1):40-51.

Chuah EL, Zakaria ZA, Suhaili Z, Abu Bakar S, Desa M. Antimicrobial activities of plant extracts against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *JMR* 2014; 4(1):6-13.

Chuwa BB, Njau LA, Msigwa KI, Shao E. Prevalence and factors associated with self- medication with antibiotics among University students in Moshi Kilimanjaro Tanzania. *Afr. Health Sci*. 2021; 21(2):633-639.

Cizman M. The use and resistance to antibiotics in the community. *Int. J. Microb. Agents.* 2003; 21:297-307.

Clinical and Laboratory Standards Institute (CLSI) (2012) CLSI Document M07-A9. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard. 19th Edition, CLSI, Wayne.

Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard, Tenth edition. CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015. Available at: https://clsi.org/media/1632/m07a10 sample.pdf. Accessed on October 10, 2021.

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty third Informational Supplement, CLSI, Wayne, PA, USA, 2013.

Cochran WG. Sampling Techniques. 2nd edition. New York: John Wiley and Sons Inc. 1963; 75.

Connolly A, Thorp JM Jr. Urinary tract infections in pregnancy. *Urol. Clin. North Am.* 1999; 26:779-787.

Correa Da Silva MG, Soares MC, Muccillo-Baisch AL. Self-medication in university students from the city of Rio Grande, Brazil. *BMC Public Health*. 2012; 12:339.

Costelloe C, Metcalfe C, Lovering A, *et al*. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ* 2010:340.

Cutillas A, Carrasco A, Martinez-gutierrez R, Tomas V, Tudela J. *Thymus mastichina* L. essential oils from Murcia (Spain): Composition and antioxidant, antienzymatic and antimicrobial bioactivities. *PLoS One* 2018;1-19.

d'Avigdor E, Wohlmuth H, Asfaw Z, Awas T. The current status of knowledge of herbal medicine and medicinal plants in Fiche, Ethiopia. *J. Ethnobiol. Ethnomedicine.* 2014; 10(1): 1-33.

Dagne E, Hailu SL, Bisrat D, Tadele W. Constituents of the essential oil of *Thymus schimperi*. *Bull. Chem. Soc. Ethiop*. 1998; 12:79-82.

Damtie D, Mekonnen Y. Antibacterial activity of essential oils from Ethiopian thyme (*Thymus serrulatus* and *Thymus schimperi*) against tooth decay bacteria. *PLoS One* 2020; 15(10): e0239775.

Daniel Nartey D, Gyesi JN, Borquaye LS. Chemical Composition and Biological Activities of the Essential Oils of Chrysophyllum albidum G. Don (African Star Apple). *Biochem. Res. Int*. 2021; 2021(9911713):11.

Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev*. 2010; 74(3):417-33.

De Silva GO, Abeysundara AT, Aponso MMW. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *Am. J. Essent. Oil*. 2017; 5(2):29-32.

Debelo N, Afework M, Debella A, Makonnen E, Ergete W, Geleta B. Histopathological and Biochemical Assessment of Chronic Oral Administration of Aqueous Leaf Extract of *Thymus Serrulatus* in Mice. *Clin. Exp. Pathol*. 2015;5.

Deetae P, Parichanon P, Trakunleewatthana P, Chanseetis C, Lertsiri S. Antioxidant and anti-glycation properties of Thai herbal teas in comparison with conventional teas. *Food Chem*. 2012; 133 (3): 953-959.

Demeke H, Hasen G, Sosengo T, Siraj J, Tatiparthi R, Suleman S. Evaluation of Policy Governing Herbal Medicines Regulation and Its Implementation in Ethiopia. *J. Multidiscip. Healthc*. 2022;15: 1383-1394.

Derbie A, Chandravanshi BS. Concentration levels of selected metals in leaves of different species of *thyme* (*T. serrulatus* and *T. vulgaris*) grown in Ethiopia. *Biol. Trace Elem. Res*. 2011; 141(1-3):317-328.

Dimitrijević M, Aleksandra Cd, Nikola S, Dragana P, Radmila Vr. Self-Medication in Primary Healthcare in the Territory of the City of Nisˇ. *Acta Medica Medianae*. 2014; 53 (3): 19-24.

Dires K, Mengist B, Lulekal E. Evaluation of acute, sub-acute and skin irritation toxicity on Essential Oil. *J. Clinical Tox.* 2018; 8:8-11.

Divya M, Bharatesh S, Vasudeva G, Varalakshmi C. Self-Medication Among Adults in Urban UdupiTaluk, Southern India. *J. Med. Public Health*. 2016; 6(3):126-129.

Dlamini MD, Turner S. *Rhamnus Prinoides L' Herit, Witwatersrand National Botanical Garden*, South African National Biodiversity Institute, Pretoria, South Africa, 2002.

Donkor GY, Dontoh E, Owusu-Ofori A. A cross-sectional study on the prevalence of antibiotic use prior to laboratory tests at two Ghanaian hospitals. *PLoS One* 2019; 14(1): e0210716.

Donmez S, Gungor K, Gov P. Knowledge, attitude and practice of self-medication with antibiotics among nursing students. *Int. J. Pharmacol.* 2018; 14(1):136-143.

Dougherty TJ, Pucci MJ. Antibiotic discovery and development. *Antibiotic Discovery and Development*. 2014; 1–1127. Available at: https://www.springer.com/gp/book/9781461413998. Accessed on 23 September 2021.

DRUGBANK. Debrisoquine. Available at: https://go.drugbank.com/drugs/DB04840. Accessed on July 12, 2022.

Du Toit A, de Wit M, Osthoff G, Hugo A. Antioxidant properties of fresh and processed cactus pear cladodes from selected Opuntia ficus-indica and O. robusta cultivars. *S. Afr. J. Bot*. 2018; 118: 44-51.

Duan L, Liu C, Wang D. The General Population's Inappropriate Behaviors and Misunderstanding of Antibiotic Use in China: A Systematic Review and Meta-Analysis. *Antibiotics* 2021; 10:497.

Durgawale PM, Shinde MB, Durgawale PP, Agarwal N. Practices of Self-Medication among Tribal Population North Maharashtra (Khandesh). *Int. J. Sci. Res*. 2014; 3: 211-215.

Dutta R, Raja D, R A, Dcruze L, Jain TPS. Self-medication practices versus health of the community. *Int J. Community Med. Public Health*. 2017; 4(8):2757-2761.

Dzoyem JP, Aro AO, McGaw LJ, Eloff JN. Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments. *S. Afr. J. Bot*. 2016; 102:70-74.

Eddouks M, Ghanimi D. The use of medicinal plants in human healthcare: a scoop on safety. *Pharm. Regul. Aff.* 2013; 3: e122.

El Zowalaty ME, Belkina T, Bahashwan SA, *et al*. Knowledge, awareness, and attitudes toward antibiotic use and antimicrobial resistance among Saudi population. *Int. J. Clin. Pharm.* 2016; 38(5):1261-1268.

Elmahi OKO, Musa RAE, Shareef AAH, Omer MEA, Elmahi MAM, Altamih RAA, *et al*. Perception and practice of self-medication with antibiotics among medical students in Sudanese universities: A cross-sectional study. *PLoS One* 2022; 17(1): e0263067.

Erb S, Frei R, Tschudin Sutter S, Egli A, Dangel M, Bonkat G, Widmer AF. Basic patient characteristics predict antimicrobial resistance in *E. coli* from urinary tract specimens: a retrospective cohort analysis of 5246 urine samples. *Swiss Med. Wkly*. 2018; 148: w14660.

Esubalew ST, Belete A, Lulekal E, Gabriel T, Engidawork E, Asres K. Review of Ethnobotanical and Ethnopharmacological Evidences of some Ethiopian Medicinal Plants traditionally used for the Treatment of Cancer. *Ethiop. J. Health Dev*. 2017; 31(3).

Ethiopian Public Health Institute (EPHIa). History of Ethiopian Public Health Institute. Available at: https://ephi.gov.et/laboratory-services/different-laboratory-services/. Accessed on August 05, 2021.

Ethiopian Public Health Institute (EPHIb). Laboratory services. Available at: https://ephi.gov.et/laboratory-services/different-laboratory-services/. Accessed on August 05, 2021.

Eticha T, Mesfin K. Self-Medication Practices in Mekelle, Ethiopia. *PLoS One* 2014; 9(5): e97464.

Etim E, Udobre A, Udoh A, Eduoku E. Evaluation of the antioxidant property of *Vernonia Cinerea (L.) LESS. (Asteraceae)* using 2.2- Diphenyl-1 Picrylhydrazine (DPPH) Assay Method. *The Pharma Innovation Journal* 2015; 4(6): 10-14.

European Medicine Agency (EMA). Guideline on non-clinical documentation in applications for marketing authorization/ registration of well-established and traditional herbal medicinal products. 2019.

Evans W. Trease and Evans' Pharmacognosy New York: Elsevier Health Sciences; 2002: 21-24.

Fainzang S. Self-medication and society-Mirages of autonomy. 2017. Routledge, London, New York.

Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. *Perspect. Medicin Chem*. 2014; 6: 25-64.

Falcone M, Paterson D. Spotlight on ceftazidime/avibactam: A new option for MDR Gram-negative infections. *J. Antimicrob. Chemother*. 2016; 71(10):2713-2722.

Farhan H, Rammal H, Hijazi A, Hamad H, Badran B. Phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese *Euphorbia macrolada schyzoceras Boiss*. *Ann. Biol. Res*. 2012; 3:149-156.

Ferdosi MFH, Khan IH, Javaid A, Saeed HM, Butt I. Gc-Ms Analysis Profile and Bioactive Components of Flowers of *Bergenia Ciliata*, A Weed of Rock Crevices. *Journal of Weed Science Research*. 2021; 27(4): 527-535.

Ferede T, Amare D, Endalamaw B, Genanew T. Rhamnus prinoides in North West Ethiopia: Production, contribution and constraints. *Recent Adv. Petrochem. Sci.* 2018; 4(5):001-006.

Fidrianny I, Ari Sri Windyaswari AS, Wirasutisna RK. Antioxidant Capacities of Various Leaves Extract from Five Colors Varieties of Sweet Potatoes Tubers Using ABTS, DPPH Assays and Correlation with Total Flavonoid, Phenolic, Carotenoid Content. *Research Journal of Medicinal Plants.* 2013; 7: 130-140.

Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol*. 2015; 13: 269-284.

Floyd RA, Lewis CA. Hydroxyl free radical formation from hydrogen peroxide by ferrous iron-nucleotide complexes. *Biochemistry* 1983; 22:2645–2649.

Food, Medicine and Health Care Administration and Control Authority of Ethiopia (FMHACA). Over-The-Counter Medicines List for Ethiopia. 2012. Second edition. June 2012. Addis Ababa, Ethiopia.

Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am. J. Med.* 2002; 113(Suppl 1A):5S–13S.

Foxman B. The epidemiology of urinary tract infection. *Nat. Rev. Urol.* 2010; 7(12): 653–660.

Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect. Dis. Clin. N. Am.* 2014; 28: 1-13.

Gadelha CAG, Costa KS, do N JM, Ju´nior, Soeiro OM, Mengue SS, da Motta ML, *et al*. Prevalence of self-medication in Brazil and associated factors. *Rev. Saude Publica*. 2016; 50: 1-11.

Gadisa E, Weldearegay G, Desta K, Tsegaye G, Hailu S, Jote K, Takele A. Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug resistant bacteria. *BMC Complement Altern. Med*. 2019; 19(1):24.

Gágyor I, Strube-Plaschke S, Rentzsch K, Himmel W. Management of urinary tract infections: what do doctors recommend and patients do? An observational study in German primary care. *BMC Infect. Dis.* 2020; 20:813.

Galato D, Galafassi L, Alano GM, Trauthman SC. Responsible self-medication: review of the process of pharmaceutical attendance. *Braz. J. Pharm Sci*. 2009; 45:625-633.

García-Sánchez F, Simón-Grao S, Martínez-Nicolás JJ, Alfosea-Simón M, *et al*. Multiple stresses occurring with boron toxicity and deficiency in plants. *J. Hazard. Mater.* 2020; 397:122713.

Gardiner BJ, Stewardson AJ, Abbott IJ, Peleg AY. Nitrofurantoin and fosfomycin for resistant urinary tract infections: old drugs for emerging problems. *Aust. Prescr.* 2019; 42:14-19.

Garofalo L, Di Giuseppe G, Angelillo IF. Self-medication practices among parents in Italy. *Biomed. Res. Int*. 2015; 2015:1–8.

Garud SK, Durgawale PM, Shinde MB, Durgawale PP. Self-medication Practice among the People Residing in Hilly Area. *Int. J. Sci. Res*. 2014; 3: 2012-2014.

Gastelurrutia MA, Larrañaga B, Garay A, de Asis Echeveste F, Fernandez-Llimos F. Impact of a program to reduce the dispensing of antibiotics without a prescription in Spain. *Pharmacy Practice*. 2013; 11:185.

Gbeasor-Komlanvi FA, Zida-Compaore WIC, Dare IH, *et al*. Medication Consumption Patterns and Polypharmacy among Community-Dwelling elderly in Lomé (Togo) in 2017. *Curr. Gerontol. Geriatr. Res.* 2020; 2020(4346035): 1-8.

Gebrekirstos NH, Workneh BD, Gebregiorgis YS, Misgina KH, Weldehaweria NB, Weldu MG, *et al*. Non-prescribed antimicrobial use and associated factors among customers in drug retail outlet in Central Zone of Tigray, Northern Ethiopia: A cross-sectional study. *Antimicrob. Resist. Infect. Control* 2017; 6:70.

Gebretekle GB, Serbessa MK. Exploration of over-the-counter sales of antibiotics in community pharmacies of Addis Ababa, Ethiopia: pharmacy professionals' perspective. *Antimicrob. Resist. Infect. Control*. 2016; 5: 2.

Gedif T, Hahn HJ. The use of medicinal plants in self-care in rural central Ethiopia. *J. Ethnopharmacol.* 2003; 87:155–61.

Geissler PW, Nokes K, Prince RJ, Achieng RO, Aagaard-Hansen J, Ouma JH. Children and medicines: self-treatment of common illnesses among Luo school children in western Kenya. *Soc. Sci. Med*. 2000; 50:1771-1783.

Gelayee DA. Self-medication pattern among social science university students in Northwest Ethiopia. *J Pharm (Cairo)*. 2017; 2017:8680714.

Getahun A. Some Common Medicinal and Poisonous Plants Used in Ethiopian Folk Medicine. *Addis Ababa, Faculty of Science, Addis Ababa University*, 1976: pp. 2–3. Available at: http://www.ethnopharmacologia.org/prelude2016/pdf/biblio-hg-07-getahun.pdf. Accessed on October 15, 2021.

Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, Teka F, *et al*. Screening of some medicinal plants of Ethiopia for their antimicrobial properties and chemical profiles. *J. Ethnopharmacol.* 2005; 97: 421-427.

Gibbons S. Anti-staphylococcal plant natural products. *Natural Product Reports*, 2004; 21(2): 263-277.

Giday M, Teklehaymanot T, Animut A, Mekonnen Y. Medicinal plants of the Shinasha, Agew-awi and Amhara peoples in northwest Ethiopia. *J. Ethnopharmacol.* 2007; 110:516-525.

Gillani AH, Chang J, Aslam F, Saeed A, Shukar S, Khanum F, Jairoun A, Nicholson A, Mohamed Ibrahim MI, Fang Y. Public knowledge, attitude, and practice regarding antibiotics use in Punjab, Pakistan: a cross-sectional study. *Expert. Rev. Anti. Infect. Ther.* 2021; 19(3):399-411.

Girma BG, Diriba AG, Abebe ZK, Derbew FB, Abera HB, Mussie GH, *et al*. Self-Medication Practices among Health Sciences Students: The Case of Mekelle University. *J. App. Pharm. Sci*. 2011; 1(10):183-189.

Godghate A, Sawant R, Sutar A. Phytochemical analysis of ethanolic extracts of roots of Carris carandus Linn. *Rasayan J. Chem*. 2012; 5(4):456-459.

Gogazeh E. Dispensing errors and self-medication practice observed by community pharmacists in Jordan. *Saudi Pharm. J.* 2020; 28 (2020):233–237.

Gomila A, Carratalà J, Eliakim-Raz N, Shaw E, Wiegand L, Vallejo-Torres L, *et al*. Risk factors and prognosis of complicated urinary tract infections caused by *Pseudomonas aeruginosa* in hospitalized patients: a retrospective multicenter cohort study. *Infect. Drug Resist*. 2018; 11: 2571-2581.

Goossens H, Ferech M, Vander Stichele R, Elseviers M. ESAC Project Group. Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. *Lancet* 2005; 365:579-587.

Gore PR, Mahavan S. Consumers' preference and willingness to pay for pharmacist counseling for non-prescription medicines. *J. Clin. Pharm. Ther*. 1994; 19:17-25.

Gou L, Zhuo C, Lu S, Guo Z. A Universal Stress Protein from Medicago falcata (MfUSP1) confers multiple stress tolerance by regulating antioxidant defense and proline accumulation. *Environ. Exp. Bot.* 2020; 178: 104168.

Griebling TL. Urinary tract infection in women. In: Urologic diseases in America. US Department of Health and Human Services, Public Health Service, National Institutes of Health. *NIDDK.* 2009; 589-617.

Grigoryan L, Burgerhof JGM, Degener JE, *et al*. Determinants of self-medication with antibiotics in Europe: the impact of beliefs, country wealth and the healthcare system. *J. Antimicrob. Chemother*. 2008; 61(5):1172-1179.

Grigoryan L, Haaijer-Ruskamp FM, Burgerhof JGM, *et al*. Self-medication with antimicrobial drugs in Europe. *Emerg. Infect. Dis*. 2006; 12:452-459.

Guesmi F, Ben AM, Barkaoui T, Tahri W, Mejri M, Ben-attia M. Effects of *Thymus hirtus sp. algeriensis Boiss. et Reut. (Lamiaceae)* essential oil on healing gastric ulcers according to sex. *Lipids Health and Dis*. 2014; 13:138.

Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, *et al*. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin. Infect. Dis.* 2011; 52: e103-120.

Gupta P, Bobhate P, Shrivastava SR. Determinants of self-medication practices in an urban slum community. *Asian J. Pharm. Clin. Res*. 2011; 4: 54-57.

Gupta S, Tang C, Tran M, Kadouri DE. Effect of Predatory Bacteria on Human Cell Lines. *PLoS One* 2016; 11(8): e0161242.

Ha TV, Nguyen AMT, Nguyen HST. Self-medication practices among Vietnamese residents in highland provinces. *J. Multidiscip. Healthc*. 2019; 12: 493-502.

Hailemariam G, Emire S. Antioxidant activity and preservative effect of *thyme* (*Thymus schimperi* R.) *Br. J. Appl. Sci. Tech*. 2013; 3(4):1311-1326.

Hanafy SA, Sallam SA, Kharboush IF, Wahdan IH. Drug Utilization Pattern During Pregnancy in Alexandria, Egypt. *EJPMR*. 2016; 3(2):19–29.

Hanish SJC, Mohamed MM, Thayashini K, Long CM, Shafeeq MF. Sociodemographic Impact and Acquaintance on Antibiotic Resistance Among Outpatients in Specialist Hospital, Malaysia. *IMJM* 2021; 20(3): 138-145.

Harborne JB. Phytochemicals Methods. Chapman and Hall Ltd., London, 1973; 49-188.

Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* 2002; 13 (10): 572-584.

Helal R, Abou-ElWafa H. Self-medication in university students from the city of Mansoura, Egypt. *J Environ. Public Health*. 2017; 2017:7.

Hilbert DW. Antibiotic Resistance in Urinary Tract Infections: Current Issues and Future Solutions, Urinary Tract Infections, Dr. Peter Tenke (Ed.), ISBN: 978-953-307-757-4, InTech. 2011. Available at: http://www.intechopen.com/books/urinary-tract-infections/antibiotic-resistance-in-urinary-tract-infectionscurrent-issues-and-future-solutions. Accessed on September 23, 2021.

Hiyeswar B, Rana K, Madhur B, Chandana D. Self-Medication Practices and its determinants among urban dwellers of Dibrugarh town, Assam. *Sch. J. App. Med. Sci*. 2016; 4(8): 3100-3106.

Horton S, Stewart A. Reasons for Self-Medication and Perceptions of Risk among Mexican Migrant Farm Workers. *J Immigrant Minority Health.* 2012; 14:664–672.

Horumpende PG, Said SH, Mazuguni FS, Antony ML, Kumburu HH, Sonda TB, *et al*. Prevalence, determinants and knowledge of antibacterial self-medication: A cross sectional study in North-eastern Tanzania. *PLoS One* 2018 (a); 13(10): e0206623.

Horumpende PG, Sonda TB, van Zwetselaar M, Antony ML, Tenu FF, Mwanziva CE, *et al*. Prescription and non-prescription antibiotic dispensing practices in part I and part II pharmacies in Moshi Municipality, Kilimanjaro Region in Tanzania: A simulated Clients approach. *PloS One* 2018 (b); 13: e0207465.

Hossain S, Sikdar S, Asif MM, Bhattacharjee O, Hakim R, Barua O, *et al*. A Survey on Awareness, Practice and Consequence of Self-medication in Chittagong City. *5th International Conference on Natural Sciences and Technology*. 2018. Pages: 70–73.

Hoxha I, Malaj A, Tako R, Malaj L. Survey on how antibiotics are dispensed in community pharmacies in Albania. *Lancet* 2005; 365:579-587.

Hughes CM, McElnay JC, Fleming GF. Benefits and risks of self-medication. *Drug Safety* 2001; 24(14):1027-1037.

Hulscher ME, van der Meer JW, Grol RP. Antibiotic use: how to improve it? *Int. J. Med. Microbiol*. 2010; 300: 351-356.

Hussain S, Malik F, Ashfaq KM, Parveen G, Hameed A, Ahmad S, *et al*. Prevalence of self-medication and health-seeking behavior in a developing country. *Afr. J. Pharm. Pharmacol.* 2011; 5:972-978.

Ibrahim N, Kebede A. *In vitro* antibacterial activities of methanol and aqueous leave extracts of selected medicinal plants against human pathogenic bacteria. *Saudi J. Biol. Sci.* 2020; 27: 2261–2268.

Isameldin E, Saeed AA, Mousnad MA. Self-medication Practice among patients living in Soba- Sudan. *Health Prim. Car.* 2020; 4: 1-5.

Izzo AA, Ernst E. Interaction between herbal medicines and prescribed drugs. *Drugs* 2009; 69:1777-1798.

Jadid N, Dewi Hidayati D, Hartanti SR, Arraniry BA, Rizka Yuanita Rachman RY, Wikanta W. Antioxidant Activities of Different Solvent Extracts of *Piper retrofractum Vahl*. using DPPH Assay. *AIP Conference Proceedings* 2017; 1854:020019.

Jaleta A, Tesema S, Yimam B. Self-medication practice Sire-town, west Ethiopia: A cross sectional study. *Cukurova Med. J*. 2016; 43:447-452.

Jalilian F, Mehdi Hazavehei SM, Vahidinia AA, Jalilian M, Moghimbeig A. Prevalence and related factors for choosing self-medication among pharmacies visitors based on health belief model in Hamadan province, west of Iran. *J. Res. Health Sci*. 2013; 13: 81-85.

James H, Handu SS, Al Khaja KA, Otoom S, Sequeira RP. Evaluation of the knowledge, attitude and practice of self-medication among first-year medical students. *Med. Princ. Pract*. 2006; 15:270-5.

Jaradat N, Hussen F, Al Ali A. Preliminary Phytochemical Screening, Quantitative Estimation of Total Flavonoids, Total Phenols and Antioxidant Activity of Ephedra alata Decne. *J. Mater Environ. Sci.* 2015; 6(6):1771-1778.

Jember E, Feleke A, Debie A, Asrade G. Self-medication practices and associated factors among households at Gondar town, Northwest Ethiopia: a cross-sectional study. *BMC Res. Notes.* 2019; 12:153.

Jernigan JA, Hatfeld KM, Wolford H, Nelson RE, Olubajo B, Reddy SC, *et al*. Multidrug-resistant bacterial infections in U.S. hospitalized patients, 2012–2017. *N. Engl. J. Med*. 2020; 382(14):1309-1319.

Jha N, Bajracharya O, Shankar PR. Knowledge, attitude and practice towards medicines among school teachers in Lalitpur district, Nepal before and after an educational intervention. *BMC Public Health.* 2013; 13: 652(2013).

Jin L, Li X, Tian D, Fang X, Yu Y, *et al*. Antioxidant properties and color parameters of herbal teas in China. *Ind. Crops Prod*. 2016; 87: 198-209.

Joos S, Glassen K, Musselmann B. Herbal medicine in primary healthcare in Germany: the patient's perspective. *Evid. Based Complement. Altern. Med.* 2012; 2012:294638.

Jose A, Kannan E, Vijay KARP, Madhunapantula SV. Therapeutic potential of phytochemicals isolated from Simarouba glauca DC for inhibiting cancers: A review. *Sys Rev Pharm.* 2019; 10(1):73-80.

Kachur K, Suntres Z. The antibacterial properties of phenolic isomers, carvacrol and thymol. *Crit Rev in Food Sci. Nutr*. 2020; 60(18):3042-3053.

Kainama H, Fatmawati S, Santoso M, *et al.* The Relationship of Free Radical Scavenging and Total Phenolic and Flavonoid Contents of *Garcinia lasoar* PAM. *Pharm. Chem. J.* 2020; 53, 1151-1157.

Kalungia AC, Burger J, Godman B, Costa JdO, Simuwelu C. Non-prescription sale and dispensing of antibiotics in community pharmacies in Zambia. *Expert Rev. Anti. Infect. Ther.* 2016; 14:1215-1223.

Kamaruding NA, Ismail N, Sokry N. Identification of Antibacterial Activity with Bioactive Compounds from Selected Marine Sponges. *Pharmacogn. J.* 2020; 12(3):493-502.

Kamoun M, Mnif M, Mnif F, *et al*. Complicated urinary tract infections associated with diabetes mellitus: pathogenesis, diagnosis and management. *Indian J. Endocrinol. Metab*. 2013; 17:442-445.

Karunakaran T, Ismail IS, Ee GCL, Nor SMM, Palachandran K, Santhanam RK. Nitric oxide inhibitory and anti-Bacillus activity of phenolic compounds and plant extracts from Mesua species. *Rev. Bras. Farmacogn*. 2018; 28(2):231-234.

Karuniawati H, Hassali MAA, Suryawati S, Ismail WI, Taufik T, Hossain MS. Assessment of Knowledge, Attitude, and Practice of Antibiotic Use among the Population of Boyolali, Indonesia: A Cross-Sectional Study. *Int. J. Environ. Res. Public Health.* 2021; 18: 8258.

Kashef N, Djavid GE, Shahbazi S. Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran. *J. Infect. Dev. Ctries*. 2010; 4:202-206.

Kassie AD, Bifftu BB, Mekonnen HS. Self-medication practice and associated factors among adult household members in Meket district, Northeast Ethiopia, 2017. *BMC Pharmacol. Toxicol*. 2018; 19:15.

Kebamo S, Dabsu R, Deressa A, Gebire M. Urinary Tract infection: bacterial etiologies, drug resistance profile and associated risk factors among diabetic patients attending NRH. *Am. J. Curr. Microbiol*. 2017; 5(1):19–32.24.

Keshari SS, Kesarwani P, Mishra M. Prevalence and pattern of self-medication practices in rural area of Barabanki. *Indian J Clin Pract.* 2014; 25(7):636–9.

Khadka K, Khadka J, Lekhak B, *et al*. Incidence of urinary tract infection among the patients visiting Western Regional Hospital, Pokhara, Nepal. *JHAS*. 2012; 2(1): 35-7.

Khalid M, Rahman SU, Bilal M, Huang D. Role of flavonoids in plant interactions with the environment and against human pathogens - A review. *J. Integr. Agric.* 2019; 18: 211-230.

Khalid S, Ali Q, Hafeez M, Malik A. Perception regarding self-medication of antibiotics in general public sector university of Southern Punjab: a comparison between medical and non-medical students. *Biol Clin Sci Res J*. 2021; 2021(1).

Khameneh B, Iranshahy M, Soheili V, Bazzaz BSF. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrob Resist Infect Control*. 2019; 8:118.

Khomarlou N, Aberoomand-Azar P, Lashgari AP, Hakakian A, Ranjbar R and Ayatollahi SA. Evaluation of antibacterial activity against multidrug-resistance (MDR) bacteria and antioxidant effects of the ethanolic extract and fractions of *Chenopodium album* (sub sp *striatum*). *Int J Pharm Sci Res*. 2017; 8(9): 3696-08.

Khoury M, Stien D, Eparvier V, Ouaini N, El Beyrouthy M. Report on the medicinal use of eleven Lamiaceae species in Lebanon and rationalization of their antimicrobial potential by examination of the chemical composition and antimicrobial activity of their essential oils. *Evid Based Complement Alternat Med*. 2016; 2016:2547169.

Kindl M, BlaDekoviT B, Bucar F, Vladimir-KneDevi TS. Antioxidant and Anticholinesterase Potential of Six Thymus Species. *Evid Based Complement Alternat Med.* 2015; 2015:10.

Kinsalin VA, KUMAR PS, Duraipandiyan V, Ignacimuthu S, Al-Dhabi NA. Antimicrobial activity of methanol extracts of some traditional medicinal plants from Tamil Nadu, India. *Asian J. Pharmaceutical Clin. Res*. 2014; 7(1):38-42.

Kiringe JW. A survey of traditional health remedies used by the Massai of South Kaijiado District, Kenya. *Ethnobot Res Appl*. 2006; 4: 61-74.

Klemenc-Ketiš Z, Kersnik J. The effect of demographic characteristics on self-medication patterns: a cross-sectional nationwide study from Slovenia. *Coll Antropol*. 2011; 35(4):1237–1242.

Klemenc-Ketis Z, Mitrovic D. Patients' use of and attitudes towards self-medication in rural and remote Slovenian family medicine practices: a cross-sectional multicentre study. *Rural Remote Health*. 2017; 17:2.

Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, *et al*. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. *Int. J. Med. Med. Sci.* 2009; 1(5):163-167.

Kotwani A, Holloway K. Trends in antibiotic use among outpatients in New Delhi, India. *BMC Infect Dis*. 2011; 11:99.

Kumar N, Kanchan T, Unnikrishnan B, Rekha T, Mithra P, Kulkarni V, *et al*. Perceptions and Practices of Self-Medication among Medical Students in Coastal South India. *PLoS One*. 2013; 8.

Kumar SR, Loveleena D, Godwin S. Medicinal property of *Murraya koenigii* - a review. *Intl Res J Biol Sci*. 2013; 2: 80-83.

Kumar V, Mangal A, Yadav G, Raut D, Singh S. Prevalence and pattern of self-medication practices in an urban area of Delhi, India. *Med J Dr Patil Univ*. 2015; 8(1):16-20.

Kurniawan K, Posangi J, Rampengan N. Association between public knowledge regarding antibiotics and self-medication with antibiotics in Teling Atas Community Health Center, East Indonesia. *Med J Indones*. 2017; 26(1):62–69.

Labiad MH, Harhar H, Ghanimi A, Tabyaoui M. Phytochemical screening and antioxidant activity of Moroccan Thymus satureioïdes extracts. *J Mat Environ Sci.* 2017; 8(6):2132-9.

Lei X, Jiang H, Liu C, Ferrier A, Mugavin J. Self-medication practice and associated factors among residents in Wuhan, China. *Int. J. Environ. Res. Public Health*. 2018; 15(1): 68.

Lemmen SW, Häfner H, Klik S, Lütticken R, Zolldann D. Comparison of the bactericidal activity of moxifloxacin and levofloxacin against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Klebsiella pneumoniae. *Chemotherapy*. 2003; 49(1-2):33-5.

Lesielle Adaptive Skincare. O-CYMEN-5-OL. Available at: https://www.lesielle.com/int/en/o-cymen-5-ol070#:~:text=O%2Dcymen%2D5%2Dol%20helps%20stabilize%20a%20product%20to,protects%20the%20skin%20from%20infections. Accessed on July 09, 2022.

Levison ME. Pharmacodynamics of antimicrobial drugs. *Infect Dis Clin N Am*. 2004; 18:451–65.

Levy SB. Factors impacting on the problem of antibiotic resistance. *J Antimicrob Chemother*. 2002; 49:25–30.

Li X, Liu J, Chang Q, Zhou Z, Han R, Liang Z. Antioxidant and Antidiabetic Activity of Proanthocyanidins from *Fagopyrum dibotrys*. *Mol*. 2021; 26: 2417.

Li X, Wu X, Huang L. Correlation between antioxidant activities and phenolic contents of *Radix angelicae sincensis (Danggui)*. *Mol*. 2009; 14(12): 5349–5361.

Loizzo MR, Tundis R, Bonesi *et al*. Radical scavenging, antioxidant and metal chelating activities of *Annona cherimola Mill.* (cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents. *J. Food Compos. Anal.* 2012; 25(2): 179–184.

Lorian V. Antibiotics in Laboratory Medicine. 5th ed. Philadelphia: Lippincott Williams and Wilkins, 2005.

Losada L, Amundsen CL, Ashton-Miller J, Chai T, Close C, Damaser M, DiSanto M, Dmochowski R, Fraser MO, *et al*. Expert Panel Recommendations on Lower Urinary Tract Health of Women Across Their Life Span. *J Womens Health*. 2016; 11:1086-1096.

Losada-Barreiro S, Bravo-Díaz C. Free radicals and polyphenols: The redox chemistry of neurodegenerative diseases. *Eur. J. Med. Chem.* 2017; 133: 379–402.

Lukovic JA, Miletic V, Pekmezovic T, Trajkovic G, Ratkovic N, Aleksic D, et al. Self-medication practices and risk factors for self-medication among medical students in Belgrade, Serbia. *PLoS One*. 2014; 9.

LynnYeen G, Vitry AI, Semple SJ, Esterman A, Luszcz MA. Self-medication with over-the-counter drugs and complementary medications in South Australia's elderly population. *BMC complement. med. ther.* 2009; 9(42): 32.

Mabilika RJ, Mpolya E, Shirima G. Prevalence and predictors of self-medication with antibiotics in selected urban and rural districts of the Dodoma region, Central Tanzania: a cross-sectional study. *Antimicrob. Resist. Infect. Control*. 2022; 11:86 (2-9).

Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. *Arter. Thromb. Vasc. Biol.* 2005; 25: 29–38.

Madhavi DL, Deshpande SS, Sulunkhe DK. Food antioxidants: Technological, toxicological and health perspectives. New York: Marcel Dekker; 1996.

Maffei ME, Gertsch J, Appendino G. Plant volatiles: Production, function and pharmacology. *Nat Prod Rep*. 2011; 28: 1359-1380.

Mahdi Yahya Mohsen S, Hamzah HA, Muhammad Imad Al-Deen M, Baharudin R. Antimicrobial susceptibility of *klebsiella pneumoniae* and *Escherichia coli* with extended-spectrum β-lactamase associated genes in hospital Tengku Ampuan Afzan, Kuantan, Pahang. *Malays J Med Sci*. 2016; 23(2): 14-20.

Mahmoud MA, Wajid S, Naqvi AA, Samreen S, Althagfan SS, Alworafi Y. Self-medication with antibiotics: a cross-sectional community-based study. *Lat Am J Pharm.* 2020; 39: 348-353.

Mahomoodally MF. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evid Based Complement Alternat Med.* 2013; 2013: 617459.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012; 18(3):268-81.

Majekodunmi SO. Review of extraction of medicinal plants for pharmaceutical research. *Merit Res J Med*. 2015; 3:521–527.

Malka R, Shochat E, Rom-Kedar V. Bistability and bacterial infections. *PLoS One*. 2010; 5: e10010.

Mamo S, Ayele Y, Dechasa M. Self-Medication Practices among Community of Harar City and Its Surroundings, Eastern Ethiopia. *J Pharm*. 2018; 2018:1–6.

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.,* 2004; 79: 727–747.

Marami LM, Dilba GM, Babele DA, Sarba EJ, Gizaw A, Bune WM, *et al*. Phytochemical Screening and in-vitro Evaluation of Antibacterial Activities of *Echinops amplexicaulis, Ruta chalepensis and Salix subserrata* Against Selected Pathogenic Bacterial Strains in West Shewa Zone, Ethiopia. *J. Exp. Pharmacol.* 2021; 13:511–520.

Marchese A, Orhan IE, Daglia M, Barbieri R, Di Lorenzo A, Nabavi SF, Gortzi O, Izadi M, Nabavi SM. Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chem*. 2016; 210:402-14.

Marinova D, Ribarova F, Atanasoval M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Univ. Chem. Technol. Metallurgy.* 2005; 40(3): 255.

Martins AP, Da Costa Miranda A, Mendes Z, Soares MA, Ferreira P, Nogueira A. Self-medication in a Portuguese urban population: A prevalence study. *Pharmacoepidemiol Drug Saf*. 2002; 11: 409–414.

Martins N, Barros L, Ferreira ICFR. In vivo antioxidant activity of phenolic compounds: facts and gaps. *Trends Food Sci Technol*. 2016; 48(0924-2244):1-12.

Mate I, Come CE, Gonc¸alves MP, Cliff J, Gudo ES. Knowledge, attitudes and practices regarding antibiotic use in Maputo City, Mozambique. *PLoS one.* 2019; 14(8): e0221452.

Mathewos T, Daka K, Bitew S, Daka D. Self-medication practice and associated factors among adults in Wolaita Soddo town, Southern Ethiopia. *Int J Infect Control*. 2021; 17: 20322.

Mazzariol A, Bazaj A, Cornaglia G. Multidrug-resistant Gram-negative bacteria causing urinary tract infections: a review. *J Chemother.* 2017; 29:2-9.

Mechal T, Hussen S, Desta M. Bacterial Profile, Antibiotic Susceptibility Pattern and Associated Factors Among Patients Attending Adult OPD at Hawassa University Comprehensive Specialized Hospital, Hawassa, Ethiopia. *Infect Drug Resist*. 2021:14: 99–110.

Mediani A, Abas F, Khatib A, Ping TC, Lajis NH. Influence of growth stage and season on the antioxidant constituents of Cosmos caudatus. *Plant Food Human Nutr*. 2012; 67:344–50.

Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, *et al*. Abiotic stress responses and microbe-mediated mitigation in plants: The omics strategies. *Front. Plant Sci.* 2017; 8.

Mekonnen B, Asrie AB, Wubneh ZB. Antidiarrheal Activity of 80% Methanolic Leaf Extract of *Justicia schimperiana*. *Evid. Based Complementary Altern. Med.* 2018; 2018 (3037120):10.

Melka AE, Makonnen E, Debella A, Fekadu N, Geleta B. Evaluation of Diuretic Activity of Methanol Crude Extract of *Thymus serrulatus* Leaves and its Solvent Fraction in Mice. *Biol Med*. 2016; 8:5-10.

Meresa A. Herbal Medicines for the Management of Diabetic Mellitus in Ethiopia and Eretria including their Phytochemical Constituents. *Am J Adv Drug Del*. 2017; 5:40-58.

Mgbeahuruike EE, Yrj¨onen T, Vuorela H, Holm Y. Bioactive compounds from medicinal plants: focus on Piper species. *S. Afr. J. Bot.* 2017; 112: 54–69.

Michiels C. Physiological and pathological responses to hypoxia. *Am. J. Pathol*. 2004; 164:1875–1882.

Michno M, Sydor A, Walaszek M, Sulowicz W. Microbiology and drug resistance of pathogens in patients hospitalized at the Nephrology Department in the South of Poland. *Pol J Microbiol*. 2018; 67(4):517–524.

Milevskaya VV, Prasad S, Temerdashev ZA. Extraction and Chromatographic Determination of Phenolic Compounds from Medicinal Herbs in the *Lamiaceae* and *Hypericaceae* Families: A Review. *Microchem. J.* 2018; 145: 1036–1049.

Mishra J, Srivastava RK, Shukla SV, Raghav CS. Antioxidants in Aromatic and Medicinal plants. *Science Tech Entrepreneur*. 2007; 1-16.

Mogana R, Adhikari A, Tzar MN, Ramliza R, Wiart C. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium Miq*. against bacterial clinical isolates. *BMC Complement Altern. Med.* 2020; 20:55.

Moise K, Bernard JJ, Henrys HJ. Evaluation of antibiotic self-medication among outpatients of the state university hospital of Port-Au-Prince, Haiti: a cross-sectional study. *Pan Afr. Med. J*. 2017; 28: 4.

Mok CZ, Sellappans R, Ee Loo JS. The prevalence and perception of self-medication among adults in the Klang Valley, Malaysia. *Int J Pharm Pract.* 2021; 29: 29–36.

Moktan D, Shehnaz SI. Factors driving self-medication with antimicrobials in Karaikal, Puducherry, India. *J Pharmacol Pharmacother*. 2020; 11:64-71.

Molla Y, Nedi T, Tadesse G, Alemayehu H, Shibeshi W. Evaluation of the in vitro antibacterial activity of the solvent fractions of the leaves of *Rhamnus prinoides* L'Herit (*Rhamnaceae*) against pathogenic bacteria. *BMC complement. med. ther.* 2016; 16(1):1-9.

Montoro P, Braca A, Pizza C, De Tommasi N. Structure antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chem*. 2005; 92: 349–355.

Morgan DJ, Okeke IN, Laxminarayan R, Perencevich EN, Weisenberg S. Non-prescription antimicrobial use worldwide: a systematic review. *Lancet Infect Dis.* 2011; 11(9):692–701.

Mortazavi SS, Shati M, Khankeh HR, Ahmadi F, Mehravaran S, Malakouti SK. Self-medication among the elderly in Iran: a content analysis study. *BMC Geriatr*. 2017; 17(1):198.

Motalleb G, Hanachi P, Kua SH, Fauziah O, Asmah R. Evaluation of Phenolic Content and Total Antioxidant Activity in Berberis vulgaris Fruit Extract. *Journal of Biological Sciences*, 2005; 5(5): 648-653.

Mouanga NY, Onanga R, Kassa KRF, Bignoumba M, Mbehang NPP, Gafou A, *et al*. Epidemiology of Community Origin *Escherichia coli* and *Klebsiella pneumoniae* Uropathogenic Strains Resistant to Antibiotics in Franceville, Gabon. *Infect Drug Resist*. 2021; 14: 585-594.

Muhammed RA, Ali Al-Ani AHHD, Yassen AO. Self-Medication with Antibiotic among Public Population in Erbil City. *JPRI*. 2021; 33(44B): 25-33.

Mukherjee S, Mishra S, Tiwari S. Aetiological Profile and Antibiogram of Urinary Isolates Causing UTI in Patients Attending a Tertiary Care Hospital of Western Odisha. *J. Evol. Med. Dent. Sci.* 2020; 9: 662–667.

Mulatu G. Antibacterial activities of *Calpurnia aurea* against selected animal pathogenic bacterial strains. *Adv Pharmacol Pharm Sci*. 2020; 2020:8840468.

Murthy PN, Moges G, Hymete A, Gebremariam T. Antimicrobial and phytochemical screening of Justicia Schimperiana. *Eth Pharm J*. 1993; 11:47–53.

Muzila A, Msagati TAM, Abegaz B, Nindi MM. Characterization of Geshodin-metal complexation by electrospray-mass spectrometry. 11th NAPRECA Symposium, Book of Proceedings, Antananarivo, Madagascar. 2010; 35-44.

Nabaweesi I, Olum R, Sekite AB, Suubi WT, Nakiwunga P, Machali A, *et al*. Antibiotic Practices, Perceptions and Self-Medication Among Patients at a National Referral Hospital in Uganda. *Infect Drug Resist*. 2021;14: 2155–2164.

Nadarajah KK. ROS homeostasis in abiotic stress tolerance in plants. *Int. J. Mol. Sci.* 2020; 21:5208.

Nadia A, Mohammad E, Sima R, Milad S, Arefeh T, Fatemeh S, *et al*. Self-Medication and Contributing Factors: A Questionnaire Survey Among Iranian Households. *Bali Medical Journal.* 2016; 5(3): 17–21.

Nagoor Meeran MF, Jagadeesh GS, Selvaraj P. Thymol attenuates altered lipid metabolism in β adrenergic agonist induced myocardial infarcted rats by inhibiting tachycardia, altered electrocardiogram, apoptosis and cardiac hypertrophy. *J. Funct. Foods*. 2015; 14:51–62.

Nagoor Meeran MF, Jagadeesh GS, Selvaraj P. Thymol, a dietary monoterpene phenol abrogates mitochondrial dysfunction in β-adrenergic agonist induced myocardial infarcted rats by inhibiting oxidative stress. *Chem. Biol. Interact*. 2016; 244, 159–168.

Nagoor Meeran MF, Javed H, Al Taee H, Azimullah S, Ojha SK. Pharmacological Properties and Molecular Mechanisms of Thymol: Prospects for Its Therapeutic Potential and Pharmaceutical Development. *Front Pharmacol*. 2017; 8:380.

Napolitano F, Izzo MT, Di Giuseppe G, Angelillo IF, Cloeckaert A. Public knowledge. attitudes and experience regarding the use of antibiotics in Italy. *PLoS one.* 2013; 8(12): e84177.

Narangerel T, Bonikowski R, Jastrzabek K, Kunicka-Styczyn´ska A, Plucin´ska A, *et al*. Chemical and Biological Characteristics of Oxytropis pseudoglandulosa Plant of Mongolian Origin. *Mol.* 2021; 26: 7573.

Nasir M, Tafess K, Abate D. Antimicrobial potential of the Ethiopian *Thymus schimperi* essential oil in comparison with others against certain fungal and bacterial species. *BMC complement. med. ther*. 2015; 15:260.

National Center for Biotechnology Information (NCBI b). PubChem Compound Summary for CID 2966,Debrisoquine.https://pubchem.ncbi.nlm.nih.gov/compound/Debrisoquine#section=Depositor-Supplied-Synonyms. Accessed on July 12, 2022.

National Center for Biotechnology Information. PubChem Compound Summary for CID 445238, Methyl beta-D-glucopyranoside. Avaliable at:

https://pubchem.ncbi.nlm.nih.gov/compound/445238#section=Use-and-Manufacturing. Accessed on July 10, 2022.

Nazir H, Aziz M, Mirani Z, Sheikh AS, Saeed MQ, Khan AA, Ruby T, Rauf N. Correlation between antibiotic resistance and phylogenetic types among multidrug-resistant *Escherichia coli* isolated from urinary tract infections. *Iran J Basic Med Sci.* 2021; 24:400-407.

Negarandeh R, Poortaghi S, Shayan SJ. Prevalence and patterns of self-medication with antibiotics among visitors of central polyclinic of Kabul, Afghanistan. *J. Pharm. Health Serv. Res*. 2021; 12 (2): 271–274.

Nepal G, Bhatta S. Self-medication with Antibiotics in WHO Southeast Asian Region: A Systematic Review. *Cureus*. 2018; 10(4): e2428.

Ngu RC, Feteh VF, Kika BT, Nerice EKF, Ayeah CM, Chifor T, Njim T, Fankem AM, Yengo FKF. Prevalence and Determinants of Antibiotic Self-Medication among Adult Patients with Respiratory Tract Infections in the Mboppi Baptist Hospital, Douala, Cameroon: A Cross-Sectional Study. *Diseases*. 2018; 6:49.

Nichols JA, Katiyar SK. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch. Dermatol. Res.* 2010*;* 302:71–83.

Nicolle LE, Bradley S, Colgan R, *et al*. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin Infect Dis*. 2005; 40(5):643–654.

Nicolle LE. Urinary tract infections in long-term-care facilities. *Infect Control Hosp Epidemiol*. 2001; 22(3):167–175.

Nigussie G, Ibrahim F, Neway S. A Phytopharmacological Review on a Medicinal Plant: *Cordia africana Lam. J Trop Pharm Chem*. 2021; 5(3):254–63.

Nigussie G. A review on traditionally used medicinal plants for scabies therapy in Ethiopia. *ADV TRADIT MED (ADTM)*. 2021; 21(2):199–208.

Nindi MM, Kgarebe BV, Wolfender JL, Abegaz BM. Electrospray liquid chromatography-mass spectrometry of the leaf extract of *Rhamnus prinoides*. *Phytochem Anal.* 1999; 10(2):69–75.

Nunes de Melo M, Madureira B, Nunes Ferreira AP, Mendes Z, Miranda Ada C, Martins AP. Prevalence of self-medication in rural areas of Portugal. *Pharm World Sci*. 2006; 28(1):19–25.

Núñez M, Tresierra M, Gil-Olivares F. Antibiotic self-medication in university students from Trujillo. *Peru Med Univ.* 2017; 18(73):205–9.

Nwonuma CO, Adelani-Akande TA, Osemwegie OO, Olaniran AF, Adeyemo TA. Preliminary *In Vitro* Antimicrobial Potential and Phytochemicals Study of Some Medical Plants. *F1000Res*. 2020; 8: 81.

O'Neill J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. UK: Wellcome Trust, 2014.

Obakiro SB, Kiyimbaa K, Paasib G, Napyo A, Anthierens S, Waako P, Royen PV, *et al*. Prevalence of antibiotic-resistant bacteria among patients in two tertiary hospitals in Eastern Uganda. *J. Glob. Antimicrob. Resist.* 2021; 25 (2021): 82–86.

Ocan M, Bwanga F, Waako P, Bbosa SG, Bagenda D, Ogwal-Okeng J, *et al*. Patterns and Predictors of Self-Medication in Northern Uganda. *PLoS one*. 2014; 9(3): 1–7.

Ocan M, Obuku EA, Bwanga F, Akena D, Richard S, Ogwal-Okeng J, Obua C. Household antimicrobial self-medication: a systematic review and meta-analysis of the burden, risk factors and outcomes in developing countries. *BMC Public Health*. 2015; 15:742.

Oluduro AO. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. *Malays. J. Microbiol.* 2012; 8: 59-67.

Omolase CO, Adeleke OE, Afolabi AO, Ofolabi OT. Self-medication amongst general outpatients in a Nigerian community hospital*. Ann Ib Postgrad Med.* 2007; 5: 64-67.

Pagan JA, Ross S, Yau J, Polsky D. Self-medication and health insurance coverage in Mexico. *Health Policy*. 2006; 75:170-7.

Panda A, Pradhan S, Mohapatra G, Mohapatra J. Drug-related problems associated with self-medication and medication guided by prescription: A pharmacy-based survey. *Indian J Pharmacol.* 2016; 48(5):515–521.

Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014; 2(5):115-119.

Panghal M, Kaushal V, Yadav JP. *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. *Ann Clin Microbiol Antimicrob.* 2011; 10:21.

Parajuli NP, Maharjan P, Parajuli H, *et al.* High rates of multidrug resistance among uropathogenic *Escherichia coli* in children and analyses of ESBL producers from Nepal. *Antimicrob Resist Infect Control.* 2017; 6(1): 9.

Parulekar M, Mekoth N, Ramesh CM, Parulekar A. Self-medication in developing countries a systematic review. *J Pharm Technol Res Manag*. 2016; 4(2):103-27.

Patel HB, Soni ST, Bhagyalaxmi A. Patel NM. Causative agents of urinary tract infections and their antimicrobial susceptibility patterns at a referral center in Western India: An audit to help clinicians prevent antibiotic misuse. *J. Fam. Med. Prim. Care*. 2019; 8: 154–159.

Pathak K, Das RJ. Herbal medicine-a rational approach in health care system. *Int J Herbal Med*. 2013; 1:86-89.

Patil AD. Self-medication: a potentially avoidable cause of antibiotic misuse and resistance. *Int J Basic Clin Pharmacol*. 2017; 2(4):2.

Patil SB, Vardhamane S, Patil B, Santoshkumar J, Binjawadgi AS, Kanaki AR. Self-medication practice and perceptions among undergraduate medical students: a cross-sectional study. *J Clin Diagn Res.* 2014; 8(12): HC20-3.

Paudel S, Aryal B. Exploration of self-medication practice in Pokhara valley of Nepal. *BMC Public Health*. 2020; 20:714.

Petersen PJ, Labthavikul P, Jones CH, Bradford PA. *In vitro* antibacterial activities of tigecycline in combination with other antimicrobial agents determined by chequerboard and time-kill kinetic analysis. *J. Antimicrob. Chemother.* 2006; 57(3):573–576.

Piddock L, Garneau-Tsodikova S, Garner C. Ask the experts: how to curb antibiotic resistance and plug the antibiotics gap? *Future Med Chem*. 2016; 8: 1027–1032.

Piddock LJ. The crisis of no new antibiotics: what is the way forward? *Lancet Infect Dis.* 2012; 12 (3): 249–253.

Piddock LJV. Reflecting on the final report of the O'Neill review on antimicrobial resistance. *Lancet Infect Dis.* 2016; 16(7):767–768.

Pizzey RL, Marquis RE, Bradshaw DJ. Antimicrobial effects of o-cymen-5-ol and zinc, alone and in combination in simple solutions and toothpaste formulations. *Int Dent J*. 2011; 61(3):33-40.

Pons EdS, Knauth DR, VigoÂ, PNAUM Research Group, Mengue SS. Predisposing factors to the practice of self-medication in Brazil: Results from the National Survey on Access, Use and Promotion of Rational Use of Medicines (PNAUM). *PLoS One*. 2017; 12(12): e0189098.

Prabhavathi RM, Prasad MP, Jayaramu M. Studies on Qualitative and Quantitative Phytochemical Analysis of Cissus quadrangularis. *Adv. Appl. Sci. Res*. 2016; 7(4):11-17.

Pradhan MR. ICTs application for better health in Nepal. *Kathmandu Univ Med J*. 2004; 2(2):157–63.

Purreza A, Khalafi A, Ghiasi A, Mojahed F, Nurmohammadi M. To identify self-medication practice among Medical Students of Tehran University of Medical Science. *Iran. J. Epidemiology*. 2013; 8(4):40-6.

Rahman AA, Sulaiman SA, Ahmad Z, Daud WNW, Hamid AM. Prevalence and Pattern of Use of Herbal Medicines During Pregnancy in Tumpat District, Kelantan. *Malays J Med Sci*. 2008; 15(3): 40.

Rahman MM, Islam MB, Biswas M, *et al. In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res Notes.* 2015; 8: 621.

Rai GK, Upreti HC, Rai SK, Shah KP, Shrestha RM. Causative agents of urinary tract infections in children and their antibiotic sensitivity pattern: a hospital-based study. *Nepal Med Coll J*. 2008; 10(2): 86-90.

Ralte L, Bhardwaj U, Singh YT. Traditionally used edible *Solanaceae* plants of Mizoram, India have high antioxidant and antimicrobial potential for effective *phytopharmaceutical* and *nutraceutical* formulations. *Heliyon*. 2021; 7(9): e07907.

Rampure R, Gangane R, Oli AK, Chandrakanth K. Prevalence of MDR-ESBL producing *Klebsiella pneumoniae* isolated from clinical samples. *J Microbiol Biotech Res.* 2013; 3:32-39.

Rather IA, Kim B-C, Bajpai VK, Park Y-H. Self-medication and antibiotic resistance: crisis, current challenges, and prevention. *Saudi J Biol Sci.* 2017; 24(4):808.

Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 1997; 2(4):304-9.

Roien R, Bhandari D, Hosseini SMR, Mosawi SH, Ataie MA, *et al*. Prevalence and determinants of self-medication with antibiotics among general population in Afghanistan. *Expert Rev Anti Infect Ther.* 2022; 20(2): 315-321.

Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. Dis Mon. 2003; 49: 71-82.

Rossiter SE, Fletcher MH, Wuest WM. Natural products as platforms to overcome antibiotic resistance. *Chem Rev*. 2017; 117(19):12415–74.

Rouis Z, Abid N, Koudja S, Yangui T, Elaissi A, Cioni PL, *et al*. Evaluation of the cytotoxic effect and antibacterial, antifungal, and antiviral activities of *Hypericum triquetrifolium* Turra essential oils from Tunisia. *BMC Complement Altern Med*. 2013; 13:24.

Rúa J, Del Valle P, de Arriaga D, Fernández-Álvarez L, García-Armesto MR. Combination of Carvacrol and Thymol: Antimicrobial Activity Against *Staphylococcus aureus* and Antioxidant Activity. *Foodborne Pathog Dis.* 2019; 16(9):622-629.

Ruiz ME. Risks of self-medication practices. *Curr Drug Saf*. 2010; 5(4):315–323.

Ruszczycky MW, Liu HW. Biochemistry: The surprising history of an antioxidant. *Nature*. 2017; 551(7678):37-38.

Sado E, Gedi T. Drug utilization at household level in Nekemte Town and surrounding rural areas, western Ethiopia: A cross sectional study. *Open Access J. Sci.* 2014; 1:1-9.

Saeidi S, Amini Boroujeni N, Ahmadi H, Hassanshahian M. Antibacterial activity of some plant extracts against extended- spectrum beta-lactamase producing *E. coli* isolates. *Jundishapur J Microbiol*. 2015; 8(2): e15434.

Saha A, Marma KKS, Rashid A, Tarannum N, Das S, Chowdhury T, et al. (2022) Risk factors associated with self-medication among the indigenous communities of Chittagong Hill Tracts, Bangladesh. *PLoS One*. 2017; 17(6): e026962.

Salem ML. Immunomodulatry and therapeutic properties of Nigella sativa L. Seed. *Int Immun Pharamacol*. 2005; 5:1749–1770.

Salgado PO, Silva LC, Silva PM, Chianca TC. Physical methods for the treatment of fever in critically ill patients: a randomized controlled trial. *Rev Esc Enferm USP*. 2016; 50(5):823-830.

Sambakunsi CS, Småbrekke L, Varga CA, Solomon V, Mponda JS. Knowledge, attitudes and practices related to self-medication with antimicrobials in Lilongwe, Malawi. *Malawi Med J.* 2019; 31(4): 225–232.

Sanchez J. Self-medication practices among a sample of Latino migrant workers in South Florida. *Front Public Health*. 2014; 2:108.

Sapkota AR, Coker ME, Rosenberg Goldstein RE, *et al*. Self-medication with antibiotics for the treatment of menstrual symptoms in Southwest Nigeria: a cross-sectional study. *BMC Public Health*. 2010; 10: 610.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga LL. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med.* 2011; 8(1):1 –10.

Sattar S, Quddus R, Saha SK. Pattern of Self-Medication Practices among Rural Population of Mymensingh. *Mymensingh Med J.* 2018; 27(4):843-850.

Seifu WD, Gebissa AD. Prevalence and antibiotic susceptibility of Uropathogens from cases of urinary tract infections (UTI) in Shashemene referral hospital, Ethiopia. *BMC Infect Dis*. 2018; 18(1):30.

Sekar S, Kandavel D. Interaction of Plant Growth Promoting *Rhizobacteria* (PGPR) and Endophytes with Medicinal Plants– New Avenues for Phytochemicals. *J. Phytol.* 2010; 2:91–100.

Shaamekhi HR, Asghari Jafarabadi M, Alizadeh M. Demographic determinants of self-medication in the population covered by health centers in Tabriz. *Health Promot Perspect*. 2019; 9(3):181-190.

Shabnam N, Azim M. Assessment of antibiotic self-medication practice among public in the northwestern region of Pakistan. *Eur J Hosp Pharm.* 2015; 24:000733.

Shafie M, Eyasu M, Muzeyin K, Worku Y, Martin-Aragon S. Prevalence and determinants of self-medication practice among selected households in Addis Ababa community. *PLoS One*. 2018; 13(3): e0194122.

Shaghaghi A, Asadi M, Allahverdipour H. Predictors of self-medication behavior: a systematic review. *Iran J Public Health*. 2014; 43(2):136-46.

Shah H, Patel R, Nayak S, Patel HR, Sharma D. A questionnaire-based cross-sectional study on self-medication practices among undergraduate medical students of GMERS Medical College, Valsad, Gujarat. *Int. J. Med. Sci. Public Health* 2018; 7(4): 249–254.

Shah SJ, Ahmad H, Rehan RB, *et al.* Self-medication with antibiotics among non-medical university students of Karachi: a cross-sectional study. *BMC Pharmacol Toxicol*. 2014; 15:74.

Shaheed KA, Alsirraj MA, Allaith SA, Noori NA, Obaid MH, Mouhsan ZM, Swedane SS. The biological activities of seeds extracts for fenugreek and black cumin and its inhibitory influences toward some pathogens. *Iraq Med J*. 2018; 2(2): 46–50.

Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects–A review. *J. Funct. Foods.* 2015; 18: 820–897.

Shakya P, Shrestha D, Maharjan E, SharmaVK, Paudyal R. ESBL Production Among *E. coli* and *Klebsiella* spp. Causing Urinary Tract Infection: A Hospital Based Study. *Open J. Med. Microbiol*. 2017; 11: 27.

Shankar PR, Partha P, Shenoy N. Self-medication and non-doctor prescription practices in Pokhara valley, Western Nepal: a questionnaire-based study. *BMC Fam Pract*. 2002; 3:17.

Sharma A, Patel VK, Rawat S, Ramteke P, Verma R. Identification of the antibacterial component of some Indian medicinal plants against *Klebsiella Pneumoniae*. *Int J Pharm Pharm Sci.* 2010; 2:123–7.

Shatalov A. Prevalence and antibiotic resistance pattern of *Escherichia coli* and *Klebsiella pneumoniae* in urine tract infections at the La Paz Medical center, Malabo, Equatorial Guinea. *Open J Med Microbiol*. 2015; 5: 177-183.

Shcherbakova L, Mikityuk O, Arslanova L, Stakheev A, Erokhin D, Zavriev S and Dzhavakhiya V (2021) Studying the Ability of Thymol to Improve Fungicidal Effects of Tebuconazole and Difenoconazole Against Some Plant Pathogenic Fungi in Seed or Foliar Treatments. *Front. Microbiol*. 12:629429.

Sherazi BA, Mahmood KT, Amin F, Zaka M, Riaz M, Javed A. Prevalence and measure of self-medication: a review. *J Pharm Sci Res.* 2012; 4(3):1774–8.

Simegn W, Moges G. Antibiotics Self-Medication Practice and Associated Factors Among Residents in Dessie City, Northeast Ethiopia: Community-Based Cross-Sectional Study. *Patient Prefer. Adherenc*e. 2022:16: 2159–70.

Sisay M, Mengistu G, Edessa D. Epidemiology of self-medication in Ethiopia: a systematic review and meta-analysis of observational studies. *BMC Pharmacol Toxicol*. 2018; 19: 56.

Skliros E, Merkouris P, Papazafiropoulou A, Gikas A, Matzouranis G, Papafragos, *et al*. Self-medication with antibiotics in rural population in Greece: a cross-sectional multicenter study. *BMC Fam Pract*. 2010; 11:58.

Sobel JD, Kaye D. Urinary tract infections. In: Mandell, Gl; Bennett JC, Dolin R, editors. Mandell, Douglas, and Bennett's: Principles and Practice of Infectious Disease. Elsevier; PA, USA: 2010; 957-985.

Sofowra A. Medicinal Plants And traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, 1993; 191-289.

Sohail A, Yaseen A, Qazi SR. Knowledge and practices of patients regarding antibiotics used for dental problems. *J Pak Dent Assoc.* 2020; 29(2):71-76.

Somily AM, Habib HA, Absar MM, *et al.* ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care hospital in Saudi Arabia. *J Infect Dev Ctries*. 2014; 8(9): 1129-36.

Soongand YY, Barlow JP. Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem.* 2004; 88(3): 411–417.

Sridhar SB, Shariff A, Dallah L, Anas D, Ayman M, Rao PG. Assessment of nature, reasons, and consequences of self-medication practice among general population of Ras Al-Khaimah, UAE. *Int J App Basic Med Res.* 2018; 8:3-8.

Stein K, Farmer J, Singhal S, Marra F, Sutherland S, Quiñonez C. The use and misuse of antibiotics in dentistry: A scoping review. *J Am Dent Assoc.* 2018; 149(10):869-884.e5.

Strzepek-Gomółka M, Gaweł-Beben K, Kukula-Koch W. Achillea Species as Sources of Active Phytochemicals for Dermatological and Cosmetic Applications. *Oxid. Med. Cell. Longev.* 2021; 2021: 6643827.

Suleman S, Ketsela A, Mekonnen Z. Assessment of self-medication practices in Assendabo town, Jimma zone, southwestern Ethiopia. *Res Social Adm Pharm.* 2009; 5(1): 76–81.

Sultana J, Cutroneo P, Trifirò G. Clinical and economic burden of adverse drug reactions. *J Pharmacol Pharmacother*. 2013; 4(1): S73.

Sun B, Ricardo-da-Silva JM, Spranger I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* 1998; 46(10): 4267–4274.

Sunny TP, Jacob R, Krishnakumar K, Varghese S. Self-medication: Is a serious challenge to control antibiotic resistance? *Natl J Physiol Pharm Pharmacol*. 2019; 9(9):821-827.

Suswardany DL, Sibbritt DW, Supardi S, Pardosi JF, Chang S, Adams J. A cross-sectional analysis of traditional medicine use for malaria alongside free antimalarial drugs treatment amongst adults in high-risk malaria endemic provinces of Indonesia. *PLoS one.* 2017; 12(3): e0173522.

Sut S, Dall'Acqua S, Sinan KI, Bene K, Kumar G, Mahomoodally MF, Picot-Allain C, Zengin G. Cola caricifolia (G. Don) *K. Schum and Crotalaria retusa L.* from Ivory Coast as sources of bioactive constituents. *Ind Crops Prod.* 2020; 147:112246.

Tambekar D, Dahikar S. Antibacterial activity of some Indian Ayurvedic preparations against enteric bacterial pathogens. *J Adv Pharm Technol Res*. 2011; 2(1):24.

Tambekar DH, Dahikar SB. Exploring antibacterial potential of some ayurvedic preparations to control bacterial enteric infections. *J. Che. & Pharm. Res*. 2010; 2(5): 494-501.

Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious diseases society of america guidance on the treatment of AmpC β-Lactamase-producing Enterobacterales, Carbapenemresistant Acinetobacter baumannii, and Stenotrophomonas maltophilia infections. *Clin. Infect. Dis.* 2022; 74: 2089–2114.

Tayade AB, Dhar P, Kumar J, Sharma M, Chauhan RS, Chaurasia OP, Srivastava RB. Chemometric profile of root extracts of *Rhodiola imbricata Edgew*. with hyphenated gas chromatography mass spectrometric technique. *PLoS One*. 2013; 8(1):e52797.

Taybeh E, Al-Alami Z, Alsous M, Rizik M, Alkhateeb Z. The awareness of the Jordanian population about OTC medications: A cross-sectional study. *Pharmacol Res Perspect*. 2020; 8(1): e00553.

Tedila H, Assefa A, Shanmugam V. *In Vitro* Antibacterial Activity of Ruta Chalepensis (Tenadam) and Justicia Shimperiana (Senel) Plants against Some Bacterial Human Pathogens. *Journal of Health, Medicine and Nursing*. 2019; 63: 24-33.

Teferra A, Alemayehu W. Self-medication in three towns of North West Ethiopia. *Ethiop. J. Health Dev*. 2001; 15(1): 25–30.

Teklehaymanot T, Giday M. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. *J. Ethnobiol. Ethnomedicine*. 2007; 3: 12.

Teklehaymanot T. Ethnobotanical study of knowledge and medicinal plants use by the people in Dek Island in Ethiopia. *J. Ethnopharmacol*. 2009; 124:69–78.

Tesfaye A, Makonnen E, Gedamu S. Hypoglycemic and antihyperglycemic activity of aqueous extract of *Justicia Schimperiana* leaves in normal and streptozotocin-induced diabetic mice. *IJPSR*. 2016; 7(2): 107-113.

Thakur S, Pokhrel N, Sharma M. Prevalence of multidrug resistant Enterobacteriaceae and extended-spectrum β-lactamase producing *Escherichia coli* in urinary tract infection. *Res J Pharm Biol Chem Sci*. 2013; 4:1615 -1624.

Tobin EA, Atulomah NO. Antibiotic Self-Medication among Adult Out-patient Clinic Attendees in Nigeria. *West Afr J Med*. 2020; 37(5):507-514.

Togoobaatar G, Ikeda N, Ali M, Sonomjamts M, Dashdemberel S, Mori R, Shibuya K. Survey of non-prescribed use of antibiotics for children in an urban community in Mongolia. *Bull. World Health Organ.* 2010; 88**:** 930-6.

Torres NF, Solomon VP, Middleton LE. Identifying the commonly used antibiotics for self-medication in urban Mozambique: a qualitative study. *BMJ open*. 2020; 10(12):e041323.

Trombetta D, Castelli F, Sarpietro MG, Venuti, V, Cristani M, Daniele C, *et al*. Mechanisms of antibacterial action of three monoterpenes. *Antimicrob Agents Chemother*. 2005; 49:2474-78.

Umer S, Asres K, Veeresham C. Hepatoprotective activities of two Ethiopian medicinal plants. *Pharm. Biol.* 2010; 48(4): 461-468.

United Nations (UN). Millennium Development Goals Indicators. IN AFFAIRS, E. A. S. (Ed.). United Nation Statistics Division. 2008.

Upadhye M, Dhiman A, Shriwaikar A. Antioxidant activity of aqueous extract of Holostemma Annulare (Roxb) K Schum. *Adv Pharmacol Toxicol*. 2009; 10:127-31.

Van Boeckel TP, Gandra S, Ashok A, *et al*. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect Dis*. 2014; 14:742-50.

Van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. *Virulence*. 2017; 8(4):460–9.

Van Ha T, Nguyen AMT, Nguyen HST. Self-medication practices among Vietnamese residents in highland provinces. *J Multidiscip Healthc*. 2019; 12: 493–502.

Verma RK, Mohan L, Pandey M. Evaluation of self-medication among professional students in North India: proper statutory drug control must be implemented. *Asian J Pharm Clin Res*. 2010; 3:60-4.

Vila J. Update on Antibacterial Resistance in Low-Income Countries: Factors Favoring the Emergence of Resistance. *Open Infect Dis J.* 2010; 4(2):38–54.

Voidăzan S, Moldovan G, Voidăzan L, Zazgyva A, Moldovan H. Knowledge, Attitudes and Practices Regarding the Use of Antibiotics. Study on the General Population of Mureş County, Romania. *Infect Drug Resist.* 2019:12 3385–3396.

Wabe NT, Ahmed D, Angamo MT. Self-medication with antibiotics and antimalarials in the community of Silte Zone, South Ethiopia. *TAF Prev Med Bull*. 2012; 11:529-36.

Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomedicine.* 2009; 16(2-3): 97–110.

Walsh TR, Toleman MA. The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. *J. Antimicrob. Chemother.* 2012; 67: 1-3.

Wattengel BA, DiTursi S, Schroeck JL, Sellick JA, Mergenhagen KA. Outpatient antimicrobial stewardship: Targets for urinary tract infections. *Am J Infect Control*. 2020; 48(9):1009-1012.

Wayne P. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. CLSI; 2012.

Widayati A, Suryawati S, de Crespigny C, *et al.* Self-medication with antibiotics in Yogyakarta City Indonesia: a cross sectional population-based survey. *BMC Res. Notes.* 2011; 4: 491.

Wijesinghe PR, Jayakody RL, Seneviratne RA. Prevalence and predictors of self-medication in a selected urban and rural district of Sri Lanka. *WHO South East Asia J Public Health*. 2012; 1:28-41.

Williams DN. Antimicrobial resistance: Are we at the dawn of the post-antibiotic era? *J. R. Coll. Physicians Edinb.* 2016; 46:150–156.

Win PS, Win T, Soe PP. Self-medication with antibiotics among youths from Myanmar. *Int J Community Med Public Health*. 2021; 8(3):1047-1051.

Wise R, Hart T, Cars O, Streulens M, Helmuth R, Huovinen P, Sprenger M. Antimicrobial resistance. Is a major threat to public health. *BMJ*. 1998; 317(7159):609-10.

Wondimu T, Asfaw Z, Kelbessa E. Ethnobotanical study of medicinal plants around “Dheeraa” town, Arsi Zone, Ethiopia. *J. Ethnopharmacol.* 2007; 112:152–61.

Worku S, G/Mariam A. Practice of self-medication in Jimma town. *Ethiop J Health Dev*. 2003; 17:111-6.

World Health Organization (WHO). New report calls for urgent action to avert antimicrobial resistance crisis. Joint News Release. 2019. Available at: https://www.who.int/news-room/detail/14-08-2021-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis. Accessed on September 23, 2021.

World Health Organization (WHO). The role of the pharmacist in self-care and self-medication. Report of the 4th WHO Consultative Group on the Role of the Pharmacist. The Hague, The Netherlands, 26-28 August 1998. WHO/DAP/98.13. Geneva: WHO, 1998.

World Health Organization (WHO). Traditional Medicine Strategy 2014–2023. Available at: https://www.who.int/publications/i/item/9789241506096. Accessed on September 30, 2021.

World Health Organization (WHO). WHO traditional medicine strategy, 2014-2023. *World Health Organization.* 2013; 1-76.

World Health Organization (WHO). World Health Statistics 2017. Geneva; 2017. Available at: https://www.who.int/gho/publications/world_health_statistics/2017/en/. Accessed on June 25, 2020.

World Health Organization (WHO). Guidelines for the Regulatory Assessment of Medicinal Products for Use in Self Medication; WHO: Geneva, Switzerland, 2000; Available online: http://apps.who.int/medicinedocs/en/d/Js2218e. Accessed on June 23, 2020.

World Health Organization (WHO). World Medicine Situation Report 2011. 2016.

World Self-medication Industry (WSMI). The story of self-care and self-medication: 40 years of progress, 1970-2010. Ferney-Voltaire, France. 2010.

Yasir M, Sultana B, Amicucci M. Biological activities of phenolic compounds extracted from Amaranthaceae plants and their LC/ ESI-MS/MS profiling. *J Funct Foods*. 2016; 26(1756-4646):645- 656.

Yeika EV, Ingelbeen B, Kemah BL, Wirsiy FS, Fomengia JN, Van der Sande MAB. Comparative assessment of the prevalence, practices and factors associated with self-medication with antibiotics in Africa. *Trop Med Int Health.* 2021; 26(8):862-881.

Yineger H, Kelbessa E, Bekele T, Lulekal E. Plants used in traditional management of human ailments at Bale Mountains National Park, South Eastern Ethiopia. *J. Med. Plant Res.* 2008; 2(6): 132–153.

Yitbarek H, Tadesse E, Alehegne W, Takele S. *In-vitro* antimicrobial effect of selected Ethiopian medicinal plants against some bacteria of veterinary importance. *JMPR*. 2010; 4(12):1230 -4.

Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. *Mol.* 2016; 21:1–18.

Yuefeng L, Keqin R, Xiaowei R. Use of and Factors associated with self-treatment In China. *BMC Public Health*. 2012; 12(995): 1471-2458.

Zafar SN, Syed R, Waqar S, Zubairi AJ, Vaqar T, Shaikh M, Yousaf W, Shahid S, Saleem S. Self-medication amongst university students of Karachi: prevalence, knowledge and attitudes. *J Pak Med Assoc.* 2008; 58(4):214.

Zarrini G, Delgosha ZB, Moghaddam KM, Shahverdi AR. Post-antibacterial effect of thymol. *Pharm Biol*. 2010; 48(6):633-6.

Zawahir S, Lekamwasam S, Halvorsen KH, Rose G, Aslani P. Self-medication Behavior with antibiotics: a national cross-sectional survey in Sri Lanka. *Expert Rev Anti Infect Ther*. 2021; 19(10):1341–1352.

Zeid W, Hamed M, Mansour N, Diab R. Prevalence and associated risk factors of self-medication among patients attending El-Mahsama family practice center, Ismailia, Egypt. *Bull Natl Res Cent.* 2020; 44:92.

Zewde D, Dawo F, Hurisa B, Mengesha A, Tadele A. Determination of Anti-Rabies Virus Activities of Crude Extracts from Some Traditionally Used Medicinal Plants in East Wollega, Ethiopia. *IJBAV.* 2019; 8(2): 28-37.

Zhang L, Tu ZC, Xie X, Lu Y, Wang ZX, Wang H. Antihyperglycemic, antioxidant activities of two Acer palmatum cultivars, and identification of phenolics profile by UPLC-QTOF-MS/MS: new natural sources of functional constituents. *Ind. Crop. Prod.*, 2016; 89: 522–532.

Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. *Chin Med*. 2018; 13:20.

Zhang Y, Cai P, Cheng G, Zhang Y. A Brief Review of Phenolic Compounds Identified from Plants: Their Extraction, Analysis, and Biological Activity. Natural Product Communications, 2022; 17(1): 1–14.

Zhou K, Raffoul JJ. Potential anticancer properties of grape antioxidants. *J Oncol*. 2012; 803294.

Annex I (PART-ONE): Information document and Consent form

Research topic: Prevalence and determinants of self-medication practice among selected households in Addis Ababa community, Ethiopia.

Dear participant!

The aim of this study is to measure the prevalence of self-medication practice; assess the knowledge about appropriate self-medication practice; identify common diseases and drugs; and factors affecting self-medication in Addis Ababa, which is expected to forward recommendations to solve the problem.

Procedure:

We invite you to participate in this project. If you are willing to participate in this project, you need to understand and sign the agreement form. Then after, you will be interviewed by data collectors. You do not need to write your name or tell your name to the data collector and all your responses and the results obtained will be kept confidentially by using a coding system whereby no one will have access to your response.

Risk/Discomfort:

By participating in this research project, you may fill that it has some discomfort especially on wasting time about 20 minutes. We hope you will participate in the study for the sake of the benefit of the research result. There is no risk in participating in this research project.

Benefits:

If you participate in this research project, there may not be direct benefit to you but your participation will likely help us to meet the research objective.

Incentives:

You will not be provided any incentives or payment to take part in this project.

Confidentiality and privacy:

The information collected from this research project will be kept confidential. Information will be stored in a file, without your name, and only a code number will be used. It will not be revealed to anyone except the principal investigator, and it will be kept locked with key.

Right to refuse or withdraw:

You have full right to refuse from participating in this research. You can choose not to respond to some or all questions if you do not want to give your response. You have also full right to withdraw from this study at any time you wish without losing any of your rights.

Person to contact:

This research project was reviewed and approved by the IRB Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. If you have any question, you can contact by the following address and you may ask any time you want.

- ☑ Contact person: Mebrahtu Eyasu.
- ☑ Telephone number: +251-913-51-80-25.
- ☑ Email address: wedidellameb@gmail.com/mebratu.eyasu@sphmmc.edu.et

Informed consent:

I hereby confirm that I understand the advantage, nature, contents of this research project, and I consent to participating voluntarily in the research project. I understand that I am at autonomy to withdraw from the project at any time.

- ☑ Signature of participant: _____
- ☑ Date: _____

Approved by:

- ☑ Name of data collector: _____
- ☑ Signature of data collector: _____

Annex-II (PART-ONE): Data Collection Questionnaire (English)

Research topic: Prevalence and determinants of self-medication practice among selected households in Addis Ababa community, Ethiopia.

Part I: Sociodemographic characteristics of study participants

1. Age: _____
2. Sex:
   A. Male B. Female
3. Religion
   A. Orthodox Christian C. Protestant
   B. Muslim D. Others (specify): _____
4. Marital status
   A. Single C. Divorced
   B. Married D. Separated
5. Ethnicity
   A. Amhara C. Tigray
   B. Oromo D. Others (specify): _____
6. Educational Status
   A. Illiterate C. Elementary
   B. Read and write but have no formal education D. Secondary school
   E. Higher education
7. Monthly Income (in Birr): _____
8. Occupation
   A. Student D. Have private owned business
   B. Government employed E. House wife
   C. Employed in private business F. Others _____
9. Family Status
   A. Father C. Child
   B. Mother D. Relative

Part II: Questions related to self-medication

1. Have you ever self-medicated within the past two months?
   A. Yes B. No
2. If No to Q1, what was your reason?
   A. Not to use wrong drug
   B. Fear of side effects
   C. Fear of wrong diagnosis
   D. Fear of wrong use of drug
   E. I had no illness in the specified time
   F. Others, please specify: _____
3. If "yes" to Q1, what type of medication do you use?
   A. Modern medicine
   B. Traditional medicine
   C. Both type
4. If your answer for Q3 is modern medicine, what was your source of information about the drugs?
   A. Health professional
   B. Experience from previous treatment
   C. Friend
   D. Book/internet
   E. Others, please specify: _____
5. If your answer is "modern medicine" for Q3, where do you get the drugs for self-medication?
   A. From pharmacies/drug shops
   B. Left over from previous treatment
   C. From neighbor
   D. Others, please specify: _____
6. How do you request the drug if the source for the drug is drug retail outlets?
   A. By mentioning the name of drug
   B. By mentioning the sign and symptom of illness
   C. By Showing drug container
   D. By showing a piece of paper on which, the name of the drug is written
   E. Others; please specify_____
7. What was the ailment that led you practice self-medication?
   A. Headache
   B. Fever
   C. Cough
   D. Abdominal pain
   E. Toothache
   F. Diarrhea
   G. Peptic ulcer disease
   H. Eye disease
   I. Constipation
   J. Other (specify)_____
8. Could you tell/show me the drug/drugs that you used for self-medication purpose?
   _____
9. Could you please tell me why you practice/prefer self-medication?
   A. Time constraint
   B. Minor illness
   C. Health institution is too far
   D. Emergency case
   E. Self-medication is cheap
   F. I know the drug before
   G. Others (please specify): _____
10. What was the perceived outcome of the self-medication?
    A. Cured from the illness
    B. Get relief from the illness
    C. No improvement
    D. Get worsened
    E. Others (Please specify):_____
11. Was there any special physiologic/pathologic condition while self-medicating?
    A. No special condition
    B. Pregnant
    C. Breastfeeding
    D. Had a chronic disease such as diabetes, hypertension, liver disease, kidney disease, etc
    E. Others (Please specify):_____

Part III: Assessment of knowledge of participant on appropriate SMP

1. Do you know that some drugs?
   A. Cannot be taken with other drugs? A. Yes, I know B. No, I don't know
   B. Cannot be taken with alcoholic drinks? A. Yes, I know B. No, I don't know
   C. Cannot be taken with some kinds of foods? A. Yes, I know, B. No, I don't know
2. Do you know that some drugs are contraindicated or:
   A. Cannot be given to children? A. Yes, I know B. No, I don't know
   B. Cannot be given to pregnants? A. Yes, I know B. No, I don't know
   C. Cannot be given to breast feeding mothers? A. Yes, I know B. No, I don't know
   D. Cannot be taken by people with chronic diseases? A. Yes, I know, B. No, I don't know
3. Do you know that the same drug can be given by oral, injection, topical or other routes?
   A. Yes, I know B. No, I don't know
4. Did you discontinue taking drugs before the date advised by the health care provider?
   A. Yes B. No
5. While taking drugs, do you usually take alcoholic drinks?
   A. Yes B. No
6. Do you share drugs with family members, friends, neighbors, etc.?
   A. Yes B. No
7. Do you believe that the same drug can be a remedy or a poison?
   A. Yes B. No
8. Do you check expiry date of drugs during purchasing or before use?
   A. Yes B. No
9. Would you tell us about your attitude towards self-medication practice?
   A. Agree
   B. Disagree
   C. Depends on the type of disease to be treated and the drug to be used
   D. No comment

Annex-III (PART-ONE): Data Collection Questionnaire (Amharic)

የመጠይቁ መለያ ቁጥር፡ _____

<u>የጥናት ቃለ-መጠይቅ ቅጽ</u>

የጥናቱ ርእስ፡ በአዲስ አበባ ከተማ ነዋሪዎች ውስጥ ራስን በራስ በመድሀኒት የማከም ልማድና ተያያዥ ጉዳዮችን ማጥናት

የዚህ ጥናት ዋና አላማ *በአዲስ አበባ ከተማ ነዋሪዎች ውስጥ ያለውን ራስን በራስ በመድሀኒት የማከም ልማድና ተያያዥ ጉዳዮችን ማጥናትና* መለየት ነው። የሚሰጡን መረጃ ጥናቱን የተሟላ ከማድረጉም በተጨማሪ የህብረተሰቡን የጤና ችግር ከመቅረፍ አንጻር ትልቅ አስተዋጽዖ ይኖረዋል። በዚህ ጥናት ላይ ለመሳተፍ ከተስማሙ ይህ ግለሰባዊ መረጃ ለጥናቱ ብቻ የሚውል ነው። ግልጽ ያልሆነ እና ማብራሪያ የሚያስፈልግዎ ከሆነ ምንም ሳያመነቱ አስተባባሪውን መጠየቅ ይችላሉ።

አሁን በጥናቱ ለመሳተፍ ፍቃደኛ ነዎት? ሀ. አዎ ለ. አይደለሁም

በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ በፊርማዎ ያረጋግጡልን። ፊርማ _____

ለፍቃደኝነትዎ እናመሰግናለን!

<u>ክፍል አንድ ፡ ማህበራዊ ጉዳዮችን የተመለከቱ ጥያቄዎች</u>

1.እድሜዎ ስንት ነው? _____

2.ጾታ ሀ. ሴት ለ. ወንድ

3.ሃይማኖትዎ ምንድን ነው? ሀ. ኦርቶዶክስ ለ. ሙስሊም ሐ. ፕሮቴስታንት መ. ሌላ (ይግለጹት _____)

4. የትዳር ሁኔታ ሀ. ያላገባ ለ. ያገባ ሐ. የፈታ /በሕግ መ. የተለያዩ /የተራራቁ

5.ብሔርዎ ምንድን ነው? ሀ. አማራ ለ. ኦሮሞ ሐ. ትግራይ መ. ሌላ (ይግለጹት_____)

6. የትምህርት ደረጃ
   ሀ. ያልተማረ
   ለ. መጻፍና ማንበብ
   ሐ. የመጀመርያ ደረጃ
   መ. ሁለተኛ ደረጃ
   ሠ. ከፍተኛ ደረጃ

7. ስራ
   ሀ. ተማሪ
   ለ. የመንግስት ሰራተኛ
   ሐ. በግል የስራ ዘርፍ የተቀጠረ
   መ. በራሱ የግል ስራ የተሰማራ
   ሠ. ቤት ስራተኛ
   ረ. ሌላ (ይግለጹት_____)

8. የቤተ-ሰብ ሁኖታ
   ሀ. አባት
   ለ. እናት
   ሐ. ልጅ
   መ. ቤተ-ሰብ

9. ወርሐዊ ገቢ (በብር)፡_____

<u>ክፍል ሁለት፡ “ ራስን በራስ ማከምን በተመለከተ የሚሰበሰብ መረጃ”</u>

1. ባለፉት ሁለት ወራት ውስጥ ታመው ራስዎን በራስዎ አክመዉ ያውቃሉ? ሀ. አዎ ለ. አላዉቅም
2. ለጥያቄ ቁ-1 መልስዎ አላዉቅም ከሆነ፤ ምክንያትዎን ቢገልፁልን?
   ሀ. የተሳሳተ መድሃኒትን እንዳልጠቀም
   ለ. የመድሃኒት የጎንዮሽ ጉዳት በመፍራት
   ሐ. በሽታውን በትክክል መለየት ስለሚከብድ
   መ. መድሃኒቱን በተሳሳተ ሁኔታ እንዳልጠቀም
   ሠ . ሌላ (ይገለፅ) _____
3. ለጥያቄ ቁ-1 መልስዎ አዎ ከሆነ፤ የምን ዓይነት ህክምና መድሐኒት ነበር የተጠቀሙት?
   ሀ. የዘመናዊ ህክምና መድሐኒት ለ. የባህላዊ ህክምና መድሐኒት ሐ. የሁለቱንም ዓይነት ህክምና መድሃኒት
4. ለጥያቄ ቁ-3 መልስዎ የዘመናዊ ህክምና መድሐኒት ከሆነ፤ ስለተጠቀሙት መድሐኒት መረጃውን ከየት ነበር ያገኙት?
   ሀ. ከጤና ባለሙያ ለ. ከበፊት ህክምና ሐ. ከጓደኛ መ. ከመፅሐፍ/ኢንተርኔት ሠ . ሌላ (ይገለፅ) _____
5. ለጥያቄ ቁ-3 መልስዎ የዘመናዊ ህክምና መድሐኒት ከሆነ፤ መድሐኒቱን ከየት ነበር ያገኙት?
   ሀ. ከፋርማሲ/ ከመድሐኒት መደብር
   ለ. ከሌላ ሕክምና ከተረፈ መድሐኒት
   ሐ. ከጎረቤት መ. ከሌላ (ግለፅ)_____
6. መድሐኒቱን የገዙት ከፋርማሲ ከሆነ እንዴት ነበር የጠየቁት?
   ሀ. የመድሐኒቱን ስም በመጥቀስ
   ለ. የሕመሙን ምልክት በመጥቀስ
   ሐ. የበፊት የመድሐኒቱን መያዥያ በመያዝ
   መ. በብጣሽ ወረቀት የመድሐኒቱን ስም በመያዝ
   ሠ . ሌላ (ይገለፅ) _____
7. መድሐኒቱ የተጠቀሙት ምን ለማከም ነበር?
   ሀ. ራስ ምታት
   ለ. ትኩሳት
   ሐ. ሳል
   መ. ለሆድ ህመም
   ሠ. ጥርስ ህመም
   ረ. ተቅማጥ
   ሰ. የጨጓራ ሕመም
   ቀ. ለዓይን ህመም
   ሸ. ለሆድ ድርቀት
   ኘ. ሌሎች /ግለፅ/ _____

8. ራስዎን በራስዎ ለማከም የተጠቀሙትን ዘመናዊ መድሃኒት ቢጠቅሱልን ?
   _____
   _____

9. ራስዎን በራስዎ እንዲያክሙ ያነሳሳዎ ምክንያት ምንድን ነበር?
   ሀ. መታከሚያ ጊዜ ስለሌለኝ
   ለ. ቀላል በሽታ ስለሆነ
   ሐ. የጤና ተቋሙ ሩቅ ስለሆነ
   መ. ህመሙ ድንገተኛ ስለሆነ
   ሠ. ርካሽ ስለሆነ
   ረ. መድሐኒቱን ስለማዉቀዉ
   ሰ. ሌላ ምክንያት ካለ (ቢገልፁልን)_____
10. ራስዎን በራስዎ ሲያክሙ ውጤቱ ምን ነበር?
    ሀ. ከህመሙ መሉ ለሙሉ ድኛለሁ
    ለ. ሕመሙ ተሸሎኛል
    ሐ. አልተሻለኝም
    መ. ሕመሙ ባሰብኝ
    ሠ. ሌላ (ቢገልፁልን)_____
11. ከህመሙ በተጨማሪ መድሐኒቱን ሲጠቀሙ የነበሩበት ሁኔታ ከነበረ ቢገልፁልን? ለምሳሌ፡ ሀ. የተለየ ነገር አልነበረኝም ለ. እርጉዝ ነበርኩ ሐ. አጠባ ነበር መ. ለመዳን የሚያስቸግሩ በሽታዎች (የደም ግፊት መጨመር፣ የሥኳር በሽታ፣ የኩላሊት በሽታ፣ወዘተ) ታማሚ ነበርኩ ሠ. ሌላ (ቢገልፁልን)_____

ክፍል ሶስት፡ ተጠቃሚዉ ስለመድሀኒት ያለዉን እዉቀት መለኪያ ክፍል

1. አንዳንድ መድሀኒቶች ከሚከተሉት ነገሮች ጋር በተመሳሳይ ሰአት እንደማይወሠዱ ያዉቃሉ?
   ሀ. ከሌሎች መድሀኒቶች ጋር አዉቃለሁ_____ አላዉቅም_____
   ለ. ከአልኮል መጠጦች ጋር አዉቃለሁ_____ አላዉቅም_____
   ሐ. ከአንዳንድ አይነት ምግቦች ጋር አዉቃለሁ_____ አላዉቅም_____
2. አንዳንድ መድሀኒቶችን የሚቀጥሉት አይነት ሠዎች እንደማይወሥዷቸዉ ያዉቃሉ?
   ሀ. ህፃናት አዉቃለሁ_____ አላዉቅም_____
   ለ. ነፍሰጡርና አዉቃለሁ_____ አላዉቅም_____
   ሐ. የሚያጠቡ እናቶች አዉቃለሁ_____ አላዉቅም_____
   መ. አንዳንድ ለመዳን የሚያስችግሩ በሽታዎች (የደም ግፊት መጨመር፣ የሥኳር በሽታ፣ የኩላሊት በሽታ፣ወዘተ) ታማሚ ሠዎች አዉቃለሁ_____ አላዉቅም_____
3. አንድ መድሀኒት በተለያየ መንገድ ለምሳሌ በቅባት መልክ ፤በመርፌ ፤በኪኒን በሽሮፕ ወዘተ ሊሠጥ እንደሚችል ያዉቃሉ? አዉቃለሁ_____ አላዉቅም_____
4. መድሀኒቶችን መዉሰድ ከጀመሩ በኋላ የጤና ባለሙያዉ ዉሰድ ካለዉ ጊዜ ቀድመዉ አቋርጠዉ ያዉቃሉ? አዉቃለሁ_____ አላዉቅም_____
5. መድሀኒት በሚወስዱበት ጊዜ የአልኮል መጠጥ ይጠጣሉ?
   ሀ. አዎ ለ. አልጠጣም
6. የሚወስዱትን መድሀኒት ለቤተሰብ አባል ፤ ለጓደኛ ፤ ለጎረቤት ይሠጣሉ (ያጋራሉ)?
   ሀ. አዎ ለ. አልሠጥም
7. አንድ መድሀኒት በሽታን የሚያድነዉን ያክል ከባድ ጉዳት ሊያመጣ (መርዝ ሊሆን) እንደሚችል መረጃ አለዎት?
   ሀ. አዎ ለ. የለኝም
8. መድሀኒቶችን ከመግዛትዎም ሆነ ከመጠቀምዎ በፊት የመጠቀሚያ ጊዜያቸዉን የማጣራት ልምድ አሎት?
   ሀ. አዎ ለ. የለኝም
9. ራስን በራስ በመድሃኒት ስለማከም ያለዎትን የግል አስተያየት ቢገልፁልን;
   ሀ. እስማማለሁ
   ለ. አልስማማም
   ሐ. እንደ ህመሙ እና እንደ መድሀኒቱ ዓይነት
   መ. የምለዉ የለኝም

PART TWO: Annex I: RESEARCH CONSENT FORM

Dear participants!

My name is _____, I am currently a member of data collectors for this research which is conducted by Mebrahtu Eyasu on the study intended to assess *"antibiotic self-medication practice among adult patients suspected with urinary tract infections in Addis Ababa, Ethiopia"* which is expected to forward recommendations to solve the problem.

Procedure:

We invite you to participate in this project. If you are willing to participate in this project, you need to understand and sign the agreement form. Then after, you will be interviewed by data collectors. You do not need to write your name or to tell your name to the data collector and all your responses and the results obtained will be kept confidentially by using coding system whereby no one will have access to your response.

Risk/Discomfort:

By participating in this research project, you may fill that it has some discomfort especially on wasting time about 20 minutes. We hope you will participate in the study for the sake of the benefit of the research result. There is no risk in participating in this research project.

Benefits:

If you participate in this research project, there may not be direct benefit to you but your participation is likely help us to meet the research objective.

Incentives:

You will not be provided any incentives or payment to take part in this project.

Confidentiality and privacy:

The information collected from this research project will be kept confidential. Information will be stored in a file, without your name, only code number is used. It will not be revealed to anyone except the principal investigator and it will be kept locked with key.

Right to refuse or withdraw:

You have full right to refuse from participating in this research. You can choose not to respond to some or all question if you do not want to give your response. You have also full right to withdraw from this study at any time you wish without losing any of your right.

Person to contact:

This research project was reviewed and approved by the Institutional Review Board (IRB) Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. If you have any question, you can contact any of the following individuals and you may ask any time you want.

- ☑ Contact address of principal investigator: Mebrahtu Eyasu Belete (B. pharm, MSc, Assistant Professor); Telephone: +251-913-51-80-25.

Email: wedidellameb@gmail.com/mebratu.eyasu@sphmmc.edu.et/mebratupharmacology@gmail.com

Informed consent: I hereby confirm that I understand the contents of this document and the nature of the research project, and I consent to participating voluntarily in the research project. I understand that I am at autonomy to withdraw from the project at any time.

Signature of participants: _____; Date: _____

Name and signature of data collector: _____; Date: _____

PART- TWO: Annex II: Data Extraction Questionnaire (English)

Research topic: Antibiotic self-medication practice of adult patients suspected of urinary Tract Infections in Addis Ababa, Ethiopia.

Part I. <u>Sociodemographic characteristics of the patients</u>

1. Age: _____
2. Sex:_____
3. Religion

A. Orthodox Christian
B. Muslim
C. Protestant
D. Others (specify): _____

4. Marital status

A. Single
B. Married
C. Divorced
D. Separated
E. Widowed
F. Other _____

5. Educational Status

A. Illiterate
B. Read and write but have no formal education
C. Elementary
D. Secondary school
E. Above secondary school

6. Income level per month (in Birr): _____
7. Occupation

A. Student
B. Government employed
C. Employed in private business
D. Have private owned business
E. House wife
F. Others _____

8. Place of residence

A. Urban B. Rural

Part II. Knowledge towards antibiotics

1. Have you heard the word antibiotics?

A. Yes B. No

2. What are antibiotics used for? (Check more than one if applicable)

A. Virus infection
B. Bacterial infection
C. Others (specify)_____

3. Which of the following statement(s) about antibiotics is (are) correct? (True/False)

A. Broad-spectrum antibiotics are better than narrow-spectrum ones A. True B. False
B. Higher doses result in faster recovery A. True B. False
C. Lower doses result in less adverse reactions A. True B. False
D. Switching antibiotics enhances drug effects A. True B. False
E. Switching antibiotics reduces adverse reactions A. True B. False
F. Intravenous is better than oral medication A. True B. False
G. Antibiotic self-medication could cause drug resistance A. True B. False
H. Antibiotic self-medication can be practiced for all drugs A. True B. False
J. Antibiotic self-medication can result into disease complication A. True B. False
I. Antibiotic self-medication can result into harmful effects A. True B. False

4. Do you know that some drugs:

A. Cannot be taken with other drugs? A. Yes, I know B. No, I don't know
B. Cannot be taken with alcoholic drinks? A. Yes, I know B. No, I don't know
C. Cannot be taken with some kinds of foods? A. Yes, I know B. No, I don't know

5. Do you know that some drugs are contraindicated or?

A. Cannot be given to children? A. Yes, I know B. No, I don't know
B. Cannot be given to pregnant? A. Yes, I know B. No, I don't know
C. Cannot be given to breast feeding mothers? A. Yes, I know B. No, I don't know
D. Cannot be taken by people with chronic diseases? A. Yes, I know B. No, I don't know

6. Do you know that the same drug can be given by oral, injection, topical or other routes?

A. Yes, I know B. No, I don't know

7. Do you check the expiry date of the drugs you purchase or before use?

A. Yes B. No

Part III. Antibiotic Self-medication behaviors of the patients

1. Did you treat yourself with antibiotics in the past 12 months?

A. Yes B. No

2. If *your response is* NO to Question number one (Q1), what was your reason?
   A. Not to use wrong drug
   B. Fear of side effects
   C. Fear of wrong diagnosis
   D. Fear of wrong use of drug
   E. I had no illness in the specified time
   F. Others (specify)_____

If your response is NO (for questioner number 1), please go to Part IV Question 1.

3. How many times did you treat yourself with antibiotics in the past one year? _____

   If your response is NO (for questioner number 1) and no self-medication within 12 months, please go to Part III Question

4. What was (were) your reason(s) of self-medication with antibiotics? (Check more than one if applicable)
   A. Time constraint
   B. Minor illness
   C. Health institution is too far
   D. Emergency case
   E. Self-medication is cheap
   F. I know the drug before
   G. Others (specify): _____

5. For which of the following complaint(s) did you use antibiotics? (check more than one if applicable)
   A. Headache
   B. Fever
   C. Cough
   D. Abdominal pain
   E. Toothache
   F. Diarrhea
   G. Peptic Ulcer Disease
   H. Eye disease
   I. Constipation
   J. Others (specify)_____

6. Your selection of antibiotics was based on… (check more than one if applicable)
   A. Recommendation by community pharmacists
   B. Opinion of family members
   C. Opinion of friends
   D. My own experience
   E. Previous doctor's prescription
   F. Others (specify)_____

7. What did you consider when selecting antibiotics? (check more than one if applicable)
   A. Type of antibiotics
   B. Brand of antibiotics C. Price of antibiotics D. Indications for use E. Adverse reactions F. Others (specify)__

8. Where did you usually obtain antibiotics from for self-medication? (check more than one if applicable)
   A. Community Pharmacists
   B. Leftover from previous prescription
   C. From neighbor
   D. Others (specify)_____

9. How do you request the drug if the source for the drug is drug retail outlets?
   A. By mentioning the name of the drug
   B. By mentioning the sign and symptom of illness
   C. By showing drug container
   D. By showing a piece of paper on which, the name of the drug is written
   E. Others (specify) _____

10. Did you ever check the instructions come with the package insert of antibiotics for self-treatment?
    A. Yes, always. B. Yes, sometimes C. Never

    If your response is Never, please, go to Question 12

11. How much did you understand the instructions?
    A. Fully understood
    B. Partly understood
    C. Did not understand at all

12. How did you know the dosage of antibiotics? (Check more than one if applicable)
    A. By checking the package insert
    B. By consulting a pharmacist
    C. By consulting family members/friends
    D. From the Internet
    E. From my previous experience
    F. By guessing the dosage by myself
    G. Other (specify)_____

13. Did you ever change the dosage of antibiotics deliberately during the course of self-treatment?
    A. Yes, always
    B. Yes, sometimes
    C. Never

If your response is never, please, go to Question 15.

14. Why did you change the dosage of antibiotics during the course of self-treatment? (Check more than one if applicable)
    A. Improving conditions
    B. Worsening conditions
    C. To reduce adverse reactions
    D. Drug insufficient for complete treatment
    E. Others (specify)_____

15. Did you ever switch antibiotics during the course of self-treatment?
    A. Yes, always
    B. Yes, sometimes
    C. Never

If your response is never, please, go to Question 17

16. Why did you switch antibiotics during the course of self-treatment? (Check more than one if applicable)
    A. The former antibiotics did not work
    B. The former antibiotics ran out
    C. The latter one was cheaper
    D. To reduce adverse reactions

E. Others (specify)_____

17. How many different antibiotics did you take maximally during a single illness? _____
18. Are you concerned that you might have taken counterfeit antibiotics?
    A. Yes, very much B. Yes, somewhat C. No
19. Have you ever found out that you had taken the same antibiotics with different names at the same time? A. Yes B. No

If *your response is No*, please, go to Question 21.

20. What did you do for the adverse reactions during taking the same antibiotics with different antibiotics? (Check more than one if applicable)
    A. Stopped taking antibiotics
    B. Switched to another antibiotic
    C. Consulted pharmacy staff
    D. Consulted family members/friends
    E. Nothing
    F. Others (specify)_____
21. When did you normally stop taking antibiotics? (Check more than one if applicable)
    A. After a few days regardless of the outcome
    B. After symptoms disappeared
    C. A few days after the recovery
    D. After antibiotics ran out
    E. At the completion of the course
    F. After consulting a doctor/pharmacist
    G. Others (specify)_____

22. Please write down the names of antibiotics you have ever taken for self-medication: _____
23. What do you think about self-medication with antibiotics for self-health care?
    A. Good practice
    B. Acceptable practice
    C. Not acceptable practice
24. What was the perceived outcome of the self-medication?
    A. Cured from the illness
    B. Get relief from the illness
    C. No improvement
    D. Get worsened
    E. Others (specify): _____
25. Do you think you can treat common infectious diseases with antibiotics successfully by yourself?
    A. Yes, I can B. Not sure C. No, I cannot

Part-IV: Clinical variables of study participants (from patient chart)

1. Co-infection_____
2. Current symptom of the patient: A. Symptomatic_____ B. Asymptomatic _____
3. History of catheterization: A. Yes_____ B. No_____
4. History of UTIs: A. Yes_____ B. No_____

PART V. URINE CULTURE AND SENSITIVITY

1. Antimicrobial susceptibility test against bacterial isolates among UTIs suspected patients (Marked as: **S**: sensitive, **I**: Intermediate and **R**: resistant).

Bacteria	No. isolates	Amp	C	Aml	SXT	CIP	CRO	F	CN	NA	KF	NOR	DO	TE	others
E. coli															
Other bacteria															

Note: Amp=Ampicillin; Aml=Amoxicillin; C=Chloramphenicol; NA=Nalidixic Acid; F=Nitrofurantoin; CN=Gentamicin; CIP=Ciprofloxacin; KF=Cephalothin; CRO=Ceftriaxone; NOR=Norfloxacin; DO=Doxycycline; SXT=Trimethoprim-Sulfamethoxazole; TE=Tetracycline and others.

PART TWO: **<u>የጥናት መጠይቅ</u>**
<u>በቅዱስ ጳውሎስ ሆስፒታል ሚሌንየም ሜዲካል ኮሌጅ</u>
<u>የጥናቱ ተሳታፊዎች የስምምነት ቅፅ</u>

የጥናት ርዕስ: በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ ውስጥ ህክምና የሚከታተሉ አዋቂ ታካሚዎች የፀረ-ተሀዋሲያን መድሐኒቶችን የተላመዱ ባክተርያዎች የሽንት ቱቦ ኢንፌክሽን ያላቸውንና የሌላቸውን ስለ የፀረ ተሀዋሲያን መድሐኒቶችን በመጠቀም ራስ በራስ የማከም ስርጭት እና ተያያዥ የተጋላጭነት ምክንያቶች ጥናት ይመለከታል።

የዚሁ ጥናት ዋና ተመራማሪ ረዳት ፕሮፌሰር መብራቱ እያሱ ይባላል፡:

እንደምንአደሩ /እንደምንዋሉ! ..... ይባላል። የፀረ ተሀዋሲያን መድሐኒቶችን የተላመዱ ባክተርያዎች የሽንት ቱቦ ኢንፌክሽን ያላቸውን የሌላቸውን ስለ የፀረ ተሀዋሲያን መድሐኒቶችን በመጠቀም ራስ በራስ የማከም ልማድና እና ተያያዥ የተጋላጭነት ምክንያቶች ጥናት በመሰብሰብ ላይ ነኝ።

ተሳትፎዎት ለቃለ መጠይቁ ፈቃደኛ በመሆን ላይ የተመሰረተ ነው። የእርስዎ ስም እና ሌሎች የግል መረጃ በዚህ ወረቀት ላይ አይመዘገብም ።: ለእኛ የሚሰጡት መረጃ በሚስጥር ይያዛል አንዲሁም ለዚህ ጥናት ዓላማ ብቻ ጥቅም ላይ ይውላል። በዚህ ጥናት ውስጥ እያንዳንዱ ተሳታፊ መለያ ኮድ ቁጥር ይሰጠዋል።. ቃለ መጠይቁ 20 ደቂቃዎች ይፈጃል እንዲሁም በፈቃደኝነት ላይ የተመሰረተ ነው።በቃለ መጠይቁ ላይ መሳተፎ በመደበኛ ሕክምና ላይ ተጽዕኖ አይኖረውም። ይህንንም ቃለመጠይቅ በማንኛውም ጊዜ ማቋረጥ መብት አሎት። ይህ ጥናት በቅዱስ ጳውሎስ ሆስፒታል ሚሊኒየም ሜዲካል ኮሌጅ የምርምር ግምገማ ቦርድ ፀድቋል.።በዚህ ጥናት ውስጥ ተሳታፊ ከሆኑ ስለ መብቶ በተመለከተ ማንኛውም ጥያቄ ካለዎት አንዲሁም የእርስዎ ደህንነት አደጋ ላይ እንደሆነ ከተሰማዎት የቅዱስ ጳውሎስ ሆስፒታል የሚሌኒየም ሜዲካል የኮሌጅ የምርምር ቢሮ ከታች በተጠቀሰው ስልክ ቁጥር ማነጋገር ይችላሉ::

ስልክ፡ + 251-11-2-73-26-39 (መብራሀቱ አብርሃ)

ይሄን ጥናት በተመለከተ ማንኛውም ጥያቄዎች ቢኖሮት ወይም የተሻለ ማብራሪያ ካስፍለጎት እኔን ((ስልክ: + 251-913518025)) በተጠቀሰው ስልክ ቁጥር ደውለው መጠየቅ ይችላሉ ።

ጥያቄ ቁጥር አንድ በጥናቱ ላይ ለመሳተፍ ፈቃደኛነህ?

ሀ. ነኝ ለ. አይደለሁም

ጥያቄ ቁጥር አንድ የእርስዎ ምላሽ ነኝ ከሆነ በቀረበው ቦታ ላይ ስሞት እና ፊርማዎት በመፃፍ ያረጋግጡ:

_____

ከሰላምታ ጋር,

PART-TWO: Annex III: Data Collection Questionnaire (Amharic)
<u>የጥናት መጠይቅ</u>

<u>ክፍል 1 ፡ ማህበራዊ ጉዳዮችን የተመለከቱ ጥያቄዎች</u>

1.እድሜዎ ስንት ነው? _____
2.ጾታ፡ ሀ. ወንድ ለ. ሴት
3.ሃይማኖትዎ ምንድን ነው? ሀ. ኦርቶዶክስ ለ. ሙስሊም ሐ. ፕሮቴስታንት መ. ሌላ (ይግለጹት _____)
4. የትዳር ሁኔታ ሀ. ያላገባ ለ. ያገባ ሐ. የፈታ /በሕግ መ. የተለያዩ /የተራራቁ ሠ. የመተበት
5. የትምህርት ደረጃ
ሀ. ያልተማረ
ለ. መጻፍና ማንበብ የሚችል ብቻ
ሐ. የመጀመርያ ደረጃ
መ. ሁለተኛ ደረጃ
ሠ. ከሁለተኛ ደረጃ በላይ
6. ስራ
ሀ. ተማሪ
ለ. የመንግስት ሰራተኛ
ሐ. በግል የስራ ዘርፍ የተቀጠረ
መ. በራሱ የግል ስራ የተሰማራ
ሠ. ቤት ስራተኛ
ረ. ሌላ (ይግለጹት_____)
7. ወርሐዊ ገቢ (በብር)፡_____
8. የመኖርያ ቦታ ሀ. ከተማ ለ. ገጠር

<u>ክፍል 2 ፡</u> " **<u>ራስን በራስ በፀረ-ተሓዋስያን መድሃኒቶች ማከምን በተመለከተ ያሉት ዕውቀት የሚዳስስ መጠይቅ</u>**

1. ፀረ-ተሓዋስያን መድሃኒቶች ሲባል ታቃቸዋለህ? ሀ. አዎ ለ. አይደለም
(መልስዎ አይደለም ከሆነ ምሳሌ <u>መድሃኒቶች</u> ይሰጠዋል)
2. ፀረ-ተሓዋስያን መድሃኒቶችን ለምን ይጠቀማሉ? ሀ. የቫይረስ በሽታ ለማከም; ለ. የባክተርያ በሽታ ለማከም; ሐ. ሌላ (ግለፅ): _____
3. ለሚከተሉት ጥያቄዎች እውነት = 1 ወይም ውሸት = 2 በል።
ሀ. ብዙ ተሓዋሰያን የሚገድሉ መድሃኒቶች ከጥቂት ዝርያ ከሚገድሉ መድሃኒቶችን የተሻሉ ናቸው ::
ለ. ትልቅ የመድሃኒቶችን መጠን መጠቀም ቶሎ እንድታገግም ያደርጋል።
ሐ. ትንሽ የመድሃኒቶችን መጠን መውሰድ ትንሽ የመድሃኒቶችን የጎንዮሽ ጉዳት ያደርሳል።
መ. የፀረ-ተሓዋስያን መድሃኒቶችን መቀያየር የመድሃኒቱ ውጤት ይጨምራል።
ሰ. የፀረ-ተሓዋስያን መድሃኒቶችን መቀያየር የመድሃኒቶችን የጎንዮሽ ጉዳት ይቀንሳል።
ሠ. በደም ስር የሚሰጡ የፀረ-ተሓዋስያን መድሃኒቶችን በአፍ ከሚሰጡ መድሃኒቶችን የተሻሉ ናቸው።
ረ. በፀረ-ተሓዋስያን መድሃኒቶች ራስዎትን በራሰዎት ማከም ተሃዋስያን መደሃኒቶችን እንዲቋቋሙ ያደርጋል።
ሸ. በፀረ-ተሓዋስያን መድሃኒቶችን ራስዎትን በራሰዎት ማከም በሁሉም ፀረ-ተሓዋስያን መድሃኒቶችን ማድረግ ይችላል።
ቀ. በፀረ-ተሓዋስያን መድሃኒቶችን ራስዎትን በራሰዎት ማከም ህመምዎት ወደ ከፋ ደረጃ እንዲደርስ ሊያደርገው ይችላል።
ቸ. በፀረ-ተሓዋስያን መድሃኒቶችን ራስዎትን በራሰዎት ማከም የከፋ ጉዳት ማድረስ ይችላል።
4. ዋና ዋና የፀረ-ተሓዋስያን መድሃኒቶችን የጎንዮሽ ጉዳት ግልጽ።_____
5. አንዳንድ መድሀኒቶች ከሚከተሉት ነገሮች ጋር በተመሳሳይ ሰአት እንደማይወሠዱ ያዉቃሉ?
ሀ. ከሌሎች መድሀኒቶች ጋር ; አዉቃለሁ_____ አላዉቅም_____
ለ. ከአልኮል መጠጦች ጋር; አዉቃለሁ _____ አላዉቅም_____
ሐ. ከአንዳንድ አይነት ምግቦች ጋር; አዉቃለሁ _____ አላዉቅም_____
6. አንዳንድ መድሀኒቶችን የሚቀጥሉት አይነት ሠዎች እንደማይወሥዷቸዉ ያዉቃሉ?
ሀ. ህፃናት; አዉቃለሁ_____ አላዉቅም_____
ለ. ነፍሰጡርና; አዉቃለሁ _____አላዉቅም_____
ሐ. የሚያጠቡ እናቶች; አዉቃለሁ _____ አላዉቅም_____

7. አንዳንድ ለመዳን የሚያስቸግሩ በሽታዎች (የደም ግፊት መጨመር፣ የሥኳር በሽታ፣ የኩላሊት በሽታ፣ወዘተ) ታማሚ ሠዎች አዉቃለሁ_____ አላዉቅም_____
8. አንድ መድሀኒት በተለያየ መንገድ ለምሳሌ በቅባት መልክ ፤በመርፌ ፤በኪኒን በሽሮፕ ወዘተ ሊሠጥ እንደሚችል ያዉቃሉ? አዉቃለሁ_____; አላዉቅም_____

9. መድሀኒቶችን ከመግዛትዎም ሆነ ከመጠቀምዎ በፊት የመጠቀሚያ ጊዜያቸዉን የማጣራት ልምድ አሎት?
ሀ. አዎ ለ. የለኝም

<u>ክፍል 3 " ራስን በራስ በፀረ ተህዋሲያን መድሃኒቶች የማከምን ባህሪ በተመለከተ የቀረበ መጠይቅ</u>

1. በፀረ ተህዋሲያን መድሃኒቶች (በባለፈው አንድ ዓመት) ራስዎን በራስዎ አክመዉ ያውቃሉ? ሀ. አዎ ; ለ. አላዉቅም
መልስዎ አላዉቅም ከሆነ ወደ ጥያቄ ቁ-**2** ይሂዱ
መልስዎ አዎ ከሆነ ወደ ጥያቄ ቁ-**3** ይሂዱ
2. ለጥያቄ ቁ-**1** መልስዎ አላዉቅም ከሆነ፤ ምክንያትዎን ቢገልፁልን?
ሀ. የተሳሳተ መድሃኒትን እንዳልጠቀም
ለ. የመድሃኒት የጎንዮሽ ጉዳት በመፍራት
ሐ. በሽታውን በትክክል መለየት ስለሚከብድ
መ. መድሃኒቱን በተሳሳተ ሁኔታ እንዳልጠቀም
ሰ. ሌላ : _____

መልስዎ ለጥያቄ ቁ-**1** አላዉቅም ከሆነ በቀጥታ ወደ <u>ክፍል **4**</u> መጠይቅ ቁጥር አንድ ይሂዱ ::

3. በባለፈዉ አንድ ዓመት ምን ያክል ግዜ ራስዎት በራስዎት አክመዉ ያውቃሉ? _____
4. ራስዎን በራስዎ እንዲያክሙ ያነሳሳዎ ምክንያት ምንድን ነበር?
ሀ. መታከሚያ ጊዜ ስለሌለኝ
ለ. ቀላል በሽታ ስለሆነ
ሐ. የጤና ተቋሙ ሩቅ ስለሆነ
መ. ህመሙ ድንገተኛ ስለሆነ
ሠ. ርካሽ ስለሆነ
ረ. መድሐኒቱን ስለማዉቀዉ
ሰ. ሌላ (ቢገልፁልን)_____
5. መድሐኒቱ የተጠቀሙት ምን ለማከም ነበር?
ሀ. ራስ ምታት
ለ. ትኩሳት
ሐ. ሳል
መ. ለሆድ ህመም
ሠ. ጥርስ ህመም
ረ. ተቅማጥ
ሰ. የጨጓራ ሕመም
ቀ. ለዓይን ህመም
ሸ. ለሆድ ድርቀት
ኘ. ሌሎች /ግለፅ/ _____
6. የፀረ ተህዋሲያን መድሃኒቶች ራስዎን በራስዎ ለማከም **መረጃውን** ከየት ነበር ያገኙት?
ሀ. በፋርማሲሰቶች ምክር
ለ. በቤተሰቦቼ ምክር
ሐ. የጓደኖቼ ምክር
መ. ከራሴ ልምድ
ሠ. ካለፈው የሓኪም ማዘዣ
ረ. ሌሎች /ግለፅ/ _____
7. የፀረ ተህዋሲያን መድሃኒቶችን ስመርጡ ምንም መሰረት አድርገው ይመርጣሉ?
ሀ. የፀረ ተህዋሲያን መድሃኒቶችን አይነት
ለ. የፀረ ተህዋሲያን መድሃኒቶችን የንግድ ስም
ሐ. የፀረ ተህዋሲያን መድሃኒቶችን ዋጋ
መ. የፀረ ተህዋሲያን መድሃኒቶችን ጥቅም
ሠ. የፀረ ተህዋሲያን መድሃኒቶችን የጎንዮች ጉዳት
8. ስለተጠቀሙት የፀረ ተህዋሲያን መድሐኒት ፡ መድሐኒቱን ከየት ነበር ያገኙት?
ሀ. ከፋርማሲ/ ከመድሐኒት መደብር
ለ. ከሌላ ሕክምና ከተረፈ መድሐኒት
ሐ. ከጎረቤት
መ. ከሌላ (ግለፅ)_____
9. መድሐኒቱን የገዙት ከፋርማሲ ከሆነ እንዴት ነበር የጠየቁት?
ሀ. የመድሐኒቱን ስም በመጥቀስ
ለ. የሕመሙን ምልክት በመጥቀስ
ሐ. የበፊት የመድሐኒቱን መያዥያ በመያዝ
መ. በብጣሽ ወረቀት የመድሐኒቱን ስም በመያዝ
ሠ. ሌላ (ይገለፅ) _____
10. በመድሃኒቱ ውስጥ የሚገኘው የመድሃኒቱን መረጃ የያዘ ወረቀት የአጠቃቀም መመርያው ታነበዋለህ?
ሀ. አዎ ሁሌም ; ለ. አድሪጌውም አላዉቅም ; ሐ. አዎ አንዳዴ
መልስዎ አድሪጌውም አላዉቅም ከሆነ ወደ ቀጣይ ጥያቄ 12 ያምሩ።
11. መመርያው ምን ያክል ይረዱታል?
ሀ. ሙሉ በሙሉ; ለ. በከፊል (_____ ፐርሰንት); ሐ. ሙሉ በሙሉ አይገባኝም
12. የመዳሃኒቶችን የሚወስዱበት መጠን እንዴት ነው የሚያውቁት?
ሀ. በመድሃኒቱ ውስጥ ከሚገኘው የመድሃኒቱን የአጠቃቀም መመርያ
ለ. ፋርማሲስቶችን በማማከር
ሐ. ቤተሰቦችና ጓደኞቼን በማማከር
መ. ኢንተርኔት
ሠ. ከራሴ ልምድ
ሰ. በመገመት
ሸ. ሌሎች /ግለፅ/ _____
13. ሆን ብለው ሲወስዱት የነበረውን የመድሃኒቶች መጠን ራስዎትን በራስዎ በሚያክሙበት ወቅት ቀይረው ያውቃሉ?
ሀ. አዎ ሁሌም ለ. አድሪጌውም አላዉቅም ሐ. አዎ አንዳዴ

መልስዎ አድሪጌውም አላዉቅም ከሆነ ወደ ቀጣይ ጥያቄ 15 ያምሩ።

14. ለምንድ ነው የመድሃኒቶችን መጠን የቀየሩት?
    ሀ. መሻሻል ስለታየብኝ
    ለ. የከፋ ሁኔታ ስለገጠመኝ
    ሐ. የመድሃኒቶችን የጎንዮሽ ጉዳት ለመቀነስ
    መ. የመድሃኒቶችን መጠን ለሕክምናው ግዜ በቂ ስለ ስላልሆነ
    ሠ. ሌሎች /ግለፅ/ _____

15. ሆን ብለው ሲወስዱት የነበረውን የመድሃኒቶች ዓይነት ራስዎትን በራስዎ በሚያክሙበት ወቅት ቀይረው ያውቃሉ?
    ሀ. አዎ ሁሌም ለ. አድሪጌውም አላዉቅም ሐ. አዎ አንዳዴ
    መልስዎ አድሪጌውም አላዉቅም ከሆነ ወደ ቀጣይ ጥያቄ 17 ያምሩ ።

16. ለምንድ ነው የመድሃኒቶችን ሌላ ዓይነት የቀየሩት?
    ሀ. የመጀመርያው መድሃኒት ስላልሰራ
    ለ. የመጀመርያው መድሃኒት ስላለቀ
    ሐ. ሁለተኛው መድሃኒት ዋጋው ርካሽ ስለሆነ
    መ. የመጀመርያው መድሃኒት የጎንዮሽ ጉዳት ለመቀነስ
    ሠ. ሌሎች /ግለፅ/ _____

17. በአንድ ህመም ራስዎትን በራስዎት ለማከም ስንት ያህል መድሃኖቶች ይጠቀማሉ? _____

18. የምትጠቀምባቸው የፀረ-ተሃዋስያን መድሃኒቶች ጥራታቸው ያለጠበቁ እንዳይሆኑ ታሰባልህ?
    ሀ. አዎ እጅግ በጣም; ለ. አዎ በተወሰነ መልኩ; ሐ. አላሰብም

19. መቼ ነው የምጠቀሙት ፀረ-ተሓዋሰያን መድሃኒቶችን የሚያቆሙት?
    ሀ. ከጥቂት ቀናት ብኃላ በውጤቱ ብዙ ሳልጨነቅ
    ለ. የሕመም ምልክቶችን ከተወኝ ብኃላ
    ሐ. ከጥቂት ቀናት ካገገመኩኝ ብኃላ
    መ. መድኃኒቱን ካለቀ ብኃላ
    ሠ. የመድሃኒቱ የሕክምና ግዜ ካለቀ ብኃላ
    ረ. ፋርማሲስቶችን ካማከርኩኝ ብኃላ
    ሸ. ሌሎች /ግለፅ/ _____

20. ተመሳሰይ ፀረ-ተሓዋሰያን መድሃኒቶችን በተለያየ ስያሜ በአንድ ግዜ ወስደው ያውቃሉ ።
    ሀ. አዎ (ግለፅ_____); ለ. አይደለም
    መልስዎ አይደለም ከሆነ ወደ ቀጣይ ጥያቄ 22 ያምሩ።

21. የመድሃኒቶችን የጎነዮሽ ጉዳት በሚገጥሞት ግዜ በሚገጥሞት ግዜ ምን ያደርጋሉ?
    ሀ. መድሃኒት መውሰዴን አቆማሉሁ።
    ለ. ሌላ መድሓኒት እቀይራሉሁ ።
    ሐ. ፋረማሲስቶችን አማክራሉሁ ።
    መ. ቤተ-ሰቦቼ ወይም ጓደኞቼ አማክራሉሁ።
    ሠ. ምንም አላደርግም ።
    ሰ. ሌሎች /ግለፅ/ _____

22. የተጠቀምክባቸው መድሃቶችን ጻፍ:_____

23. በፀረ-ተሓዋስያን የመድሃኒቶችን ራስን በራስን ማከም እንዴት ታሰባዋለህ?
    ሀ. ጥሩ ልማድ; ለ. ተቀባይነት ያለው ልማድ ነው። ሐ. ተቀባይነት ያለው ልማድ አይደለም።

24. በፀረ-ተሓዋስያን መድሃኒቶች ራሰዎትን በራስዎት ማከምዎ ውጤቱ ምን ነበር ?
    ሀ. ከህመሙ መሉ ለሙሉ ድኛለሁ
    ለ. ሕመሙ ተሸሎኛል
    ሐ. አልተሻለኝም
    መ. ሕመሙ ባሰብኝ
    ረ. ሌላ (ግለፅ) _____

25. በራስዎት ላይ ተላላፊ በሽታዎችን ቢያጋጥምዎት በተሳካ መልኩ ራስዎትን በራሰዎት ማከም ይችላሉ።
ሀ. አዎ እችላሎሁ ። ለ. እርግጠኛ አይደለሁም ። ሐ. አልችልም ።

<u>ክፍል 4 “</u>: ክሊኒካል መረጃ

1) ሌላ በሽታ ካለህ/ሽ ተናገሪ/ር? _____
2) በአሁኑ የሽንት ቱቦ እንፌክሽን ምልክት አለው? ሀ. አዎ ለ. አይደለም
3) ካቴተር ገብቶልህ/ሽ ያቃል? ሀ. አዎ ለ. አይደለም
4) ከዚህ በፊት የሽንት ቱቦ እንፌክሽን ይዞህ/ሽ ያውቃል?ሀ. አዎ ለ. አይደለም

<u>መረጃ ሰብሳቢው የሚሞላው ፥</u>

*** የላባራቶሪ የሽንት ካልቸር ውጤት (Organism found and antimicrobial susceptibility: Attach laboratory result)

****

<u>Note</u>: ፀረ-ተሐዋስያን መድሃኒቶችን የሚባሉ: Ampicillin; Amoxicillin; Chloramphenicol; Nitrofurantoin; Gentamicin; Ciprofloxacin; Cephalothin; Ceftriaxone; Norfloxacin; Doxycycline; Trimethoprim-Sulfamethoxazole; Tetracycline; Nalidixic Acid.

Curriculum Vitea (CV)

CPERSONAL INFORMATION

Name: MEBRAHTU EYASU BELETE

Ras Emuru Building # 201, Swaziland street, Addis Ababa, 1271, Ethiopia

+251112750125 +251913518025

mebratupharmacology@gmail.com /mebratu.eyasu@sphmmc.edu.et

Face book: Anti-drug Info Ethiopia

Sex Male| Date of birth 12/09/1983 | Nationality Ethiopian.

WORK EXPERIENCE
From October 2008 to Today.

Business Sector Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.
www.sphmmc.gov.et

POSITION	*Assistant Professor of Pharmacology* **Summary**: — Still this moment, I am teaching Pharmacology and toxicology in Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. — I thought modular course in SPHMMC to the undergraduate medical students, Addis Ababa, Ethiopia. — *Postgraduate courses for Maxillofacial and Oral surgery residents; and Gynaecology and Obstetrics residents.* — *Postgraduate courses for Nursing in MSC program (2 programs)* — *Undergraduate: 5 nursing programs*

WORK EXPERIENCE

2008-2010

- St. Paul's Hospital Millennium Medical College, as pharmacist enrolled with
- Head of special pharmacy
- Drug and Therapeutics' Committee Secretary
- Trainer of some pharmacy topics like warehouse management, safe injection and supply management, etc both in Addis Ababa and outside Addis Ababa city
- Drug formulary chief editor

January, 2013- 10 May, 2015:

Lecturer of Pharmacology,

- Head of College registrar, SPHMMC, Addis Ababa, Ethiopia
- Secretary of Senate and Academic commission

- Module four coordinator
- Module six coordinator
- Teaching all undergraduate students
- Mentor/supervisor of some Medical Students
- Researcher

From 10 May, 2015 – current Assistance Professor of Pharmacology

- Currently, Head of department of Pharmacology, SPHMMC
- Teaching modular based curriculum for undergraduate and
- Teaching postgraduate students (Gyni/obs residents, maxillofacial surgery, and anaesthesiology residents; MSc respiratory in nursing)
- Andragogy trainer in SPHMMC, trained on PBL assisted teaching (NMI schools)
- Research activities
- Member of Curriculum Review Committee, SPHMMC
- Member of other standing committee of the College

EDUCATION AND TRAINING

PhD student from (2017/18) *Complutense University of Madrid, Spain.*

Dates (from – to)	August 2010 – December 2012
Name and type of organization providing education and training	Addis Ababa University, Higher institution
Principal subjects/occupational skills covered	Teaching and Doing research in Pharmacology
e of qualification awarded	Master of Science in Pharmacology
• Dates (from – to)	Oct. 2003-July 2007
• Name and type of organization providing education and training	Jimma University
• Principal subjects/occupational skills covered	*Pharmacist (five years course plus research)*
• Title of qualification awarded	Degree of Bachelor of Science in Pharmacy

Replace with dates (from - to) 2015
Best Basic Science Instructor Award of SPHMMC

• Dates (from – to)	Sep. 10 – 21, 2014
• Name and type of organization providing education and training	Ethio-Canada Microresearch training initiative
• Principal subjects/occupational	

skills covered	Research proposal and report writing
• Title of qualification awarded	Micro-research Certificate
• Dates (from – to)	August 29-30, 2013
• Name and type of organization providing education and training	Center for African Leadership Studies
• Principal subjects/occupational skills covered	Basic knowledge on Leadership
• Title of qualification awarded	Certificate on Fundamentals of Leadership
• Dates (from - to)	Nov. 11-15, 2013
• Name and type of organization providing education and training	PRIME
• Principal subjects/occupational skills covered	Pedagogy/Foundations of Teaching and Learning
• Title of qualification awarded	Certificate on Foundations of Teaching and Learning
• Dates (from - to)	July 15-16, 2013
• Name and type of organization providing education and training	University of Michigan and University of Ghana
• Principal subjects/occupational skills covered	Research Methods
• Title of qualification awarded	Certificate on Research Methodology

MOTHER TONGUE(S)	Tigrigna (s)				
Replace with language	AMHARIC	UNDERSTANDING	SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
	Excellent	Excellent	Excellent	Excellent	Excellent
Replace with language	English				
	Excellent	Excellent	Excellent	Excellent	Excellent

Computer skills

— Application of different Soft ware's (Ms word, Ms Excel, Ms Access,
— Ms power point, statistical soft ware packages like SPSS, epi-info etc., and certified for basic computer skills, and internet browsing. All
— These were acquired through short term training (three months)
— From BIDKEM Computer training center, Addis Ababa, Ethiopia.

Other skills: Poetry writing and published a book.

Job-related skills: I develop the art of teaching, supervision of the medical students and doing a research proposal to win grants. Till now, I win around nine grants from SPHMMC.
I train staffs about andragogy

Communication skills: Good communication skill, good listener, Honest and good in keeping team sprit gained through my experience as College Registrar Head, Head of Department of Pharmacology, Module coordinator for four years and secretary of senate and Academic commission through these different positions after graduation and the fact that I am a teacher

Driving licence: ▪ 278036 (Addis Ababa)

PUBLICATIONS

1. Eyasu M, Worku Y, Ababaw B, Berhan Y. COVID-19 Preventive Practices among Bus Station Workers in Ethiopia. *Am J Trop Med Hyg.* 2021 Nov 5; 106(1):114-120.
2. Getinet T, Eyasu M, Shafie M. Factors Associated With Folic Acid Supplements Resistant Spina Bifida In Addis Ababa, Ethiopia: A Case-Control Study. *Ethiop Med J*, 2021; 59(1):31-37.
3. Mensur Shafi, Mebratu Eyasu, Kedija Muzeyin, Yoseph Worku, Sagrario Martin-Aragon. Prevalence and determinants of self-medication practice among selected households in Addis Ababa community. PLOS ONE 2018, 13(3): e0194122. https://doi.org/ 10.1371/journal.pone.0194122.
4. Eyasu M, Dida T, Worku Y, Worku S, Shafie M. Acute poisonings during pregnancy and in other non-pregnant women in emergency departments of four government hospitals, Addis Ababa, Ethiopia: 2010-2015. *Tropical Medicine & International Health*. 2017;22(10):1350–60.
5. Shafie M., Eyasu M. Safety and efficacy of artemisinin and its derivatives in the treatment of severe *p. Falciparum* malaria infection: a review. *WJPPS*. 2016; 5(4):30-54.
6. Geleta B., Eyasu M., Kebamo S., Debela A., Makennon E, Abebe Abiy. In vitro vasodilatory effect of aqueous leaf extract of Thymus serrulatus. *Asian Pacific Journal of Tropical Biomedicine*. 2015; 5(1):15-18.
7. Eyasu M. Antimalarial Drug Resistance: In the Past, Present and Future Perspectives. *British Journal of Pharmacology and Toxicology*. 2015; 6(1):1-15.
8. Eyasu M., Berhane B., Yohannes S. Spectrum of Opportunistic Infections and their Associated Risk Factors among Antiretroviral therapy Experienced HIV/AIDS Patients of Addis Ababa, Ethiopia. *WJPPS*. 2015;4(10):347-366.
9. Geleta B, Eyasu M, Fekadu N, Debella A Challa F. Evaluation of Diuretic Activity of Hydro-Ethanolic Extract of *Moringa Stenopetala* Leaves in Swiss Albino Mice. *Clin Exp Pharmacol*. 2015;5: 190. doi:10.4172/2161-1459.1000190.
10. Eyasu M., Shibeshi W., Giday M., In vitro antimalarial activity of hydromethanolic leaf extract of calpurnia aurea (fabaceae) in mice infected with Chloroquine sensitive *plasmodium berghei*. *International Journal of Pharmacology and Pharmacy*. 2013;2(9) : 131-142.
11. Geleta B., Eyasu M., Debela A., Mengistu M. Review on the effects of *Moringa Stenopetala* leaves on Hypertension. *Proceedings*. 2014. Accessed on April 1, 2015. Available at: www.ephi.gov.et.

Unpublished Articles:

1. Eyasu M. *Assessment of traditional herbs used to treat active tuberculosis in Jimma Zone, Agaro town, Southern East Ethiopia, Ethiopia, 2007.* [Undergraduate paper].

GRANTS:

1. Prevalence of Diabetic Foot Ulcer and Associated Risk Factors in Diabetic Patients attending in St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. SPHMMC grant.
2. Psychoactive Substances Use among St. Paul's Hospital Millennium Medical College Undergraduate Medical Students, Addis Ababa, Ethiopia: Periodic prevalence, Predictors and consequential problems. SPHMMC grant
3. Assessment of prevalence and associated factors of herbal medicine use in Addis Ababa; Ethiopia. SPHMMC grant
4. Clinical epidemiology and predisposing factors of folic acid resistant neural tube defects in Addis Ababa, Ethiopia: Case-control study. SPHMMC grant
5. Prevalence of community acquired pneumonia and associated risk factors among children admitted in Pediatrics Ward, SPHMMC, Addis Ababa, Ethiopia. SPHMMC grant
6. Characterization of Acute Poisonings During Pregnancy and in Women of Reproductive Age in Emergency Departments of Four Government Hospitals, Addis Ababa, Ethiopia: 2010-2015. SPHMMC grant.
7. Prevalence and Determinants of Self-medication practice in Addis Ababa, Ethiopia. SPHMMC grant
8. Prevalence of community acquired pneumonia and associated risk factors among children admitted in Pediatrics Ward, SPHMMC, Addis Ababa, Ethiopia. MICRO-research project, CANADA.
9. Spectrum of Opportunistic Infections and Associated Risk Factors among HIV/AIDS Patients under the First and Second Line Antiretroviral Drugs Therapy in SPHMMC ART Clinic, Addis Ababa, Ethiopia. 2014. SPHMMC

Conference presentations:

- Acute poisonings during pregnancy and in other non-pregnant women in emergency departments of four government hospitals, Addis Ababa, Ethiopia: 2010-2015 in SPHMMC, 3 rd Annual meeting (ORAL PRESENTATION)
- Spectrum of Opportunistic Infections and their Associated Risk Factors among Antiretroviral therapy Experienced HIV/AIDS Patients of Addis Ababa, Ethiopia in SPHMMC, first Annual meeting (ORAL PRESENTATION)
- Review on the effects of *Moringa stenopetala* leaves on hypertension. Proceedings of the Consultative Workshop on *Moringa stenopetala* to maximize its Potential Use, May 22-23, 2014, Bishoftu, Ethiopia, pp.16.