UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE FARMACIA

TESIS DOCTORAL

Plantas medicinales en farmacia comunitaria: de la validación farmacológica al *DNA-Barcoding*

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Marta Sánchez Gómez-Serranillos

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MADRID, 2021

A mis padres, Marcial y Pilar

A mi hermana Raquel, Laurent y Alicia

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I. RESUMEN

I. RESUMEN

Plantas medicinales en farmacia comunitaria: de la validación farmacológica al *DNA-Barcoding*

Introducción

A lo largo de los años, numerosos hechos han puesto en manifiesto el papel esencial de las plantas medicinales para la salud humana como fuente molecular con potencial terapéutico y base para la obtención de nuevos medicamentos. La Organización Mundial de la Salud (OMS) establece que la terapéutica basada en plantas medicinales es una parte importante y con frecuencia subestimada de la atención al paciente. Las plantas medicinales fueron declaradas como "fuente inagotable de nuevos fármacos" en el año 1978 por la OMS. En la actualidad, millones de personas en el mundo consumen plantas medicinales con fines terapéuticos; debido a ello, la OMS ha diseñado la denominada Estrategia 2014/2023 cuyo fin es reforzar el papel de la medicina tradicional, resaltando la importancia de promover e incluir el uso de plantas medicinales en los sistemas de salud de los países miembros.

El interés por las plantas medicinales ha ido aumentando en los últimos años. Se estima que en los países desarrollados aproximadamente el 80% de la población utiliza estos productos como primera estrategia terapéutica. Dentro de la Unión Europea, alrededor del 50% de la población consume plantas medicinales, a excepción de en Alemania, donde este porcentaje es mayor. El creciente interés por las plantas medicinales queda patente en el aumento de publicaciones científicas sobre este tema en los últimos años.

En España, la disponibilidad de estudios sobre usos actuales de plantas medicinales, así como sus tendencias a futuro es escasa en comparación con la de otros países.

Objetivos

En base a lo anteriormente expuesto y a las líneas de trabajo de nuestro grupo de investigación, en el presente trabajo se ha planteado, como objetivo general, aportar nuevos conocimientos sobre el empleo de las plantas medicinales por la población, abordando todos aquellos aspectos que garantizan su calidad, seguridad y eficacia.

Los objetivos específicos del trabajo de investigación se definen a continuación:

1. Realizar un estudio sobre el consumo de plantas medicinales por la población de la Comunidad Autónoma de Madrid, determinando su patrón de uso y evaluando posibles efectos secundarios e interacciones.
2. Realizar una validación farmacológica actualizada de las plantas medicinales identificadas como de mayor consumo.
3. Emplear el código de barras de ADN (DNA *Barcoding*) como método para la identificación y autentificación de las especies vegetales
4. Identificar y cuantificar los principios activos presentes en las especies estudiadas mediante Cromatografía liquida de ultra alta eficacia acoplada a espectrometría de masas (UHPLC-MS).

Material y métodos

El trabajo se ha desarrollado en diferentes fases:

En una primera etapa, se han realizado encuestas presenciales voluntarias a una muestra representativa de pacientes consumidores de plantas medicinales que acuden a Oficinas de Farmacia y centros sanitarios en la Comunidad Autónoma de Madrid. Para ello se ha diseñado un cuestionario que incluye una primera parte sobre características sociodemográficas (sexo, edad, nivel de estudios, ocupación, zona de residencia) y una segunda parte centrada en el uso de las plantas medicinales (tipo de plantas consumidas, frecuencia, propósito, lugar de adquisición, origen de la recomendación, resultado terapéutico, y conocimiento sobre efectos secundarios).

Una vez recopilados y gestionados los datos obtenidos, se ha procedido al análisis de los mismos utilizando el programa estadístico Sigmaplot versión 14.0.

En la fase dos de la Tesis Doctoral se ha llevado a cabo una revisión y análisis de los usos actuales farmacológicos de las plantas medicinales más utilizadas en la Comunidad de Madrid según la encuesta poblacional previamente realizada. Se ha determinado el conocimiento, la percepción y las actitudes de la población frente al consumo de plantas medicinales, así como la forma de consumo y/o posibles interacciones.

La tercera fase se ha centrado en realizar la validación farmacológica de los usos populares de las plantas medicinales más consumidas en la Comunidad de Madrid, revisando las bases de datos (PubMed/MEDLINE, Science Direct y Scopus) y fuentes de referencia (European Scientific

Cooperative on Phytotherapy (ESCOP); Commission E, Organización Mundial de la Salud, Agencia Europea de Medicamentos (EMA).

En la cuarta etapa se han combinado los métodos basados en la reacción en cadena de la polimerasa (PCR) con las técnicas de secuenciación del ADN, mediante la tecnología de código de barras de ADN (DNA *Barcoding*), para la identificación molecular de las especies más utilizadas.

En la quinta y última etapa, se ha determinado la presencia de los principales compuestos bioactivos de cada especie estudiada, utilizando Cromatografía liquida de ultra alta eficacia acoplada a espectrometría de masas (UHPLC-MS).

Resultados

Un total de 543 personas participaron en el estudio poblacional. La mayoría de ellas afirmó consumir plantas medicinales en los últimos 12 meses con fines terapéuticos entre los que destacan los trastornos digestivos y los relacionados con el sistema nervioso central. El perfil del consumidor de plantas medicinales es mujer, con edad entre 18-44 años, en activo y con estudios superiores. Se identificaron un total de 78 plantas medicinales, siendo las más consumidas *Matricaria recutita, Valeriana officinalis, Aloe vera, Tilia spp y Camellia sinensis*.

En relación con los datos de consumo, el VU (Valor de Uso) más alto se encontró para *Mentha pulegium* (0,130), seguido de *Aloe vera* (0,097) y *Vaccinium macrocarpon* (0,080). El Nivel de Fidelidad (FL) fue calculado para las 10 plantas medicinales más usadas, siendo los valores más altos para *Eucalyptus* spp. (FL 90,5%, problemas respiratorios), *Matricaria recutita* L. y *Mentha pulegium* L. (85,5% y 84,1% problemas digestivos, respectivamente) y *Valeriana officinalis* (76,4%) para insomnio. En último lugar, se calculó el Factor de consenso de los informantes (FCI) cuyo resultado más alto correspondió al empleo de plantas medicinales para el metabolismo y la depresión (FCI = 1), seguido de dolor (FCI = 0,97), insomnio (FCI = 0,96) y ansiedad (FCI = 0,95). Se ha podido concluir, además, que las farmacias son el lugar más habitual de obtención de las plantas medicinales y, la infusión es la forma de consumo más común. En cuanto a la seguridad de las plantas medicinales y de los productos a base de las mismas, más de la mitad de los participantes consideran que son bastante seguras ya que se trata de productos “naturales” e “inocuos”. Dentro de este trabajo se identificaron algunos casos de reacciones adversas que habían sufrido algunos encuestados. Además también se identificaron interacciones en personas que consumen plantas medicinales junto con medicamentos convencionales.

La revisión y el análisis de las investigaciones recientes sobre actividad farmacológica realizadas mediante estudios *in vitro*, *in vivo* y ensayos clínicos de las especies más utilizadas por la población de la Comunidad Autónoma de Madrid para el tratamiento de afecciones o problemas de salud, indican que, en el caso del té, las patologías más investigadas fueron diabetes y obesidad. El té verde ha sido el más estudiado y, de los compuestos aislados destacan los trabajos que evalúan los efectos de la epigalocatequina-3-galato. El extracto de *Aloe vera* y sus compuestos aislados aloe-emodina y aloína muestran un prometedor papel como agentes citotóxicos, antitumorales, anticancerígenos y antidiabéticos. Los estudios más recientes sobre valeriana profundizan sobre sus propiedades beneficiosas a nivel del sistema nervioso central, actividad que se atribuye principalmente a los ácidos volvalerenal K y valerénico. Los estudios recientes sobre *Matricaria recutita* indican que es eficaz frente a hipertensión, diabetes mellitus tipo II y para el tratamiento del dolor. No se encontraron estudios recientes sobre actividad farmacológica de *Tilia* spp.

El análisis molecular a través del método *DNA barcoding* se realizó a 33 muestras comerciales obtenidas en diferentes puntos de venta (supermercados, farmacias y herbolarios) de las especies *Matricaria recutita, Tilia spp, Camellia sinenis y Valeriana officinalis*. Se consiguió aislar ADN en 23 de las muestras, generándose 18 nuevas secuencias *matK* que fueron alineadas con 38 secuencias que estaban disponibles en *GenkBank.* El 36,4% de las muestras fueron identificadas de forma correcta y el 15,2% como sustituciones. El análisis metabolómico, realizado mediante Cromatografía líquida de ultra alto rendimiento acoplada en tándem a espectrometría de masas (UHPLC-MS/MS), permitió cuantificar el contenido de los principales compuestos bioactivos responsables de la actividad farmacológica. Así, en la manzanilla el contenido medio de apigenina-7-glucósido fue de 0,003%; en las muestras de valeriana el contenido de ácido valerénico fue muy variable, desde 0,048% a 0,167%, y la concentración media de ácido acetoxi valerénico ha sido de 0,025%. En el té, el contenido medio de epigalocatequina fue de 2,85% y el de tilirosido osciló entre 0,008% y 0,043% en las muestras de tila.

Conclusiones

a) Un 89,6% de los encuestados manifiesta emplear plantas medicinales, de forma ocasional o frecuente, con fines terapéuticos. El principal uso terapéutico de las plantas medicinales en la población encuestada es para tratar problemas digestivos, seguido de trastornos del sueño y ansiedad. El uso que se realiza de las mismas es correcto en relación con las enfermedades para las que son eficaces.

b) El perfil más habitual del consumidor son mujeres con edad entre 18-44 años, nivel superior de estudios y en activo. Las farmacias comunitarias son el principal lugar de adquisición de las plantas medicinales y la infusión, la forma de consumo más habitual.

c) Se ha determinado el nivel de Fidelidad, el Valor de Uso y el Factor de consenso de los informantes.

d) Un 3,5% de la población encuestada manifiesta haber sufrido alguna reacción adversa, como ansiedad, taquicardia y mareos. El 21,1% ha consumido plantas medicinales junto con medicamentos convencionales.

e) Se ha realizado la revisión y el análisis de los estudios sobre actividad farmacológica, como fuente de validación, de las especies más consumidas.

f) Se logró aislar ADN con calidad para ser secuenciado en el 69,7 % de las muestras comerciales, alineando las secuencias generadas con las secuencias disponibles en GenBank. El empleo de maturasa k (*matk)* como marcador para la identificación molecular, ha permitido la asignación correcta respecto a su etiquetado en el 36,4 % de las muestras estudiadas; se han detectado sustituciones en el 15,2 %.

g) El análisis cualitativo y cuantitativo de los compuestos responsables de la actividad farmacológica por UHPLC/MS ha permitido determinar el contenido medio de los compuestos bioactivos en las muestras. El trabajo pone de manifiesto que el empleo de técnicas como DNA *Barcoding* y UHPLC para el análisis y control de calidad de productos comercializados a base de plantas medicinales es una herramienta eficaz para garantizar la calidad, efectividad y seguridad del paciente.

II. SUMMARY

I. SUMMARY

Medicinal plants in community pharmacy: from pharmacological validation to *DNA-Barcoding*

Introduction

Over the years, numerous events have demonstrated that medicinal plants play an essential role in human health as a molecular source of therapeutic potential and a source to develop new medicines. The World Health Organization (WHO) states that herbal medicine is an important and often underestimated part of patient care. Medicinal plants were declared an "inexhaustible source of new drugs" in 1978 by the WHO. Currently, millions of people in the world consume medicinal plants for therapeutic purposes; because of this, the World Health Organization has designed the so-called Strategy 2014/2023, which aims to strengthen the role of traditional medicine, highlighting the importance of promoting and including the use of medicinal plants in the health systems of member countries.

Interest in medicinal plants has been increasing in recent years, and in developed countries it is estimated that approximately 80% of the population uses these products as their first therapeutic strategy. Within the European Union, approximately 50% of the population in most countries consume medicinal plants, with the exception of Germany, where this percentage is higher. The growing interest in medicinal plants is evidenced by the increase of published scientific articles on this topic in recent years. In Spain, the number of studies on current uses of medicinal plants as well as their future trends is scarce compared to other countries.

Objectives

According to the information previously mentioned as well as the area of expertise of our research groups, the general objective of this study is to provide new and updated information on the use of medicinal plants by the population, addressing all of the aspects that guarantee their quality, safety and efficacy.

The specific objectives of this study are defined below:

1. To carry out a study on the consumption of medicinal plants by the population of the Autonomous Community of Madrid, determining their pattern of use and evaluating possible side effects and interactions.
2. To carry out an updated pharmacological validation of the medicinal plants identified as the most widely consumed.

3. To use DNA barcoding as a method for the identification and authentication of plant species.
4. To identify and quantify the active principles present in the species studied by Ultra High Performance Liquid Chromatography coupled to Mass Spectrometry (UHPLC-MS).

Material and methods

The study has been developed in different phases:

In the first stage, voluntary face-to-face surveys have been carried out as a representative sample of patients who consume medicinal plants and who go to pharmacies and health centers in the Autonomous Community of Madrid. For this purpose, a questionnaire was designed that included a first part with demographic characteristics (sex, age, level of studies, occupation, area of residence) and a second part centered on the use of medicinal plants (type of plants consumed, frequency, purpose, place of acquisition, origin of the recommendation, therapeutic result, knowledge about side effects).

Once the data obtained was collected and managed, it was analyzed using the Sigmaplot version 14.0 statistical program.

In phase two of the Doctoral Thesis, a review and analysis of the current pharmacological uses of the most commonly used medicinal plants in the Community of Madrid has been carried out according to the previously conducted population survey. The knowledge, perception, and attitude of the population towards the consumption of medicinal plants was studied, as well as the manner of consumption and/or possible interactions.

The third phase was focused on conducting pharmacological validation of popular uses of the most consumed medicinal plants in the Community of Madrid, reviewing databases (PubMed/MEDLINE, Scholar google and SCOPUS) and reference sources (European Scientific Cooperative on Phytotherapy (ESCOP); Commission E, World Health Organization, European Medicines Agency (EMA).

In the fourth stage, methods based on polymerase chain reaction (PCR) have been combined with DNA sequencing techniques, using DNA barcoding technology, for the molecular identification of the most commonly used species.

In the fifth and final stage, the presence of the main bioactive compounds of each species studied has been determined, using Ultra High Performance Liquid Chromatography coupled to Mass Spectrometry (UHPLC-MS).

Results

A total of 543 people participated in the population study. Most of them stated to consume medicinal plants in the last 12 months with therapeutic purposes, among which digestive disorders and those related to the central nervous system stand out. The consumer profile of the medicinal plants is typically female, aged between 18-44 years, active and possessing a higher education title. A total of 78 medicinal plants were identified, the most consumed being *Matricaria recutita, Valeriana officinalis*, *Aloe vera, Tilia spp* and *Camellia sinensis*.

In relation to the consumption data, the highest use value (UV) was found for *Mentha pulegium* (0.130), followed by Aloe vera (0.097) and *Vaccinium macrocarpon* (0.080). The fidelity level (FL) was calculated for the 10 most used medicinal plants, where the highest values were represented by *Eucalyptus* spp. (FL: 90.5%, respiratory problems), *Matricaria recutita* L. and *Mentha pulegium* L. (FL: 85.5% and 84.1% digestive problems, respectively) and *Valeriana officinalis* (FL: 76.4%) for insomnia. Finally, the informant consensus factor (ICF) was calculated, with the highest result corresponding to the use of medicinal plants for metabolism and depression (ICF = 1), followed by pain (ICF = 0.97), insomnia (ICF = 0.96) and anxiety (ICF = 0.95).

It has also been concluded that pharmacies are the most common place to obtain medicinal plants and that infusions are the most common form of consumption. Regarding the safety of medicinal plants and plant products, more than half of the participants consider them to be fairly safe as they are "natural" and "harmless" products. As part of this work, cases of adverse reactions were identified in some of the participants of this study. In addition, interactions were also identified in individuals who consumed medicinal plants along with conventional medicines.

Recent research on the pharmacological activity of the medicinal plants most used by the population of the Autonomous Community of Madrid for the treatment of health conditions or illnesses has been carried out through *in vitro*, *in vivo* studies and clinical trials. Review and analysis of this research indicate that in the case of tea, the most investigated pathologies were diabetes and obesity. Green tea has been by far the most studied type of tea and, of the isolated compounds, the research evaluating the effects of epigallocatechin-3-galate is particularly noteworthy. *Aloe vera* extract and its isolated aloe-emodine and aloin compounds show a promising role as cytotoxic, anti-tumour, anti-cancer and anti-diabetic agents. The most recent studies on valerian have looked more closely at its beneficial properties aimed towards the central nervous system, an activity which is mainly attributed to volvalerenal K and valerenic acids. Recent studies on *Matricaria recutita* indicate that it is effective against hypertension, type II diabetes mellitus and for the treatment of pain. No recent studies on pharmacological activity of *Tilia* spp. were found.

The molecular analysis using the *DNA barcoding* method was carried out on 33 commercial samples obtained in different points of sale (supermarkets, pharmacies, and herbalists) of the species *Matricaria recutita, Tilia spp, Camellia sinenis and Valeriana officinalis*. DNA was isolated in 23 of the samples, generating 18 new matK sequences that were aligned with 38 sequences that were available in GenkBank. A total of 36.4% of samples were correctly identified and 15.2% of them were identified as replacements. The metabolomic analysis, carried out by Ultra High Performance Liquid Chromatography coupled to mass spectrometry (UHPLC-MS/MS), allowed the content of the main bioactive compounds responsible for the pharmacological activity to be quantified. Thus, in camomile the average content of apigenin-7-glucoside was 0.003%; in valerian samples the content of valerenic acid was very variable, from 0.048% to 0.167%, and the average concentration of acetoxy valerenic acid was 0.025%. In tea, the average epigallocatechin content was 2.85% and the tiliroside content varied between 0.008% and 0.043% in lime blossom samples.

Conclusions

a) 89.6% of participants reported the use of medicinal plants, occasionally or frequently, for therapeutic purposes. The main therapeutic use of medicinal plants in the population surveyed was to treat digestive problems, followed by sleep disorders and anxiety. Their use is correct in relation to the diseases for which they are effective.
b) The most common consumer profile is women, aged between 18-44 years, with a higher level of education and currently employed. Community pharmacies are the main location of purchase of medicinal plants and infusions are the most common form of consumption.
c) The level of fidelity, use value and consensus factor of the informants has been determined.
d) 3.5% of the population surveyed stated that they had suffered some adverse reaction such as anxiety, tachycardia, and dizziness. 21.1% of the population have consumed medicinal plants along with conventional medicines.
e) The review and analysis of studies on pharmacological activity, as a source of validation, of the most consumed species has been carried out.
f) High quality DNA was isolated to be sequenced in 69.7% of the commercial samples, aligning the sequences generated with the sequences available in GenBank. The use of

maturase k (matk) as a marker for molecular identification has allowed the correct allocation with respect to its labelling in 36.4% of the samples studied; substitutions have been detected in 15.2%.

g) The qualitative and quantitative analysis of the compounds responsible for pharmacological activity by UHPLC/MS has made it possible to determine the average content of bioactive compounds in the samples. The work shows that the use of techniques such as DNA Barcoding and UHPLC for the analysis and quality control of products marketed on the basis of medicinal plants is an effective tool for guaranteeing the quality, efficacy and safety of the patient.

III. INTRODUCCIÓN

III. INTRODUCCIÓN

Las plantas se han usado por sus propiedades terapéuticas durante milenios y siguen utilizándose ampliamente en la actualidad. Los datos de la Organización Mundial de la Salud (OMS) y otros organismos internacionales muestran que millones de personas emplean las plantas medicinales en todo el mundo (World Health Organization, 2013). En los países en vías de desarrollo se estima que alrededor del 80% de la población depende de las plantas para la atención primaria de su salud (Thomford et al., 2018; Joharchi & Amiri, 2012; World Health Organization, 2011). Además, aproximadamente el 25% de los medicamentos en general y hasta el 60% de los fármacos antitumorales derivan de productos naturales (Ahamed et al., 2020; Newman & Cragg, 2012; Brower, 2008). Sin olvidar que sólo el 15% de las aproximadamente 300.000 especies de plantas identificadas en el mundo han sido evaluadas para determinar su potencial farmacológico (De Luca et al., 2012). El creciente interés por las plantas medicinales queda patente en el aumento de publicaciones científicas sobre este tema (Giang & Otsuka, 2018; Mankga et al., 2013; Butnariu & Coradini, 2012; Gurib-Fakim, 2006).

En Alemania, el 90% de la población utiliza productos naturales con fines terapéuticos, mientras que en otros países europeos, el porcentaje de la población que usa plantas medicinales es del 50%. En Estados Unidos, más de la mitad de la población adulta sana consume productos a base de plantas como complemento a sus dietas (Bailey et al., 2013) y en Canadá, el 70% de la población los ha consumido al menos una vez (World Health Organization, 2013; World Health Organization, 2011). En China, se estima que entre el 30% y el 50% de los medicamentos consumidos son a base de plantas medicinales o derivados. Así pues, los medicamentos a base de plantas (fitomedicamentos) constituyen una importante parte del mercado farmacéutico en Europa, norte de América y países de Oriente, incluyéndose en la práctica médica habitual.

Los factores que han impulsado a este crecimiento son el aumento de la confianza de la población por incluir plantas medicinales en los programas de prevención de la salud, las innovaciones de los laboratorios de productos naturales, el avance en el conocimiento de sus actividades y mecanismos de acción, la aplicación de técnicas innovadoras en su estudio y la publicación de las Buenas Prácticas de Fabricación actuales (CGMP) para los suplementos dietéticos por parte de organismos reguladores como la *Federal Drug Administration* (FDA). A ello hay que añadir la importancia social adquirida de un estilo de vida saludable. Así pues, en la actualidad, el consumo de plantas medicinales varía según el país o la región, principalmente en función de factores como la sensibilización de los consumidores, la disponibilidad de los productos, las formas de distribución y las reglamentaciones regionales. El principal grupo de consumidores lo forman mujeres de mediana edad que tienen una mayor conciencia de la importancia de cuidar la salud y una mayor preocupación por la dieta.

A medida que el mercado de plantas medicinales ha ido creciendo, los episodios de contaminación (i.e. insecticidas, plaguicidas, drogas sintéticas y metales pesados) o de adulteración o falsificación (adición o sustitución de una planta por otra de forma intencionada o por identificación errónea) también han aumentado, lo que da lugar a una mayor preocupación por la seguridad, eficacia y calidad de los productos a base de plantas, factores directamente vinculados al control de calidad de las materias primas (Rubio et al., 2018; Govindaraghavan et al., 2012; Betz et al., 2011.). Hay que recordar que, hasta la fecha, no todos los materiales de partida se someten a pruebas rutinarias de autenticidad o adulteración, salvo los destinados a la elaboración de medicamentos (Figura 1).

La OMS ha publicado directrices para la regulación y registro de productos a base de plantas (World Health Organization, 2011; World Health Organization, 1998). Además, varios países han elaborado sus propias directrices para garantizar el control de calidad de los medicamentos y productos a base de plantas, incluida la importancia de la adhesión a las buenas prácticas agrícolas y de recolección (BPA), las buenas prácticas de fabricación (BPF) y las buenas prácticas de laboratorio (BPL). Según la OMS, de 103 países que participaron en una encuesta sobre reglamentación de los medicamentos a base de plantas, el 65% posee legislación y reglamentos al respecto. En general, podemos decir que los países europeos integrados en la OMS tienen alto nivel de compromiso con la calidad, seguridad y eficacia de los medicamentos a base de plantas (Coutinho Moraes et al., 2015).

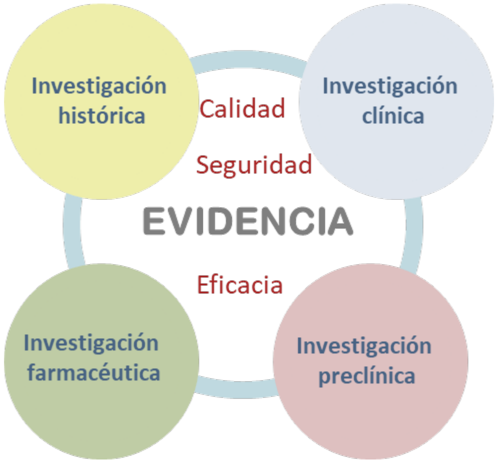

Figura 1. Evidencia para los medicamentos a base de plantas.

III.1. Evolución histórica del empleo de plantas medicinales

El término Fitoterapia fue acuñado por el médico francés H. Leclerc (1874-1955) en el tratado *Précis de Phytothérapie*; es un neologismo formado por dos vocablos de origen griego: *phytón* (planta) y *therapeía* (tratamiento) que etimológicamente significa *"tratamiento de enfermedades con plantas*" o bien *"terapéutica de plantas"*. Podemos definirla como *"ciencia que estudia la utilización de los productos de origen vegetal con finalidad terapéutica, ya sea para prevenir, atenuar o curar un estado patológico"* (Li et al., 2019; Castillo & Martínez, 2015).

Así, los orígenes de la Fitoterapia, en cuanto a la utilización de plantas medicinales refiere, son tan antiguos como la Humanidad. Las primeras evidencias datan de hace aproximadamente 60.000 años. Se han encontrado huesos humanos neandertales con restos de polen procedentes de plantas medicinales con actividad estimulante, diurética, astringente y emética. Además, las primeras pruebas sobre cultivo de plantas como manzanilla, adormidera o valeriana también son antiguas (aproximadamente 35.000 años).

Los primeros documentos escritos sobre uso de plantas se conservan en el Museo Británico; se trata de tablillas sumerias de arcilla, escritas con caracteres cuneiformes (datan del siglo VI a.C) y de documentos sumerios y babilónicos en los que se mencionan drogas como opio y beleño.

El documento más importante de la historia antigua relacionado con el empleo de plantas medicinales es el denominado Papiro de Ebers (adquirido por el Profesor G.M. Ebers). Este documento, que data de hace 1500 años a.C., recoge más de 700 remedios naturales, citando de forma específica el uso de unas 160 drogas vegetales.

En la Grecia antigua surgió Hipócrates (padre de la medicina), Teofrasto (padre de la Botánica) y Dioscórides (padre de la Farmacognosia). Es a partir de esta época cuando se da un carácter más científico al conocimiento de las drogas vegetales, alejándose en gran medida de las concepciones religiosas y mágicas de civilizaciones anteriores.

En la civilización romana, destaca Dioscórides quien aportó sus propios conocimientos y recopiló información existente sobre medicamentos en el tratado "De Materia Medica" traducido al latín en el s. XV. Este tratado (5 volúmenes) contiene unas 600 drogas vegetales y algunas de origen mineral y animal de la flora de Asia Menor, Grecia, Egipto e Italia, con el modo de uso y las indicaciones terapéuticas. Además, se incluyen métodos físicos y químicos para identificar, conservar y preservar la calidad de las drogas vegetales. Esta obra se extendió entre romanos y árabes, ejerciendo gran influencia hasta finales de la Edad Media. Fue traducida al español por Andrés Laguna en el s. XVI.

En el s. II surge C. Galeno, gran conocedor de la Flora Medicinal, quien señala la importancia de la procedencia botánica y las adulteraciones, basándose en la procedencia geográfica, caracteres organolépticos y potencia farmacológica para distinguir la calidad de los productos.

Tras la caída del Imperio romano, la Europa Occidental atraviesa un período de oscurantismo que perdura hasta el s. XI, en el que la magia y la brujería dominan el uso de las plantas. En esta época cabe citar a Carlomagno. Además, en el mundo árabe destacan las figuras de Avicena y Averroes. Fruto de la influencia árabe en el mundo cristiano surgen las escuelas de Salerno (creada por Carlomagno) y Montpellier como centros científicos de aprendizaje.

A partir de entonces hasta el s. XVIII, la alquimia y la magia reinan en casi toda Europa Occidental, deteniéndose el avance de la medicina. Sin embargo, el descubrimiento de las nuevas rutas marítimas de las Indias y América, permitieron conocer en Europa nuevas drogas como cacao, quina o coca.

La figura más atrayente del Renacimiento, en lo que a las ciencias médicas refiere, es Paracelso, quien fomentó la experimentación química al hablar de la *quintaesencia* (principio activo de la planta). Además, observó la relación existente entre la cantidad que se administra y la acción que produce.

En el s. XVIII, nace C. Linneo, quien llevó a cabo una importante modificación de la botánica, estableciendo la nomenclatura binaria. En el mismo siglo, A. Lavoisier abordó la concepción científica de la química, creando la nueva nomenclatura. A finales de este siglo, C. W. Scheele separó los primeros compuestos químicos (ácidos orgánicos) a partir de drogas vegetales

Posteriormente, a principios del s. XIX se aíslan diversos compuestos como morfina por Serturner, estricnina y quinina por Pelletier y Caventou, atropina por Geise y Hesse y, salicina por Leroux (Schmitz, 1985).

En España, los estudios sobre plantas medicinales conocen su período de esplendor a finales del siglo XVIII y comienzos del siglo XIX, cuando los expedicionarios en Perú regresaron a la metrópoli. En esta época son importantes los estudios de Hipólito Ruiz sobre el género *Cinchona*, recogidos en *Quinología* (Madrid, 1792), *Suplemento a la Quinología* (Madrid, 1801, firmado conjuntamente con J. Pavón) y *Compendio histórico-médico-comercial de las quinas* (inédito hasta 1992). Además, destacan *Lecciones de Historia Natural* (Agustín Yáñez y Girona, 1820) y *Tratado de Materia Farmacéutica* (Manuel Jiménez Murillo, 1838) que estudian las drogas desde un punto de vista micrográfico y de composición química.

Con el desarrollo de la Fisiología, F. Magendie y su discípulo C. Bernard comenzaron a estudiar en animales de experimentación la actividad farmacológica y mecanismo de acción de alcaloides aislados (emetina, morfina y estricnina).

Es en la segunda mitad del s. XIX cuando se generaliza la caracterización de materiales farmacéuticos de acuerdo con su actividad fisiológica. *Tratado de Materia Farmacéutica Vegetal* (Madrid, 1883), redactado por el profesor J. R. Gómez Pamo, fue el texto que gozó de más aceptación con reediciones en los comienzos del s. XX.

Se van delimitando, así, los tres aspectos del estudio de las plantas medicinales: examen botánico de las drogas, estudio de su composición química y estudio de su actividad fisiológica que marcará su empleo en terapéutica.

Es a partir de la segunda mitad del s. XX cuando el uso de preparados vegetales con fines terapéuticos aumenta. Varios son los factores que contribuyen a este resurgimiento incluidos el interés de la población por todo lo natural, la aparición de problemas de seguridad derivados de reacciones adversas en fármacos de síntesis, el avance en el conocimiento de las drogas vegetales (i.e. composición química y estudios farmacológicos), el desarrollo de productos galénicos a base de plantas medicinales y la aplicación de métodos analíticos como control de calidad de los preparados a base de plantas medicinales.

Al desarrollo de la fitoterapia también contribuye la creación de sociedades científicas, el desarrollo de programas específicos de la OMS y la creación de un comité específico en la Agencia Europea del Medicamento (EMA). Todos ellos tienen como objetivo elaborar documentos sobre evaluación de la calidad, seguridad y eficacia de los preparados a base de plantas medicinales y desarrollar monografías sobre los mismos.

III.2. Organización Mundial de la Salud (OMS)

La OMS establece que la terapéutica basada en plantas medicinales es una importante parte y con frecuencia subestimada de la atención al paciente; se puede considerar tanto un pilar fundamental de la prestación de servicios de salud a la población como un complemento de la misma.

A día de hoy muchos son los países que reconocen la necesidad de crear un enfoque congruente e integral de la atención sanitaria, con el objetivo de facilitar que los gobiernos, los profesionales sanitarios y, finalmente, los usuarios de los servicios sanitarios dispongan de manera segura, correcta, accesible y eficiente acceso a la medicina tradicional. Una estrategia global que promueve la integración, reglamentación y supervisión adecuada para ayudar a los países a desarrollar políticas proactivas en la atención sanitaria (OMS, 2013).

La estrategia de la OMS sobre medicina tradicional 2014-2023, que se desarrolló en repuesta a la resolución WHA62.13 de la Asamblea Mundial de la Salud sobre medicina tradicional del año 2009, marca como objetivo estratégico el de promover y regular el uso seguro y eficaz de las plantas medicinales. Particularmente se establece la necesidad de promover la seguridad, eficacia y calidad de la medicina tradicional promoviendo el conocimiento y orientación sobre normas de reglamentación y garantía de calidad (Dickinson et al. 2019).

Esta estrategia sobre medicina tradicional 2014-2023 de la OMS ayudará a las autoridades sanitarias a encontrar soluciones que favorezcan mejorar la salud y autonomía de los pacientes a través de la reglamentación, investigación e incorporación de productos, prácticas y profesionales. Estos son los objetivos estratégicos concretos:

1) desarrollo de una base de conocimientos y formulación de políticas nacionales.

2) fortalecimiento de la seguridad, la calidad y la eficacia mediante la reglamentación.

3) fomento de la cobertura sanitaria universal por medio de la integración de servicios de medicina tradicional y la auto atención de salud en los sistemas nacionales de salud.

III.3. Marco legislativo de las plantas medicinales

Si bien las plantas medicinales han sido utilizadas en terapéutica desde la más remota antigüedad, la regulación de los medicamentos a base de plantas en un entorno legal no se introdujo hasta el siglo XX. El objetivo general de esta regulación es el de proteger la salud pública asegurando calidad, eficacia y seguridad.

La Directiva europea 2001/83/CE que establece un código comunitario sobre medicamentos para uso humano ha sido modificada por la Directiva 2004/247/CE, en lo que refiere a medicamentos a base de plantas medicinales, con el fin de crear consenso entre los Estados miembros para garantizar seguridad, eficacia y calidad y, facilitar su comercialización dentro de la Unión europea.

Esta Directiva 2004/247/CE ha sido incorporada en España a los textos legislativos Real Decreto 1345/2007 y Real Decreto Legislativo 1/2015.

<u>1º.- Real Decreto 1345/2007, de 11 de octubre por el que se regula el procedimiento de autorización, registro y condiciones de dispensación de los medicamentos de uso humano fabricados industrialmente.</u>

Se incorpora la autorización de un medicamento tradicional a base de plantas (MTP) de forma rápida y sencilla mediante registro simplificado. Los fabricantes deben proporcionar revisiones bibliográficas o informes de expertos que demuestren seguridad y eficacia, en base a su uso tradicional y no a ensayos clínicos, durante un período mínimo de 30 años y, al menos 15 años en la Unión Europea.

Un MTP debe incluir en su etiquetado, prospecto y publicidad destinada al público, que tiene consideración de medicamento tradicional a base de plantas y, que su eficacia para la indicación aprobada se basa exclusivamente en el uso tradicional.

Además, los MTP pueden contener vitaminas o minerales; la acción de estos componentes debe ser secundaria a la actividad de las sustancias activas vegetales para las indicaciones específicas autorizadas.

Así pues, según la normativa vigente una planta medicinal puede estar disponible en el mercado como Medicamento a base de plantas (MP), Medicamento tradicional a base de plantas (MTP) y Producto de plantas de venta libre (PPL), dependiendo de su uso y no de su especie. En el caso de las MP y MTP, su venta se realizará exclusivamente en farmacias comunitarias, mientras que los productos de plantas de venta libre pueden adquirirse en variedad de establecimientos incluidos farmacias comunitarias, herbolarios, tiendas de dietética y supermercados. Estos productos de plantas de venta libre no podrán incluir referencias sobre propiedades terapéuticas, diagnósticas o preventivas. Además, en ningún caso se permite la venta a granel ni la venta ambulante.

- Medicamentos a base de plantas (MP): *el medicamento que contenga exclusivamente como principios activos, sustancias vegetales, preparados vegetales o combinaciones de estos*.

Se entiende como sustancias vegetales o drogas vegetales a "*las plantas, principalmente enteras, fragmentadas o cortadas, las partes de plantas, algas, hongos y líquenes no tratados, normalmente en forma seca pero también frescos y determinados exudados que no han sido sometidos a un tratamiento específico*".

Se entiende como preparados vegetales a "*los que se obtienen sometiendo las sustancias vegetales a tratamientos como extracción, destilación, prensado, fraccionamiento, purificación, concentración o fermentación, sustancias vegetales trituradas o pulverizadas, las tinturas, los extractos (fluidos, blandos, secos), los aceites esenciales, los zumos exprimidos y los exudados tratados*".

- Medicamento tradicional a base de plantas (MTP). Para obtener un registro simplificado de un medicamento tradicional a base de plantas se deben cumplir las siguientes condiciones:

a) Que los medicamentos tengan indicaciones apropiadas exclusivamente para medicamentos tradicionales a base de plantas, que por su composición y finalidad, estén destinados y concebidos para su utilización sin el control de un médico a efectos de diagnóstico, prescripción o seguimiento de un tratamiento.

b) Que se administren siempre de acuerdo con una dosis o posología determinada.

c) Que se trate de preparados para uso por vía oral, externo o por inhalación.

d) Que haya transcurrido el periodo de uso tradicional, consistente en un periodo mínimo de treinta años, de los cuales al menos quince, se haya utilizado en la Unión Europea.

e) Que la información sobre uso tradicional sea suficiente y en particular que el producto demuestre no ser nocivo en las condiciones de uso establecidas y la acción farmacológica o la eficacia del medicamento a base de plantas, se pueda deducir de la experiencia en la utilización tradicional.

- Producto de plantas de venta libre (PPL). Cuando no hagan referencia a propiedades terapéuticas, diagnósticas o preventivas. En este caso, se podrán comercializar como complementos dietéticos, especies vegetales para uso en infusiones o bajo otra denominación que el fabricante considere. Serán de venta libre a través de distintos canales.

2º.- Real Decreto Legislativo 1/2015, de 24 de julio, por el que se aprueba el texto refundido de la Ley de Garantías y Uso Racional de los Medicamentos y Productos Sanitarios. (Ley 29/2006, de 26 de julio).

El capítulo V de la Ley de Garantías y Uso Racional de los Medicamentos y Productos Sanitarios refiere a los medicamentos especiales, entre los que se incluyen a las plantas medicinales. El artículo 51 regula el marco de comercialización de los productos a base de plantas medicinales.

a) *Artículo 51.1. Las plantas y sus mezclas, así como los preparados obtenidos de plantas en forma de extractos, liofilizados, destilados, tinturas, cocimientos o cualquier otra preparación galénica que se presente con utilidad terapéutica, diagnóstica o preventiva seguirán el régimen de las fórmulas magistrales, preparados oficinales o medicamentos industriales, según proceda y con las especificidades que reglamentariamente se establezcan*.

b) Artículo 51.2. El Ministerio de Sanidad y Consumo establecerá una lista de plantas cuya venta al público estará restringida o prohibida por razón de su toxicidad.

c) Artículo *51.3. Establece que se podrán vender libremente al público aquellas plantas tradicionalmente consideradas como medicinales que se ofrezcan sin referencia a propiedades terapéuticas, diagnósticas o preventivas, quedando prohibida su venta ambulante.*

Por otro lado el artículo 3.6 de esta Ley 29/2006, de 26 de julio establece que la custodia, conservación y dispensación de estos medicamentos se reserva a farmacias comunitarias o a servicios de farmacia hospitalaria.

Por último, la orden SCO/190/2004, de 28 de enero, contiene un listado de plantas cuya venta al público queda prohibida o restringida por razones de toxicidad (salvo por motivos ornamentales, industriales o cosméticos). Se incluyen un total de 197 especies y géneros botánicos que por razones de seguridad su uso queda restringido a la fabricación de medicamentos, cepas homeopáticas e investigación. Esta Orden fue recurrida por AFEPADI y anulada por Sentencia A.N. (Sala de lo Contencioso-Administrativo, Sección 4.ª) el 27 de junio de 2005. Posteriormente, una sentencia del Tribunal Supremo (9 de junio de 2008), desestimó un recurso de casación interpuesto por el Estado.

III.4. Estudios sobre consumo de plantas medicinales.

En los últimos años ha aumentado el interés por el uso terapéutico de productos naturales, en especial por derivados de plantas (Awortwe et al., 2019; Izzo et al., 2018), entre otras razones por el uso abusivo y/o incorrecto de drogas sintéticas que pueden producir efectos secundarios y reacciones adversas, por el hecho de que un gran porcentaje de la población mundial no tiene acceso a tratamientos farmacológicos convencionales y, por el convencimiento de que los productos "naturales" son inofensivos. Se estima que más del 50% de la población mundial consume preparados de plantas y/o medicinas alternativas. En Europa, el consumo de plantas medicinales ha experimentado un incremento medio anual del 5%. Así, en Alemania, el 70% de su población ha manifestado utilizar "medicinas naturales", siendo las plantas medicinales las más consumidas (Encuesta Allensbach, 2017). En este país, en el año 2015, se vendieron medicamentos a base de plantas por valor de 1.360 millones de euros (Zahn et al., 2019; Jütte et al., 2017; Vargas-Murga, 2011). Por otro lado, en Estados Unidos, el uso de preparados de plantas ha crecido del 3% en 1990 al 12% en 1997 y hasta el 30% en 2008. De igual manera se ha observado un aumento en el consumo de suplementos dietéticos del 6,8% en Estados Unidos y Europa (Zahn et al., 2019; Izzo et al., 2016; García-Alvarez et al., 2014).

Actualmente en España existen pocos estudios relacionados con el consumo de plantas medicinales por parte de la población española. El informe *European Advisory Services* recoge que el consumo de plantas medicinales en España aumentó más del 50% durante el periodo 1997-2005 y se estima que seguirá creciendo en torno a un 2-5% anual (Batanero-Hernan et al., 2017).

En el año 2015, el Centro de Investigación en Fitoterapia (INFITO) reveló que 1 de cada 3 españoles (67% de la población) ha consumido plantas medicinales con fines terapéuticos. El principal consumidor eran mujeres (3 de cada 4; un 71,4%) con estudios superiores o universitarios. Además, 1 de cada 4 personas tomaba plantas medicinales por tradición familiar. También, se observó que el consumo era mayor con la edad. Así, el consumo semanal de plantas medicinales era de un 57,5% para los consumidores mayores de 51 años, de 47,1% para los consumidores menores de 35 años y del 53,8% para los consumidores entre 35 y 50 años. El principal lugar de adquisición de las plantas medicinales fueron herboristerías (84%), seguido de farmacias (45%), parafarmacias y supermercados (INFITO, 2015).

En otro trabajo realizado en centros de salud de atención primaria de Barcelona se concluyó que el 59,6% de los entrevistados consumían plantas medicinales, siendo este consumo de forma diaria o semanal en más del 40% de los participantes (Baulies Romero et al., 2009).

En un reciente estudio sobre consumo de plantas en la población anciana española, se determinó que un 88,3% de la población de la tercera edad consume preparados de plantas medicinales, principalmente en forma de infusión (media de 2,1 infusiones/día). Además, el 47,6% de la población anciana utiliza preparados comerciales de plantas medicinales. De estos últimos, los más utilizados son los preparados dermatológicos de aloe en crema, seguidos de valeriana en cápsulas y crema de árnica (Batanero-Hernan et al., 2017).

Dentro de la medicina tradicional y complementaria, las plantas medicinales ocupan un destacado lugar como opción terapéutica para personas con problemas médicos particulares. Así, según una encuesta del año 2013, las plantas medicinales eran las más utilizadas como medicina complementaria en pacientes con cáncer (Posadzki et al., 2013). Además, en otra encuesta realizada en Reino Unido, un 34% de pacientes con cáncer manifestaron consumir suplementos a base de plantas que incluían 101 productos diferentes (Alsanad, 2016). En un estudio realizado en Estados Unidos se recoge que las plantas medicinales son de frecuente uso entre enfermos con cefaleas y migrañas (21,7%) (Zahn et al., 2017). En otra encuesta de Reino Unido realizada en mujeres postmenopáusicas, el 70,4% utilizaba especies como el aceite de prímula (*Primula vulgaris* Huds.)(48,6%) y de cimicifuga (*Cimicifuga racemosa* (L.) Nutt) (30,3%) para aliviar los síntomas del climaterio (Gentry-Maharaj et al., 2015).

La escasez de tratamientos farmacológicos nuevos y eficaces para combatir enfermedades crónicas como demencia y cáncer, o las infecciones resistentes a antibióticos ha vuelto a centrar la atención en las plantas medicinales como fuente potencial de nuevos medicamentos. Así, del total de 1.562 nuevas moléculas aprobadas por la FDA entre 1981 y 2014, el 27% eran totalmente sintéticas y más de la mitad derivaban de compuestos de origen natural (Newman y Cragg, 2012). La mayoría de estas moléculas proceden de plantas superiores como los agentes anticancerígenos paclitaxel y sus derivados (*Taxus* spp), la camptotecina y sus análogos (*Camptotheca acuminata* Decne), la homoharringtonina (*Cephalotaxus harringtonia* K. Koch), los agentes anticolinesterásicos como galantamina obtenida de *Galanthus nivalis* L. o el agente antimalárico artemisinina, extraído de la especie vegetal utilizada en la medicina tradicional china, *Artemisia annua* L. y cuyo descubrimiento mereció, en 2015, el premio Nobel de Medicina y Fisiología otorgado a la científica china Tu YouYou.

De forma paralela al uso de principios activos aislados, también se utilizan los extractos de plantas. Algunos de ellos, evaluados en ensayos clínicos controlados aleatorios, son los extractos de ginkgo (*Gingko biloba* L), salvia (*Salvia officinalis* L.), romero (*Rosmarinus officinalis* L.) y melisa (*Melissa officinalis* L.) para mejorar las propiedades cognitivas y memoria, la hierba de San Juan (*Hypericum perforatum* L.) para la depresión, la valeriana (*Valeriana officinalis* L.) para el insomnio, árnica (*Arnica montana* L.) para el dolor y la inflamación, la canela (*Cinnamomum verum*) para la reducción de la presión arterial y la menta (*Mentha x balsamea* Willd.) para el síndrome del intestino irritable (Farah et al., 2019; Dai et al., 2018).

En una revisión sistemática sobre características demográficas y factores que se relacionan con el estado de salud y el uso de medicinas tradicionales o complementarias se concluyó que la mayoría de los estudios de consumo se han realizado en Estados Unidos (64% de 110 estudios) seguido de Europa [13% de los estudios, la mayoría en Escandinavia (7%) y Reino Unido (5%)] (García-Álvarez et al., 2014; Bishop et Lewith, 2010). Los estudios se han visto limitados por la heterogeneidad de las definiciones utilizadas, la diversidad en los diseños de los estudios y los objetivos, lo que dificulta la comparación de los resultados y la extrapolación de las conclusiones. Así, la ambigüedad de categorías como "medicina natural", "remedios a base de plantas" o "medicina a base de plantas" y qué constituye un "suplemento dietético" dificulta el conocer con fiabilidad la prevalencia del uso de plantas medicinales con fines terapéuticos o como suplementos dietéticos (García-Alvarez et al., 2014; Vargas-Muga et al., 2011; Harrison et al., 2004).

Las plantas medicinales se comportan como verdaderos fármacos; sus constituyentes químicos pueden ejercer una actividad biológica en los seres humanos. Por esta razón, la administración conjunta de drogas convencionales y plantas medicinales puede producir reacciones adversas a medicamentos (RAM), algunas con graves consecuencias (Awortwe et al., 2019; de Souza Silva et al., 2014; Gonzalez-Stuart, 2011). El riesgo de RAM aumenta con la edad, puesto que las personas mayores tienen mayor número de comorbilidades lo que lleva a tomar un mayor número de medicamentos (Agbabiaka et al., 2017; Izzo et Ernst, 2009). Este tipo de interacciones pueden originarse por mecanismos farmacocinéticos, si afectan a procesos de absorción, distribución, metabolismo y excreción (Feltrin & Oliveira Simoes, 2019; Na et al., 2011) o farmacodinámicos, si afectan al resultado de su acción farmacológica (Orellana-Paucar et Vintimilla-Rojas, 2020; Lougheed et al., 2010), pero en muchos casos, la población tiene una percepción errónea de la inocuidad de estos productos. Así pues, el consumo de plantas medicinales es un aspecto muy importante a tener en cuenta en la revisión y control de los tratamientos farmacológicos. Conocer estas interacciones y orientar al paciente mejorará la atención farmacéutica y la seguridad del tratamiento.

IV. REFERENCIAS BIBLIOGRÁFICAS

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V. DISEÑO DEL ESTUDIO

V. DISEÑO DEL ESTUDIO

El presente proyecto de Tesis Doctoral se desarrollará en las siguientes fases:

Fase 1.- Realización de encuestas sobre consumo y empleo de plantas medicinales por la población de la Comunidad Autónoma de Madrid.

Fase 2.- Revisión y análisis de los usos farmacológicos actuales de las plantas medicinales más utilizadas.

Fase 3.- Validación farmacológica de los usos tradicionales de las plantas medicinales más usadas.

Fase 4.- Investigación encaminada al proceso de normalización descriptiva y estandarización para establecer códigos moleculares de barras para la identificación de especies, lo que se denomina DNA *Barcoding*.

Fase 5.- Estudio analítico cuantitativo de los principios activos responsables de la actividad de las especies vegetales más consumidas por cromatografía de líquidos de ultra-alta resolución con espectrómetro de masas (UHPLC-MS), de acuerdo con Farmacopea.

Fase 1 – Encuestas

Se realizarán encuestas personales voluntarias (presenciales) a pacientes consumidores de plantas medicinales que acuden a Oficinas de Farmacia y centros sanitarios de la Comunidad Autónoma de Madrid.

El cuestionario precodificado incluirá una primera parte con características demográficas (sexo, edad, nivel de estudios, ocupación y lugar de residencia) y una segunda parte centrada en el uso de las plantas medicinales (tipo de plantas consumidas, frecuencia, propósito, lugar de adquisición, origen de la recomendación, conocimiento sobre efectos secundarios e identificación de interacciones).

Se elaborará una base de datos para recopilar y gestionar los datos obtenidos en las encuestas y se realizará un posterior análisis de los datos utilizando un programa estadístico (Sigmaplot versión 14.0).

Fase 2 – Usos farmacológicos actuales

En esta fase de la Tesis Doctoral se llevará a cabo una revisión de los usos actuales farmacológicos de las plantas medicinales más utilizadas en la Comunidad Autónoma de Madrid, analizando el conocimiento, percepciones y actitudes e incluyendo información relativa a la identificación de riesgos y precauciones asociadas a su consumo concomitante con medicamentos convencionales.

Fase 3 – Validación farmacológica

Esta fase se centrará en la validación farmacológica de los usos populares de las plantas medicinales más consumidas en la Comunidad Autónoma de Madrid. Para esta validación se revisarán las monografías de las siguientes agencias oficiales internacionales:

- *European Scientific Cooperative on Phytotherapy (ESCOP)*: organización que agrupa y representa a las sociedades nacionales de fitoterapia en Europa. Publica monografías inicialmente en formato de libro y desde el año 2011 en formato online (70 monografias publicadas).
- *Commission E*: Monografías de la Comisión de Alemania que dejó de funcionar en el año 1994. Incluye un total de 385 monografías.
- *Organización Mundial de la Salud (OMS)*: ha publicado un total de cuatro volúmenes de monografías (volumen 1 con 28 monografías; volumen 2 con 30 monografías; volumen 3 con 31 monografías y volumen 4 con 28 monografías).
- *La Agencia Europea de Medicamentos (EMA)*: el Comité de medicamentos a base de plantas (HMPC) se encarga de la elaboración de las monografías de plantas medicinales y productos de las mismas como los aceites esenciales.

Se realizará una exhaustiva revisión sistemática en bases de datos bibliográficas disponibles en Internet, concretamente PubMed, Science Direct y Scopus.

Fase 4 – Identificación molecular por *DNA barcoding*

Los avances en la secuenciación del ADN han promovido el empleo del código de barras de ADN (*DNA barcoding*) en la identificación y autentificación de plantas medicinales. Estos métodos genómicos se basan en la diversidad de secuencias de regiones cortas y estándar de ADN (400-800 pares de bases) que permiten la identificación precisa y fiable a nivel de especie.

Emplearemos la tecnología de código de barras de ADN para identificar las plantas medicinales más utilizadas. Para ello combinaremos los métodos basados en la reacción en cadena de la polimerasa (PCR) con las técnicas de secuenciación del ADN, comparando las secuencias obtenidas tras la secuenciación con bases de datos que incluyan las secuencias de los marcadores en las diferentes especies. Las bases de datos utilizadas serán *Barcode of Life*

Database (BOLD) de la Universidad de Guelph en Ontario y la base de datos colaborativa internacional de secuencia de nucleótidos (*International Nucleotide Sequence Database Collaborative*), una colaboración entre *GenBank* (Estados Unidos), *Nucleotide Sequence Database* (Europa) y *DNA Data Bank* (Japón). Para el registro de las secuencias obtenidas se seguirán las normas acordadas por el CBOL (*Consortium for the Barcode of Life*).

Fase 5 – Identificación y cuantificación de los principios activos responsables de la actividad farmacológica

En la última etapa se identificarán y cuantificarán los principales principios activos responsables de la actividad farmacológica de las plantas medicinales más consumidas. Para ello se empleará la cromatografía de líquidos de ultra-alta resolución con espectrómetro de masas (UHPLC-MS).

VII. JUSTIFICACIÓN

VI. JUSTIFICACIÓN

El uso terapéutico y registro de los medicamentos tradicionales a base de plantas se basa en gran medida en su empleo tradicional a lo largo de los años. Desde el año 2004, el comité para medicamentos a base de plantas (HMPC) de la EMA ha contribuido con la aplicación de la directiva de los productos medicinales herbales tradicionales (THMP), evaluando las evidencias científicas de un gran número de productos utilizados tradicionalmente como medicamentos y publicando las correspondientes monografías.

No obstante, sería deseable una evaluación que vaya más allá de los usos populares. Con el fin de asegurar que esta terapia cumple con los requisitos de la medicina basada en evidencias/pruebas, es esencial que todos los datos no sean sólo los especificados en las disposiciones reglamentarias (30 años, referencia del producto), sino que sean científicamente valorados y evaluados.

Este trabajo de Tesis Doctoral se ha realizado en base a una encuesta poblacional realizada en la Comunidad Autónoma de Madrid sobre consumo de plantas medicinales que ha permitido identificar cuáles son las especies vegetales más utilizadas, y realizar una exhaustiva revisión bibliográfica, con el fin de garantizar "la mejor evidencia científica posible", que es el rango distintivo de la medicina basada en la evidencia.

En una segunda parte del trabajo de Tesis Doctoral, hemos llevado a cabo un estudio por cromatografía de líquidos de ultra-alta resolución con espectrómetro de masas (UHPLC-MS) de los principales constituyentes de las plantas más consumidas para, finalmente aplicar la tecnología más novedosa y actual, el DNA *barcoding*, al estudio de especies vegetales de uso terapéutico. Todo ello permite garantizar la calidad, seguridad y eficacia exigible a todo medicamento.

VII. OBJETIVOS

VII. OBJETIVOS

Los objetivos de la presente Tesis Doctoral se definen a continuación:

1. Realizar un estudio poblacional sobre consumo de plantas medicinales en la Comunidad Autónoma de Madrid, analizando el conocimiento, percepciones y actitudes e identificando reacciones adversas y potenciales interacciones, con el fin de analizar la importancia farmacológica y, por consiguiente, cultural y socioeconómica de las especies vegetales utilizadas actualmente por la población.

2. Revisar y validar la información farmacológica de las plantas medicinales identificadas como de mayor consumo.

3. Aplicar la tecnología de código de barras de ADN (DNA *Barcoding*) para identificar las plantas medicinales más utilizadas, combinando los métodos basados en la reacción en cadena de la polimerasa (PCR) con las técnicas de secuenciación del ADN.

4. Identificar y cuantificar los principios activos responsables de la actividad presentes en las plantas medicinales más consumidas mediante cromatografía de líquidos de ultra-alta resolución con espectrómetro de masas (UHPLC-MS).

El objetivo final de este trabajo es aportar nuevos conocimientos sobre el empleo de las plantas medicinales para tratar problemas de salud, abordando todos aquellos aspectos que garanticen su calidad, seguridad y eficacia.

VIII. PUBLICACIONES

ARTICULO I

Current uses and knowledge of medicinal plants in the Autonomous Community of Madrid (Spain): a descriptive cross-sectional study

Marta Sánchez, Elena González-Burgos, Irene Iglesias, Rafael Lozano , María Pilar Gómez-Serranillos.

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BMC Complementary Medicine and Therapies

RESEARCH ARTICLE Open Access

Current uses and knowledge of medicinal plants in the Autonomous Community of Madrid (Spain): a descriptive cross-sectional study

Marta Sánchez[1], Elena González-Burgos[1], Irene Iglesias[1], Rafael Lozano[2] and María Pilar Gómez-Serranillos[1*]

Abstract

Background: The usage of medicinal plants as a key component of complementary and alternative medicine, has acquired renewed interest in developed countries. The current situation of medicinal plants in Spain is very limited. This paper provides new insights and greater knowledge about current trends and consumption patterns of medicinal plants in the Autonomous Community of Madrid (Spain) for health benefits.

Methods: A descriptive cross-sectional study was designed for a population-based survey on medicinal plants. The data were collected (May 2018 to May 2019) using semi-structured face-to-face interviews in independent pharmacies, hospital centers and primary care health centers in the Autonomous Community of Madrid. The survey had 18 multiple choice and open-ended questions. Quantitative indices were calculated: Fidelity Level (FL), Use Value (UV) and Informants Consensus Factor (ICF). Chi-square test was used for data analysis.

Results: Five hundred forty-three people were interviewed. The majority of the participants (89.6%) have used medicinal plants to treat health disorders in the past 12 months, mainly for digestive problems, sleep disorders and central nervous system diseases. A total of 78 plants were recorded, being *Matricaria recutita*, *Valeriana officinalis*, *Tilia* spp. and *Aloe vera* the most used. The highest UV was found for *Mentha pulegium* (UV 0.130) followed by *Aloe vera* (UV 0.097) and *Vaccinium macrocarpon*. (UV 0.080). The highest FL values were for *Eucalyptus* spp. (FL 90.47%) for respiratory conditions and, *Matricaria recutita* (85.55%) and *Mentha pulegium* (84.09%) for digestive problems. The highest ICF corresponded to metabolism and depression (ICF = 1), pain (ICF = 0.97), insomnia (ICF = 0.96) and anxiety (ICF = 0.95). Participants mostly acquired herbal medicines from pharmacies, herbal shops and supermarkets. Some side effects (tachycardia, dizziness and gastrointestinal symptoms) and potential interactions medicinal plants-drugs (*V. officinalis* and benzodiazepines) were reported.

Conclusion: Many inhabitants of the Autonomous Community of Madrid currently use herbal products to treat minor health problems. The most common consumer pattern are young women between 18 and 44 years of age with higher education. In order to confirm the pattern, further research should be focused to investigate current uses of medicinal plants in other Spanish regions.

Keywords: Medicinal plants, Autonomous Community of Madrid (CAM), Spain, Consumption patterns

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Background

Complementary and alternative medicines (CAMs) represent different resources that complement or replace conventional therapies [1]. The World Health Organization's (WHO) strategy, 2014–2023, aims to strengthen the role of traditional medicine, emphasizing the importance of promoting and including the utilization of medicinal plants in the health systems of its member countries [2].

The use of medicinal plants has acquired a renewed interest in developed countries and constitutes the first therapeutic strategy for 80% of developing countries. The majority of the global population (87.5%) uses traditional herbal medicine to treat health difficulties [3, 4]. Moreover, the growing interest in the employ of medicinal plants is evidenced by the increase of systematic reviews and prevalence surveys about herbal medicines in the last 15 years [5]. In Europe and throughout the Mediterranean area, both wild-collected and purchased from herbalists, supermarkets and pharmacies, is re-emerging. This renewed interest in traditional herbal medicine in more developed societies must be seen in the context of changes in the lifestyle, in which it enhances the concept of real and natural products. This leads consumers to perceive herbal medicine as a softer option for health issues [5–7].

Previous studies on medicinal plants in Spain are alternatively based on their traditional use [6, 7]. All these preceding works aim to study the relationships between plants and human beings in the present and in the past, based on the understanding of herbal remedies which were traditionally used to treat disorders in different health situations [8]. However, the available information on current perspectives and uses of medicinal plants in Spain is very limited compared to other European countries and USA [9, 10] and additionally very restrictive to specific areas [11, 12].

On the other hand, there exists a widespread belief among population that herbal products, being from natural origin, are not harmful to health [13]. However, medicinal plants can interact with other drugs and thus cause adverse reactions [13, 14]. The complete monographs of the German Commission E: *Therapeutic Guide to Herbal Medicines* includes more than 100 plants historically employed for their therapeutic properties but they are no longer recommended, since scientific evidence has shown potential toxicity or inefficiency [15].

Therefore, based on the state of the art, the aim of this study is to comprehend and deepen the current uses (consumption patterns, perceptions and attitudes) of medicinal plants in different regions of the Autonomous Community of Madrid (Spain), identifying the risks and precautions associated with its use and/or concomitant with conventional drugs.

Methods

Study area

The Autonomous Community of Madrid is the most densely populated territory in Spain (676 inhabitants per km^2), it hosts the capital of Spain (Madrid). Most of the population is concentrated in Madrid Capital City and in its surrounding metropolitan areas. Even rural areas have Madrid as their referent in the urban lifestyle. The Autonomous Community of Madrid has a very diverse population in terms of its origin (being most of it from other Autonomous Communities), its cultural and socio-economic terms [16]. This study has tried to represent different random localities with different social environments. In order to determine if the sample surveyed was representative of the population, the latest statistical data available on the website of Institute of Social Sciences (http://www.madrid.org/iestadis/) related to sex, age and occupation were analyzed.

Study setting

A descriptive cross-sectional study was designed for a population-based survey on medicinal plants. This research (PR016/04) was approved on November 2016 by the Ethics and Animal Experimentation Committee, Faculty of Pharmacy, University Complutense of Madrid (Spain).

Questionnaire

The questionnaire (Additional file 1), developed in Spanish language and designed for this study, was based on previous works on medicinal plants [9, 17, 18] and reviewed by experts in traditional plant-based medicines and pharmacognosy and agreed with experts in public health in order to evaluate the structure, relevancy and clarity of the questions. Before gathering research data, a pilot study was conducted on a sample of 50 people to validate the degree of acceptance and understanding of the questionnaire. Minor modifications, based on the pilot survey, were made in the questionnaire. The final version of the questionnaire consisted on five differentiated parts with a total of 18 multiple choice and open-ended questions to achieve a better understanding of the knowledge and use of medicinal plants for health-seeking behavior. The first part with five questions collected information on demographic data, including age, gender, educational level, area of residence and occupation. The second part, with four issues, focused on the utilization of herbs for medicinal or health purposes (disease categories, frequency, therapeutic uses, types of medicinal plants – excluding multi-herbal drug combinations - and forms of administration). This part of the questionnaire included a definition of medicinal plants: "*Plants that contain properties or compounds that can be used for therapeutic purposes or those that synthetize* *metabolites to produce useful drugs*" [19] and, being respondents allowed freely to comment which medicinal plants they use to prevent or treat pathologies (open list of medicinal plants). Moreover, regarding the frequency of consumption, it has been considered frequent when the interviewee consumes medicinal plants at least once a month. The third part had three questions about where the consumer acquired the medicinal plants and information on their therapeutic uses. The fourth and the fifth sections containing both 3 questions, were related to the knowledge of potential side effects and identification of concomitant consumption of medicinal plants with conventional medicines, respectively.

Data collection and sample size

Data were collected on a Tablet computer by a research group from May 2018 to May 2019 using a face-to-face interview technique. Participants were recruited directly in a total of 30 independent pharmacies, hospitals and primary care health centers of different districts of the Capital City of Madrid and municipalities of the Autonomous Community of Madrid. The average number of interviewees from each place was from 15 to 20. Sample population interviewed was voluntary, randomly selected and previously informed (Fig. 1). Over the period of data collection, we conducted a total of 543 surveys. This sample size, based on population size, provides a margin error of 4% at 95% confidence level [20–22].

Quantitative indices

The quantitative indices Fidelity Level (FL), Use Value (UV) and Informants Consensus Factor (ICF) were calculated.

Fidelity Level (FL)

FL corresponds to the percentage of informants that use a certain medicinal plant to treat a specific condition and it is calculated as FL (%) = (Np/N) × 100 (Np: number of informants citing a certain medicinal plant to treat a specific condition and N: number of informants citing a medicinal plant to treat any given disease) [23]. This index is used to identify the most frequently used plants to treat a disease or condition.

Use Value of species (UV)

UV measures the relative importance of a medicinal plant to the informants and it is calculated as UV =

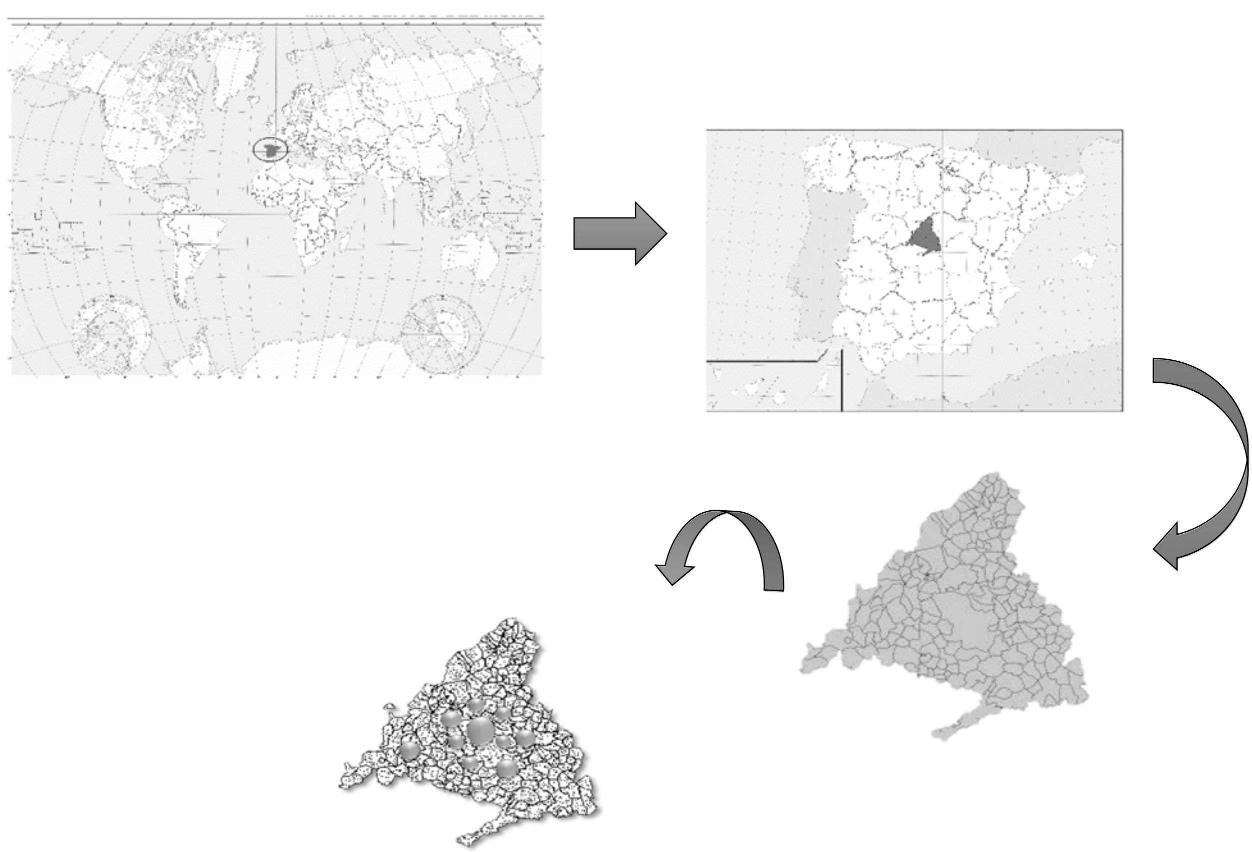

Fig. 1 a Location of the Autonomous Community of Madrid in Spain. **b** Location of the municipalities within the Community of Madrid where the surveys were carried out

Ui/N (Ui: number of citations for each medicinal plant and N: total number of informants). It is a quantitative parameter that indicates the relative importance of the different plant species in a community. It is useful to determine plants with the greatest use (most frequently used) in the treatment of a condition. It also allows knowing the confidence in the use and pharmacological characteristics of related plants [17, 24].

Informants Consensus Factor (ICF)

ICF estimates the user variability of medicinal plants and it is calculated as $(N_{ur} - N_t)/(N_{ur} - 1)$ (N_{ur}: number of used citations in each ailment category, and Nt: number of medicinal plants reported in each ailment category). This index is used to indicate to what extent the information is homogenous. The ranges obtained for this factor vary between 0 and 1. A value close to 1 indicates a relatively high use of the medicinal plant, while a low value close to 0 shows that this plant species is not used by informants for the treatment of an ICF condition. This factor was originally developed by Trotter and Logan (1986) [25] and then readapted by Heinrich et al. 1998, 2000 [26, 27].

Data analysis

All data were entered and stored in an Excel Spreadsheet. Frequencies and percentages were calculated using Microsoft Excel. Statistical analysis was performed using chi-square tests in Sigmaplot version 14.0, to analyze data with correlations between the frequency of medicinal plants and certain demographic characteristics. The level of statistical significance was $p < 0.05$.

Results

Demographic information

Socio-demographic characteristics are shown in Table 1. Most participants were women ($n = 382$; 70.3%). The most frequent age from the respondents was 18–44 years ($n = 340$; 62.6%), followed by 45–64 years old ($n = 151$; 27.8%) and finally by those over 65 years ($n = 52$; 9.6%). Regarding the level of education, the majority of interviewees had higher education ($n = 417$; 76.8%) while 3.2% of participants had basic education or vocational training (i.e. auto repair, plumbing). In reference to the occupation, more than half of participants were employees ($n = 293$; 54%), followed by students ($n = 167$; 30.8%), pensioners ($n = 56$; 10.3%) and unemployed ($n = 15$; 2.8%).

Table 1 Demographic characteristics of participation sample

Population characteristics	Answers (*N* = 543) n (%)
Gender	
Male	161 (29.7%)
Female	382 (70.3%)
Age (years)	
18–44	340 (62.6%)
45–64	151 (27.8%)
≥ 65	52 (9.6%)
Educational level	
Basic education	56 (10.3%)
Vocational training	70 (12.9%)
Higher education	417 (76.8%)
Occupation	
Student	167 (30.8%)
Employee	293 (54%)
Unemployed	15 (2.8%)
Pensioner	56 (10.3%)
Does not answer	12 (2.2%)

Uses and consumption patterns of medicinal plants

The majority of the population interviewed ($n = 491$, 89.6%) used specifically medicinal plants to treat a disease or a health disorder, from which 20.1% ($n = 110$) were habitual (more than 4 times/month) consumers and 69.5% ($n = 381$) were occasional users (1–4 times/month). Only 10.4% of respondents ($n = 57$) had never consumed medicinal plants in the last 12 months (Table 2).

A total of 78 medicinal plants used for health problems, were identified in this study (Table 3). The average consumption was 2.3 medicinal plants by participant. The ten most commonly used medicinal plants were *Matricaria recutita* L. (24.8%), *Valeriana officinalis* L. (20.5%), *Tilia* spp. (13.6%), *Aloe vera* L. (9%), *Camellia sinensis* (L.) Kuntze (7.1%), *Mentha pulegium* L. (6.9%), *Eucalyptus* spp. (5.8%), *Passiflora incarnata* L. (5.2%), *Rosa eglanteria* L. (4.8%) and *Vaccinium macrocarpon* Ait. (3.7%) (Fig. 2). Some of these plants were also consumed in combined preparations, such as *Valeriana officinalis*, *Passiflora incarnata* and *Eschscholzia californica* Cham; however, these mixtures have not been taken into account in the study.

The uses of the medicinal plants were grouped into 12 categories. The most common therapeutic use (Table 4a)

Table 2 Frequency of use of herbal products (referring to the last 12 months) with therapeutically purposes among participation sample, according to range of age

Frequency of use of herbal products	Age		
	18–44 n (%)	45–64 n (%)	≥ 65 n (%)
Frequently[a]	48 (8.8%)	39 (7.1%)	23 (4.2%)
Occasionally[b]	256 (46.7%)	102 (18.6%)	23 (4.2%)
Never	35 (6.4%)	13 (2.4%)	9 (1.6%)

[a] > 4 times/month
[b] 1–4 times/month

Table 3 List of reported medicinal plants used (botanical name, family, disease/problem, mode of use, FL and UV)

Medicinal plant	Family	Common name	Disease/problem	Mode of use	FL (%)	UV
Aesculus hippocastanum L.	Hipocastanaceae	Horse chestnut	Vascular problems	Extract/Capsule	66.66	0.0055
Ajuga chamaepitys (L.) Schreb.	Lamiaceace	Bugle	Digestive problems	Infusions	100	0.0018
Aloe vera (L.) Burm.f.	Asparagaceae.	Aloe vera	Calm pain, healing	Cream/gel	55.55	0.097
Aloysia triphylla (L'Hér.) Britton	Verbenaceae	Herb louisa	Digestive problems / Calm pain	Infusion / capsule	75	0.022
Althaea officinalis L.	Malvaceae	Marshmallow	Respiratory problems	Infusion/Capsule	50	0.0036
Arctium lappa L.	Compositae	Burdock	Digestive problems	Capsule	100	0.0018
Argania spinosa (L.) Skeels	Sapotaceae	Argan	Healing	Essential oil	66.66	0.0055
Arnica montana L.	Compositae	Arnica	Calm pain	Cream	66.66	0.016
Aspalathus linearis (Burm. f.) R. Dahlgren	Leguminosae	Rooibos	Digestive problems	Infusion	100	0.0055
Calendula officinalis L.	Compositae	Calendula	Calm pain	Cream	12.5	0.014
Camellia sinensis (L.) Kuntze.	Theaceae	Tea plant	Digestive problems	Capsule/ Infusion	64.58	0.072
Cassia angustifolia Vahl.	Leguminosae	Senna	Digestive problems	Capsule	33.33	0.016
Centella asiatica L. Urb.	Apiaceae	Asiatic pennywort	Healing	Cream	33.33	0.0055
Cinnamomum verum J. Presl.	Lauraceae	Cinnamon	Control sugar, respiratory problems	Infusion	33.33	0.0055
Citrus sinensis (L.) Osbeck	Rutaceae	Sweet Orange	Sleep disorders	Infusion	66.66	0.055
Coffea arabica L.	Rubiaceae	Arabica Coffe	Others (stimulant)	Infusion	75	0.0073
Crataegus monogyna Jacq.	Rosaceae	Hawthorn	Anxiety/nervousness states	Capsule	42.85	0.012
Cuminum cyminum L.	Apiaceae	Cumin	Digestive problems	Infusion	100	0.0018
Curcuma longa L.	Zingiberaceae	Turmeric	Digestive problems, calm pain	Capsule	71.42	0.012
Cynara scolymus L.	Compositae	Globe artichoke	Digestive problems	Extract/Capsule	88.88	0.016
Echinacea purpurea (L.) Moench	Compositae	Echinacea	Respiratory problems	Infusion	46.15	0.023
Eleutherococcus senticosus (Rupr. & Maxim.) Maxim.	Araliaceae	Siberian ginseng	Others (stimulant, anti-stress)	Extract/Capsule	100	0.00368
Equisetum arvense L.	Equisetaceae	Field horsetail	Digestive problems	Infusion/Extract	68.96	0.033
Eschscholzia californica Cham.	Papaveraceae	California poppy	Sleep disorders	Capsule	40	0.0092
Eucalyptus globulus Labill.	Myrtaceae	Eucalyptus	Respiratory problems	Capsule/vapors/ ointment	90.47	0.071
Euphrasia rostkoviana Hayne	Orobanchaceae	Eyebright	Digestive problems	Infusion	100	0.0018
Foeniculum vulgare Mill.	Apiaceae	Fennel	Digestive problems	Infusion	100	0.020
Fucus vesiculosus L.	Fucaceae	Bladder wrack	Others (laxative)	Capsule/Extract	66.66	0.0055
Garcinia cambogia (Gaertn.) Desr.	Clusiaceae	Garcinia cambogia	Others (weight loss)	Capsule	66.66	0.0055
Ginkgo biloba L.	Ginkgoaceae	Ginkgo	Vascular problems	Capsule	57.14	0.012
Glycine max (L.) Merr.	Leguminosae.	Soyabean	Others (menopause)	Capsule	0	0.0073
Glycyrrhiza glabra L.	Leguminosae.	Licorice	Digestive problems	Capsule	72.72	0.020
Grindelia robusta Nutt.	Compositae	Gumweed herb.	Respiratory problems	Syrups	100	0.0018
Hamamelis virginiana L.	Hamamelidaceae	Witch Hazel	Vascular problems	Capsule	50	0.0036
Harpagophytum procumbens (Burch.) DC. ex Meisn.	Pedaliaceae	Devil's Claw	Calm pain	Capsules	85.71	0.012
Hibiscus sabdariffa L.	Malvaceae	Roselle	Respiratory problems	Infusions	100	0.0036
Hypericum perforatum L.	Hyperidaceae	St. John's wort.	Depression	Capsules	40	0.0092
Jasminum grandiflorum L.	Oleaceae	Jasmin	Anxiety/nervousness states	Infusions	100	0.0018
Laurus nobilis L.	Lauraceae	Bay laurel	Digestive problems	Infusions	50	0.0036
Lavandula angustifolia Mill.	Lamiaceae	Lavander	Sleep disorders	Infusions	50	0.014
Malva sylvestris L.	Malvaceae	Common mallow	Respiratory problems	Infusions	30	0.018
Matricaria recutita L.	Compositae	German chamomille	Digestive problems	Infusions	85.55	0.044
Melaleuca linariifolia Maiden & Betche	Myrtaceae	Tea tree	Skin disorders	Essential oil	50	0.0036
Melissa officinalis L.	Lamiaceae	Lemon balm	Sleep disorders	Infusions	64.70	0.038
Mentha pulegium L.	Lamiaceae	Pennyroyal	Digestive problems	Infusions	84.09	0.13
Mentha × piperita L.	Lamiaceae	Peppermint	Digestive problems	Infusions	72.41	0.035
Monascus spp (Yeast rice)	Monascaceae	Red yeast rice	Others (cholesterol)	Capsules	100	0.0073
Oenothera biennis L.	Onagraceae	Evening primrose	Others (premenstrual syndrome)	Capsules	50	0.011
Origanum vulgare L.	Lamiaceae	Oregano	Digestive problems	Infusions	50	0.014
Panax ginseng C.A.Mey.	Araliaceae	Asiatic Ginseng	Others (stimulant, anti-stress)	Capsules	100	0.0092
Passiflora incarnata L.	Passifloraceae	Purple passionflower	Sleep disorders	Capsules/extract	83.33	0.054
Paullinia cupana Kunth	Sapindaceae	Guarana	Others (stimulant)	Capsules	100	0.0018
Peumus boldus Molina	Momiaceae	Boldo	Digestive problems	Infusions / capsules	87.5	0.014
Pimpinella anisum L.	Apiaceae	Aniseed	Digestive problems	Infusions	85	0.032
Plantago major L.	Plantaginaceae	Broadleaf plantain	Respiratory problems	Infusions	50	0.0036
Plantago ovata Phil.	Plantaginaceae	Spogel plantain	Digestive problems	Capsules/oral suspension powder	85.71	0.012
Rhamnus purshiana DC	Rhamnaceae	Cascara buckthorn	Digestive problems	Extract/ capsules	100	0.0092
Rhamnus frangula L.	Rhamnaceae	Alder buckthorn	Digestive problems	infusions/capsules	100	0.0018
Rheum palmatum L.	Polygonaceae	Chinese rhubarb	Digestive problems	Capsules/infusions	100	0.0018
Rosa eglanteria L.	Rosaceae	Sweet briar	Healing	Essential oil	83.87	0.055
Rosa micrantha Borrer	Rosaceae	Smallflower sweetbrier	Others	Capsules	100	0.0018
Rosmarinus officinalis L.	Lamiaceae	Rosemary	Calm pain	Essential oil	27.27	0.038
Ruscus aculeatus L.	Asparagaceae	Butcher's-broom	Vascular problems	Capsules	62.5	0.014
Serenoa repens (W. Bartram) Small.	Arecaceae	Saw palmetto	Others (benign prostatic hyperplasia)	Capsules	33.33	0.0055
Silybum marianum (L.) Gaertn.	Compositae	Variegated thistle	Digestive problems	Extract/capsules	87.5	0.014
Simmondsia chinensis (Link) C.K. Schneid	Simmondsiaceae	Jojoba	Healing	Essential oil	100	0.0036
Syzygium aromaticum (L.) Merr. & L.M. Perry	Myrtaceae	Clove	Digestive problems	Essential oil	100	0.0018
Taraxacum officinale (L.) Weber ex F.H. Wigg.	Compositae	Dandelion	Digestive problems	Infusions / capsules	100	0.0036
Thymus vulgaris L.	Lamiaceae	Thyme	Respiratory problems	Infusions	60.86	0.035
Tilia cordata Mill.	Malvaceae	Lime	Anxiety/nervousness states	Infusions	70.83	0.052
Tribulus terrestris L.	Zygophyllaceae	Bendy-eye	Hormone enhancer/ Others (weight loss)	Capsules	100	0.0018
Turnera diffusa Willd. ex Schult.	Passifloraceae.	Damiana	Tonic/digestive problems	Capsules	100	0.0018
Urtica dioica L.	Urticaceae	Stinging nettle	Calm pain	Capsules	16.66	0.011
Vaccinium macrocarpon Aiton	Ericaceae	American cranberry	Genitourinary problems	Capsules	76	0.08
Valeriana officinalis L.	Caprifoliaceae	Common Valerian	Anxiety/nervousness states/sleep disorders	Capsules	76.38	0.034
Verbascum thapsus L.	Schrophulariaceae	Mullein	Respiratory problems	Infusions	100	0.0018
Vitis vinifera L.	Vitaceae	Grapevine	Vascular problems	Capsules	80	0.0092
Zingiber officinale Rosc.	Zingiberaceae	Ginger	Digestive problems	Infusions	54.54	0.02

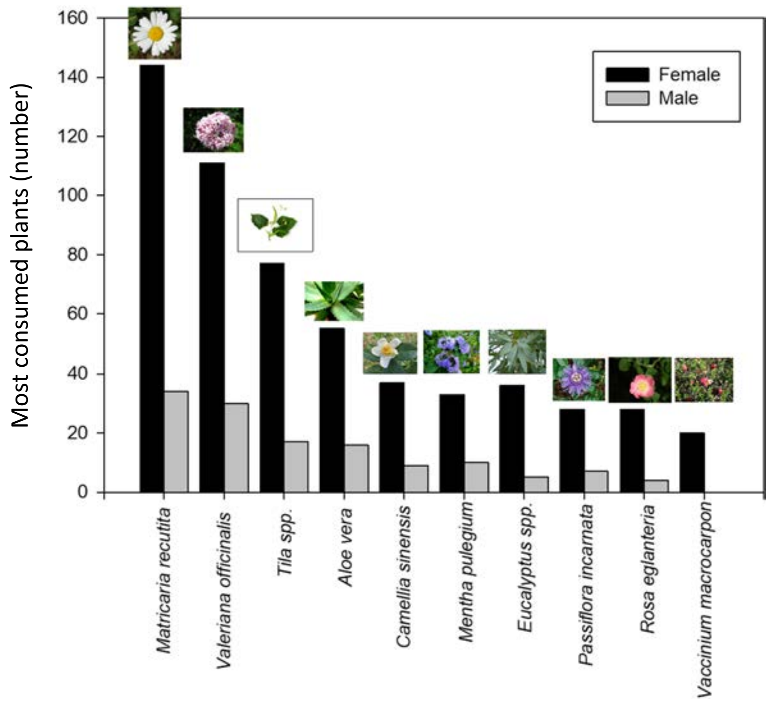

Fig. 2 The ten most consumed medicinal plants by the population of the Autonomous Community of Madrid in Spain according to the gender (n total = 543; n female = 382 and n male = 161)

was for digestive problems such as intestinal gas and stomach cramps (*n* = 252, 69%), followed by sleep disorders (*n* = 211, 57.8%), anxiety and nervousness states (*n* = 166, 45.5%) and, respiratory problems such as bronchitis and common cold (*n* = 93, 25.5%). Other less common therapeutically uses were genitourinary problems (*n* = 42, 11.5%), vascular problems (*n* = 31, 8.5%), blood pressure control (*n* = 12, 3.3%), blood sugar levels (*n* = 8, 2.2%) and depression (*n* = 8, 2.2%).

The most popular form of consumption was as herbal infusion (*n* = 369, 75.8%), followed by tablets/capsules (*n* = 210, 43.1%) and creams (*n* = 121, 24.8%) (Table 4b).

Quantitative indices

The Fidelity Level (FL)

The results of the Fidelity Level for the 10 most cited medicinal plants showed that the highest values were for *Eucalyptus* spp. (FL 90.47%) for respiratory conditions followed by *Matricaria recutita* (85.55%) and *Mentha pulegium* (84.09%) for digestive problems treatment and, *Valeriana officinalis* (76.38%) for insomnia (Table 5).

The Use Value (UV)

UV calculations revealed that the highest value was found for *Mentha pulegium* (UV 0.130) followed by *Aloe vera* (UV 0.097) and *Vaccinium macrocarpon*. (UV 0.080). These were followed by *Camellia sinensis* (UV 0.072) and *Eucalyptu*s spp. (UV 0.071) (Table 6).

Informant Consensus Factor (ICF)

The highest ICF value found corresponds to metabolism and depression (ICF = 1) followed by pain (ICF = 0.97), insomnia (ICF = 0.96) and anxiety (ICF = 0.95) (Table 7).

Place of acquisition preferences and therapeutic resources

Regarding to the place where herbal products were acquired, almost half of the participants preferred pharmacies (*n* = 253, 51.9%) followed by herbal shops (*n* = 209, 42.9%) and supermarkets (*n* = 170, 34.9%), being. The internet resulted in the last position (2.7%) (Table 8).

Most interviewers initiated the consumption of medicinal plants for prevention and treatment following the recommendations of friends and family (*n* = 226, 46.4%), being less who started by their own initiative (*n* = 216, 44.3%) (Table 8). The information concerning the therapeutically uses of medicinal plants came mainly from family and friends (*n* = 234, 48.1%), followed by pharmacist (*n* = 210, 43.1%) and the internet (*n* = 160, 32.8%) (Table 8).

Subjective perception of risks and precautions of medicinal plants

Half of the respondents (*n* = 227, 46.6%) believed that medicinal plants could cause adverse reactions such as conventional drugs do while the other half of the sample population did not (*n* = 260, 53.4%). Moreover, it was

Table 4 (A) Main uses for medicinal plants among the survey sample population. (B) Herbal products most used for therapeutic purposes among the population interviewed. Several possible answers were possible for both questions

	Age		
	18–44 n, (%)	**45–64 n, (%)**	**≥ 65 n, (%)**
A)			
Clinical purposes			
Anxiety / nervousness states	118 (32.3%)	38 (10.4%)	10 (2.7%)
Blood pressure control	4 (1.1%)	5 (1.4%)	3 (0.8%)
Calm the pain	45 (12.3%)	20 (5.5%)	6 (1.6%)
Control sugar	3 (0.8%)	2 (0.5%)	3 (0.8%)
Depression	4 (1.1%)	2 (0.5%)	2 (0.5%)
Digestive problems	141 (38.6%)	85 (23.3%)	26 (7.1%)
Genitourinary problems	24 (6.6%)	14 (3.8%)	4 (1.1%)
Wound healing	54 (14.8%)	16 (4.4%)	1 (0.3%)
Others	54 (14.8%)	39 (10.7%)	10 (2.7%)
Respiratory problems	55 (15.1%)	34 (9.3%)	4 (1.1%)
Sleep disorders	132 (36.2%)	65 (17.8%)	14 (3.8%)
Vascular problems	12 (3.3%)	17 (4.7%)	2 (0.5%)
B)			
Forms of consumption of medicinal plants		**Answers n, (%)**	
Creams (i.e. *Aloe vera*, *Rosa eglanteria*, *Arnica montana*)		121 (24.8%)	
Essential oils (i.e. *Eucalyptus spp.*)		84 (17.2%)	
Herbal teas (i.e. *Equisetum arvense*)		369 (75.8%)	
Syrups *(i.e. Eleutherococcus senticosus)*		37 (7.6%)	
Tablets, capsules (i.e. *Valeriana officinalis*, *Vaccinium macrocarpon*, *Passiflora incarnata*)		210 (43.1%)	

investigated if any of the respondents had suffered any side effect when consuming herbal products for therapeutic purposes. Of those respondents, 17 (3.5%) reported that they had suffered some adverse reaction such as anxiety, tachycardia, dizziness and gastrointestinal symptoms (Table 9).

The potential risk in respect of interactions between medicinal plants and conventional drugs was also investigated. Several respondents have consumed medicinal plants along with conventional medicines ($n = 103$; 21.1%) (Table 10a). Generally, patients do not perceive the need to separate medicinal plants consumption from other drugs. Moreover, interviewees have acknowledged not have received information from health institutions about potential medicinal plants and conventional drugs interactions.

It is revealed that there are several different medicinal plants which were concomitantly consumed with

Table 5 Fidelity Level (FL) of the ten most consumed medicinal plants

Medicinal Plants	Main therapeutic uses	No. of claimed uses reports	FL (%)
Valeriana officinalis L.	Insomnia	110	76.38
Aloe vera L.	Wound healing	40	55.55
Matricaria recutita L.	Digestive problems (i.e. flatulence, stomatitis, and gastrointestinal spasms)	154	85.55
Tilia spp.	Anxiety	68	70.83
Rosa eglanteria L.	Wound healing	26	36.11
Camellia sinensis (L.) Kuntze	Asthenia	27	49.09
Mentha pulegium L.	Digestive problems (i.e. flatulence, dyspepsia)	37	84.09
Eucalyptus spp.	Common cold	38	90.47
Passiflora incarnata L.	Anxiety	22	59.45
Vaccinium macrocarpon Ait.	Cystitis	19	76

Table 6 Use Value (UV) of the ten most consumed medicinal plants

Plant specie	Common name	Part(s) used	Methods of use	Reported uses (per claimed respondents)	UV
Valeriana officinalis L.	Valerian	Root	Oral, infusion	Anxiety / nervousness states, blood pressure control, gastrointestinal disorder, sleep disorders	0.034
Aloe vera L.	Aloe vera	Gel	Topical	Anxiety / nervousness states, calm pain, gastrointestinal disorders, sleep disorders, vascular problems, wound healing,	0.097
Matricaria recutita L.	Chamomile	Flower	Infusion	Anxiety / nervousness states, blood pressure control, calm pain, gastrointestinal disorders, genitourinary problems, sleep disorders, wound healing.	0.044
Tilia spp,	Tila	Leaves	Infusion	Anxiety / nervousness states, blood pressure control, calm pain, gastrointestinal disorders, sleep disorders.	0.052
Rosa eglanteria L.	Rose Hip	Oil	Topical	Anxiety / nervousness states, sleep disorders, vascular problems wound healing.	0.055
Camellia sinensis (L.) Kuntze	Thea	Leaves	Infusion	Anxiety / nervousness states, gastrointestinal disorders, sleep disorders,	0.072
Mentha pulegium L.	Pennyroyal	Summit	Infusion	Anxiety / nervousness states, blood pressure control, calm pain, gastrointestinal disorders, sleep disorders.	0.13
Eucalyptus spp.	Eucalyptus	Leaves	Topical	Anxiety / nervousness states, gastrointestinal disorders, respiratory disorders,	0.071
Passiflora incarnata L.	Passiflora	Aerial part	Infusion	Anxiety / nervousness states, sleep disorders	0.054
Vaccinium macrocarpon Ait	Red blueberry	Fruit	Oral	Calm pain, genitourinary problems	0.08

conventional drugs (ibuprofen, levonorgestrel/ethinylestradiol, paracetamol and omeprazole) (Table 10b). It is concluded that *Matricaria recutita* and *Valeriana officinalis* were the medicinal plants most commonly consumed together with conventional drugs.

The percentage of patients who did not inform doctors or pharmacists of medicinal plants consumption while using other medicines was 65.3% (Table 10a).

Discussion

This work reveals new insights and greater knowledge about the main reasons and current consumption mode of medicinal plants in the population of the Autonomous Community of Madrid for health benefits.

The Community of Madrid has a very varied population and it is very densely populated. Therefore, data from our study were compared with those available from the Institute of Social Sciences to find out whether the surveyed population is representative of the population of this Spanish region. As evidenced demographic parameters are representative (i.e. active population percentage which is 43.6% and range of age which are 55.3% for 18–44, 27.3% for 45–64 and 17.3% for ≥65 years) [16].

Regarding medicinal plants, it was unconcluded that it was higher than the one estimated for other Spanish cities [12]. The main reasons for this finding are the consumer's perception of efficacy and safety as well as the easy access. In this study, the most common consumption pattern of medicinal plants is young women, between 18 and 44 years of age, with higher education. There is statistically significant differences in

Table 7 Informant Consensus Factor (ICF) per medicinal plant category

Ailment category	Number of claimed medicinal plants	Number of claimed citations	ICF
Anxiety / nervousness states	9	166	0.95
Blood pressure control	3	12	0.81
Calm the pain	3	71	0.97
Control Sugar	1	8	1
Depression	1	8	1
Gastrointestinal disorder	32	252	0.87
Genitourinary problems	5	42	0.90
Respiratory disorders	17	93	0.82
Sleep disorders	8	211	0.96
Vascular problems	5	31	0.86
Wound healing	5	71	0.94

Table 8 People who have recommended the use of medicinal plants, place of acquisition and sources of information among the general population surveyed. Several possible answers were possible for both questions

Questions	Possible responses	n (%)
Who recommends the medicinal plants you use?	Doctor recommendation	67 (13.7)
	Own initiative	216 (44.3)
	Pharmacists advice	162 (33.3)
	Recommended by friends / family / acquaintances	226 (46.4)
Where do you acquire mainly medicinal plants?	Supermarkets	170 (34.9)
	Herbal shops	209 (42.9)
	Internet	13 (2.7)
	Others (i.e. street market)	36 (7.4)
	Pharmacy	253 (51.9)
Where do you mainly get information about the uses of medicinal plants?	Doctor	71 (14.6)
	Family and friends	234 (48.1)
	Internet	160 (32.8)
	Other means of communication (magazines, TV ...)	82 (16.8)
	Pharmacist	210 (43.1)

consumption frequency related to gender respondents, being higher in women ($P < 0{,}001$). This high prevalence in the preference of medicinal plants by the female gender has been also confirmed in previous studies [28]. As surveys have been conducted in different health centers, the fact that participants were predominantly women may be due that visits to pharmacies, nurses and doctors in Spain are more frequent in women [29] alongside satisfaction with complementary and alternative medicines [30]. Moreover, a statistically significant finding related to age ranges was found [respondents aged 18–44 consumed medicinal plants more often than those in 45–64 age range ($P = 0{,}010$) and even more often than those ≥65 years ($P < 0{,}001$)]. This pattern, contrasts with studies performed in other parts of Europe where the frequency of consumption is higher in older people rather than in younger people [31]. Moreover, studies from the USA found that medicinal plants consumption is more frequent in middle-aged people [10]. These differences may lie in the area where study was conducted, economic level and consumer trends. Particularly, the Autonomous Community of Madrid has the highest Gross Domestic Product per capita in Spain. In addition, it is one of the Spanish regions most influenced by urbanization and where there is not such a strong connection to traditional use of medicinal plants as in other areas of Spain. Furthermore, there is a growing trend, especially amongst younger people with higher educational level, to use natural products to succeed a healthy lifestyle and mentality [3, 31].

Table 9 Survey responses related to side effects of medicinal plants among the general population surveyed

Questions	Possible responses	n (%)
Do you think that medicinal plants may cause side effects?	Yes	227 (46.6)
	No	260 (53.4)
Have you had any reaction or side effect when consuming medicinal plants?	Yes	17 (3.5)
	No	470 (96.5)
If the previous answer is **YES:**	With which medicinal plant or herbal product? / What side effect or reaction?	
Chamomile / vomiting		
Dandelion / dizziness		
Ginseng / nervous, diarrhea and tachycardia		
Guarana / tachycardia Sen / diarrhea and tachycardia		
St. John's wort/ interaction similar to the shock		
Tea / anxiety, palpitations		
Valerian / sleepiness the next day		

Table 10 (A) Survey responses related to concomitant consumption of medicinal plants and conventional drugs. (B) Main conventional drugs and medicinal plants that are consumed concomitantly (n = number of there associations have been reported in the survey)

A)

Questions	Possible responses	n (%)
Do you usually consume medicines and medicinal plants concomitantly?	Yes	103 (21.1)
	No	297 (61.0)
	Sometimes	87 (17.9)
Do you communicate to your doctor / pharmacist that you consume conventional drugs and medicinal plants concomitantly?	Yes	66 (34.7)
	No	124 (65.3)

B)

Medicinal plant	***Conventional drugs***
Aloe vera	Ibuprofen (n=3), levonorgestrel/ethinylestradiol (n=1), metronidazole (n=1), paracetamol (n=2), tamoxifen (n=1)
Alosya triphylla	Omeprazole (n=1)
Articum lappa	Ibuprofen (n=1)
Camellia sinensis	Acetylsalicylic acid (n=1), amoxicillin (n=2), atenolol (n=1), iron (n=1), ibuprofen (n=5), loratadine (n=1), naproxen sodium (n=1), omeprazole (n=2), paracetamol (n=3)
Cassia angustifolia	Aripiprazole (n=1), quetiapine (n=1)
Centella asiatica	Ebastine (n=1), ibuprofen (n=1)
Cynara scolymus	Diosmin (n=1), omeprazole (n=1), salbutamol (n=1), tiotropium bromure (n=1)
Echinacea purpurea	Alprazolam (n=1), clomipramine (n=1), ibuprofen (n=1), metamizol (n=1), paracetamol (n=1)
Equisetum arvense	Atorvastatin (n=1), clorazepate (n=1), diazepam (n=1), ibuprofen (n=2), levonorgestrel/ethinylestradiol (n=1), lorazepam, (n=1), valsartan/hydrochlorothiazide (n=1)
Eschscholtzia californica	Iron (n=1), salmeterol/fluticasone propionate (n=1)
Eucalyptus spp.	Atorvastatin (n=1), ibuprofen (n=2), paracetamol (n=3), valsartan/hydrochlorothiazide (n=1)
Foeniculum vulgare	Levonorgestrel/ethinylestradiol (n=1), omeprazole (n=1), tryptizol (n=1)
Ginkgo biloba	Ibuprofen (n=1)
Harpagophytum procumbens	Ibuprofen (n=1),levotiroxine (n=1), paracetamol (n=1), vitamin D (n=1)
Laurus nobilis	Ibuprofen (n=1)
Malva sylvestris	Levonorgestrel/ethinylestradiol (n=2)
Matricaria recutita	Almagate (n=1), atenolol (n=1), atorvastatin (n=1), dutasteride/tamsulosin (n=1), enalapril (n=2) , irbesartan (n=1), iron (n=1), levonorgestrel/ethinylestradiol (n=2), lormetazepam (n=1), mesalazine (n=1), naproxen sodium (n=1) , omeprazole (n=1), paracetamol (n=14), pravastatin (n=1), simethicone (n=1), simvastatin (n=1), sitagliptin/metformin (n=1), tiotropium (n=1), valsartan/hydrochlorothiazide (n=1)
Melissa officinalis	Alprazolam (n=1), paracetamol (n=3), ibuprofen (n=2)
Mentha pulegium	Almagate (n=1), ibersartan/hydrochlorothiazide (n=1), ibuprofen (n=6), levonorgestrel/ethinylestradiol (n=2), paracetamol (n=4), rosuvastatine (n=1)
Panax ginseng	Amoxicillin (n=1), ibuprofen (n=1)
Passiflora incarnata	Clorazepate (n=1), budesonide/formoterol (n=1), ibuprofen (n=3), iron (n=1), salmeterol/fluticasone propionate (n=1)
Paullinia cupana	Amoxicillin (n=1), ibuprofen (n=1)
Peumus boldus	Atorvastatin (n=1), hydrochlorothiazide (n=1), valsartan (n=1)
Pimpinella anisum	Levonorgestrel/ethinylestradiol (n=2)
Rhamnus frangula	Ethinylestradiol/drospirenone (n=1)
Rhamnus purshiana	Aripiprazole (n=1), ethinylestradiol/drospirenone (n=1), quetiapine (n=1)
Rosa eglanteria	Levonorgestrel/ethinylestradiol (n=1)
Ruscus aculeatus	Chondroitin sulfate (n=1)
Sabal serrulata	Paracetamol (n=1)
Tilia spp.	Acetylsalicylic acid (n=1), buproprion (n=1), enalapril (n=1), ibuprofen(n=1), irbersartan (n=1), paracetamol (n=8), pravastatin (n=1), sitagliptin/metformin (n=1), topical corticosteroids (n=1)
Urtica dioica	Ibuprofen (n=1), paracetamol (n=1)
Vaccinium myrtillus	Losartan (n=1)
Vaccinium oxycoccus	Fosfomycin (n=2), ciprofloxacin (n=2), levonorgestrel/ethinylestradiol (n=2)
Valeriana officinalis	Anastrozol (n=1), atenolol (n=1), doxilamine (n=1), dutasteride/tamsulosin (n=1), escitalopram (n=1), heparin (n=1), ibuprofen (n=3), iron (n=1), levonorgestrel/ethinylestradiol (n=1), levotiroxine (n=1), lorazepam (n=1), lormetazepam (n=1), tiotropium bromure (n=1), omeprazole (n=2), paracetamol (n=4), ranitidine (n=1), salmeterol/fluticasone propionate (n=1), topical corticosteroid (n=1), trazodone hydrochloride (n=1), vitamin D (n=1)
Vitis vinifera	Diosmin (n=1), mesalazine (n=1)

One of the limitations found in former published studies on prevalence of medicinal plants consumption, unlikely to the one presented, is on the one hand that "medicinal plants" concept is not properly defined, and on the other hand, a list of medicinal plants is providing limiting the knowledge of their use [5]. Of the 78 identified plants, women reported using 72 while men reported 49. Moreover, most people surveyed use them appropriately in relation to diseases for which they are found to be effective. There were no significant differences ($p = 0.242$) in medicinal plants consumption between female and male. However, preferences for some medicinal plants were found among gender. *Melissa officinalis* L, *Cynara scolymus* L., *Echinacea angustifolia* DC, *Equisetum arvense* L. and *Mentha piperita* L. were preferred by women whereas *Vitis vinifera* L. and *Tribulus terrestris* L. were preferred by men. Moreover, in this study, *Vaccinium macrocarpon* Ait. Consumption was exclusive to women in order to prevent uncomplicated acute lower urinary tract infections recurrence. Women's urethra is shorter than that of men's allowing bacteria rapid access to the urinary bladder [32].

It is necessary to emphasize that some of the medicinal plants consumed by the population of the Autonomous Community of Madrid are considered as threatened/vulnerable/endangered by the IUCN Red List. These plant species include in this Red list are *Aesculus hippocastanum* (vulnerable), *Arnica montana* (least concern), *Coffea arabica* (endangered), *Ginkgo biloba* (endangered), *Laurus nobilis* (least concern), *Rhamnus purshiana* (least concern) and *Tilia cordata* (least concern). Particularly, those plant species classified as least concern are not considered to be at threat from extinction and, the future conservation actions are aimed at controlling agriculture practices and include an international legislation. However, *Aesculus hippocastanum* is classified as vulnerable because this plant species suffer from severe defoliation by the invasive insect pest *Cameraria ohridella.* The conservation actions consists on *Cameraria ohridella* control and research, ex situ cultivation and to reduce human impacts. On the other hand, *Coffea arabica* and *Ginkgo biloba* are endangered plant species. The main threats to *Coffea arabica* are pests (i.e. *Hypothenemus hampei*), diseases (i.e. Coffee Berry Disease), deforestation (mainly in Ethiopia) and climate change (i.e. high temperatures). There are several conservation actions for *Coffea arabica* such as ex-situ conservation and, education and awareness programs. Finally, *Ginkgo biloba* is threatened because its logging and wood harvesting. The conservation action for this specie has been widespread in cultivation. It is therefore important that investigations with these species follow the guidelines "IUCN Policy Statement on Research Involving Species at Risk of Extinction" that guarantee the increase and survival of these plant species, bearing in mind that the conservation of these research sources is of clear scientific interest, and in the case of our study, of great therapeutic interest [33–39].

Regarding forms of consumption, the effectiveness of medicinal plants depends on the correct use and preparation. Decoction and infusion are the main preparation methods for herbal teas of roots, barks and seeds. Herbal teas are closely linked to self-medication, being this form of administration not suitable for active principles with narrow therapeutic margin. Tablets/capsules are commonly used for medicinal plants oral administration because of good bioavailability, therapeutic adherence and patient comfort [40].

Concerning accessibility to medicinal plants, most of the herbs are freely available in different places for its acquisition, even at supermarkets (i.e. *Matricaria recutita*, *Camellia sinensis* and *Mentha pulegium*) whereas there are other medicinal plants that are only available in local pharmacies and herbal shops (i.e. *Verbascum thapsus* and *Ajuga chamaepitys*). Participants' perception is that medicinal plants dispensed in pharmacies have better quality and efficiency than those from other acquisition places; however, medicinal plants bought in pharmacies are more expensive than in other sales establishments. This explains why the purchase of medicinal plants in supermarkets and herbal shops is very high. This pattern of herbal products acquisition for therapeutic purposes has also been observed in other countries [41]. However, within Spain, patients from a social security primary health care center in Barcelona bought medicinal plants first in herbal shops, then in supermarket and in pharmacies in third place [12]. The role of the pharmacist is consolidated as the health professional and expert in medicinal plants and pharmacy offices as a reference in the dispensation of medicinal plants, offering quality guarantees.

Due to the wide traditional utilization of medicinal plants and the limited existing clinical trials, there is a lack of scientific evidence on the efficacy and safety of medicinal plants [4]. Adverse drug reactions is defined as "all noxious and unintended responses to a medicinal product" [42, 43]. There is a common perception of safety of medicinal plants as "natural" and "harmless", which could lead to an under-reporting of adverse reactions. Adverse reactions may be due both to medicinal plants and to other factors (i.e. adulteration, lack of botanical identification) [44]. Studies conducted on natural products' perception for health, show an increase in the demand for information about medicinal plants [45, 46]. It is necessary to include medicinal plants consumption in the usual medical history to identify possible adverse reactions and drug interactions [47]. Many health professionals have not received academic preparation on medicinal plants during their Degree studies [48]. In Spain, only pharmacists receive university education on medicinal plants. This lack of knowledge is a limiting factor when health professionals recommend medicinal plants and identify possible adverse reactions and interactions. The need to include medicinal plants in undergraduate training to the rest of health professionals is presumed.

Currently, there are a paucity of robust data on interactions between medicinal plants and conventional medicines [49]. However, it has been found that certain plants can lead to therapeutic inefficiency or drug toxicity. There is evidence of interactions for *Hyperycum perforatum* L. with digoxin, indinavir and cyclosporines [50]. Moreover, *Ginkgo biloba* L. Mant. Pl. can increase insulin elimination or interfere with omeprazole [51]. Furthermore, and in relation to the medicinal plants, that are more consumed concomitantly in this study, there are evidences of pharmacodynamics interactions between *M. recutita* and lormetazepam, *M. officinalis* and alprazolam, and *V. officinalis* and lormetazepam, increasing hypnotic effect of these benzodiazepines [51]. The clinical effects of the interactions depend on patient (age, genetic and pathologies), medicinal plants (species, dose and duration) and concomitant medication (dose, activity and posology) making it difficult to detect interactions if health personnel do not know its use.

Finally, several participants told that neither they reported medicinal plants consumption to these health professionals nor did they ask. This leads to a potential underreporting of adverse reactions and interactions with medicinal plants and, supports the need in the academic training of health sciences personnel to include subjects of medicinal plants in undergraduate degree.

Conclusions

In this paper, we have explored medicinal plant uses, consumption patterns and attitude towards medicinal plants of the population of the Autonomous Community of Madrid that attend health-related centers. This study shows that although the Autonomous Community of Madrid is not a region of Spain with a long tradition in the use of medicinal plants, many inhabitants currently use herbal products (i.e. *M. recutita*, *V. officinalis*, *Tilia*, *A. vera*. and *C. sinensis*) to treat, mainly, minor health problems (i.e. digestive problems and sleep disorders). All the reported medicinal plants have been extensively used in different countries, not identifying neither new records nor new therapeutic activities. These medicinal plants are mainly acquired in pharmacies, herbal shops and supermarkets. The most common consumer pattern of medicinal plants are young women between 18 and 44 years of age with higher education. It has been proved that one of the main reasons for the use of medicinal plants is that the surveyed population has the perception that being natural means harmless.

Moreover, in the present work a correct use of medicinal plants-therapeutic benefits has been detected. However, the high percentage of self-medication may increase the problem of lack of adverse reaction registration and/or drug interactions. Medicinal plants consumption is a matter to consider in the control of pharmacological treatments of the patients. This will guarantee safety, efficacy and quality in the use of medicinal plants, thus constituting an integral health system. According to the results of the study, the need for studies and research to predict the future use of medicinal plants is verified to ensuring the best quality of traditional herbal remedy.

Furthermore, taking into account that studies on current uses of medicinal plants in Spain are very limited, it would be interesting in future research to approach other regions in Spain to have deeper knowledge of the current situation, using and adapting the tolls of this work.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12906-020-03089-x.

Additional file 1. Survey on medicinal plants. The questionnaire was developed in Spanish language and designed for this study.

Abbreviations

CAM: Complementary and alternative medicine; FL: Fidelity Level; UV: Use Value; ICF: Informants Consensus Factor

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Authors' contributions

MS and EGB contributed to the study conception, investigation and writing the original draft. MPGS was responsible to the study conception and the supervision of the study as well as for the writing (review and editing). RL and II contributed to writing (review and editing). All authors read and approved the manuscript.

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Availability of data and materials

The data will be accessible by contacting the corresponding author of this study.

Ethics approval and consent to participate

This research (PR016/04) was approved on November 2016 by the Ethics and Animal Experimentation Committee, Faculty of Pharmacy, University Complutense of Madrid (Spain). Informed consent was verbal according to Spanish legislation (Ley 41/2002).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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	CONSUMPTION AND KNOWLEDGE SURVEY OF MEDICINAL PLANTS AND RELATED PRODUCTS IN THE AUTONOMOUS COMMUNITY OF MADRID

1. **Gender:** Female ☐ Male ☐
2. **Age**: 18-44 ☐ 45-64 ☐ $\geq$ 65 ☐
3. **Educational level :** Basic education ☐ Vocational training ☐ Higher education ☐
4. **Place of residence:** Postal code ☐
5. **Occupation:** Student ☐ Employers ☐ Unemployed ☐ Pensioners ☐ No answer ☐

Medicinal plants definition: *"Plants that contain properties or compounds that can be used for therapeutic purposes or those that synthetize metabolites to produce useful drugs"*

6. **Frequency with which you consume medicinal plants (referring to the last 12 months):**
   Frequently* ☐ Occasionally** ☐ Never ☐
   ** > 4 times/month* *** 1-4 times/month*

7. **What do you use medicinal plants for therapeutic purposes?** (more than one possible option)
   - O Respiratory problems
   - O Digestive problems
   - O Sleep disorders
   - O Anxiety and nervousness states
   - O Calmar dolor
   - O Blood pressure control
   - O Depression
   - O Vascular problems
   - O Genitourinary problems
   - O Sugar levels
   - O Healing
   - O Others: ☐

8. **What medicinal plants have you used?**

   ☐

9. **Forms in which you have consumed medicinal plants:**
   - O Herbal teas
   - O Tablets/capsules
   - O Essential oils
   - O Creams
   - O Syrups

10. Who recommends the medicinal plants you use?:

- O Doctor recommendation
- O Pharmacist advice
- O Friends and family recommendations
- O Own initiative

11. Where do you acquire mainly medicinal plants?:

- O Pharmacy
- O Herbal shops
- O Internet
- O Supermarkets
- O Others (i.e. Street markets)

12.Where do you mainly get information about the uses of medicinal plants?:

- O Doctor
- O Pharmacist
- O Family and friends
- O Internet
- O Other means of communications (i.e. magazines, TV)

13. Do you think that medicinal plants may cause side effects / adverse reactions?:
YES ☐ NO ☐

14. Have you had any side effects / adverse reactions when consuming medicinal plants? YES ☐ NO ☐

15. If the previous answer is YES:

- **What side effects / adverse reactions?** ☐

- **With which medicinal plant?** ☐

16. Do you usually consume medicines and medicinal plants concomitantly? YES ☐ NO ☐ SOMETIMES ☐

17.If the previous answer is "YES" or "SOMETIMES", please, indicate the name of the medicine and the medicinal plants that you consume concomitantly:

Name of the medicine	Name of the medicinal plant

18.If the answer to question number 17 is "YES" or "SOMETIMES":

- **Do you tell your doctor / pharmacist that you are consuming concomitantly medicines and medicinal plants?**
YES ☐ NO ☐

ARTÍCULO II

The Pharmacological Activity of *Camellia sinensis* (L.) Kuntze on Metabolic and Endocrine Disorders: A Systematic Review.

Marta Sánchez , Elena González-Burgos , Irene Iglesias , Rafael Lozano , M Pilar Gómez-Serranillos.

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Review

The Pharmacological Activity of *Camellia sinensis* (L.) Kuntze on Metabolic and Endocrine Disorders: A Systematic Review

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Abstract: Tea made from *Camellia sinensis* leaves is one of the most consumed beverages worldwide. This systematic review aims to update *Camellia sinensis* pharmacological activity on metabolic and endocrine disorders. Inclusion criteria were preclinical and clinical studies of tea extracts and isolated compounds on osteoporosis, hypertension, diabetes, metabolic syndrome, hypercholesterolemia, and obesity written in English between 2014 and 2019 and published in Pubmed, Science Direct, and Scopus. From a total of 1384 studies, 80 reports met inclusion criteria. Most papers were published in 2015 (29.3%) and 2017 (20.6%), conducted in China (28.75%), US (12.5%), and South Korea (10%) and carried out with extracts (67.5%, especially green tea) and isolated compounds (41.25%, especially epigallocatechin gallate). Most pharmacological studies were *in vitro* and *in vivo* studies focused on diabetes and obesity. Clinical trials, although they have demonstrated promising results, are very limited. Future research should be aimed at providing more clinical evidence on less studied pathologies such as osteoporosis, hypertension, and metabolic syndrome. Given the close relationship among all endocrine disorders, it would be of interest to find a standard dose of tea or their bioactive constituents that would be beneficial for all of them.

Keywords: *Camellia sinensis*; metabolic disorders; endocrine disorders; tea

1. Introduction

The incidence and prevalence of metabolic and endocrine disorders such as obesity and type 2 diabetes mellitus are dramatically increasing due to sedentary lifestyle, food intake, and endocrine disruptors, among others [1,2]. In the year 2030, it is estimated that many of these metabolic and endocrine diseases will be responsible for one of the leading causes of death worldwide [3].

Camellia sinensis (L.) Kuntze (Theaceae family) is a tree that mainly grows in tropical and subtropical climates. Tea made from the leaves of *Camellia sinensis* is one of the most consumed beverages in the world. Teas can be classified depending on the degree of fermentation as green tea (unfermented tea), white tea and yellow tea (lightly fermented), oolong tea (semi-fermented tea), black tea (fermented tea), and pu-erh tea (post-fermented tea). Black tea is the most produced and consumed tea worldwide (78% of total tea, especially in Western countries) followed by green tea (20%, especially in China, India, and Japan) and oolong tea (<2%) [4]. Flavanols (primary catechin compounds such as epigallocatechin gallate), flavonols and glycosyl derivatives (i.e., apigenin, myricetin, quercetin, rutin), teaflavins and thearubigins have been identified as main bioactive compounds in the leaves of *Camellia sinensis*.

The type and amount of these compounds is determined by the degree of fermentation of the leaves. Epigallocatechin-3-gallate is the major compound in green tea and theaflavins are produced during the processing of black tea, providing the characteristic flavor [5–7]. The health benefits of *Camellia sinensis* teas include antioxidant, anti-inflammatory, anti-cancer, cholesterol lowering, and cardiovascular protection properties, among others [4,8].

The present systematic review aims to update the pharmacological activity of *Camellia sinensis* (L.) Kuntze on metabolic and endocrine disorders (osteoporosis, hypertension, diabetes, metabolic syndrome, hypercholesterolemia, and obesity).

2. Method

2.1. Search Strategy

The systematic review included preclinical and clinical studies of *Camellia sinensis* on endocrine and metabolic disorders. The literature search was conducted using combination of the following keywords "*Camellia sinensis*", "osteoporosis", "hypertension", "diabetes", "metabolic syndrome", "hypercholesterolemia", and "obesity" on Pubmed, Science Direct, and Scopus databases. The search years were from 2014 to 2019.

2.2. Inclusion and Exclusion Criteria

The inclusion criteria were preclinical (*in vitro* and *in vivo*) and clinical studies, written in English, focused on the pharmacological activity of isolated compounds and extracts of *Camellia sinensis* on metabolic and endocrine disorders. The excluded criteria were case reports, review articles, conference proceedings, and editorial letters. Moreover, studies involving medicinal plant mixtures, galenic formulations, *Camellia* species different than *Camellia sinensis*, functional foods with tea, and comorbidities associated with endocrine and metabolic diseases were excluded.

The literature research was performed by two independent researchers (E.G.-B. and M.S.) and consisted of an initial identification in the above-mentioned databases, followed by duplicated works elimination and, finally, an exclusion of studies that did not meet inclusion criteria established in this systematic review. The research was verified by a third reviewer (M.P.G.-S.) using a predefined spreadsheet designed by the authors.

3. Pharmacological Activity. Description of the Data

Initially, a total of 1384 studies were identified in Pubmed (n = 170), Science Direct (n = 1177), and Scopus (n = 37). However, 40 reports were deleted when appearing in two or more databases (duplicated). Then, 1264 articles were excluded after title and abstract analysis (n = 1237) and after full-text analysis (n = 27), 80 articles finally being included in this systematic review (Figure 1). Five studies of these 80 reports carried out both *in vitro* and *in vivo* experiments and one study *in vitro* and clinical trials.

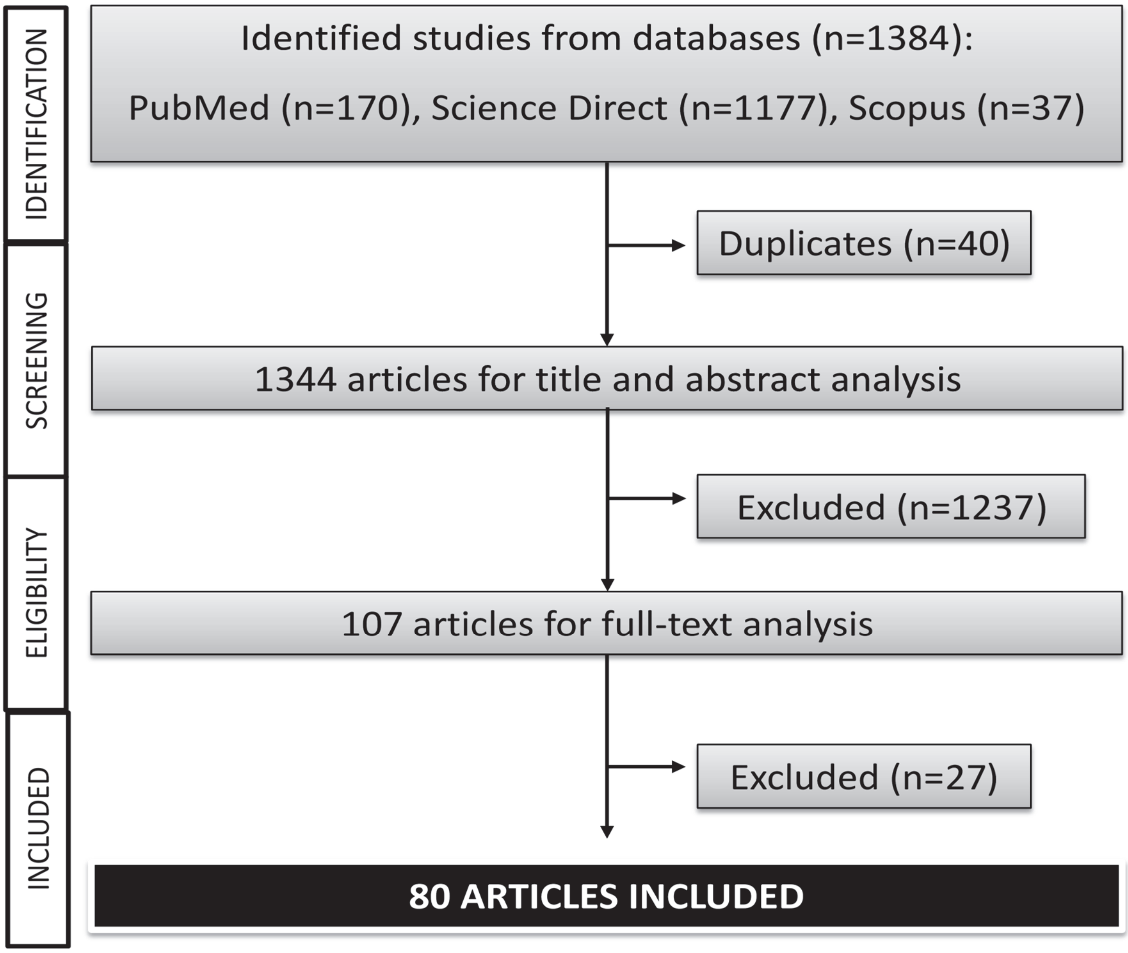

Figure 1. Flowchart of the literature research (*in vitro*, *in vivo*, and clinical trials studies) of *Camellia sinensis*.

Appendix A (Table A1) and Appendix B (Table A2) include *in vitro* and *in vivo* pharmacological studies, respectively, and these studies were grouped based on disease, extract/isolated compound, experimental model, treatments, major findings, and references. Appendix C (Table A3) includes clinical trials and the main information contained was study (author, year, and country), study design, sample size, population, type of plant, intervention, duration of treatments, and results. Most papers were published in 2015 (n = 27, 29.3%) and 2017 (n = 19, 20.6%) (Figure 2A). All works included in this systematic review were conducted by research groups of 23 countries, the majority of them from China (n = 23, 28.75%), United States (n = 10, 12.5%), and South Korea (n = 8, 10%) (Figure 2B). These studies were carried out with extracts (n = 54, 67.5%) and isolated compounds (n = 33, 41.25%) from *Camellia sinensis*. Particularly, extracts were the part of the plant most studied in *in vivo* studies and clinical trials whereas isolated compounds were in the *in vitro* studies. Regarding endocrine and metabolic disorders, diabetes and obesity were the most studies pathologies (n = 35 and n = 33, respectively) followed by hypercholesterolemia (n = 9), osteoporosis (n = 6), hypertension (n = 5) and metabolic syndrome (n = 4). Particularly, diabetes was the most study disease in *in vitro* studies (n = 16) and obesity in *in vivo* studies (n = 20), and clinical trials (n = 6). In several *in vitro* and *in vivo* studies, the effect of extracts and isolated compounds of *Camellia sinensis* on two different pathologies were studied in the same research work [9–16]. This review is divided into six sections, based on the pathologies, diabetes, hypercholesterolemia, hypertension, metabolic syndrome, obesity, and osteoporosis. Within each pathology, pharmacological activities of tea isolated compounds and extracts was classified in terms of signal transduction, redox system, and changes of biomarkers.

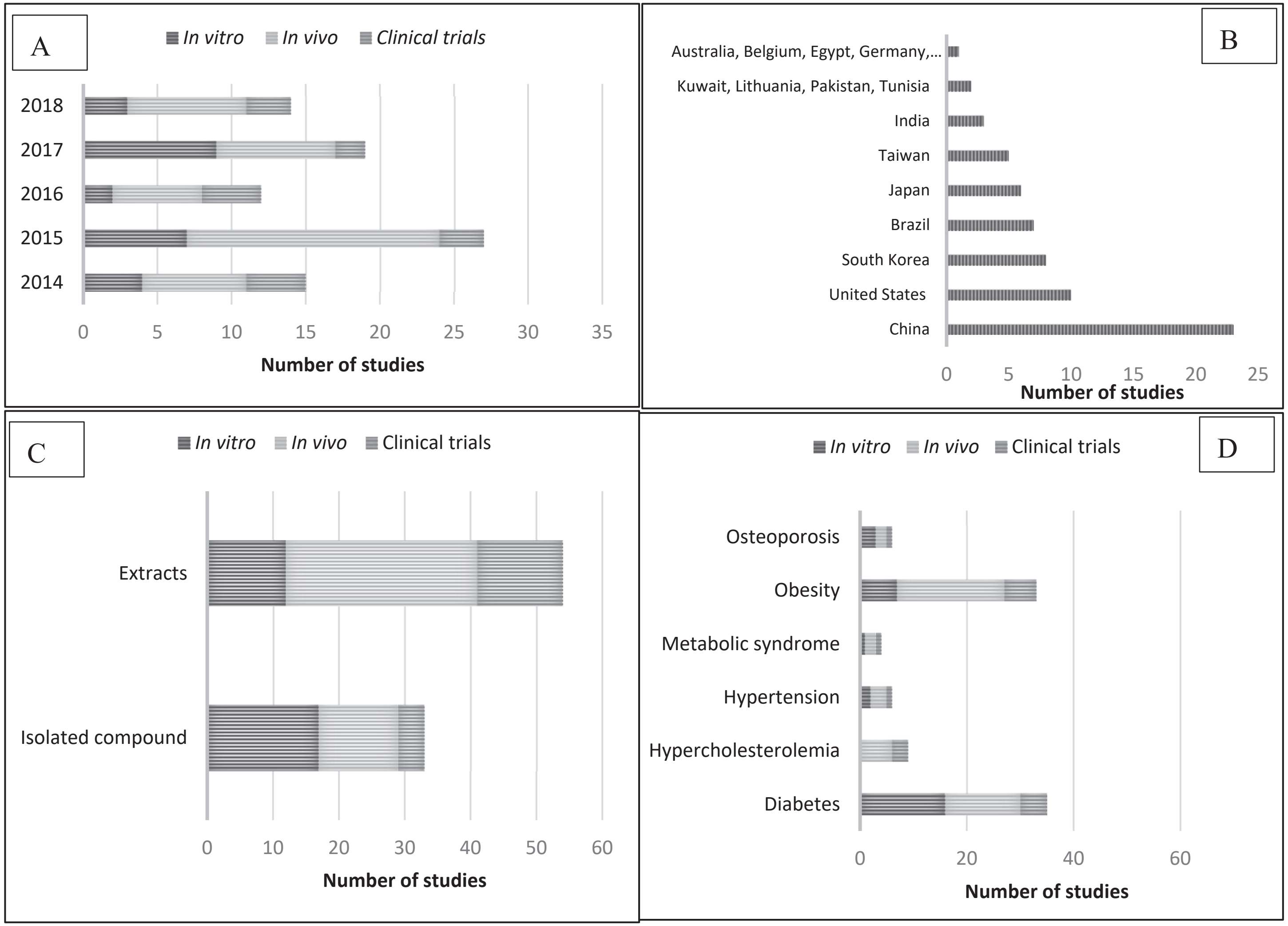

Figure 2. Main characteristics of papers published on pharmacological activity of *Camellia sinensis.* (**A**) Year of publication. (**B**) Research group country. (**C**) Part of the plant used for research. (**D**) Diseases studied in *in vitro*, *in vivo*, and clinical trials studies.

3.1. *Camellia sinensis* and Diabetes

Diabetes mellitus is a chronic metabolic disease that causes abnormally high levels of blood sugar (hyperglycemia) due to a failure in insulin production by pancreas or when the body cannot use insulin effectively [17]. Diabetes mellitus affects about 425 million adults aged between 20 and 79, and it is estimated that in 2025 there will be 629 million. Diabetes mellitus is especially prevalent in low and middle income countries such as those of South-East Asia (82 million) and Western Pacific (159 million) [18]. There are three diabetes mellitus types: type 1, type 2, and gestational. Type 1 diabetes mellitus (insulin-dependent diabetes) is an autoimmune condition that commonly affects individuals during childhood and accounts for around 5% of diabetes mellitus diagnosed cases [17,19]. Type 2 diabetes mellitus (adult onset diabetes) is the most common of the diabetes types (90%–95% of all diagnosed cases worldwide) and it is mainly associated with excess body fat, sedentary lifestyle, and aging [17]. Gestational diabetes mellitus occurs during pregnancy (second or third trimester) because of glucose intolerance; the main risk factors for gestational diabetes mellitus include obesity, ethnicity, age at childbearing, and family history of type 2 diabetes mellitus [20,21].

Most *in vitro* diabetes studies with *Camellia sinensis* are based on the ability of their isolated compounds and extracts to inhibit α-amylase and α-glucosidase activity. In addition, there are several *in vitro* studies with cellular models, mouse 3T3-L1 pre/adipocytes and HepG2 cell lines being the most common. Moreover, for *in vivo* studies, preclinical diabetic animal models (Kunming mice, Sprague-Dawley, and Wistar rats) commonly used to investigate the anti-diabetic properties of tea are streptozotocin and alloxan-induced diabetic animals [10,16,22,23]. Furthermore, the nematode *Caenorhabditis elegans* has been also investigated as diabetic model [24].

Molecular targets in signaling pathways is one of the most successful therapeutic approaches in antidiabetic therapy [25]. Recent studies have determined that tea and its active metabolites can have a therapeutic effect against diabetes through different signaling pathways. Hence, studies on rat islet RIN-5F cell tumor demonstrated that a type II arabinogalactan (200 μg/mL) isolated from green tea leaves increased glucose-stimulated insulin secretion targeting cAMP/PKA [26]. This cAMP/PKA signaling pathway plays a key role in the regulation of glucose homeostasis through gluconeogenesis process and glycogen synthesis and breakdown [25]. Another signal transduction pathway on which tea polysaccharides from green tea (200, 400, and 800 mg/kg b.w. per day for 4 weeks) have been shown to act is the PI3K/Akt signal pathway which stimulates GLUT 4 translocation and activation [10]. Moreover, epigallocatechin gallate promoted glucose uptake by increasing GLUT4 translocation via PI3K/AKT in L6 skeletal muscle cells [15]. Another anti-hyperglycemic strategy is to inhibit sodium glucose transporters such as intestinal SGLT1 and renal SGLT2 that are involved in glucose absorption and to promote GLUT2 and GLUT 4 transporters which facilitate glucose movement across membranes. The acute administration (30 min) and chronic administration (6 weeks) of green tea decoction (50 g/L) and a combination of 4 mg epigallocatechin gallate (EGCG) and 2 mg epigallocatechin (EGC) inhibited SGLT-1 activity and increased *GLUT2* mRNA levels in the jejunum mucosa and *GLUT4* mRNA levels in adipose tissue in Wistar rats fed a high fat diet [12]. Moreover, epigallocatechin gallate inhibited GLUT4-dependent insulin-like growth factor I and II, and stimulated glucose transport in 3T3-L1 adipocytes [27]. Another antidiabetic mechanism has been shown for tea polypeptides from green tea (1000 mg/kg bw/day, p.o. for 5 weeks) which reduced blood glucose and ameliorated diabetic nephropathy in a streptozocin-induced mice model by stimulating the AGEs/RAGE/TGF-β1 signaling pathway and inhibiting the NF-κB pathway [28]. Moreover, pu-erh tea ameliorated insulin resistance by inhibiting IL-6 induction via signal transducer and activator of transcription 3 (STAT3) in C57BL/6J mice [9]. Finally, Chen et al. (2019) found that non-catechin flavonoids (500, 1000, 2000 ppm, for 72 h) ameliorated TNF-α induced insulin resistance by stimulating glucose uptake and inhibiting p38 and JNK pathways in HepG2 cells [29].

The ability of different types of teas and their bioactive compounds to inhibit the enzymes α-amylase and α-glucosidase has been extensively studied in recent years. The enzyme α-amylase, found in saliva and pancreas, catalyzes the hydrolysis of alpha 1–4 bonds of glycogen and starch to form simple sugars (oligosaccharides and disaccharides). Then, α-glucosidase enzyme catalyzes alpha 1–4 bonds of oligosaccharides and disaccharides to form glucose in the small intestine. Both enzymes are a therapeutic target for diabetes mellitus treatment [30]. Yang and Kong (2016) [31] investigated the α-glucosidase inhibitory activity of green tea, black tea, and oolong tea, oolong tea having the lowest IC_{50} value (1.38 μg/mL). Moreover, Oh et al. (2015) [32] compared α-glucosidase inhibitory activity of tea water extracts and tea pomace extracts obtained from green, oolong, and black tea; this research demonstrated that there were no differences between tea water extracts and tea pomace extracts and that green tea was the most active of all assayed type teas (IC_{50} = 2040 μg/mL for tea water extracts and IC_{50} = 1950 μg/mL for tea pomace extracts). Furthermore, the aqueous extract of black tea leaves inhibited α-glucosidase enzyme activity (IC_{50} = 2400 μg/mL for sucrose and IC_{50} = 2800 μg/mL for maltase) but not α-amylase activity [33]. Additionally, black and green teas inhibited α-amylase activity with IC_{50} = 589.86 μg/mL and IC_{50} = 947.80 μg/mL, respectively, and α-glucosidase activity with IC_{50} = 72.31 μg/mL and IC_{50} = 100.23 μg/mL, respectively. Differing chemical composition of these three teas may explain, at least in part, their different effects on diabetes-related enzyme activity. Oolong tea stands out for having dimeric flavan-3-ols (theasinensins), green tea has epigallocatechin-3-gallate as major catechin, and black tea is rich in theaflavins and thearubigins [34,35]. Moreover, the differences in activity for the same type of tea may be due to the fact that the chemical composition is highly influenced by the nature of the green shoots and the procedures to manufacture tea in the producing countries [36]. Apart from studies on black, green, and oolong tea, different ages of pu-erh tea polysaccharide have demonstrated inhibition of α-glucosidase activity, specially 3-year old and 5-year old tea (IC_{50} = 0.583 and 0.438 μg/mL, respectively), however no inhibitory activity was found against α-amylase [37]. In a similar work, Xu et al. (2014) [38] found that pu-erh tea polysaccharides with aging for 3 years and 5 years resulted in inhibition of α-glucosidase enzyme activity with same potency as acarbose (3 years aging) and three times more potently than acarbose (5 years aging). Besides, water extract of pu-erh tea moderately inhibited sucrose activity (IC_{50} = 14.4 μg/mL) and maltase (IC_{50} = 11.4 μg/mL), the compound epigallo-catechin-3-*O*-gallate having the greatest inhibitory activity with IC_{50} = 32.5 μM against sucrose and IC_{50} = 1.3 μM against maltase [14]. In another study, the ethyl acetate fraction from Qingzhuan tea extracts showed significant α-glucosidase inhibitory potential (IC_{50} = 0.26 μg/mL), attributing this activity to the compounds epigallocatechin gallate and epicatechin gallate. Epicatechin gallate has shown to inhibit α-amylase activity (IC_{50} = 45.30 μg/mL) and α-glucosidase activity (IC_{50} = 4.03 μg/mL) and epigallocatechin gallate inhibited α-glucosidase with IC_{50} = 19.5 μM [15,39]. Moreover, the isolated compound amelliaone A from YingDe black tea inhibited more potently α-glucosidase enzyme activity (IC_{50} = 10.2 μM) than the reference compound acarbose (IC_{50} = 18.2 μM) [40]. Furthermore, Hua et al. [41] investigated the inhibitory activity of flavone and flavone glycosides of green tea (Lu'an GuaPian) on α-glucosidase and α-amylase enzymes; 7 kaempferol monoglycoside was the most active against α-glucosidase (IC_{50} = 40.02 μM) and kaempferol diglycoside against α-amylase (IC_{50} = 0.09 μM). Based on IC_{50} values of the isolated compounds, epigallocatechin gallate and 7 kaempferol monoglycoside resulted as the most promising α-glucosidase inhibitory agents and kaempferol diglycoside the most interesting α-amylase inhibitor.

Oxidative stress (reactive oxygen species/antioxidant imbalance) contributes to the development of diabetes mellitus and its associated complications. Black tea aqueous extract (2.5%) reduced lipid peroxidation levels and increased GSH content in diabetic rats [22]. Moreover, tea polysaccharides from green tea (200, 400, and 800 mg/kg b.w. per day for 4 weeks) increased superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities in diabetic Kunming mice [10]. Furthermore, in another study epigallocatechin-3-gallate demonstrated reduction of lipid peroxidation, protein oxidation, and superoxide level and increased antioxidant enzymatic activity and GSH content in diabetic rats [23].

Moreover, several studies have identified changes in relevant biomarkers for diabetes mellitus after tea extract supplementation. Hence, epigallocatechin-3-gallate (2 mg/kg, p.o., alternative days, 1 month) reduced glucose levels and glycosylated hemoglobin and increased insulin [23]. Moreover, green tea powder (10%) and ethanolic extract of green tea (5%) for 8 weeks reduced glucose levels in Sprague-Dawley rats [16]. Furthermore, green tea extract and pu-erh tea extract (both at doses of 0.8 g/kg with a content of 30% catechin and 10% caffeine) but not epigallocatechin-3-gallate (at a dose of 0.24 g/kg) reduced blood glucose levels in BALB/c mice which suggests that caffeine is essential in the hypoglycemic effect of tea [41]. The doses and time treatments could explain the differences in the effectiveness of epigallocatechin-3-gallate [23,41]. Finally, both black and green teas suppressed the increased production of advanced glycosylation end products in 3T3-L1 preadipocytes [42].

Clinical trials were randomized, double-blind, and placebo-controlled and they evaluated the hypoglycemic effect of green tea (mainly) and black tea. Most of these works included patients of both sexes (except one with overweight women) and aged between 30 and 80 years. The duration of the treatments varied from weeks to months and the doses/day administered were also different in each clinical trial (i.e., 1 g/day; 2.5 g/three times day; 560 mg tea polyphenols/two times day; 200 mg tea extract/day). The parameters measured were different, being analyzed from biochemical parameters such as blood glucose levels to oxidative stress markers. Doses of 1 g of dry extract of green tea and 2.5 g/three times day of black tea for 12 weeks were effective to improve glycemic control even better than the reference drug metformin [43,44]. Moreover, both 560 mg tea polyphenols/two times day for 20 weeks and 200 mg tea extract/day for 9–18 months had an antioxidant effect as evidenced in an increase of superoxide dismutase activity and a decrease of lipid peroxidation [45,46].

3.2. *Camellia sinensis and Hypercholesterolemia*

Hypercholesterolemia (blood cholesterol values > 200 mg/dL) affects over 39% of people worldwide, Europe and America being the most affected continents [44].

Green tea extracts have demonstrated in *in vivo* studies reduced total cholesterol, LDL, and tryglicerides [13,16,47] which is mainly attributed to epigallocatechin gallate and flavonols [48,49]. Moreover, Chungtaejeon aqueous extracts, which is a Korean fermented tea, has shown to decrease hepatic cholesterol, total serum cholesterol, and LDL cholesterol in high fat atherogenic Wistar rats [50].

Clinical trials on the anti-hypercholesterolemia action of black tea and green tea were investigated in patients with high cholesterol levels in randomized, double-bind, and placebo studies. The cholesterol-lowering effect of tea extracts was evaluated by measuring biochemical parameters (i.e., LDL content and total cholesterol) and antioxidant content. Both clinical studies with black tea demonstrated its effectiveness of reducing LDL/HDL ratio, total cholesterol, apolipoprotein B, and oxidative stress. In one of these clinical trials, the effective dose was 2.5 g black tea and phytosterol mixture which contains 1 g plant sterols for 4 weeks [51]. However, for the other study, a specific dose is not specific, but five cups of black tea per day for two 4-week treatment periods [52]. On the other hand, the consumption of "Benifuuki" green tea, which is rich in methylated catechins (3 g of green tea extract/three times daily for 12 weeks) contributed significantly to reduce serum total cholesterol and serum LDL cholesterol compared to "Yabukita" green tea or barley infusion (placebo tea) consumers [53].

3.3. *Camellia sinensis and Hypertension*

Hypertension (blood pressure of ≥ 130/85 mm Hg) is one of the most common cardiovascular diseases which affects around 1.13 billion people worldwide. Endocrine hypertension occurs when there is a hormone imbalance as example in Cushing syndrome, primary aldosteronism, and pheochromocytoma [54,55].

Angiotensin I-Converting Enzyme converts angiotensin I into angiotensin II (vasoconstrictor properties). Infusions and decoctions of four black tea samples (Doors tea, Siliguri tea, Guwahati tea, and Nilgiri tea) (15 µg/mL) were investigated for their ability to inhibit angiotensin I converting enzyme. In general, decoctions were more active than infusions and Nilgiri tea showed the highest inhibitory activity. Antihypertension properties are mainly attributed to thearubigin and theaflavin [56,57]. In another *in vitro* study, pretreatments with black tea extract (0.3–5 µg/mL) and theaflavin-3,3′-digallate (0.03–0.5 μg/mL) for 30 min improved endothelium dependent relaxations in homocysteine (endoplasmic reticulum stress inductor) treated cultured rat aortic endothelial cells [58]. Moreover, San Cheang et al. (2015) [58] also investigated the effect of black tea extract (15 mg/kg/day for 2 weeks) in a rat model of angiotensin II. This study revealed that black tea extract prevented elevated plasma homocysteine levels and downregulated endoplasmic reticulum stress markers. Furthermore, Nomura et al. (2017) [59] investigated the protective effect of three different cultivars of *Camellia sinensis* ("Yabukita", "Sofu" and "Sunrouge") in a model of hypertensive rats fed with a high salt diet. All these tea cultivars reduced urinary NO metabolite and, moreover, "Yabukita" and "Sofu" increased soluble guanylate cyclase expression.

Finally, a single clinical trial has been identified in which the effect of tea, compared with coffee, on blood pressure was evaluated. This study (1352 subjects aged 18–69 years) stratified population in three groups (non-consumers, ≤3 dL/d consumers, and >3 dL/d consumers of tea or coffee). Results showed that consumption of 1 dL/day of tea was associated with lower systolic blood pressure (by 0.6 mm Hg) and lower pulse pressure (by 0.5 mm Hg) [60].

3.4. Camellia sinensis and Metabolic Syndrome

The metabolic syndrome is a cluster of metabolic disorders (obesity, hypertension, hypercholesterolemia, and diabetes) that favor cardiovascular disease development [61,62]. It is estimated that around a billion people worldwide suffer from metabolic syndrome [63].

Yang et al. (2014) [64] demonstrated that green tea extract (0.2%–0.5%, w/v) inhibited lipid accumulation during adipogenesis in 3T3-L1 preadipocytes by reducing expression of transcription factors C/EBPα and PPARγ.

The atypical antipsychotic drug olanzapine is associated with severe metabolic side effects through H1 receptor antagonism, 5-HT2 C receptor antagonism, D2 receptor antagonism, and muscarinic (M3) receptor antagonism [65,66]. Green tea aqueous extract (25, 50, and 100 mg/kg/day for 11 days) showed to reduce body weight gain, hypertension, and hyperleptinemia, to decrease blood glucose, triglycerides, total cholesterol, and LDL, and to increase HDL in adult male Wistar rats olanzapine induced [67]. In another *in vivo* study, Xu et al. (2018) [68] investigated the effect of large yellow tea manufactured in the Anhui Province of China on metabolic syndrome in high fat diet treated C57BL/6 mice. This work revealed that yellow tea improved metabolic abnormalities (changes in lipid profile, hyperglycemia, and body weight).

In a clinical trial, patients with metabolic syndrome who received decaffeinated green tea extracts capsules (500 mg green tea extract providing 400 mg catechins; two capsules/time/day for 12 weeks) had lower adiponectin and visfatin concentration levels than control patients who received water [64].

3.5. Camellia sinensis and Obesity

Obesity (body mass index ≥ 30) and overweight (body mass index ≥ 25) are increasing due to an augmented intake of energy-dense foods and sedentary lifestyle; it affects more than 1.9 billion adults worldwide. This disease causes about 4 million deaths globally [69] and it is related to other prevalent pathologies including hypertension, type 2 diabetes mellitus, stroke, obstructive sleep apnea, and several cancers [70].

The mouse adipocyte 3T3-L1 cell line has been extensively used to study the *in vitro* effect of tea extracts and its isolated compounds [71–73]. In obesity *in vivo* studies, C57BL/6J mice are the most widely animal model since they are susceptible to high fat diet-induced obesity [74,75]. Moreover, other experimental animal models have been used to investigate the effects of different kind of teas and isolated compounds on obesity including Wistar rats, Sprague-Dawley rats, and Swiss mice fed with a high fat diet [76,77].

Several signaling pathways associated with obesity development have been described. The cAMP/PKA pathway participates in adipogenesis process regulation. The stimulation of the serine/threonine kinase protein kinase A (PKA) activity inhibits adipogenesis whereas the inhibition of PKA activity favors the adipogenic process [78]. Green tea polyphenols (epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin, gallocatechin, catechin, and gallocatechin gallate) increased norepinephrine-induced lipolysis via protein kinase A-dependent pathway in the differentiated mouse adipocyte 3T3-L1 cell line [71]. Moreover, the mitogen activated protein kinases (MAPKs) signaling pathways (extracellular signal regulated kinases (ERKs), Jun amino terminal kinases (JNKs), and stress activated protein kinases (p38/SAPKs)) are involved in adipocyte differentiation and in adipogenesis regulation [79]. Green tea polyphenols, gallocatechin gallate, and epigallocatechin-3-gallate decreased MAPK pathway activation as evidenced in downregulation of adipogenic factor expression (CCAAT element binding protein α (C/EBPα), peroxisome proliferator-activated receptor gamma (PPARγ), and sterol regulatory element-binding protein-1c (SREBP-1c)) in a differentiated mouse adipocyte 3T3-L1 cell line [72,73]. Furthermore, the compound gallocatechin gallate has also demonstrated inhibition of inflammation through NF-κB signaling; adipose tissue inflammation is involved in several pathologies associated with obesity such as diabetes mellitus type 2 [73].

In addition to acting on these signaling pathways, several *in vitro* studies focused on the inhibitory effect on the digestive enzyme pancreatic lipase which hydrolyzes triglycerides into monoglycerides and free fatty acids. The ethanol extract of *Camellia sinensis* has demonstrated to suppress lipase activity (IC_{50} = 0.5 mg/mL) [80] and among isolated compounds, highlight in order of pharmacological potency theaflavin-3,3'-digallate (IC_{50} = 1.9 μM), theaflavin-3-gallate (IC_{50} = 3.0 μM), theaflavin-3'-gallate (IC_{50} = 4.2 μM), theaflavin (IC_{50} > 10 μM), epigallo-catechin-3-*O*-gallate (IC_{50} = 13.3 μM), and catechin-3-*O*-gallate (IC_{50} = 13.6 μM) [81,82].

Oxidative stress plays a key role in the development of comorbidities (insulin resistance, cardiovascular problems, diabetes) associated with obesity. The compounds gallocatechin gallate and epigallocatechin-3-gallate at a concentration of 10 μg/mL act as potent antioxidant by decreasing ROS production [73].

Moreover, using experimental *in vivo* models, the effect of tea extracts and their bioactive compounds on changes in relevant biomarkers for obesity have been demonstrated. Green tea extract supplementation has anti-obesity effects by reducing body weight, white adipose tissue fat, liver fat accumulation, and serum triglyceride levels and by increasing lysophospholipids levels and energy expenditure [83–91]. These anti-obesity properties of green tea are mainly attributed to its polyphenols (i.e., epigallocatechin gallate and epigallocatechin) and polysaccharides [12,15]. Moreover, Heber et al. (2014) [74] compared the effects of decaffeinated polyphenol extracts from green tea, black tea, and oolong tea in in male C57BL/6J mice fed a high fat diet. This study revealed that all teas reduced body weight, total visceral fat volume, and liver lipid weight and, that green tea polyphenols and oolong tea polyphenols also increased adiponectin gene expression. Besides, oolong tea reduced body weight and fat accumulation by regulating fatty acid oxidation and energy expenditure [75]. Moreover, oolong tea, white tea, yellow tea, pu-erh tea, and black tea have also showed to decrease body weight, plasma triglycerides, white fat accumulation, and fatty acid synthesis and to increase energy expenditure, fatty acid oxidation, and fecal triglycerides excretion in Wistar rats, Sprague-Dawley rats and Swiss mice fed with a high fat diet [76,77]. Furthermore, other studies showed that pu-erh tea and Traditional Korean Chungtaejeon, which are fermented teas, possess anti-obesity properties by ameliorating hepatic lipid metabolism and decreasing fat mass [9,92]. Studies on bioactive compounds isolated from tea extracts have shown that epigallocatechin-3-gallate is responsible of reducing body weight, fat infiltration in liver tissue, and of increasing serum lipid profiles [11,93] and teasaponin reduced body weight gain and improved gut microbiota alteration and cognitive decline in obese C57BL/6J mice [14].

Clinical studies evaluate the anti-obesity activity of green tea and its main secondary metabolite epigallocatechin-3-gallate. These clinical trials were mainly randomized, double-blind and placebo-controlled, except one which was single-blind and another one which was longitudinal. The duration of these clinical trials was mainly weeks (6, 8, and 12 weeks), although there is a study with 12-month intervention. There is also variability in the administered doses from 300 mg/day of EGCG to 856.8 mg/day. Analyzing the different clinical trials, it has been observed that high doses of EGCG (856.8 mg for 6–12 weeks) do have anti-obesity activity, reducing weight in women and men with a body mass index (BMI) ≥ 27 kg/m^2 [94,95]. However, in another study in which a dose of 843 mg of EGCG was administered for 12 months, it had no effect on reductions in adiposity nor body mass index. Compared with the previous cited studies, this lack of efficacy can be attributed to the fact that this last study has only been performed with postmenopausal obese women [96]. On the other hand, low doses of EGCG (300 mg/day and 560 g/day for 12 weeks) do not have any beneficial effect on weight control [97,98]. Finally, another clinical trial elucidated that the possible mechanism of action by which EGCG exerts its anti-obesity activity is by increasing HIF1-alpha (HIF1-α) and rapamycin-insensitive companion of mTOR (RICTOR) [99].

3.6. Camellia sinensis and Osteoporosis

Osteoporosis is a very common metabolic skeletal disorder characterized by bone formation reduction (decrease in osteoblast number) and bone resorption increase (sex hormone lack). As a consequence of this disequilibrium between both processes, there is a bone mass loss and a bone tissue deterioration leading to an increase risk of fractures especially hip and vertebral fractures [100]. Osteoporosis is a multifactorial disease, with age being the most common risk. Other risk factors include environmental (i.e., alcohol consumption, smoking, vitamin D and calcium deficiencies, low physical activity), metabolic (estrogen deficiency), and genetic factors (i.e., cathepsin K, sclerostin, chloride channel 7, high-risk ethnic groups) [101]. Osteoporosis affects around 200 million people worldwide (30% of women, 12% of men) [101].

Osteoporosis *in vitro* models commonly employ mouse macrophage-like RAW264.7cell lines. Receptor activator of nuclear factor-κB ligand (RANKL) is used to differentiate macrophage cells into mature osteoclasts [68]. Moreover, for *in vivo* studies, the most common animal model is ovariectomized rats. Ovariectomized rat bone loss has many similarities to those of postmenopausal bone loss such as bone resorption increases more than bone formation and intestinal calcium absorption reduction; these related characteristics make ovariectomized rats an ideal animal model to study osteoporosis pathogenesis [102].

Green tea extract (25, 50, and 100 µg/mL, 48 h) has shown to inhibit RANKL-induced osteoclast formation in the mouse macrophage-like cell line RAW264.7 related to NFATc1, cathepsin K, C-Fos, and MMP-9 gene levels reduction [103]. Moreover, the isolated compound gallocatechin gallate (oxidation product of epigallocatechin-3-gallate) at 10 µM concentration inhibited osteoclastogenesis more potently than epigallocatechin-3-gallate through gene and protein downregulation (TRAP, c-Src, β3-Integrin, cathepsin K, and MMP-9) and master transcriptional regulators downregulation (NFATc1 and c-Fos) in RAW264.7 cells [104].

Redox imbalance is also involved in the pathogenesis of bone loss. Overproduction of ROS increases osteoclast activity and inhibits mineralization [105]. Flavones from tea have demonstrated to act as antioxidants. Particularly, epicatechin isolated from Huangshan Maofeng tea (green tea produced in Anhui province of China) has shown to protect against oxidative stress in a hydrogen peroxide-induced model on C2C12 mouse myoblast cells [106].

Moreover, *in vivo* evidence has demonstrated that tea exerts a protective effect on osteoporosis as evidenced in relevant biomarkers. Hence, green tea extracts (dose of 370 mg/kg for 13 weeks) increase cortical and trabecular bone mass in ovariectomized female Wistar rats [107]. Furthermore, green tea polyphenols supplementation (4 months) improved bone properties (alleviate bone loss and favored bone microstructure restructuring) in obese rats fed with a high fat diet and a high fat diet followed by a caloric restricted diet [108].

Patients with diabetes mellitus have low bone mass which increase fracture risk. Therefore, de Amorim et al. (2018) [104] conducted a double-blind, randomized, placebo-controlled clinical trial to evaluate the effect of green tea extract on bone mass of diabetic patients. This clinical trial revealed that those subjects with diabetes who received 1120 mg of green tea extract containing 560 mg of polyphenols/day for 20 weeks increased their bone mineral content.

4. Conclusion

This systematic review unified publications on the pharmacological effect (preclinical and clinical studies) of teas made from the leaves of *Camellia sinensis* and its isolated compounds on metabolic and endocrine disorders. Most pharmacological studies were *in vitro* and *in vivo* studies focused on diabetes and obesity. Clinical trials, although they have demonstrated promising results, are very limited. The most studied tea and isolated compounds have been green tea and epigallocatechin gallate, respectively. For almost each pathology, research has been focused on investigating the effect on different signaling pathways, oxidative stress, and relevant biomarkers. Among the types of teas, differences in pharmacological action may be explained by differing chemical compositions. Moreover, differences in activity for the same type of tea can be observed because chemical composition is highly influenced by the nature of the green shoots and the procedures to manufacture tea in the producing countries. Regarding clinical trials, the different doses, treatment duration, and subjects included in each study explain the differences in the activity of tea and bioactive compounds. Future research should be aimed at providing more clinical evidence on less studied pathologies such as osteoporosis, hypertension, and metabolic syndrome. Given the close relationship among all endocrine disorders, it would be of interest to find a standard dose of tea or their bioactive constituents that would be beneficial for all of them.

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Appendix A

Table A1. *In vitro* pharmacological studies for *Camellia sinensis*.

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Diabetes	Amelliaone A	α-Glucosidase model	-	α-Glucosidase inhibition: IC_{50} = 10.2 μg/mL	[40]
	Arabinogalactan	Rat islet tumor RIN-5F cells	50 or 200 μg/mL, 2 h	↑ Insulin secretion	[26]
	Black and green teas	Mouse 3T3-L1 preadipocytes	10 μg/mL, 24 h	↑ SOD, CAT, and GPx activities ↓ Protein glycation ↓ α-Amylase and α-glucosidase activities	[42]
	Black tea aqueous extract	α-Glucosidase model α-Amylase model Caco-2 cells	-	↓ α-Glucosidase activity No effect on GLUT2 and SGLT1 uptake	[33]
	Black, green, and dark tea extracts	Human liver HepG2 cancer cells	-	↓ α-Glucosidase, aldose reductase, advanced glycation end-products ↑ Glucose uptake (dark tea extracts)	[109]
	Epicatechin gallate	α-Amylase model α-Glucosidase models	-	↓ α-Amylase activity (IC_{50} = 45.30 μg/mL) ↓ α-Glucosidase activity (IC_{50} = 4.03 μg/mL)	[39]
	Epigallocatechin gallate	Mouse 3T3-L1 adipocytes	20 μM, 2 h	↓ IGF-I and IGF-II stimulation	[27]
	Epigallocatechin-3-O-gallate	Rat skeletal muscle L6 cells	0, 20, 40, 50, and 60 μM, 48 h	↓ α-Glucosidase activity (IC_{50} = 19.5 μM) ↑ Glucose uptake Promotion GLUT4 translocation to plasma	[15]
	Flavanols	α-Glucosidase model	-	↓ Sucrase activity and maltase activity (EGCG IC_{50} = 32.5 and 1.3 μM, respectively)	[14]
	Flavone and flavone glycosides	α-Glucosidase model α-Amylase model	-	↓ α-Glucosidase activity (kaempferol monoglycoside IC_{50} = 40.02 μM) ↓ α-Amylase activity (kaempferol diglycoside IC_{50} = 0.09 μM)	[41]
	Green tea polyphenols Green, black, and oolong tea extracts	α-Glucosidase model	-	↓ α-Glucosidase activity (green tea polyphenols IC_{50} = 2.33 μg/mL, green tea IC_{50} = 2.82 μg/mL, black tea IC_{50} = 2.25 μg/mL, and oolong tea IC_{50} = 1.38 μg/mL)	[31]

Table A1. *Cont.*

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Diabetes (continued)	Green, oolong, and black water and pomace tea extracts	Rat intestinal α-glucosidase activity	-	↓ α-glucosidase activity (tea water extract IC_{50} = 2040 μg/mL and tea pomace extract IC_{50} = 1950 μg/mL)	[32]
	Non-catechin flavonoids	Human liver HepG2 cancer cells	Insulin (5 μM) Insulin + TNF-α (30 ng/mL) Insulin + TNF-α + NCF (2000 ppm) Insulin + TNF-α + NCF (1000 ppm) Insulin + TNF-α + NCF (500 ppm) 2, 4, and 6 h	↑ TNF-α induced insulin resistance ↓ Glucose levels	[29]
	Pu-erh tea polysaccharides	α-Glucosidase model α-Amylase model	-	↓ α-glucosidase activity No α-amylase inhibition	[37]
	Qingzhuan dark tea	α-Glucosidase model	IC_{50} 2270 μg/mL for ethyl acetate fraction	↓ α-Glucosidase activity (ethyl acetate fraction, EGCG, ECG)	[110]
	Tea polysaccharides	α-Glycosidase model	-	↑ α-Glycosidase inhibitory activities (polysaccharides with 5 years aging)	[38]
Hypertension	Black tea aqueous extracts Thearubigin, theaflavin, catechin, epicatechin, epigallocatechin gallate, gallic acid, caffeine	Angiotensin converting enzyme model	Aqueous tea extract (15 μg/mL) Isolated compounds (37 μM)	↑ ACE inhibitory activity (Thearubigin, theaflavin, catechin)	[56]
	Black tea extract Theaflavin-3,3′-digallate	Endothelial cells from rat thoracic artery	Black tea (0.3–5 μg/mL), 30 min TF3 (0.03–0.5 μg/mL), 30 min	Endothelium dependent relaxations restored ↓ ROS production	[57]
Metabolic Syndrome	Green tea extract	Mouse 3T3-L1 preadipocytes	Green tea extract (0.2%–0.5%, w/v), 2 days	↓ Adipogenesis induced lipid accumulation ↓ C/EBPα and PPARγ expression	[64]

Table A1. *Cont.*

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Obesity	Black tea theaflavins	Pancreatic lipase model	-	Pancreatic lipase inhibition Theaflavin-3,3′-digallate IC_{50} = 1.9 µM Theaflavin-3′-gallate IC_{50} = 4.2 µM Theaflavin-3-gallate IC_{50} = 3.0 µM Theaflavin IC_{50} > 10 µM	[81]
	Ethanol tea extracts	Porcine pancreatic lipase type II	5 mg/mL ethanol	Antilipase activity (IC_{50} = 500 µg/mL)	[80]
	Flavanols	Lipase model	-	↓ Lipase activity ECG (IC_{50} = 16.0 µM) CG (IC_{50} = 13.6 µM) Epiafzelechin-3-O-gallate (IC_{50} = 19.8 µM) EGCG (IC_{50} = 13.3 µM)	[14]
	Gallocatechin gallate Epigallocatechin gallate	Mouse 3T3-L1 preadipocytes	Gallates 0–20 µg/mL	Anti-adipogenic activity ↓ Intracellular lipid droplets (GCG, EGCG) ↓ PPAR γ, SREBP-1c and C/EBP α adipogenic transcription factors (GCG, EGCG) ↓ ROS levels (GCG) ↓ NF-κB activation (GCG) ↓ IL-6 production (GCG)	[73]
	Green tea catechins	Mouse 3T3-L1 preadipocytes	Green tea catechins with/without norepinephrine (0.1 or 1 µM) for 6 or 24 h	↑ Lipolysis via PKA-dependent pathway	[71]
	Green tea polyphenols Epigallocatechin-3-gallate	Mouse 3T3-L1 preadipocytes	Green tea polyphenols (1, 10, and 100 µg/mL) EGCG (6.8 µg/mL)	↓ Triglyceride accumulation ↓ Adipogenic factor C/EBPα, SREBP-1c, and PPARγ expression	[72]
	Traditional Korean Chungtaejeon	Mouse 3T3-L1 preadipocytes	Traditional Korean Chungtaejeon (250 µg/mL)	↓ Lipid accumulation ↓ PPARγ expression ↓ Adipocyte lipid-binding protein	[92]
Osteoporosis	Green tea extract	Mouse macrophage RAW 264.7 cells treated with RANKL (50 ng/mL)	25, 50, or 100 µg/mL for 48 h	↓ mRNA expression osteoclast-associated genes ↓ NFATc1, c-Fos, c-src and cath-K protein levels	[103]
	Gallocatechin gallate Epigallocatechin-3-gallate	Mouse macrophage RAW 264.7 cells	10 µM, 20 min	↓ RANKL-induced osteoclast differentiation ↓ F-actin ring formation ↓ Osteoclastogenesis-related marker genes and proteins expression, especially gallocatechin gallate	[104]
	Flavones	Rat osteoblastic cells C2C12 mouse myoblast cell line	From 3.125 to 50 µg/mL, 48 h	↑ Alkaline phosphatase activity (epicatechin) ↑ Hydroxyproline content (epicatechin) ↑ Area of mineralized bone nodules (epiafzelechin)	[106]

Appendix B

Table A2. *In vivo* pharmacological studies for *Camellia sinensis*.

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Diabetes	Black tea aqueous extract	GK rats	Group 1: black tea 31.3, 62.5, and 250 mg/kg Group 2: acarbose 0.1, 0.3, and 3.0 mg/kg Group 3: acarbose 0.3 mg/kg + black tea 31.3 mg/kg	↓ Plasma glucose levels	[33]
	Black tea aqueous extract	Alloxan-induced diabetic rats	Group 1: control Group 2: alloxan Group 3: black tea extract (1 mL/100 g body w/d for 10 days before alloxan injection and 35 days after alloxan injection) Group 4: black tea extract (35 days) Group 5: diabetic insulin group (twice a day/subcutaneous injection of three units of insulin)	↑ Plasma antioxidant potential ↓ Lipid peroxidation levels ↑ GSH levels	[22]
	Epigallocatechin-3-gallate	C57BL/6J mice	Group 1: low fat diet Group 2: high fat diet Group 3: high fat diet + EGCG (25 mg/kg) Group 4: high fat diet + EGCG (75 mg/kg)	↓ Plasma glucose ↓ Insulin level ↓ Advanced glycation end products	[11]
	Epigallocatechin-3-gallate	Wistar rats streptozotocin-nicotinamide-induced diabetic rats	Group 1: control Group 2: EGCG (2 mg/kg body wt) Group 3: diabetic control group Group 4: diabetic control group + EGCG 1 month	↓ Glucose, glycosylated hemoglobin, HOMA-IR and lipid profile level ↑ Insulin levels ↑ GSH levels and SOD and CAT activities	[23]
	Green tea decoctions Epigallocatechin gallate Epigallocatechin	Wistar rats	Group 1: water Group 2: green tea decoctions Group 3: EGCG, EGC	↓ SGLT-1 activity ↑ GLUT2 activity ↑ Glucose tolerance	[12]
	Green tea ethanol extracts	Sprague-Dawley rats	Group 1: hyperglycemic rats Group 2: hyperglycemic rats + tea extract 10% Group 3: hyperglycemic rats + tea extract 5% 8 weeks	↓ Serum glucose	[16]
	Green tea extract	Nematode *Caenorhabditis elegans*	0.1%, 48 h	↓ Glucose induced damage	[24]

Table A2. *Cont.*

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Diabetes (continued)	Green tea extract	Rat model High sodium diet	Group 1: high sodium diet Group 2: high sodium diet + 2 g green tea extract in kg diet Group 3: high sodium diet + 4 g green tea extract in kg diet 6 weeks	↓ Insulin level and homeostatic model assessment	[13]
	Green tea polysaccharides	Kunming mice	Group 1: high fat diabetic control Group 2: rosiglitazone Groups 3, 4, and 5: green Tea polysaccharides (200, 400, and 800 mg/kgb.w. per day) 4 weeks	↓ Insulin resistance PI3K/Akt signal pathway	[10]
	Pu-erh tea and green tea	BALB/c mice	Group 1: glucose (2000 mg/kg) Group 2: glucose (2000 mg/kg) + pu-erh tea (800 mg/kg) Group 3: glucose (2000 mg/kg) + green tea (800 mg/kg) Group 4: glucose (2000 mg/kg) + EGCG (240 mg/kg) Group 5: glucose (2000 mg/kg) + EGCG (240 mg/kg) + caffeine (80 mg/kg) Group 6: caffeine (80 mg/kg)	↓ Blood glucose levels	[111]
	Pu-erh tea polysaccharides (TPS)	ICR mice	Group 1: control Group 2: acarbose (5 mg kg^{-1}) Group 3: TPS (1 mg kg^{-1}) Group 4: TPS (5 mg kg^{-1})	↓ Blood glucose levels	[37]
	Pu-erh tea extract	C57BL/6J mice	Group 1: normal chow diet Group 2: high fat diet Group 3: normal chow diet + pu-erh tea extract (5 mg/mL, 17 weeks) Group 4: high fat diet + pu-erh tea extract (5 mg/ml, 17 weeks)	↓ Gluconeogenesis related genes expression	[9]
	Tea polypeptides from green tea	High fat diet/streptozocin induced (30 mg/kg bw) diabetic mice	1000 mg/kg bw/day, p.o., 5 weeks	↓ Total urinary protein, creatinine, and urine nitrogen	[28]
	Tea water extract and tea pomace extract of green and black tea	Sprague-Dawley rats	Group 1: sucrose Group 2: tea extracts (0.5 g/kg body wt)	↓ Glucose level	[32]

Table A2. *Cont.*

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Hypercholes-terolemia	Chungtaejeon aqueous extracts	Wistar rats high fat atherogenic diet (HFAD)	Group 1: normal basal diet Group 2: HFAD Group 3: 100 mg/kg day tea extract + HFAD Group 4: 200 mg/kg day tea extract + HFAD Group 5: 400 mg/kg day tea extract + HFAD	↓ LDL cholesterol ↓ Total serum cholesterol ↓ Hepatic cholesterol	[50]
	Epigallocatechin-gallate	Wistar rats Fluoride-induced oxidative stress mediated cardiotoxicity	Group 1: normal saline Group 2: EGCG (40 mg/kg BW/day) Group 3: sodium fluoride (25 mg/kg body weight/day, 4 weeks) Group 4: EGCG (40 mg/kg BW/day) + sodium fluoride (25 mg/kg body weight/day, 4 weeks)	↓ Lipid peroxidative markers ↓ Plasma total cholesterol ↓ Triglycerides ↓ Phospholipids ↓ LDL cholesterol ↑ HDL cholesterol	[48]
	Green tea ethanol extracts	Sprague-Dawley rats	Group 1: hypercholesterolemic rats Group 2: hypercholesterolemic rats + diet containing green tea extracts 5% Group 3: hypercholesterolemic rats + diet containing tea powder 10% 8 weeks	↓ LDL ↓ Triglycerides ↓ Cholesterol	[16]
	Green tea extracts	Rat model High sodium diet	Group 1: high sodium diet Group 2: high sodium diet + 2 g green tea extract in kg diet Group 3: high sodium diet + 4 g green tea extract in kg diet 6 weeks	↓ Total cholesterol, LDL, cholesterol serum concentrations	[13]
	Green tea polysaccharides	Kunming mice	Group 1: high fat diabetic control Group 2: rosiglitazone Groups 3, 4, and 5: green tea polysaccharides (200, 400, and 800 mg/kgb.w. per day) 4 weeks	↓ Total cholesterol ↓ LDL cholesterol	[47]
	Tea flavonols ("Sofu" green tea leaves and "Yabukita" tea leaves)	Mice model High cholesterol diet induced	Group 1: high cholesterol diet Group 2: high cholesterol diet + water Group 3: high cholesterol diet + "Sofu" green tea Group 4: high cholesterol diet + "Yabukita" tea	↓ Plasma oxidized LDL level	[59]
Hypertension	Black tea extract	Sprague-Dawley rats Angiotensin II induced	Group 1: control Group 2: angiotensin II (50 ng/kg/min) Group 3: angiotensin II + black tea extract (15 mg/kg/day, 14 days)	↑ Endothelium-dependent relaxations ↓ Endoplasmic reticulum stress markers levels ↓ ROS production	[57]
	Green tea from three cultivars "Yabukita", "Sofu" and "Sunrouge"	Hypertensive rats High salt diet	Group 1: high salt water Group 2: high salt water + Yabukita Group 3: high salt water + Sofu Group 4: high salt water + Sunrouge	↓ Urinary NO metabolite ↑ Soluble guanyilate cyclase expression (Yabukita and Sofu)	[59]

Table A2. *Cont.*

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Metabolic Syndrome	Green tea aqueous extract	Olanzapine induced Wistar rats	Group 1: control Group 2: olanzapine (5 mg/kg/day) Groups 3, 4, and 5: green tea aqueous extract (25, 50, and 100 mg/kg/day) + olanzapine Groups 6, 7, and 8: green tea aqueous extract (25, 50, and 100 mg/kg/day)	↓ Body weight gain ↓ Average food and water intake Improved changes in lipid profile ↓ Hyperleptinemia and hypertension	[67]
	Yellow tea	C57BL/6 male mice High fat diet	Group 1: low fat diet Group 2: high fat diet Group 3: high fat diet + 2.5% yellow tea Group 4: high fat diet + 0.5% yellow tea 12 weeks	↓ Body weight, liver weight, and adipose tissue weight ↓ Serum glucose, TC, TG, LDL-C, and HDL-C ↓ Glucose intolerance and insulin resistance	[68]
Obesity	Black tea and green tea decoctions	Male Wistar rats	Group 1: high fat diet Group 2: green tea decoction Group 3: black tea decoction 10 weeks	↑ Fecal triglycerides excretion ↓ Liver triglycerides ↓ Plasma triglycerides ↓ Body weight ↓ Glucose	[76]
	Decaffeinated green tea extract rich in EGCG	Male Swiss mice	Group 1: control diet + water (0.1 mL/day) Group 2: control diet + EGCG (50 mg/kg/day) Group 3: hyperlipidic diet + water Group 4: hyperlipidic diet + EGCG 16 weeks	↓ Body weight ↓ Insulin level ↓ Liver fat accumulation ↑ Glucose uptake	[90]
Obesity (continued)	Decaffeinated polyphenol extracts (green tea, black tea, and oolong tea)	C57BL/6J mice High fat/high sucrose	Group 1: low fat/high sucrose diet Group 2: high fat/high sucrose diet Group 3: high fat/high sucrose diet + green tea polyphenols Group 4: high fat/high sucrose diet + black tea polyphenols Group 5: high fat/high sucrose diet + oolong tea polyphenols	↓ Body weight ↓ Total visceral fat volume ↓ Liver lipid weight ↓ Food intake (green tea polyphenols)	[74]
	Decaffeinated green tea extract rich in EGCG	Swiss mice High fat diet	Group 1: control diet Group 2: high fat diet Group 3: control diet + placebo Group 4: high fat diet + placebo Group 5: control diet + EGCG Group 6: high fat diet + EGCG 8 weeks	↓ Body weights ↓ Serum triglyceride levels ↓ Adipocyte area	[91]
	Epigallocatechin 3-gallate	C57BL/6J mice	Group 1: low fat diet (negative control) Group 2: high fat diet (positive control) Group 3: high fat diet + EGCG (25 mg/kg) Group 4: high fat diet + EGCG (75 mg/kg)	↓Body weight ↓ Liver and kidney weight	[11]

Table A2. *Cont.*

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Obesity (continued)	Epigallocatechin-3-gallate	C57BL/6 mice	Group 1: high fat diet Group 2: high fat diet + EGCG (20 mg/kg)	↓ Body weight ↓ Fat infiltration in liver tissue ↑ Serum lipid profiles	[93]
	Fermented green tea extract	C57BL/6 mice	Group 1: normal diet Group 2: high fat diet Group 3: high fat diet + fermented green tea extract	↓ Body weight gain ↓ Fat mass ↓ Glucose intolerance ↓ Fatty liver symptoms	[84]
	Green tea	C57BL/6J mice	Group 1: normal diet Group 2: high fat (60% energy as fat) Group 3: high fat + 0.25% (w/w) Green tea 12 weeks	↓ Body weight gain ↑ Energy expenditure ↓ Adiposity	[85]
Obesity (continued)	Green tea	C57BL/6J mice	Group 1: normal diet Group 2: high fat diet Group 3: high fat diet + 0.25% (w/w) green tea extract	↑ Lysophospholipids levels	[87]
	Green tea decoctions Epigallocatechin gallate Epigallocatechin	Wistar rats	Group 1: normal diet Group 2: high fat diet Group 3: high fat diet + green tea decoctions	↓ Body weight ↓ Triglycerides ↓ Cholesterol	[12]
	Green tea extract	C57BL/6J mice	Group 1: green tea extract (77 mg/g) Group 2: voluntary exercise Group 3: green tea extract + voluntary exercise	↑ Adipose tissue conversion into brown fat like adipose	[83]
	Green tea extracts	C57BL/6 mice	Group 1: control diet Group 2: high fat diet Group 3: high fat diet + 0.5% polyphenolic green tea extracts 8 weeks	↓ Adiposity ↓ High diet inflammation ↓ Adipocyte size ↓ Lipid droplet size	[86]
	Green tea extract	Sprague–Dawley rats	Group 1: normal diet control Group 2: high fat diet control Group 3: orlistat control (50 mg/kg/d + high fat diet) Group 4: green tea extract (100 mg/kg/d + high fat diet) 50 days	↓ Body weight ↓ White adipose tissue fat	[89]
	Oolong tea water extract	C57BL/6J mice	Group 1: normal diet Group 2: high fat diet Group 3/4/5: oolong tea (different storage years) 6 weeks	↓ Body weight ↓ Fat accumulation ↓ Triglyceride levels ↓ LDL cholesterol ↑ HDL cholesterol level	[75]
	Polyphenol-rich green tea extract	C57BL/6 mice	Group 1: fed a standard diet + gavage with water Group 2: standard diet + gavage with 500 mg/kg GT Group 3: HFD + gavage with water Group 4: HFD+ gavage with GT 16 weeks	↓ Body weight ↓ Body adiposity ↓ Inflammation ↑ Insulin sensitivity	[88]

Table A2. *Cont.*

Disease	Extract/Isolated Compound	Experimental Model	Treatments	Major Findings	References
Obesity (continued)	Polysaccharides, polyphenols and caffeine from green tea	Sprague-Dawley rats High fat rats	Groups control, polysaccharides, polyphenols, and caffeine at two doses (low and high)	↓ Body weight and fat accumulation ↑ Antioxidant levels ↓ Leptin levels ↓ Fatty acids absorption	[112]
	Pu-erh tea extract	C57BL/6J mice	Group 1: normal chow diet Group 2: high fat diet Group 3: normal diet + tea extract (5 mg/mL, 17 weeks) Group 4: high fat diet + tea extract (5 mg/mL, 17 weeks)	↓ Obesity ↓ Hepatic steatosis and liver inflammation ↓ Liver injury	[9]
	Teasaponin	High fat diet C57BL/6 male mice	High fat diet (8 weeks) + oral teasaponin (0.5%) with high diet (6 weeks)	↓ Neuroinflammation ↑ Brain derived neurotrophic factor ↑ Glucose tolerance ↓ Body weight gain	[82]
	Traditional Korean Chungtaejeon	C57BL6J-Lep ob/ob mice	Traditional Korean Chungtaejeon (200 or 400 mg/kg body weight, 10 weeks)	↓ Body weight gain ↓ Fat mass ↓ Food efficacy ratio ↓ Levels of plasma triglyceride and total cholesterol	[92]
	Water extract of white tea, yellow tea, oolong tea, green tea, white tea, and raw pu-erh tea	High fat diet induced obese mice	Group 1: untreated Group 2: atorvastatin-treated (oral daily at 10 mg/kg body weight) Group 3: green tea Group 4: yellow tea Group 5: black tea Group 6: white tea Group 7: raw pu-erh tea Group 8: oolong tea Teas: daily oral 1000 mg/kg body weight for 9 weeks	↓ Body weight ↓ White fat accumulation ↑ Energy expenditure and fatty acid oxidation (white, yellow, and oolong teas) ↓ Fatty acid synthesis (green, white, and raw pu-erh teas) Best tea: white tea	[77]
Osteoporosis	Green tea aqueous extract	Ovariectomized female rats	GTE (60, 120, and 370 mg/kg, 13 weeks)	↑ Bone mass ↓ Trabecular bone loss	[103]
	Green tea polyphenols	Sprague-Dawley	Group 1: high fat diet Group 2: caloric restricted diet Group 3: high fat diet + 0.5% green tea polyphenols Group 4: caloric restricted diet + 0.5% green tea polyphenols	↑ Femoral mass and strength ↑ Trabecular thickness and number ↑ Cortical thickness of tibia ↓ Trabecular separation ↓ Formation rate and eroded surface at proximal tibia ↓ Insulin-like growth factor-I and leptin	[108]

Appendix C

Table A3. Clinical trials for *Camellia sinensis.*

Study (Author, Year, Country)	Study Design	Sample Size	Population	Type of Plant	Intervention	Duration of Treatment	Results
				DIABETES			
Alves Ferreira et al., 2017 [43] Brazil	Randomized, double-blind, placebo-controlled study	120	Women (20–45 years) abnormal glucose values	Green tea capsules	Group 1: control (cellulose) Group 2: green tea (1 g) Group 3: metformin (1 g) Group 4: green tea (1 g) + metformin (1 g)	12 weeks	Improving glycemic and lipid profile ↓ Fasting glucose ↓ Total cholesterol and LDL
Lasaite et al., 2014 [113] Lithuania	Randomized double-blind placebo-controlled study	56	Patients (37–78 years) with diabetes mellitus type II and diabetic retinopathy, nephropathy or neuropathy	Green tea extract	Group 1: placebo Group 2: *Gingko biloba* dry extract Group 3: green tea extract For extracts: one capsule twice a day (9 months) and one capsule three times a day (9 months)	18 months	No statistically significant differences in HbA1c level, antioxidant state, and psychological data
Mahmoud et al., 2016 [44] Kuwait	Randomly assigned	34	Male and female type 2 diabetics	Black tea infusions	Group 1: three cups black tea daily (600 mL) Group 2: one cup black tea daily (200 mL)	12 weeks	↓ HbA1c levels ↑ Regulatory T cells ↓ Pro-inflammatory
Spadiene et al., 2014 [45] Lithuania	Randomized, double-blind, placebo-controlled study	45	Patients (35-80 years) with diabetes mellitus type II and diabetic retinopathy, nephropathy or neuropathy	Green tea extract	Group 1: green tea extract Group 2: placebo	9–18 months	↓ Lipid peroxidation
Vaz et al., 2018 [46] Brazil	Randomized, double-blind, placebo-controlled study	60	Patients with diabetes	Green tea extract	Group 1: green tea extract (two capsules/day, containing 560 mg of polyphenols/each) Group 2: cellulose (two capsules/day)	20 weeks	No effect on total antioxidant capacity, glycemic control markers, and renal function ↑ SOD activity
				HYPERCHOLESTEROLEMIA			
Imbe et al., 2016 [53] Japan	Randomized, double-blind, placebo-controlled trial	155	Healthy volunteers High LDL cholesterol levels Aged 20–80 years	"Benifuuki" green tea	Group 1: "Benifuuki" Group 2: "Yabukita" Group 3: barley infusion drinker	12 weeks	↓ LDL cholesterol levels ↓ Lectin-like oxidized LDL receptor-1 containing LAB level
Orem et al., 2017 [51] Canada	Randomized, double-blind, placebo-controlled study	125	Subjects 25–60 years hypercholesterolemia	Black tea	Group 1: placebo Group 2: instant black tea Group 3: functional black tea	4 weeks	Functional black tea: ↓ Total cholesterol ↓ LDL ↓ Oxidative stress index ↑ Total antioxidant status
Troup et al., 2015 [52] United States	Randomized, double-blind, crossover trial	57	45–65 years, hypercholesterolemia	Black tea	Group 1: controlled low flavonoid diet plus five cups per day of black tea Group 2: Placebo	4 weeks	↓ LDL/HDL ratio ↓ Total cholesterol

Table A3. *Cont.*

Study (Author, Year, Country)	Study Design	Sample Size	Population	Type of Plant	Intervention	Duration of Treatment	Results
				HYPERTENSION			
Alkerwi et al., 2015 [60] Luxembourg	National cross-sectional stratified sample	1352	18–69 years	Tea	Group 1: nonconsumers Group 2: ≤ 3-dL/d consumers (tea/coffee) Group 3: > 3-dL/d consumers (tea/coffee)	-	↓ Systolic BP and pulse pressure
				METABOLIC SYNDROME			
Yang et al., 2014 [64] China	-	134	Metabolic syndrome	Green tea extract	Group 1: green tea extract (500 mg). Two capsules/time/day Group 2: control (water)	45 days	↑ Adiponectin serum concentrations ↓ Visfatin levels
				OBESITY			
Chen et al., 2016 [94] Taiwan	Randomized, double-blind trial	102	Women BMI ≥ 27 kg/m^2 Waist circumference ≥ 80 cm	EGCG	Group 1: placebo Group 2: high dose green tea	12 weeks	↓ Weight ↓ Waist circumference ↓ Total cholesterol and LDL plasma levels
Dostal et al., 2016 [96] USA	Randomized, double-blind, placebo-controlled clinical trial	937	Postmenopausal women aged 50–70 with high breast density and overweight/obese	Green tea extract	Group 1: placebo Group 2: EGCG (843 mg), four capsules daily	12 months	No ↓ adiposity No improvements in BMI ↓ Tissue fat and gynoid fat
Huang et al., 2018 [95] Taiwan	Randomized, double-blind, crossover, placebo-controlled	90	Women (18 - 65 years) BMI ≥ 27 kg/m^2 LDL-C ≥ 130 mg/dL	Green tea extract	Group 1: placebo Group 2: one capsule 30 min after meal, three times a day, green tea extract	6 weeks	↑ Leptin ↓ LDL
Janssens et al., 2015 [98] The Netherlands	Randomized, placebo-controlled, single-blind design	60	Caucasian men and women with body mass index from 18 kg/m^2, age: 18–50	Green tea extract	Group 1: placebo Group 2: green tea (capsules > 0.06 g EGCG and 0.03–0.05 g caffeine)	12 weeks	No effect on fecal energy content, fecal fat content, resting energy expenditure, respiratory quotient, and body composition
Mielgo-Ayuso et al., 2014 [97] Spain	Randomized, double-blind, parallel design	83	Obese (30 kg/m^2. BMI, 40 kg/m^2) premenopausal women	EGCG	Group 1: placebo (lactose) Group 2: EGCG (300 mg/d)	12 weeks	No changes in body weight No changes in adiposity
Nicoletti et al., 2019 [99] Brazil	Longitudinal interventional study	11	Women (18–60 years) (BMI) > 40 kg/m^2	EGCG	Group 1: eutrophic women Group 2: decaffeinated green tea capsules with 450.7 mg of EGCG, two capsules/day	8 weeks	↑ RICTOR ↑ HIF1-α expression
				OSTEOPOROSIS			
Amorim et al., 2018 [114] Brazil	Double-blind, randomized, controlled clinical trial	35	≥ 18 years old Diabetes for more than 5 years.	Green tea extract	Group 1: cellulose Group 2: 1120 mg of green tea extract contains 560 mg of polyphenols/day	10 and 20 weeks	↑ Bone mineral content

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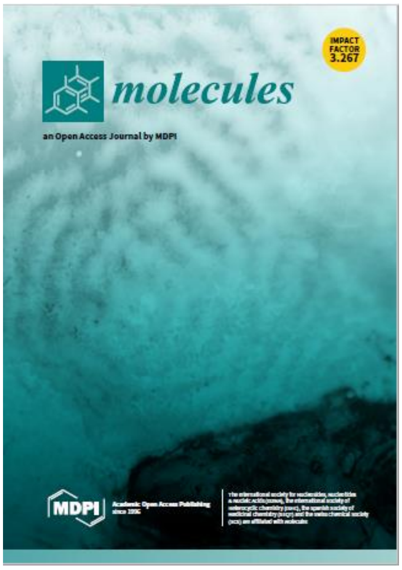

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Review

Pharmacological Update Properties of *Aloe Vera* and its Major Active Constituents

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Abstract: *Aloe vera* has been traditionally used to treat skin injuries (burns, cuts, insect bites, and eczemas) and digestive problems because its anti-inflammatory, antimicrobial, and wound healing properties. Research on this medicinal plant has been aimed at validating traditional uses and deepening the mechanism of action, identifying the compounds responsible for these activities. The most investigated active compounds are aloe-emodin, aloin, aloesin, emodin, and acemannan. Likewise, new actions have been investigated for *Aloe vera* and its active compounds. This review provides an overview of current pharmacological studies (in vitro, in vivo, and clinical trials), written in English during the last six years (2014–2019). In particular, new pharmacological data research has shown that most studies refer to anti-cancer action, skin and digestive protective activity, and antimicrobial properties. Most recent works are in vitro and in vivo. Clinical trials have been conducted just with *Aloe vera*, but not with isolated compounds; therefore, it would be interesting to study the clinical effect of relevant metabolites in different human conditions and pathologies. The promising results of these studies in basic research encourage a greater number of clinical trials to test the clinical application of *Aloe vera* and its main compounds, particularly on bone protection, cancer, and diabetes.

Keywords: *Aloe vera*; pharmacology; extracts; isolated compounds

1. Introduction

Aloe vera (*Aloe barbadensis* Miller, family Xanthorrhoeaceae) is a perennial green herb with bright yellow tubular flowers that is extensively distributed in hot and dry areas of North Africa, the Middle East of Asia, the Southern Mediterranean, and the Canary Islands. *Aloe vera* derives from "Allaeh" (Arabic word that means "shining bitter substances") and "Vera" (Latin word that means "true"). The colorless mucilaginous gel from *Aloe vera* leaves has been extensively used with pharmacological and cosmetic applications. Traditionally, this medicinal plant has been employed to treat skin problems (burns, wounds, and anti-inflammatory processes). Moreover, *Aloe vera* has shown other therapeutic properties including anticancer, antioxidant, antidiabetic, and antihyperlipidemic. *Aloe vera* contains more than 75 different compounds, including vitamins (vitamin A, C, E, and B12), enzymes (i.e., amylase, catalase, and peroxidase), minerals (i.e., zinc, copper, selenium, and calcium), sugars (monosaccharides such as mannose-6-phosphate and polysaccharides such as glucomannans), anthraquinones (aloin and emodin), fatty acids (i.e., lupeol and campesterol), hormones (auxins and gibberellins), and others (i.e., salicylic acid, lignin, and saponins) [1–3].

In this review, we summarize an update of the pharmacological activities (in vitro, in vivo, and clinical trials) of *Aloe vera*. Publications (original papers) were published in English in the years 2014 to 2019 in peer-reviewed scientific journals of the Pubmed database. Those articles that included *Aloe vera* combined with other plants or *Aloe* species other than *Aloe vera* were excluded from this review.

This review is structured into different activities, which include in vitro, in vivo, and clinical trials, published in the last six years. The order of activities is based on the interest and importance of studies for *Aloe vera*. The Table 1 (in vitro studies), Table 2 (in vivo studies), and Table 3 (clinical trials) summarize the main pharmacological findings for *Aloe vera* and its isolated compounds (Figures 1 and 2).

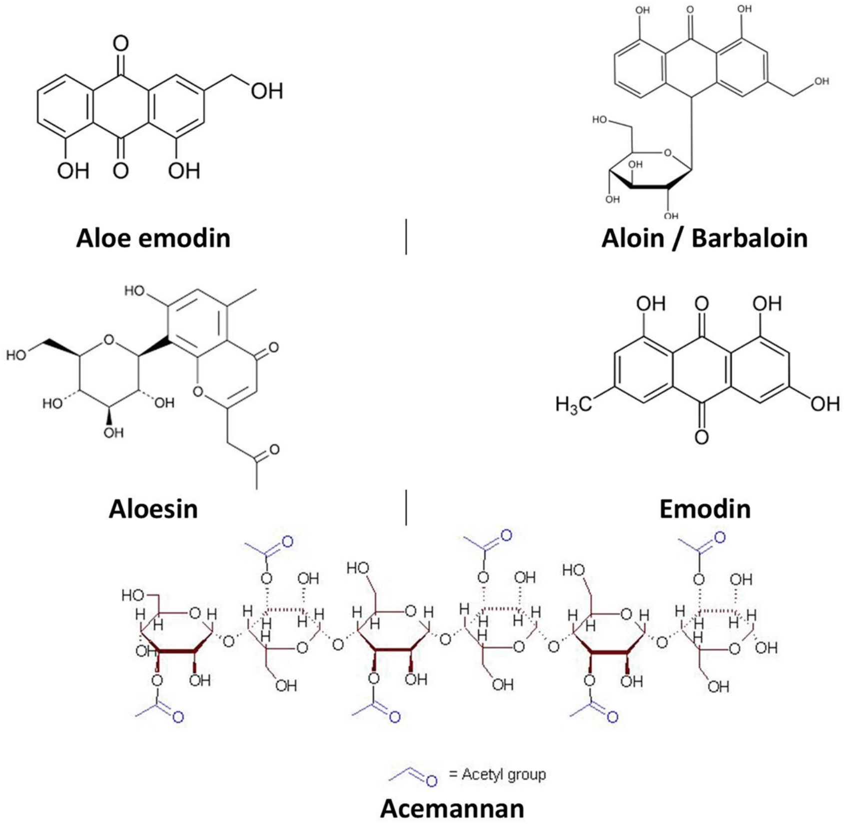

Figure 1. Chemical structure of compounds isolated from *Aloe vera* with pharmacological activity.

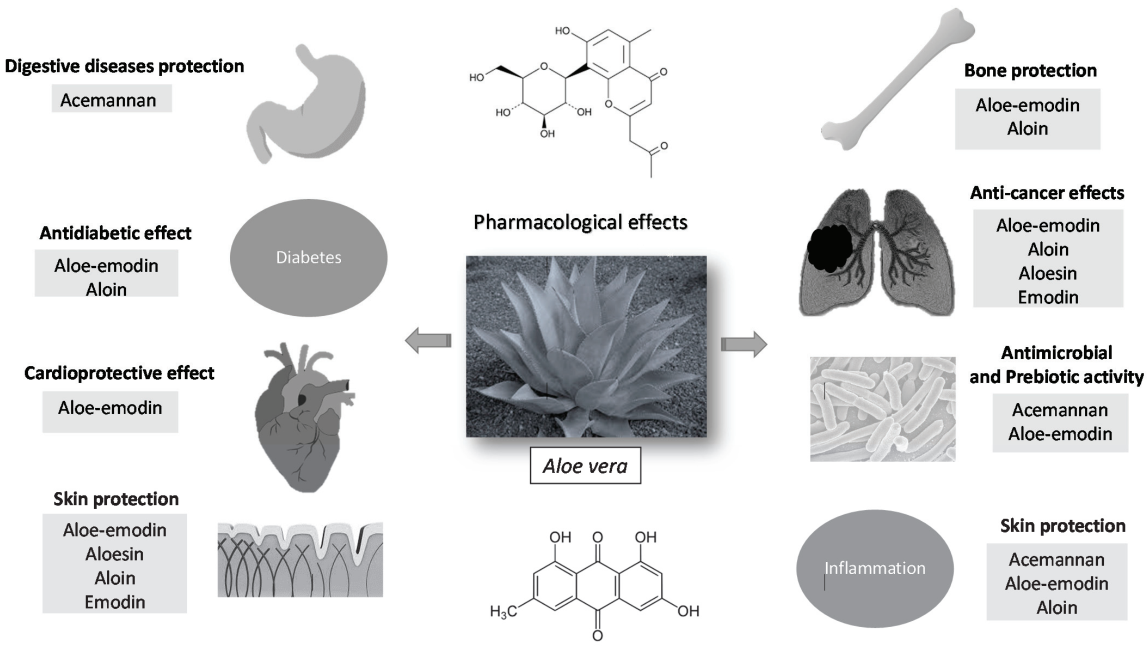

Figure 2. Pharmacological effects of the main constituents of *Aloe vera*.

2. Digestive Diseases Protection

Aloe vera extract (50%) increased cell viability of dental pulp stem cells being useful for avulsed broken teeth [4]. This effect is attributed to polysaccharides, mainly acemannan, by inducing osteogenic-specific gene expressions, DNA synthesis, growth factor, and JAK-STAT pathway [5,6]. Moreover, *Aloe vera* (225 mg/kg) exerted a radioprotective effect against salivary gland dysfunction in a rat model as evidenced in an increase of salivary flow rate [7].

Periodontitis is a serious and common dental affliction in which gums are infected and become inflamed, causing tissue and bone destruction. Gingivitis is the initial phase of periodontitis and is caused by dental plaque. Significant clinical evidence has demonstrated that *Aloe vera* mouthwash and gel are effective in the prevention and treatment of gingivitis and periodontitis by reducing gingival index, plaque index, and probing depth and by increasing bone fill and regeneration [8–14]. *Aloe vera* has proven to be as effective as other usual treatments such as chlorhexidine, alendronate, and chlorine dioxide [8,10,11,13].

In a randomized placebo double-blind study with 20 healthy adults, Fallahi et al. [15] investigated the effect of *Aloe vera* mouthwash on postoperative complications after impacted third molar surgery. *Aloe vera* gel significantly reduced swelling and postoperative pain. In another work, Kalra et al. [16] evaluated the efficacy of *Aloe vera* gel and mineral trioxide aggregate as pulpotomy agents in primary molar teeth. The overall success rates at 3, 6, 9, and 12 months was high for patients treated with mineral trioxide aggregate. Moreover, a cross-sectional randomized interventional study revealed that *Aloe vera* gel promoted wound healing and reduced pain in patients that required atraumatic tooth extractions, and its effectiveness was higher than that of traditional analgesics [17]. Furthermore, *Aloe vera* resulted to be a promising cavity disinfecting agent in minimally invasive dentistry in a randomized clinical trial with 10 patients [18].

Oral mucositis/stomatitis is an inflammatory and/or ulcerative condition that occurs as a debilitating complication of chemotherapy and radiotherapy treatments and affects quality of life of oncological patients. *Aloe vera* mouthwash alleviated radiation-induced mucositis severity in patients with head and neck cancers similarly to the reference benzydamine mouthwash [19]. Moreover, *Aloe vera* mouthwash has also demonstrated to be efficient in the treatment of stomatitis (mean intensity and pain) associated with radiotherapy in patients with acute myeloid leukemia and acute lymphocytic leukemia [20].

Oral submucous fibrosis is a precancerous condition of the oral cavity characterized by abnormal collagen deposition. This malignant disorder is mainly caused by chewing areca nut and it is most frequent in India and Southeast Asia. Anuradha et al. [21] evaluated the efficacy of *Aloe vera* (systemic as juice and topical as gel) in the treatment of oral submucous fibrosis. Clinical evidence demonstrated that *Aloe vera* reduced burning sensation and increased cheek flexibility, mouth opening, and tongue protrusion similar to the reference treatment hydrocortisone, hyaluronidase, and antioxidant supplements. In another study on oral submucous fibrosis, the combination of *Aloe vera* gel with physiotherapy was more efficient in decreasing burning sensation and increasing tongue protrusion, mouth opening, and cheek flexibility than the combination of antioxidant capsules with physiotherapy [22].

Gastroesophageal reflux disease is a common chronic digestive disease in which gastric acids move up into the esophagus. *Aloe vera* syrup (10 mL/day) for 4 weeks reduced the frequency of symptoms of gastroesophageal reflux diseases including heartburn, food regurgitation, dysphagia, flatulence, belching, nausea, and acid regurgitation without causing adverse effects (only one case of vertigo and another of stomach ache were reported) [23].

Gastritis is an inflammation of mucous membrane layer of the stomach. *Aloe vera* gel protected in a Balb/c mouse model of alcohol-induced acute gastritis by increasing matrix metalloproteinase-9 inhibitory activity [24].

The topical administration of *Aloe vera* 3% ointment alleviated the symptoms of diarrhea and fecal urgency in patients with acute radiation proctitis induced by radiotherapy of the pelvic area [25]. Moreover, *Aloe barbadensis* extract (AVH200®) reduced, but not significantly, the severity of gastrointestinal symptoms in patients with irritable bowel syndrome compared to a control group [26]. Lin et al. [5] revealed that *Aloe* polysaccharide (15 mg/kg) protected rats from 2,4,6-three nitrobenzene sulfonic acid colitis induced by increasing JAK2, p-JAK2, STAT-3, and p-STAT3 protein expression. Furthermore, *Aloe vera* cream applied three times daily for 6 weeks reduced chronic anal fissure pain and hemorrhaging after defection and promoted wound healing in a prospective double blind clinical trial [27].

3. Skin Protection

Most in vitro studies on skin protection study the ability of *Aloe vera* and active compounds in wound healing. The immortalized human keratinocyte HaCaT cell line, the primary normal human epidermal keratinocytes HEKa cell line, and fibroblasts cell lines are the most used. These studies have revealed that *Aloe vera* and its major compounds (aloesin, aloin, and emodin) exert their protective action mainly through antioxidant and anti-inflammatory mechanisms. Hence, *Aloe vera* up-regulated TFGβ1, bFGF, and Vegf-A expression in fibroblasts and increased keratinocyte proliferation and differentiation by lysosomal membrane stability [28–32]. Moreover, *Aloe vera* solution could accelerate corneal wound closure at low concentrations (≤175 μg/mL) by increasing type IV collagen-degrading activity in a cellular model of primary cultures of corneal epithelial cells [33]. Furthermore, aloin exerted skin protection by reducing IL-8 production, DNA damage, lipid peroxidation, and ROS generation and by increasing GSH content and SOD activity [34]. The compound aloesin resulted in promoting wound healing by increasing cell migration via phosphorylation of Cdc42 and Rak1, cytokines, and growth factors [35]. In addition to this healing activity, it has been seen that *Aloe* polysaccharide (20, 40, and 80 μg/mL for 24 h) could be a beneficial agent in psoriasis as evidenced in the inhibition of TNF-α levels and IL-8 and IL-12 protein expression in human keratinocyte HaCaT cell line.

As for in vivo studies, the most common models are genetically modified animals (BALB/c mice, HR-1 hairless mice and SKH-1 hairless mice) and UV and X-ray skin damage in animals. Most of these in vivo studies have been done with *Aloe vera* extracts and gel. Application of topical *Aloe vera* favored wound healing in animal models with dermal incisions by reducing inflammatory cell infiltration, increasing CD4+/CD8+ ratio lymphocytes, and improving epidermal thickness and collagen deposition [36–39]. In another study conducted in Indonesia with several medicinal plants, the effect of *Nigella sativa* oil gel and *Aloe vera* gel to treat diabetic ulcers was investigated. *Aloe vera* resulted to be more efficient in improving wound healing on alloxan-induced diabetes in Wistar rats with wounds on dorsum as evidenced by a decrease of necrotic tissue and inflammation and an improvement of re-epithelialization [40]. Furthermore, a UV-induced mice model revealed that *Aloe vera* gel powder increased epidermal growth factor and hyaluronan synthase and reduced matrix metalloproteinases expression (types 2, 9, and 13) [41,42]. *Aloe* sterols are involved in this UV protection [43]. Likewise, it has been observed that *Aloe vera* protected against X-radiation through antioxidant mechanisms (increased antioxidant enzyme activity and GSH content and reduced ROS production and lipid peroxidation) [44,45]. Among isolated compounds, investigations with the compounds aloe-emodin and aloesin have shown that their healing activity is due to angiogenic properties [46,47].

In the last 6 years, several clinical trials have also been carried out. Some of these have been aimed at evaluating the effectiveness of *Aloe vera* on ulcers. Hence, the administration of *Aloe vera* gel twice daily for 3 months improved and accelerated wound healing as well as reduced hospitalization time [48,49]. Moreover, in a randomized, triple-blind clinical trial with 80 patients hospitalized in the orthopedic ward, Hekmatpou et al. [50] demonstrated that *Aloe vera* gel twice daily for 10 days prevented the development of pressure ulcers on the areas of hip, sacrum, and heel. Moreover, clinical trials have demonstrated that *Aloe vera* facilitated rapid tissue epithelialization and granulation in burns [51], promoted healing of cesarean wound [52], and accelerated wound healing of split-thickness skin graft donor sites [53]. Furthermore, *Aloe vera* has been investigated in randomized, double-blind, placebo-controlled studies for its benefits to maintain healthy skin. Therefore, the daily oral intake of 40 μg of *Aloe* sterol (cycloartenol and lophenol) for at least 12 weeks improved skin elasticity in men under 46 years exposed to the sunlight but do not use sunscreen to protect themselves [54], reduced facial wrinkles in Japanese women over 40 years old by stimulating hyaluronic acid and collagen production [55], and increased gross elasticity, net elasticity, and biological elasticity in women aged 30–59 [56]. However, despite clinical evidence on the protective role of *Aloe vera* in the skin, there are clinical trials that have not yet found effectiveness of this medicinal plant, particularly in decreasing radiation-induced skin injury. Two clinical trials have been published between 2014 and 2019 in relation to this effect. Both studies found that topical administration of *Aloe vera* as gel or cream did not reduce the prevalence and severity of radiotherapy-induced dermatitis and skin toxicity in breast cancer patients compared to control group [57,58].

4. Anti-Inflammatory Activity

Most recent studies on anti-inflammatory activity of *Aloe vera* are focused on the action mechanism of isolated compounds in murine macrophage RAW264.7 cells and mice stimulated with LPS. Hence, the potential anti-inflammatory effect of aloin is related to its ability to inhibit cytokines, ROS production, and JAK1-STAT1/3 signaling pathway [59,60]. Moreover, aloe-emodin sulfates/glucuronides (0.5 μM), rhein sulfates/glucuronides (1.0 μM), aloe-emodin (0.1 μM), and rhein (0.3 μM) inhibited pro-inflammatory cytokines and nitric oxide production, iNOS expression, and MAPKs phosphorylation [61].

In another study, Thunyakitpisal et al. [62] demonstrated that acemannan increased IL-6 and IL-8 expression and NF-κB/DNA binding in human gingival fibroblast via a toll-like receptor signaling pathway. Since there is a relation between high IL-1β levels and periodontal diseases, Na et al. [63] investigated the anti-inflammatory properties of aloin in human oral KB epithelial cells stimulated with saliva from healthy volunteers. This study revealed that those saliva samples with high content in IL-1β stimulated IL-8 production in KB cells, and pretreatments with aloin inhibited IL-8 production by decreasing p38 and extracellular signal-regulated kinases pathway.

In addition to isolated compounds, Ahluwalia et al. [64] evaluated the activity of AVH200®, a standardized *Aloe vera* extract which contains alin and acemannan on the activation, proliferation, and cytokine secretion of human blood T cells obtained from healthy individuals aged 18–60, and they found that it decreased CD25 and CD3 expression on CD3(+) T cells. Moreover, AVH200® exhibited concentration-dependent T cell proliferation suppression and IL-2, IFN-γ, and IL-17A reduction. Moreover, the anti-inflammatory effect of *Aloe vera* has also been investigated in an acetaminophen-induced hepatitis (inflammatory condition of the liver) mice model. The results of this study revealed that *Aloe vera* (150 mg/kg) reduced hepatic MDA, IL-12, and IL-18 levels and ALT and increased GSH content [65].

5. Anticancer Effects

Studies conducted in the years of the review of this work focusing on cancer are mostly in vitro and in vivo studies. In vitro studies have the main purpose of identifying potential molecules with cytotoxic activity for later evaluation in in vivo studies and clinical trials. In addition, in vitro studies allow elucidating the mechanism of action by identifying promising pharmacological targets. In vivo studies allow us to understand the pharmacological activity and behavior in living organisms prior to their study in humans. Since clinical trials are very limited, and as it is not possible to confirm the anti-cancer activity of *Aloe vera* and its bioactive principles, it would be interesting for future research to focus on this activity based on the promising in vitro and in vivo results.

In vitro and in vivo studies included in the present review are aimed at evaluating cytotoxic and antitumor activity against a variety of cancer types using a diversity of cell lines and animal models (breast and gynecological cancers such as cervical cancer and ovarian cancer, malignant conditions of the gastrointestinal tract (i.e., oral cavity, esophagus, colon) and accessory digestive organs (pancreas), osteosarcomas, and melanoma). One clinical trial focused on the efficacy of *Aloe vera* on ocular surface squamous neoplasia; this clinical trial has been included at the end of this section.

MCF-7 cells, which express estrogen receptor, are the most popular breast cancer cell line, and the immortal HeLa cell line are the oldest and most used cervical cancer cells [66,67]. *Aloe vera* crude extracts (40%, 50%, and 60% for 6, 24, and 48 h) reduced cell viability of cancer cell lines (human breast MCF-7 and cervical HeLa) through apoptosis induction (chromatin condensation and fragmentation and apoptotic bodies appearance in sub-G0/G1 phases) and modulation of effector genes expression (an increase in cyclin D1, CYP1A1, and CYP1A2 expression and a decrease in p21 and bax expression) [68]. Moreover, the isolated compound aloe-emodin has resulted to be an effective anticancer agent against both MCF-7 cells and HeLa cells by inducing mitochondrial and endoplasmic reticulum apoptosis and inhibiting metastasis oxidative stress [69–72]. Furthermore, a recent study demonstrated that *Aloe vera* extract (300 mg/kg) and training (swimming) combined exerted a protective anticancer effect in mice with breast cancer by inhibiting the COX pathway (COX-2 reduction levels) and prostaglandin E2 production [73]. Finally, aloesin reduced tumor growth in in vitro and in vivo models of ovarian cancers by inhibiting the MAPK signaling pathway [74].

For malignant conditions of gastrointestinal tract and accessory digestive organs, emodin (10, 20, 30, 40 μM for 24 and 48 h) decreased cell proliferation and Bcl-2 protein levels and increased caspase-3 protein expression and Bax protein levels in human oral mucosa carcinoma KB cells [75]. Moreover, aloe-emodin has been shown to effectively suppress esophageal TE1 cancer cells in a concentration-dependent manner (from 2.5 μM to 20 μM concentrations assayed) through inhibiting AKT and ERK phosphorylation and reducing the number of cells in the S phase [76]. Furthermore, *Aloe* polysaccharide induced autophagy alone and in combination with radiation in pancreatic carcinoma BxPC-3 cells as evidenced in ULK1 mRNA expression upregulation and BECN1 and BCL-2 mRNA expression downregulation [77]. Finally, several in vitro and in vivo studies were performed to evaluate the potential anticancer properties of *Aloe vera* and its isolated compounds in colon cancer (fourth most common cancer and the third leading death cause) [78]. Chen et al. [79] exhibited cytotoxic properties of aloe-emodin on colon cancer cells at 10, 20, and 40 μM concentrations through activating the apoptotic pathway, increasing ROS production, and cytosolic calcium levels and up-regulating ER stress-related proteins. Moreover, *Aloe vera* powder and extract 1% and 3% protected C57BL/6J mice from aberrant crypt foci colorectal cancer by increasing hepatic phase II enzyme glutathione S-transferase mRNA levels [80]. Furthermore, *Aloe vera* gel (200 or 400 mg/kg/day orally) reduced inducible NO synthase and COX2 expression, NF-kB activation, and cell cycle progression, inducing cellular factors in BALB/c female mice with induced colitis-associated colon carcinogenesis.

Osteosarcomas are uncommon bone tumors in which malignant cells produce osteoid [81]. Aloe-emodin has also resulted to be a promising photosensitive agent against the human osteosarcoma MG-63 cell line via ROS/JNK signaling pathway as evidenced in an increase of caspases, cytochrome c, CHOP, and GRP78 expression [82,83].

For melanoma (malignant transformation of melanocytes), aloe-emodin protected against metastatic human melanoma cells by decreasing cell proliferation, increasing cell differentiation, and transamidating activity of transglutaminase and dabrafenib antiproliferative activity [84,85].

Regarding clinical trials conducted in recent years on anticancer activity, Damani et al. [86] reported the efficacy of *Aloe vera* eye drops 3 times daily for 3 months in the regression of ocular surface squamous neoplasia in a 64-year-old Hispanic woman. On the other hand, Koo et al. [87] stated that aloe polysaccharide could reduce tobacco associated diseases such as cancer due to its ability to increase urinary excretion of benzo(a)pyrene and cotinine.

6. Antidiabetic Effect

Diabetes is a chronic disease presenting with high levels of glucose in blood because of an insulin resistance or an insulin deficiency. Studies on the effect of *Aloe vera* in diabetes and related complications have been investigated mainly in animal models induced by streptozotocin. Consistent evidence supports that oxidative stress is a main cause of the beginning and the progression of diabetes complications such as nephropathies and neuropathies. Hence, using this experimental model, *Aloe vera* showed to reduce blood glucose levels, to increase insulin levels, and to improve pancreatic islets (number, volume, area, and diameter) [88], and this medicinal plant protected from oxidative stress-induced diabetic nephropathy and anxiety/depression-like behaviors [89]. Moreover, *Aloe vera* topical administration (60 mg/mL, four times daily for 3 days of eye drops) favored corneal re-epithelialization in streptozotocin-induced diabetic Wistar rats with corneal alkali burn injury [90]. Furthermore, experiments with genetically modified animals have revealed that *Aloe vera* polysaccharides (100 μg/g for 3 weeks) are responsible for the decrease of blood glucose levels [91]. A recent in vitro study showed that the action mechanism of *Aloe vera* polysaccharides antidiabetic effect is related to its ability to inhibit apoptosis and endoplasmic reticulum stress signaling [91]. In another in vitro study using a high-glucose-induced toxicity cell model, the compound aloe-emodin (20 μM) protected RIN-5F cells derived from rat pancreatic β-cells from glucotoxicity through an apoptotic and anti-inflammatory effects [92]. Lastly, the intake of *Aloe vera* (300 mg twice day for 4 weeks) decreased fasting blood glucose in pre-diabetic subjects [93].

7. Antioxidant Properties

Antioxidants are compounds that prevent or slow down biomolecule oxidative damage caused by ROS through free radical scavenging, metal chelation, and enzyme regulation [94]. Kumar et al. 2017 [95] investigated the potential antioxidant activity of crude methanolic extracts of *Aloe vera* from six agro-climatic zones of India using different in vitro methods (i.e., DPPH, metal chelating, and reducing power assay). Antioxidant activity was higher in those species collected in Northern India than in Southern India, which is related to a high content in alkaloids, glycosides, phenolic compounds, flavonoids, and saponin glycosides. Moreover, *Aloe vera* ethanol extract protected, particularly human microvascular endothelial cells, against hydrogen peroxide and 4-hydroxynonenal-induced toxicity by reducing ROS production and HNE-protein adducts formation [96]. The antioxidant activity of *Aloe vera* is, at least in part, due to anthraquinones and related compounds (10 μM) which possess peroxyl radical scavenging activity and reducing capacity [97].

Apart from these in vitro assays, in a clinical trial with 53 healthy volunteers, the intake of *Aloe vera* gel extract (14 days) increased total antioxidant capacity of plasma of subjects [98].

8. Bone Protection

In vitro studies with isolated *Aloe vera* compounds have been aimed at studying the potential protective effect on bone pathogenesis. Aloe-emodin induced chondrogenic differentiation on clonal mouse chondrogenic ATDC5 cells which is related to bone formation through BMP-2 and MAPK-signaling pathway activation [99]. Moreover, aloin has resulted to be beneficial in osteoporosis and osteopenia disorders by suppressing receptor activator of NFkB ligand (RankL) induced through NF-κB inhibition in mouse macrophage RAW 264.7 cells [100,101].

9. Cardioprotective Effect

In vivo models of ischemia-reperfusion injury are commonly employed to evaluate the cardioprotective activity of *Aloe vera*. *Aloe vera* administered with gastric gavage previous to abdominal aorta and spinal cord ischemia increased antioxidant enzymes activity (SOD, CAT, and GPx) and reduced lipid peroxidation level (MDA content), edema, hemorrhage, and inflammatory cell migration in Wistar albino rats [102,103]. Moreover, barbaloin, also known as aloin, (20 mg/kg/day, 5 days) administered intragastrically reduced myocardial oxidative stress and inflammatory response and increased AMPK signaling in Sprague-Dawley rats in a myocardial ischemia/reperfusion injury [104]. Esmat et al. [105] demonstrated that this compound (50 mg/kg body weight, twice weekly over 2 weeks), administered intramuscularly, had non-atherogenic activity and iron chelating properties. Another compound isolated from *Aloe vera* and investigated for its cardioprotective properties is aloe-emodin. In an in vitro model of heme protein (hemoglobin), it was demonstrated that aloe-emodin (100 μM) had its maximum activity as an anti-aggregatory agent as evidenced in structural alterations of β sheet and the appearance of α helices [106]. On the other hand, an in vivo study revealed that aloe-emodin could alleviate hyperlipidemia by reducing total cholesterol and low-density lipoprotein-cholesterol levels at doses of 50 and 100 mg/kg for 6 weeks in male Wistar rats [107]. Regarding clinical studies, a double-blind randomized controlled trial showed that *Aloe vera* 300 mg and 500 mg/twice day for 4 and 8 weeks reduced HbA1C, total cholesterol, LDL, and triglyceride levels in pre-diabetic patients [92]. Furthermore, the oral gavage administration of *Aloe vera* (30 mg/kg/day for 1 month) resulted to decrease ischemic fiber degeneration by preventing the formation of lipid peroxides, increasing antioxidant enzymes, and up-regulating the transcription factor NRF1 in Wistar albino rats [108].

10. Antimicrobial and Prebiotic Activity

Different studies have been carried out to evaluate the antimicrobial activity of *Aloe vera* and its main constituents. Most of these studies are in vitro and focus on the antibacterial activity. One of the most studied bacteria are *Staphlococcus aureus* and *Pseudomonas aeruginosa*. Hence, *Aloe vera* aqueous extract reduced growth and biofilm formation against methicillin resistant *Staphylococcus aureus* [109]. Moreover, this bacteria has also been inhibited by *Aloe vera* gel (50% and 100% concentrations), along with other oral pathogens obtained from patients with periapical and periodontal abscess including *Actinobacillus actinomycetemcomitans*, *Clostridium bacilli*, and *Streptococcus mutans* using disc diffusion, micro-dilution, and agar dilution methods [110]. One of the compounds attributed to antibacterial activity against *Staphylococcus aureus* is aloe-emodin which acts by inhibiting biofilm development and extracellular protein production [111]. In the case of *Pseudomonas aeruginosa*, *Aloe vera* extracts have shown to inhibit the growth of multidrug-resistant *Pseudomonas aeruginosa* isolated from burned patients with wounds infections at MIC_{50} and MIC_{90} values of 200 μg/mL [112]. *Pseudomonas aeruginosa* growth and biofilm formation inhibition has been also demonstrated for *Aloe vera* inner gel. This *Aloe vera* inner gel also inhibited other Gram-negative bacteria (*Helicobacter pylori* and *Escherichia coli*) as well as the fungus *Candida albicans* [113]. Moreover, in another study, *Aloe vera* hydroalcoholic extract showed antibacterial activity against *Enterococcus faecalis*, an infecting microorganism of the root canals of teeth, with inhibition zones of 13 mm (saturated) and 9.6 mm (diluted) [114]. Furthermore, concentrations up to 1 mg/mL of *Aloe vera* aqueous extracts could inhibit *Mycobacterium tuberculosis* growth, which is the pathogen responsible for causing tuberculosis, one of the most lethal infectious diseases worldwide [115]. Finally, in a clinical trial with 53 healthy volunteers, the daily drinking of *Aloe vera* gel extract for 14 days exerted an antimicrobial activity as shown in a reduction of *Lactobacillus* spp. number [98].

Antiviral activity of *Aloe vera* has been investigated for herpes simplex virus type 1 and H1N1 subtype influenza virus. *Aloe vera* extract gel (concentrations from 0.2% to 5%) showed antiviral activity against herpes simplex virus type 1 on Vero cells by inhibiting its growth [116]. On the other hand, in vitro studies have demonstrated that *Aloe* polysaccharides decreased H1N1 subtype influenza virus replication and viral adsorption period by interacting with influenza virus particles. Moreover, in vivo studies with SPF BALB/c mice infected with PR8(H1N1) improved clinical symptoms and lung damage [117].

The parasite *Plasmodium falciparum* is the main causative agent of malaria, in its most aggressive and lethal form. Kumar et al. [118] investigated the activity of *Aloe vera* crude aqueous extracts collected in six different climatic regions of India (highland, semi-arid, arid, humid subtropical, tropical wet and dry, and humid subtropical climate) against a chloroquine-sensitive strain of *Plasmodium falciparum*. This study showed that those *Aloe vera* from colder climatic regions possessed the highest antiplasmodial activity which was related to the highest aloin and aloe-emodin content (EC50 value of 0.289 μg/mL).

Finally, there are other studies which support the prebiotic potential of *Aloe vera* defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit". *Aloe vera* mucilage (rich in acemannan) could improve gastrointestinal health by increasing short chain fatty acids and modifying bacterial composition [119]. Moreover, acemannan and fructans from *Aloe vera* increased bacterial growth, especially *Bifidobacterium* spp. population [120].

11. Other Effects

Aloe vera has also been investigated for treating reproductive health care problems. The results of these works carried out with experimental animals are contradictory. While Asgharzade et al. [121] demonstrated that *Aloe vera* ethanol extract (150 and 300 mg/kg) had negative effects on spermatogenesis and sperm quality in Wistar rats, Erhabor and Idu [122] observed that *Aloe vera* ethanol extract (400 mg/kg) improved male sexual behavior (mount frequency and latency, intromission frequency and latency, and testosterone levels) and Behmanesh et al. [123] that *Aloe vera* extract increased body and testis weights, spermatocyte and spermatids quantity, and seminiferous tubule diameter and height.

Aloe vera processed gel prevented of ovoalbumin-induced food allergy by exerting an anti-inflammatory action (histamine, mast cell protease-1, and IgE reduction) [124].

At the blood level, the oral administration of *Aloe vera* gel prevented and restored lymphopenia and erythropenia as well as IgA secretion on cyclophosphamide-induced genetically modified mice [125]. Moreover, *Aloe vera* ethanol extract (200 mg/kg, 400 mg/kg, and 600 mg/kg) normalized levels of white blood cells, red blood cells, and platelet count through antioxidant mechanisms [126].

Regarding diseases of the musculoskeletal system, aloe-emodin showed to reduce viable cell numbers (concentrations $\geq$10 μM) and to induce apoptosis by arresting G2/M phase (concentrations $\geq$20 μM) in MH7A human synovial fibroblast-like cells, aloe-emodin being a promising agent to treat rheumatoid arthritis and a complementary treatment to methotrexate [127]. Moreover, *Aloe vera* lyophilized extract ointment reduced tendon lesions and increased non-collagenous proteins in Wistar rats with partial transection of the calcaneal tendon [128].

The dose of 10 mg/kg of *Aloe vera* aqueous extract (3 times daily for a week) resulted to be the most effective in morphine withdrawal syndrome in morphine-dependent female rats as shown in agitation, disparity, and floppy eyelids reduction [129].

Finally, highlighting the protective effect of *Aloe vera* gel extract (seven weeks, 500 mg/kg b.w. daily) on pulmonary tissue of cigarette smoke induced in Balb/c mice by reducing mucin production, citrulline and NO levels, and peroxidative damage [130].

12. Conclusions

Aloe vera has been traditionally used to treat skin injuries (burns, cuts, insect bites, and eczemas) and digestive problems because of its anti-inflammatory, antimicrobial, and wound healing properties. Research on this medicinal plant has been aimed at validating traditional uses and deepening the mechanism of action, identifying the compounds responsible for these activities. Likewise, new actions have been investigated for *Aloe vera* and its active compounds, especially highlighting its promising role as a cytotoxic, antitumoral, anticancer, and antidiabetic agent. In the last 6 years, most pharmacological studies have been in vitro and in vivo works. Among in vitro studies, antimicrobial, anti-inflammatory, cytotoxic, antitumor, anticancer, and skin protection activities are the most studied in number. It should be especially noted that among in vitro studies there are several works that evaluate the protective action of *Aloe vera* in bone diseases such as osteoporosis. The results on bone protection are promising; however, it is necessary to perform them with experimental animals and humans. Regarding in vivo studies, these are aimed at evaluating cardioprotective effect, cytotoxic, antitumor and anticancer activities, and skin protection activities. Compared to in vitro and in vivo assays, clinical trials are limited and focus on digestive and skin protective effects. In addition, these clinical trials have been conducted just with *Aloe vera*, but not with its isolated compounds; therefore, it would be of interest to study the clinical effect of relevant metabolites in different human conditions and pathologies. Among the major active compounds, research in the last six years focused on aloe-emodin, aloin, aloesin, amodin, and acemannan. Of these, aloe-emodin and aloin have been the most studied ones. Particularly, aloe–emodin has resulted to be a promising agent as an antimicrobial, antidiabetic, cytotoxic, cardiprotective, and bone protective (in in vitro studies) as well as anti-inflammatory and skin protective compound (in in vivo studies). Aloin was effective in inflammatory process and bone diseases (in vitro studies) and in cancer and cardiovascular diseases (in vivo studies). The promising results of basic research encourage a greater number of clinical trials to test the clinical application of *Aloe vera* and its main compounds, particularly on bone protection, cancer, and diabetes.

Table 1. In vitro pharmacological studies for *Aloe vera*.

Aloe Vera Composition	Experimental Model	Major Findings	References
Digestive Diseases Protection			
Acemannan	Human deciduous pulp cells	↑ Pulp cell proliferation ↑ ALP ↑ Type I collagen ↑ BMP-2, BMP-4, vascular endothelial growth factor and dentin sialoprotein expression	[6]
Aloe polysaccharide	HT-29 cells LPS and TNF-α induced	↑ JAK2 and STAT-3 expression ↓ JAK2, p-JAK2, STAT-3 and p-STAT3 protein expression Ulcerative colitis protection	[5]
Aloe vera extract	Dental pulp stem cells from rabbits	↑ Cell viability	[4]
Skin Protection			
Aloe polysaccharide	HaCaT cells	↓ TNF-α levels ↓ IL-8 and IL-12 expression levels ↓ p65 expression ↑ *IkB*-alpha protein expression Psoriasis protection	[49]
Aloe vera	HEKa and NFDH cells	↑ Cell viability ↑ Cell proliferation ↑ Cell migration ↑ Wound healing	[31]
Aloe vera	HaCaT cells	↓ Photodamage Membrane integrity maintenance ↑ Lysosomal stability	[32]
Aloe vera ethanolic extract	c147 cells	↑ Fibroblast migration ↑ VEGF-A gene expression ↑ Wound healing	[30]
Aloe vera gel	Mouse embryonic fibroblast cells	↑ TFGβ1 and bFGF factor expression ↑ Wound healing	[29]

Table 1. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera gel	HEKa Human skin equivalent model	↑ Cell number ↑ Wound healing ↑ Keratinocyte proliferation and differentiation ↑ Cell surface expression of adhesion molecules (β1-integrin, α6-integrin, β4-integrin and E-cadhesin) ↑ Wound healing	[28]
Aloesin	HaCaT cells	↑ Cell migration ↑ Cytokines and growth factors ↑ Wound healing	[35]
Aloin	Hs 68 cells Heat stress-mediated oxidative stress	↑ GSH ↑ SOD activity ↓ Lipid peroxidation ↓ 8-OH-dG ↑ Cell viability ↓ ROS	[34]
Aloin	κβ cells	↓ IL-8 production	[63]
Emodin	THP-1 cells and HaCaT cells	↑ VEGF ↑ MCP-1 Burn wound protection	[5]
Pure *Aloe vera* gel	Primary cultures of corneal epithelial cells and fibroblasts	↑ Corneal epithelial cell wound closure (*Aloe vera* concentrations ≤ 175 µg/mL) ↑ Type IV collagen-degrading activity	[33]
Anti-Inflammatory Activity			
Acemannan	Human gingival fibroblasts	↑ IL-6 and IL-8 expression ↑ NF-Kβ/DNA binding	[62]
Aloe vera extract (AVH200®Batch: 2013016)	Peripheral blood mononuclear cells	↓ CD25 and CD28 expression Suppression of T cell proliferation ↓ IL-2, IFN-γ and IL-17A secretion	[61]

Table 1. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe-emodin sulfates/glucuronides, rhein sulfates/glucuronides, aloe-emodin and rhein	RAW 264.7 cells stimulated with LPS and mouse peritoneal excluded macrophages	↓ iNOS expression ↓ TNF-α, IL-12, and NO production ↓ MAPKs phosphorylation	[64]
Aloin	RAW 264.7 cells	↓ iNOS expression ↓ IL-1β, IL-6, tumor necrosis factor alpha and NO dose-dependently ↓ JAK1-STAT1/3 activation ↓ STAT1/3 nuclear translocation ↓ ROS production	[59]
Aloin	KB cells	↓Salivary IL-1β-induced IL-8 production ↓p38 and ERK pathway	[63]
Barbaloin/aloin	RAW 264.7 cells stimulated with LPS	↓ Phosphorylation levels of IκBα and NF-κB p65 ↓ Pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) expression ↓ ROS	[60]
Anticancer Effects			
Aloe polysaccharide	BxPC-3 cells	↑ ULK1 mRNA expression ↓ BECN1 and BCL-2 mRNA expression	[77]
Aloe vera crude extract	MCF-7 cells and HeLa cells	↓ Cell viability Apoptosis induction ↓ Cyclin D1, CYP1A1 and CYP1A2 ↑ Bax and p21 expression	[68]
Aloe-emodin	Metastatic human melanoma cell lines Primary stem-like cells	↓ Cell proliferation ↑ Cell differentiation ↑ Transamidating activity of transglutaminase ↑ Dabrafenib antiproliferative activity	[85]
Aloe-emodin	TE1 cancer cells	↓ AKT and ERK phosphorylation ↓ Number cells in S phase	[76]

Table 1. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe-emodin	MCF-7 cells Photodynamic therapy	↓ Adhesion, migration and invasion of cells cytoskeleton disorganization Apoptosis: mitochondrial and endoplasmic reticulum death pathways	[70]
Aloe-emodin	HUVECs cells Photodynamic therapy	↓ Angiogenesis and Cell Metastasis MAPK Signaling Pathway activation ↓ Adhesion, migration and invasion of cells Apoptosis: mitochondrial death pathways cytoskeleton disorganization	[79]
Aloe-emodin	SW620 and HT29 cells	↓ Cell viability ↑ Apoptosis (Upregulation of CHOP and caspase 12) ↑ ROS Upregulation of unfolded protein response proteins	[79]
Aloe-emodin	HeLa cells	↓ Cell proliferation G2/M and S phase cell cycle arrest ↑ Radiosensitivity ↑ Cyclin B and γ-H2AX expression ↑ ALP activity	[69]
Aloe-emodin	MG-63 cells	↑ ROS production ↓ Mitochondrial membrane potential ↑ Caspase-3, caspase-9, caspase-12 expression ↑ Cytochrome c release	[82]
Aloe-emodin	HeLa cells	↑ Mitotic death ↓ Mitotic index ↓ G2/M phase	[72]
Aloe-emodin	Breast cancer cells (MCF-7, MDA-MB-231, MDA-MB-468, BT-474, HCC-1954)	↑ Tamoxifen cytotoxicity	[71]
Aloe-emodin	MG-63 cells	↓ Cell viability ↑ Autophagy ↑ Apoptosis ↑ ROS	[83]

Table 1. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloesin	SKOV3 cells	↓ Cell viability ↓ Cell clonality ↓ Cell cycle at S-phase ↑ Apoptosis ↓ Migration and invasion cancer	[74]
Emodin	KB cells	↓ Cell proliferation ↑ Caspase-3 upregulation ↑ Bax protein levels ↓ Bcl-2 protein levels	[75]
		Antidiabetic Effect	
Aloe vera polysaccharides	Hamster pancreatic β-cell line HIT-T15 in response to free fatty acids	↓ Number of apoptotic β-cell death Relief of endoplasmic reticulum stress signaling	[91]
Aloe-emodin	RIN-5F cells High glucose induced toxicity	↑ Cell viability ↓ ROS generation ↓ Pro-inflammatory cytokines levels (IFN-γ, IL-1β) ↑ Anti-inflammatory cytokine levels (IL-6 and IL-10) ↓ DNA fragmentation ↓ Bax, caspase 3, Fadd, and Fas expression ↑ Bcl-2 expression	[92]
		Antioxidant Properties	
Aloe vera crude methanolic extracts	In vitro antioxidant methods: DPPH, metal chelating, hydrogen peroxide scavenging, reducing power and β-carotene-linoleic	Antioxidant activity	[95]
Aloe vera ethanol extracts	Cell models (HeLa, HMEC, HaCat, and HOS) hydrogen peroxide and 4-hydroxynonenal induced	↓ ROS production ↓ HNE protein adducts HMEC cells were the most sensitive	[96]
Anthraquinone derivatives Phenolic derivatives Chromones Pyrones	Peroxyl radical scavenging Reducing capacity	Antioxidant activity	[97]

Table 1. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Bone Protection			
Aloe-emodin	ATDC5 cells	↑ Accumulation cartilage nodules ↑ Matrix proteoglycans synthesis ↑ ALP activity ↑ Chondrogenic marker genes ↑ ERKs ↑ BMP-2 protein expression	[99]
Aloin	RAW 264.7 cells	↓ RankL induced miR-21 expression ↓ Cathepsin K Osteoporosis protection	[101]
Aloin	RAW264.7 cells	↓ TRAP content ↑ F4/80 content ↓ Cathepsin K ↓ RANKL-induced NF-κB pathway ↓ DNA binding activity of NF-κB Osteoporosis protection	[100]
Cardioprotective EFFECT			
Aloe emodin	Model heme protein (hemoglobin)	↓ Hemoglobin aggregation (máximum effect at 100 μM)	[106]
Antimicrobial and Prebiotic Activity			
Acemannan and fructans	*Lactobacillus* and *Bifidobacterium* species Human fecal bacteria	↑ Bacterial growth (fructans) ↑ *Bifidobacterium* spp population ↑ Acetate concentrations	[120]
Aloe polysaccharides	H1N1 subtype Influenza A virus	↓ H1N1 subtype influenza virus replication ↓ Viral adsorption period	[117]
Aloe vera aqueous extract	Methicillin resistant *Staphylococcus aureus*	↓ Growth ↓ Biofilm formation	[109]
Aloe vera aqueous extracts	Drug resistant *Mycobacterium tuberculosis*	Inhibition zone: 60 mm (disk diffusion method) ↓ Cell growth (up to 1 mg/mL concentration) (pour plate method)	[115]
Aloe vera crude aqueous extracts	*Plasmodium falciparum*	Antiplasmodial activity	[95]

Table 1. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera extracts	Multidrug-resistant *Pseudomonas aeruginosa*	Similar MIC_{50} and MIC_{90}	[112]
Aloe vera gel	*Actinobacillus actinomycetemcomitans Clostridium bacilli*, *Streptcoccus mutans* and *Staphlococcus aureus*	Antibacterial activity against oral pathogens	[110]
Aloe vera gel	Virus herpes simplex 1	↓ HSV-1 growth	[116]
Aloe vera hydroalcoholic extract	*Enterococcus facecalis*	Antibacterial activity against *Enterococcus faecalis*	[114]
Aloe vera inner gel	Gram negative bacteria, Gram positive bacteria and *Candida albicans*	Antimicrobial and antibiofilm activities against Gram negative bacteria (*Helicobacter pylori*, *Escherichia coli* and *Pseudomonas aeruginosa*) and *Candida albicans*	[113]
Aloe vera mucilage	Intestinal microbiota from healthy donors	Prebiotic activity (↑ short chain fatty acids and modifications in bacterial composition)	[119]
Aloe-emodin	*Staphylococcus aureus*	↓ Biofilm development (initial adhesion and proliferation stages) and extracellular protein production	[111]
Other Effects			
Aloe-emodin	MH7A human RA synovial fibroblast-like cells	↓ Viable cells number ↑ Apoptosis (G2/M phase arrest) Rheumatoid arthritis protection	[127]
Aloe-emodin	ARPE-19 cells	↓ VEGF secretion ↓ VEGFA and PHD-2 mRNA expression ↓ VEGFA, HIF-1α and PHD-2 protein expression	[131]

Table 2. In vivo pharmacological studies for *Aloe vera*.

Aloe Vera Composition	Experimental Model	Major Findings	References
Digestive Diseases Protection			
Acemannan	Beagle Dogs	Mineralized Bridge Formation	[6]
Aloe polysaccharide	2,4,6-three nitrobenzene sulfonic acid colitis induced Rats	↑ JAK2, p-JAK2, STAT-3 and p-STAT3 protein expression ↓ Ulcerative colitis	[5]

Table 2. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera gel	Balb/c mouse model of alcohol-induced acute gastritis	↑ Matrix metalloproteinase-9 inhibitory activity	[24]
Aloe vera	Male Wistar rats Irradiated model	↑ Salivary flow rate	[7]
		Skin Protection	
Aloe emodin	BALB/c mice burn wound-induced	↑ Wound healing activity (↑ re-epithelialization and angiogenesis)	[6]
Aloe sterols	Female HR-1 hairless mice Ultraviolet B-induced skin photoaging	↓ Skin dryness, epidermal thickness and wrinkle formation ↓ Dermal collagen fibers degeneration ↓ Cutaneous apoptosis cells ↓ Pro-inflammatory cytokines ↓ Matrix metalloproteinases	[43]
Aloe vera	Adult male Wistar rats with incision on neck	↑ Fibroblasts ↑ TGF-β gene expression	[38]
Aloe vera	Adult female Sprague Dawley rats with a skin wound infected with methicillin-resistant *Staphylococcus aureus*	↓ Inflammatory cell infiltration ↑ Wound closure and skin tensile strength % ↑ Collagen deposition ↑ Skin tensile strength	[132]
Aloe vera aqueous gel extract	X-ray irradiated Male balb/c mice	↑ Hepatic and renal function parameters ↓ROS ↓ Lipid peroxidation ↑ GSH ↑ GR, GPx, CAT, SOD, GST ↑ Sperm count/motility and testosterone levels	[45]
Aloe vera cream	Male Sprague-Dawley rats	↓ Wound percentage ↓ Leucocytes infiltration ↓ Angiogenesis ↓ $CD8^+$ lymphocytes expression ↑ Epidermal thickness ↑ $CD4^+$ lymphocytes expression	[39]
Aloe vera extract gel	Wistar rats with a wound made by incision	↑ Organization of skin and collagen	[36]

Table 2. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera gel	Male Wistar rats Alloxan-induced diabetes with wounds	↓ Necrotic tissue and inflammation ↓ Wound areas Better reepithelialization	[40]
Aloe vera gel	Male Wistar rats	↑ Wound contraction and epithelialization ↓ Scar tissue size ↑ Alignment and organization of regenerated scar tissue	[37]
Aloe vera gel aqueous extract	X-ray irradiated Male balb/c mice	↑ Hepatic and renal function parameters ↓ ROS, ↓ Lipid peroxidation ↓Lactate dehydrogenase	[45]
Aloe vera gel powder	Ovariectomy HR-1 hairless mice UV-irradiation model	↓ Matrix metalloproteinases (MMPs) expression ↑ Epidermal growth factor ↑ Hyaluronan synthase	[41]
Aloe vera gel powder	HR-1 hairless mice UVB-induced model	↑ Skin elasticity ↓ Matrix metalloproteinase 2, 9 and 13 ↑ Hyaluronic content ↑ HA synthase 2	[42]
Aloe vera hydroalcoholic extract	Wistar rats with traumatic ulcers	No acceleration of oral wound	[133]
Aloesin	SKH-1 hairless mice	↑ Angiogenesis ↑ Collagen deposition and granulation tissue formation	[35]
Anti-Inflammatory Activity			
Aloe vera	Male ICR strain mice Acetaminophen-induced hepatitis	↓ Hepatic MDA ↓ IL-12 and IL-18 ↓ ALT transaminase ↓ Hepatitis	[65]
Aloe-emodin sulfates/glucuronides, rhein sulfates/glucuronides, aloe-emodin and rhein	LPS-induced septic mice	↓ NO level	[61]
Barbaloin	BALB/c mice LPS-induced acute lung injury	Histological analysis revealed certain protective effect	[60]

Table 2. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
		Anticancer Effects	
Aloe vera aqueous extract	Male Swiss albino mice	Mutagenic activity Cytotoxic effect Wound healing (antioxidant properties)	[47]
Aloe vera extract	Mice with breast cancer by implantind	↓ COX-2 level ↓ VEGF levels	[73]
Aloe vera powder and extract	C57BL/6J mice high-fat diet induced and azoxymethane induced aberrant crypt foci colorectal cancer	↑ Hepatic phase II enzyme glutathione S-transferase mRNA levels ↓ Cell proliferation in the colonic mucosa ↓ Number of aberrant crypt foci	[80]
Aloe vera gel	BALB/c female mice with induced colitis-associated colon carcinogenesis	↓ Multiplicity of colonic adenomas and adenocarcinomas ↓ Adenoma and adenocarcinoma development ↓ Activation of nuclear factor kappa B ↓ Inducible nitric oxide synthase and cyclooxygenase-2 expression ↓ Cell cycle progression-inducing cellular factors	[125]
Aloesin	Mice	↓ Tumor growth	[74]
Aloin	Male Swiss albino rats	↑ Erythropoiesis impairment ↑ Serum iron level No changes on serum lipid profile No changes on serum elements and kidney function parameters	[105]
Emodin	SPF BALB/c-nu nude mice	↑ Survival time of tumor ↓ Effect on transplantation tumors ↓ Oral cancer	[75]
		Antidiabetic Effect	
Aloe vera extract	Streptozotocin-induced diabetic Wistar rats	↓ Blood glucose levels ↑ Insulin levels ↑ Number, diameter, volume and area of pancreatic islets	[88]

Table 2. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera extract	Streptozotocin-induced nephropathy diabetic Wistar rats	↓ Development of nephropathy ↓ Lipid alteration ↓ Renal oxidative stress Direct renoprotective action	[89]
Aloe vera gel	Streptozotocin-induced diabetic male Wistar rats	↓ Anxiety/depression-like behaviors ↑ Exploratory and locomotor activities ↑ Memory performance ↓ Stress related behaviors ↓ Oxidative stress ↑ Neuronal loss in hippocampus	[134]
Aloe vera lyophilized extract	Wistar rats with corneal alkali-burn injury Normal rats and diabetic rats streptozotocin-induced	↑ Wound healing (especially in diabetic rats) ↓ Edema Complete and higher corneal re-epithelialization	[90]
Aloe vera polysaccharides	C57BL/KsJ-db/db male mice fed with high fat diet	↓ Fasting blood glucose levels	[91]
		Cardioprotective Effect	
Aloe vera	Wistar albino rats Spinal cord ischemia reperfusion injury model	↓ MDA levels ↓ NF-κβ and nNOS expressions ↓ Hemorrhage ↓ Edema ↓ Inflammatory cell migration ↓ Neurons	[102]
Aloe vera extract	Wistar Albino rats with ischemia—reperfusion injury of sciatic nerve	↓ Ischemic fiber degeneration ↓ MDA ↑ NRF1 level ↑ SOD activity	[108]
Aloe vera gel	Male Wistar albino rats Ischemia reperfusion injury model	↑ SOD, CAT and GPx ↓ MDA levels	[103]

Table 2. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera gel	Male Wistar Albino rats Sciatic nerve ischemia model	↓ Ischemic fiber degeneration ↓ MDA content ↑ NRF1 level and SOD activity Neuroprotection	[108]
Aloe-emodin	Male Wistar rats	↓Total cholesterol ↓ Low-density lipoprotein-cholesterol ↓ Hyperlipidemia	[107]
Aloe-emodin	Sprague Dawley rats Hypoxia conditions	↓Hypoxia-induced retinal neovascularization	[131]
Aloin	Swiss albino rats	↓ Triacylglycerols ↓ Total cholesterol ↓ Cholesteryl esters ↓ Low density lipoprotein–cholesterol ↓ Very low density lipoprotein–cholesterol ↓ Urea ↓ Creatinine ↓ Blood hemoglobin concentration ↑ Serum iron level	[105]
Barbaloin/aloin	Sprague-Dawley rats Myocardial ischemia/reperfusion injury model	↓ I/R induced myocardial oxidative stress and inflammatory response ↑ AMPK signaling	[104]
Antimicrobial and Prebiotic Activity			
Aloe polysaccharides	PR8(H1N1) virus infection SPF BALB/c mice	Clinical symptoms improvement Lung damage improvement	[117]
Other Effects			
Aloe vera aqueous extract	Female Wistar albino rats Morphine dependent model	↓ Agitation, disparity and floppy eyelids	[129]
Aloe vera aqueous extract	Wistar rats drug-induced sleeping and anesthesia and analgesia	↑ Loss of righting reflex ↓ Locomotion activity Changes in total sleep time, percent of REM sleep and percent of NREM sleep	[135]

Table 2. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera ethanol extract	Wistar rats	↑ Mounting frequency ↑ Intromission frequency ↓ Mount and intromission latencies ↑ Ejaculatory latency ↑ Testosterone and cholesterol concentrations	[122]
Aloe vera ethanol extract	Rats	↓ TNF-α levels ↓ NK cells ↓ Th17 cells percentage Hepatoprotective	[136]
Aloe vera gel	C57BL/6 female mice C3H/HeJ mice	↑ Lymphocyte and erythrocytes number Lymphopenia and erythropenia restoration IgA secretion restoration	[125]
Aloe vera gel extract	Wistar rats Bisphenol A Induced Testicular Toxicity	↑ Body and testis weights ↑ Seminiferous tubule diameter and height of seminiferous epithelium ↑ Quantity of spermatocyte and spermatids ↓ MDA ↑ GSH	[123]
Aloe vera gel extract	Balb/c mice pulmonary tissue of cigarette smoke induced	↓ Degree of histoarchitectural alterations ↓ Mucin production ↓ NO levels ↓ Citrulline levels ↓ Peroxidative damage ↓ Serum LDH activity	[130]
Aloe vera lyophilized extract ointment	Wistar rats with partial transection of the calcaneal tendon	↑ Non-collagenous proteins ↑ Content and arrangement of glycosaminoglycans ↓ Tendon lesions	[128]
Aloe vera ethanol	Albino rabbits	Normalized levels of white blood cells, red blood cells, platelet count, packed cell volume, mean cell volume and haemoglobin values ↓ MDA ↑ CAT	[126]

Table 2. *Cont.*

Aloe Vera Composition	Experimental Model	Major Findings	References
Aloe vera gel ethanol extract	Wistar rats	↓ Testes weight ↓ Serum testosterone ↓ Sperm count ↓ Fertility ↑ Serum NO Negative effects on spermatogenesis and sperm quality	[121]
Aloe vera processed gel	BALB/c mice on ovalbumin -induced food allergy	↓ Serum concentrations of type 2 helper T cell (Th2) cytokines (Interleukin-(IL)-4, IL-5, and IL-13) ↓ Histamine ↓ Mast cell protease-1 ↓ Immunoglobulin IgE ↑ IL-10 production ↑ Population of type 1 regulatory T (Tr1) cells ↓ Allergy	[124]
Aloin	F344/N Rats	↑ Incidences and severities of mucosal and goblet cell hyperplasia ↑ Shifts in gut microbiota structure	[137]

Table 3. Clinical trials with *Aloe vera*.

Reference	Study Design	Number of Patients	Intervention	Results
Digestive Diseases Protection				
Anuradha et al. (2017) [21] India	-	74	Group 1: *Aloe vera* juice (30 mL/twice daily) and *Aloe vera* gel (5 mg/3 times daily) for 3 months Group 2: intralesional injection of hydrocortisone (25 mg/mL) and hyaluronidase (1500 IU) weekly for 6 weeks with antioxidant supplements for 3 months	Oral submucous fibrosis: ↓ Burning sensation ↑ Mouth opening, cheek flexibility, and tongue protrusion
Ashouri Moghaddam et al. (2017) [12] Iran	Single-blind clinical trial	20	Group 1: *Aloe vera* gel Group 2: Placebo (distilled water)	Chronic periodontitis: ↓ Gingival index ↓ Probing depth

Table 3. *Cont.*

Reference	Study Design	Number of Patients	Intervention	Results
Fallahi et al. (2016) [15] Iran	Randomized double blind	20	Group 1: *Aloe vera* Group 2: Placebo	↓ Swelling ↓ Postoperative pain
Gupta et al. (2014) [8] India	Double blind randomized control trial	300	Group 1: *Aloe vera* mouthwash group Group 2: chlorhexidene group Group 3: Placebo	↓ Plaque index and gingival index
Ipshita et al. (2018) [13] India	-	90	Group 1: Placebo Group 2: 1% alendronate gel Group 3: *Aloe vera* gel	Chronic periodontitis: ↑ defect depth reduction
Kalra et al. (2017) [16] India	-	48	Group 1: *Aloe vera* gel Group 2: mineral trioxide aggregate	Pulpotomy: Success rates was higher in mineral trioxide aggregate than in *Aloe vera* gel
Kurian et al. (2018) [14] India	Randomized, single-center, longitudinal, triple-blinded, parallel arm y	90	Group 1: Placebo Group 2: 1% metformin gel Group 3: *Aloe vera* gel 6–12 months	Chronic periodontitis: ↓ Pocket probing depth ↓ Clinical attachment level ↑ Bone fill and regeneration
Mansouri et al. (2016) [20] Iran	Randomized controlled clinical trial	64	Group 1: *Aloe vera* solution (5 mL/two minutes wash, three times daily) Group 2: ordinary mouthwashes. 14 days	Stomatitis: ↓ Chemotherapy-induced Stomatitis in patients with lymphoma and leukemia
Nimma et al. (2017) [17] India	Cross-sectional randomized interventional study	40	Group 1: analgesics (7 days) and socket healing Group 2: *Aloe vera* gel (7 days) and socket healing	Ulcers: ↓ Pain ↑ Healing
Panahi et al. (2015) [23] Iran	Pilot, randomized controlled, open-label, trial	79	Group 1: *Aloe vera* syrup (10 mL/day) Group 2: omeprazole capsule (20 g/day) Group 3: Ranitidine tablet (150 mg twice daily). 4 weeks	↓ Frequency symptoms gastroesophageal reflux disease
Prabhakar et al. (2015) [18] India	Experimental, in vivo intergroup split mouth, randomized clinical trial	10	Group 1: Distilled water Group 2: Propolis extract Group 3: *Aloe vera* extract	Antimicrobial: ↓ Bacterial counts Disinfection

Table 3. *Cont.*

Reference	Study Design	Number of Patients	Intervention	Results
Pradeep et al. (2016) [9] India	Single center, randomized, longitudinal, triple masked, interventional study	60	Group 1: Placebo Group 2: *Aloe vera* gel	Chronic periodontitis: ↓ Plaque index ↓ Modified sulcus bleeding index ↓ Probing depth ↑ Clinical attachment level Patients with type 2 diabetes and chronic periodontitis
Rahmani et al. (2014) [27] Iran	Prospective observational clinical trial	60	Cream of 0.5% *Aloe vera* juice powder (3 times daily) 6 weeks	↓ Chronic anal fissure pain and hemorrhaging upon defection ↑ Wound healing
Sahebjamee et al. (2015) [19] Iran	Triple-blind randomised and controlled interventional	26	Group 1: *Aloe vera* mouthwash Group 2: benzydamine mouthwash 0.15%	Oral mucositis: ↓ Severity of radiation-induced mucositis in patients with head and neck cancers
Sahebnasagh et al. (2017) [25] Iran	Double-blind placebo-controlled trial	20	Group 1: *Aloe vera* gel 3% Group 2: Placebo	Proctitis: Improvement of diarrhea, fecal urgency, clinical presentation total, Radiation Therapy Oncology Group total and lifestyle
Singh et al. (2016) [22] India	-	40	Group 1: *Aloe vera* gel (three times daily) + physiotherapy Group 2: Antioxidant capsules (twice daily) + physiotherapy 3 months	Oral submucous fibrosis: ↓ Burning sensation ↑ Mouth opening, cheek flexibility, and tongue protrusion
Størsrud et al. (2015) [26] Sweden	Randomized, double-blind, placebo controlled study	68	Group 1: *Aloe vera* extract (AVH200®) Group 2: Placebo 4 weeks	Irritable bowel syndrome: ↓ severity of the gastrointestinal symptoms
Vangipuram et al. (2016) [10] India	Randomized controlled trial	390	Group 1: *Aloe vera* mouth wash Group 2: Chlorhexidine (0.12%) mouth wash Group 3: Placebo	Gingivitis: *Aloe vera* has equal effectiveness than chlorhexidine ↓ Plaque index and gingival index

Table 3. *Cont.*

Reference	Study Design	Number of Patients	Intervention	Results
Yeturu et al. (2016) [11] India	Randomized single-center, single-blind, parallel group, controlled trial	85	Group 1: *Aloe vera* mouth wash Group 2: chlorine dioxide mouth wash Group 3: chlorhexidine mouth wash 10 mL of mouth rinse for 1 min, twice daily for 15 days	↓ Mean plaque and gingival scores
		Skin Protection		
Ahmadloo et al. (2017) [57] Iran	Prospective randomized controlled clinical trial	100	Group 1: *Aloe vera* gel twice daily Group 2: control	Dermatitis: No positive effect on prevalence or severity of radiation dermatitis
Avijgan et al. (2016) [48] Iran	-	60	Group 1: *Aloe vera* gel twice daily Group 2: Conventional treatment 3 months	Ulcers: wound healing
Burusapat et al. (2018) [53] Thailand	Double-blind, randomized, controlled trial	12	Group 1: *Aloe vera* gel Group 2: Placebo	↑ Split-thickness skin graft donor-site healing No pain relief
Hekmatpou et al. (2018) [50] Iran	Triple-blind randomized clinical trial	80	Group 1: pure *Aloe vera* gel twice daily Group 2: Placebo (gel or water and starch) 10 days	Ulcers: ↓ pressure ulcers
Hoopfer et al. (2015) [58] Canada	Three-Arm Randomized Phase III Trial	248	Group 1: *Aloe vera* cream Group 2: Placebo	No ↓ skin reaction severity in breast cancer radiation therapy
Irani and Varaie (2016) [51] Iran	Randomized clinical trial	30	Burned area: nitrofurazone 2% Symmetry burned area: *Aloe vera* gel	Burns: Earlier epithelialization and granulation tissue
Molazem et al. (2014) [52] Iran	Prospective randomized double-blind clinical trial	90	Group 1: *Aloe vera* gel Group 2: dry gauze alone	↑ Cesarean wound healing

Table 3. *Cont.*

Reference	Study Design	Number of Patients	Intervention	Results
Tanaka et al. (2015) [55] Japan	Randomized, double-blind, placebo-controlled study	58	Group 1: *Aloe* sterol (5 tablets/daily) Group 2: Placebo 12 weeks	↓ Facial wrinkles
Tanaka et al. (2016) [54] Japan	Randomized, double-blind, placebo-controlled study	48	Group 1: *Aloe* sterol (5 tablets/daily) Group 2: Placebo 12 weeks	↑ Skin elasticity in photodamaged skin
Tanaka et al. (2016) [54] Japan	Randomized, double-blind, placebo-controlled study	64	Group 1: *Aloe* sterol-supplemented yogurt Group 2: Placebo	↑ Gross elasticity, net elasticity, biological elasticity, skin fatigue area, collagen content
		Anticancer Effects		
Damani et al. (2015) [86] USA	Case report	1	*Aloe vera* eye drops 3 times daily	Ocular surface squamous neoplasia: Lesion regressed
Koo et al. (2019) [87] South Korea	Randomized	40	Group 1: *Aloe* polysaccharide (600 mg/day) Group 2: Propolis (600 mg/day) Group 3: *Aloe* polysaccharide + propolis 4 weeks Group 4: Placebo	Cancer: ↑ Urinary excretion of benzo(a)pyrene and cotinine ↓ Creatinine, glucose, and total bilirubin levels ↓ Risk of cancer associated with tobacco
		Antidiabetic Effect		
Alinejad-Mofrad et al. (2015) [93] Iran	Double blind randomized controlled trial Pre-diabetes	72	Group 1: Placebo Group 2: *Aloe vera* 300 mg/twice day (AL300) Group 3: *Aloe vera* 500 mg/twice day (AL500) 4 and 8 weeks	Diabetes: ↓ Fasting blood glucose (AL300, 4 weeks)
		Antioxidant Properties		
Prueksrisakul et al. (2015) [98] Thailand	-	53	*Aloe vera* gel extract daily 14 days	Antioxidant: ↑ Plasma total antioxidant capacity

Table 3. *Cont.*

Reference	Study Design	Number of Patients	Intervention	Results
Cardioprotective Effect				
Alinejad-Mofrad et al. (2015) [93] Iran	Double blind randomized controlled trial Pre-diabetes	72	Group 1: Placebo Group 2: *Aloe vera* 300 mg/twice day (AL300) Group 3: *Aloe vera* 500 mg/twice day (AL500) 4 and 8 weeks	↓ HbA1C (AL300, 8 weeks) ↓ Total cholesterol (AL500, 8 weeks) ↓ LDL-C (AL500, 8 weeks) ↓ Triglyceride level (AL500, 4 weeks)
Antimicrobial and Prebiotic Activity				
Prueksrisakul et al. (2015) [98] Thailand	-	53	*Aloe vera* gel extract daily 14 days	Antimicrobial: ↓ *Lactobacillus* spp.

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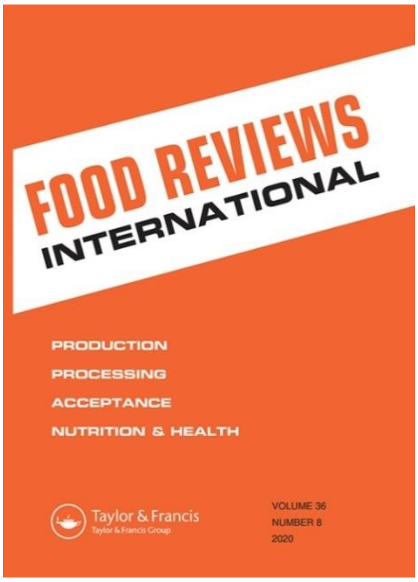
ARTÍCULO IV



The pharmacology and clinical efficacy of *Matricaria recutita* L.: a systematic review of in vitro, in vivo studies and clinical trials.

Marta Sánchez, Elena González-Burgos & M. Pilar Gómez-Serranillos.

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REVIEW

The pharmacology and clinical efficacy of *matricaria recutita* L.: a systematic review of *in vitro, in vivo* studies and clinical trials

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ABSTRACT

This systematic review shows the effectiveness of *Matricaria chamomilla* for improving health. Original papers and case reports published during 2014–2018 in PubMed, Science Direct and Scopus were included. Most studies were *in vitro*, performed with extracts, in 2018, and originated from Iran. *In vitro* assays focused mainly on antimicrobial, antiparasitic and antioxidant activities. *In vivo* studies specially investigated organ and tissue protective activity of chamomile. Clinical trials have validated some of these *in vitro* and *in vivo* pharmacological activities (diabetes mellitus, hypertension and pain). Future preclinical and clinical investigations should be aimed at deeping into pure compounds responsible for activities.

KEYWORDS

Matricaria chamomilla; *in vitro*; *in vivo*; clinical trials; pharmacology

Introduction

Chamomile (*Matricaria chamomilla* L., *Matricaria recutita* L.), belonging to the Asteraceae family, is an annual herbaceous plant native to Europe and Western Asia and it has also been colonized the American and oceanic continents. This common plant stands out for its numerous uses in therapeutic and cosmetic and its high nutritional value. Hence, *M. chamomilla* has been widely used in traditional medicine and in alternative medical systems (i.e. unani, homeopathy) for its digestive relaxant, anti-inflammatory and healing actions.[1] In cosmetics, essential oils and infusions from aerial parts of *M. chamomilla* have been used as ingredients of hair products, soaps and perfumes for its coloring, flavoring and aromatic properties.[2] Moreover, *M. chamomilla* is one of the most popular herbal tea.[3] These properties are attributed to its active compounds among which flavonoids [i.e. apigenin (16.8%), quercetin (9.9%), luteolin (1.9%)], coumarins (i.e. herniarin 0.1%) and sesquiterpenes [i.e. α-bisabolol and bisabolol oxide (≤78%), chamazulene (1–15%)].

The current interest of the food industry related to disease prevention and health promotion lies in the use of natural compounds instead of synthetic products.[4,5]*M. chamomilla* extracts and essential oils have been investigated as food preservative for its antioxidant and antimicrobial properties in cottage cheese, yogurts and bakery products.[6–8] This systematic review aimed to update previous reviews [,13] to assess the pharmacological (*in vitro* and *in vivo*) properties and clinical evidence (clinical trials) of extracts, essential oils and active compounds isolated from *M. chamomilla*.

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Supplemental data for this article can be accessed on the publisher's website.

Material and methods

Search strategy

A systematic review was conducted through the databases PubMed, Science Direct and Scopus using a combination of the following keywords: *Matricaria recutita, Matricaria chamomilla, in vivo, in vitro*, clinical trials and pharmacology (Supplementary file).

Study selection

The selection of the manuscripts was performed by two independent researchers based on the title, abstract and finally through an analysis of the full-text publication.

The inclusion criteria were pre-clinical studies (both *in vitro* and *in vivo*) and clinical trials involving the pharmacological properties of extracts, essential oils and active compounds isolated from *Matricaria recutita*/*Matricaria chamomilla*. There were selected those original papers and case reports written in English, French, Spanish and German published in the last five years (2014–2018).

The exclusion criteria for this systematic review were those studies that include plants mixtures, other varieties of *Matricaria* and synthetic drug combinations with chamomile. Moreover, those works on ecology, phytochemical analysis and galenic formulations were also excluded. Furthermore, other review articles, editorials/letters, conference proceedings and meta-analysis were similarly excluded.

Data extraction

The following information was included in the *in vitro* and *in vivo* assays: administration methods, activity, extract/essential oil/isolated compound, experimental model, treatments, major findings, first author and year of publication.

In clinical trials, findings were divided into administration modes and the obtained information in each column activity, first author, year of publication and country of the origin of the paper, study design, sample size, population, type of plants, intervention, treatment duration and results.

Results

The primary search on pharmacology activity of chamomile identified a total of 633 manuscripts (Pubmed n = 204, Science Direct n = 100 and Scopus n = 329). There were 179 papers which were duplicates because they appeared in two or more databases and 368 papers which were excluded after title and abstract analysis (n = 322) and after complete paper analysis (n = 46). Finally, a total of 86 works met inclusion criteria established in this systematic review, of which, one paper reported both *in vitro* and *in vivo* activity and another paper reported both *in vivo* activity and clinical trials (n = 46 *in vitro*, n = 20 *in vivo* and n = 22 clinical trials). The flow chart of paper selection process is shown in Fig. 1. Tables 1, 2 and Table 3 summarize the main information for each *in vitro* studies, *in vivo* studies and clinical trials, respectively.

The majority of works on *Matricaria chamomilla* pharmacological activity was published in the year 2015 (n = 14; 16.1% *in vitro*, n = 4; 4.6% *in vivo* and n = 5; 5.7% clinical trials) and in the year 2018 (n = 16; 18.4% *in vitro*, n = 8; 9.2% *in vivo* and n = 6; 6.9% clinical trials). All these studies were performed by research groups of 24 different countries located mainly in Asia and European continents. Most of these were originated from Iran (n = 33; 37.9%) followed by Brazil (n = 8; 9.2%), Tunisia (n = 6; 6.9%) and India (n = 5; 5.7%). Moreover, most of the pharmacological studies with *Matricaria chamomilla* have been done with extracts (n = 32 *in vitro*, n = 18 *in vivo* and n = 16 clinical trials), followed by essential oils (n = 8 *in vitro*, n = 3 *in vivo* and n = 7 clinical trials) and finally isolated compound (n = 12 *in vitro* and n = 1 *in vivo*). There is not any clinical trial with isolated compound. Of the 46 *in vitro* studies, single activity has been studied in 30 of them while two or more activities have

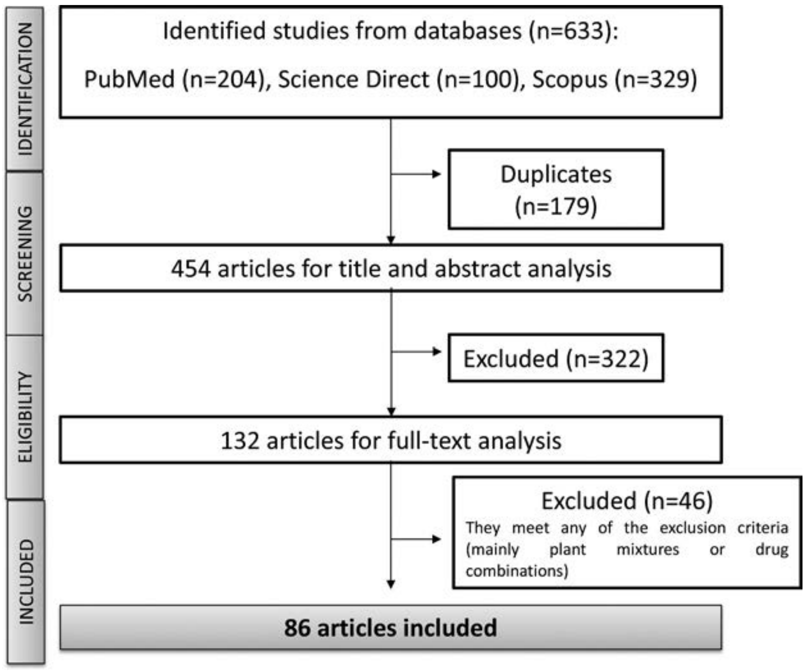

Figure 1. Flowchart of the literature research on pharmacological (*in vitro* and *in vivo*) properties and clinical evidence (clinical trials) of extracts, essential oils and active compounds isolated from *M. chamomilla.*

been evaluated in 16 reports. The most studied activities correspond to antibacterial (n = 15; 68.2%), antioxidant (n = 13; 59%), antifungal (n = 9; 40.9%) and antiparasitic (n = 6; 27.3%). Of the 21 *in vivo* studies, there are 19 reports on a single activity and 2 reports on two different activities. Most of these *in vivo* studies focused on protection activity (n = 12; 57.1%), mainly neuroprotection and hemato-protection. In clinical trials, a variety of syndromes or pathologies have been studied, being oral diseases (n = 5; 22.7%), anxiety (n = 2; 9.09%), carpal tunnel syndrome (n = 2; 9.09%), diabetes mellitus (n = 2; 9.09%), premenstrual syndrome (n = 2; 9.09%) and sleeps (n = 2; 9.09%) the most studied Fig. 2.

Discussion

In vitro studies

Antimicrobial

The inappropriate and irrational use of antibiotics has led bacteria to develop mechanisms of resistance through extrusion by efflux and target site modification. This bacteria resistance to antibiotics available in the market is one of the greatest public health problems.[92] It is estimated that around 70% of bacteria responsible for hospital infections are resistant to at least one of the most common antibiotics used in clinics.[93] Therefore, the search for new antibiotics, especially among medicinal plants, constitutes one of the main research challenges to deal with antibiotic resistance.

All studies included in the present systematic review that focus on evaluating the antimicrobial activity of *Matricaria chamomilla* have investigated its antibacterial action. Of the 15 studies of antibacterial activity, 12 of them have been performed with extracts and 3 of them with essential oils. The antibacterial effect of essential oils has been investigated in a total of fifteen different Gram positive and Gram negative bacteria. Kazemi[9] demonstrated that especially Gram positive bacteria are sensitive to essential oils of *M. chamomilla*. This can be due to outer membrane of bacteria; Gram positive bacteria have a thick peptidoglycan wall whereas Gram negative bacteria have a complex and

Table 1. *In vitro* pharmacological studies for *Matricaria chamomilla.*

Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Antibacterial	Essential oil α-bisabolol oxide, camphene, camphor, 1,8-cineol, limonene, α-pinene, sabinene	Bacteria: gram-positive (*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus*) and gram-negative (*Pseudomonas aeruginosa, Proteus* sp., *Shigella shiga, Shigella sonnei*)	1.0 μg/mL for essential oil and isolated compounds	Active against *B. cereus* The most active compounds: α-bisabolol oxide and bisabolol	[9]
Antibacterial	Essential oils	*Enterococcus faecalis, Methicillin Resistant Staphylococcus aureus, Staphylococcus aureus, Staphylococcus epidermidis, Vancomycin-resistant Enterococcus*	Concentration range (4% to 0.016%).	No antibacterial action	[10]
Antibacterial	Ethanol (different percentages) and ethyl acetate extracts	*Helicobacter pylori*	0.98 to 1000 μg/mL	MIC = 31.3–62.5 μg/ ml highlight ethyl acetate and ethanol extracts, respectively	[11]
Antibacterial	Infusion (2%, 4% and 8%)	*Streptococcus mutans*	Infusion (2%, 4% and 8%)	No antibacterial activity (infusion)	[12]
Antibacterial Antifungal	Aqueous and alcohol extracts	*Staphylococcus aureus, Escherichia coli* *Candida albicans*	5, 10, 20, 40% extract concentrations	Aqueous extract has the lowest inhibitory effect on microbial growth (1.64 mm)	[13]
Antibacterial Antifungal	Aqueous extract (15% and 25%)	*Enterococcus faecalis* *Candida albicans*	48 h	Low antimicrobial activity (25%)	[14]
Antibacterial Antifungal	Chamomilla extract-incorporated Ag nanoparticles	*Escherichia coli, Staphylococcus aureus* *Candida albicans*	6.25–400 ppm	↑ antimicrobial activity of chamomile extract-incorporated Ag nanoparticles than *M. chamomilla* extract itself	[15]
Antibacterial Antifungal	Essential oils	Bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella species, Staphylococcus aureus*) Fungi (*Aspergillus* spp., *Trichophyton* spp.)	15.75–250 mg/mL	No antibacterial activity No antifungal activity	[16]

(*Continued*)

Table 1. (Continued).

Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Antibacterial Antifungal	Methanol extract	*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Staphylococcus epidermidis, Streptococcus pneumonia, Streptococcus pyogenes* *Aspergillus niger, Aspergillus terreus, Candida albicans, Microsporum canis, Penicillium expansum, Saccharomyces cerevisiae, Trichoderma horzianum, Trichoderma viride*	-	Maximum zone formation was against *P. mirabilis* ↑ active against *A. terreus*	[17]
Antibacterial Antifungal	Silver nanoparticles with extract	*Staphylococcus aureus* *Candida albicans*	-	Antibacterial and antifungal activity	[18]
Antibacterial Antifungal Antioxidant Anticancer	Chamomile ligulate flowers extracts obtained by different methods	*Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus* *Aspergillus niger, Candida albicans*, Radical scavenging activity (DPPH and reducing power models) Human rhabdomyosarcoma cell line, human cervix carcinoma – HeLa derivative and murine fibroblast cell line	Different concentrations of extracts for each assay	Antimicrobial activity against *E. coli* and *A. niger* for subcritical water extraction ↑ Antioxidant effect for subcritical water extraction ↓ Cell viability cancer (highlight subcritical water extraction)	[19]
Antibacterial Antifungal Antioxidant	Methanol extract	*Lactobacillus acidophilus, Staphylococcus aureus, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Pseudomonas aeruginosa* *Candida albicans, Candida krusei, Candida parapsilosis, Candida tropicalis* Radical scavenging activity (DPPH and ABTS models)	-	Antibacterial activity except against *P. aeruginosa* Better result against *S. mutans* DPPH activity (85%) ABTS activity (79.58%)	[20]

(*Continued*)

Table 1. (Continued).

Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Antibacterial Antioxidant	Aqueous extracts	*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* Radical scavenging activity (DPPH model) Folin-Ciocalteu method and aluminum chloride colorimetric assays	-	Antimicrobial activity (specially leaf and stem extracts better than stem extract) DPPH radical scavenging activity	[21]
Antibacterial Antioxidant	Ethanol and metanol extract	*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* Radical scavenging activity (DPPH model)	Ethanol extract (2.5, 5, 10 and 20 µg/ml) and methanol extract (1.5, 3, 6 and 12 µg/ml)	Antibacterial activity against *S. aureus* and *E. coli* (methanol extract) DPPH radical scavenging activity (specially methanol extract)	[22]
Antibacterial Antioxidant	Methanol extract	*Bacillus subtilis, Escherichia coli, Kocuria, Propionibacterium acnes, Pseudomonas aeruginosa, Staphylococcus aureus* Radical scavenging activity (DPPH model)	50,100, 150 and 200 µg/ml for antioxidant assay	Significant activity against *P. acnes* Good antioxidant activity	[23]
Anticancer	Hydroalcoholic extract	Breast cancer cell lines (MCF-7 and MDA-MB-468)	50–1300 µg/mL (24, 48, and 72 h)	↑ Apoptosis and necrosis ↓ Cellular invasion or migration	[24]
Anticancer	Hydroalcoholic extract	*Saccharomyces cerevisiae*	250 µg/ml, 1000 µg/ml, and 3000 µg/ml	↓ ERG9 gene expression	[25]
Anticancer	Methanol extract	Skin cancer melanoma cell lines (A375.S2 and WM136.1A)	6.25–100 µg/ml, 72 h	↓ melatonic WM1361A proliferation	[26]
Anticancer	Root extract	Breast cancer cell line (MCF-7)	200 µg / ml −2000 µg / ml (24, 48 and 72 h)	↑ Cell death	[27]
Anticancer	Silver nanoparticles of aqueous extract	A549 lung cancer cells	0–100 µg/mL (24 h and 48 h)	↓ cell viability ↑ apoptotic effect	[28]
Antidiabetic	Apigenin, apigenin-7-*O*-glucoside, (*Z*) and (*E*)−2-hydroxy-4-methoxycinnamic acid glucosides	Human α-amylase and rat α-glucosidase inhibition assays *Caco-2/TC7 cells*	Several treatments in function of assay and compound	↓ α-amylase and maltase activities ↓ glucose transport (interaction with GLUT2 transport)	[29]
Antidiabetic	Extract Apigenin, apigenin-7-O-glucoside, cis- and trans-ferulic acid hexoside	Enzymatic models (human alfa-amylase and maltase)	-	↓ Salivary human α-amylase and maltase (highlight apigenin)	[30]

(*Continued*)

Table 1. (Continued).

Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Antifungal	Essential oils	Fluconazole resistant, fluconazole susceptible *Candida albicans*	1000 to 3000 $\mu g.ml^{-1}$	MIC values = 1700 $\mu g.ml^{-1}$ for fluconazole resistant and 1550 $\mu g.ml^{-1}$ for fluconazole susceptible strains	[31]
Anti-inflammatory	***Apigenin-7-glucoside***	Bone Marrow Derived Macrophages (BMDMs) from C57BL/6 mice	3–300 µg/ml	↓ TNF- α production	[32]
Anti-inflammatory	Chamazulene Matricine	Human microvascular endothelial cells (HMEC-1)	10–75 µM	↓ TNF-α and LPS-induced expression of the adhesion molecule ICAM-1 (Matricine) ↓ NF-κB signaling effect (Matricine)	[33]
Antioxidant Anti-inflammatory Antiarthritic	Aqueous extract	Live tissue Anti-coagulated human blood Collagen from the fore limb cartilages of chicken	-	↓ Lipid peroxidation ↓ Protein denaturation Lysosomal membrane stabilisation ↓ Collagen denaturation	[1]
Antioxidant	Chamazulene	Radical scavenging activity (DPPH and ABTS models)	Different concentrations of extracts to determine IC50 values	Total antioxidant activity of chamazulene higher than antioxidant reference compounds ABTS radical scavenging activity	[34]
Antioxidant	Chamomile polyphenolic-polysaccharide conjugates	Radical scavenging activity (DPPH and ABTS models) Blood plasma samples	0.5–300 µg/mL	DPPH and ABTS radical scavenging activity ↓ Lipid oxidation and protein oxidation	[35]
Antioxidant	Copper oxide nanoparticleswith chamomilla flower extract	Radical scavenging activity (DPPH model)	7.5–60 ppm	DPPH radical scavenging activity	[36]
Antioxidant	Essential oil Methanol extract	DPPH radical scavenging activity FRAP assay	1 mg/10 mL	DPPH activity (methanol extract > essential oils) FRAP test (methanol extract ≈ essential oils)	[37]
Antioxidant Antidiabetic	Ethanol and hexane extracts	*In vitro* assays (α-amylase, lipase and glycation) Radical scavenging activity (DPPH, ORAC and FRAP models)	0–10 µg/ml (α-amylase assay) 0–1200 µg/ml (lipase assay) 0–250 µg/ml (DPPH assay) 0–300 µg/ml (glycation assay)	↓ α-amylase and lipase activities DPPH radical scavenging activity Anti-glycation activity	[38]

(*Continued*)

Table 1. (Continued).

Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Antioxidant Antidiabetic	Methanol extract Isolated compounds: apigenin, apigenin-7-O-β-D-glucuronide, apigenin-7-O-β-D-glucoside, 3,5-O-di-caffeoylquinic acid, luteolin, luteolin-7-O-β-D-glucoside, luteolin-7-O-β-D-glucuronide	Radical scavenging activity (DPPH models) Rat lens	3–75 µg/ml extract and 15–75 µg/ml isolated compounds (DPPH radical scavenging) 1–10 µg/ml extract and isolated compounds (rat lens aldose reductase) 20–200 µg/ml extract and 10–50 µg/ml isolated compounds (advanced glycation end products)	Rat lens aldose reductase and advanced glycation end products inhibition DPPH radical scavenging activity	[39]
Antiparasitics	Aqueous and methanol extracts	Parasite eggs from fecal samples of infected lambs and larvae *Haemonchus contortus*	1–1024 µg/ml (egg hatch test) and 1365–0.66 µg/ml (larval development test) for methanol extract 50–1563 µg/ml (egg hatch test) and 40–0.019 mg/ml (larval development test) for aqueous extract	↓ *In vitro* ovicidal and larvicidal activity	[40]
Antiparasitics	Aqueous and organic extract Essential oil	*Acanthamoeba castellanii* Neff. Radical scavenging activity (ABTS, DPPH and FRAP models) Murine macrophages (J774A.1)	Different concentrations to determine IC50 values	Inhibition of *A. castellanii* Antioxidant effects (specially ABTS radical scavenging activity)	[41]
Antiparasitics Antioxidant	Aqueous, chloroform, hexanol, methanol extracts	Eggs or adult stages from faeces and abomasum of *Barbarine donor* lambs infected of *Haemonchus contortus* Radical scavenging activity (DPPH and ABTS models)	Methanol (IC50 = 1.559 mg/ml) and aqueous (IC50 = 2.559 mg/ml) extracts for anthelmintic activity Concentrations from 0–100 mg/mL of extracts for antioxidant assays	↓ Egg-hatching and ↓ motility adult worms of *H. contortus* (methanol and aqueous extracts) DPPH and ABTS radical scavenging activity (methanol and aqueous extracts)	[42]
Antiparasitics	Essential oil (-)-α-bisabolol	Promastigotes and amastigotes of *Leishmania infantum* and *Leishmania amazonensis* J774A.1 murine macrophage cell line	Essential oil (IC50 value 10.8 µg/mL for *L. amazonensis* and 10.4 µg/mL for *L. infantum*) at 96 h (−)α-bisabolol (16 µg/mL and 5.9 µg/mL for *L. amazonensis*, 9.5 µg/mL and 4.8 µg/mL for *L. infantum* promastigotes and amastigotes, respectively, and 31.9 µg/mL for J774A.1)	↓ strains growth and activity ↑ apoptotic effect (phosphatidylserine externalization, membrane damage, ↓ mitochondrial membrane potential, ↓ total ATP levels)	[43]

(*Continued*)

Table 1. (Continued).

Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Antiparasitics	Essential oils	Promastigotes of *Leishmania amazonensis* L6 cell monolayers	IC50 60.16 µg/mL, 24 h for *L. amazonensis* CC50 173.04 µg/mL, 24 h for L6 cells	↓ Parasite growth	[44]
Antiparasitics	α-bisabolol	*Acanthamoeba castellani* Neff	IC50 20.839 µg/ml IC90 46.601 µg/ml	↑ Apoptosis ↑ Permeability of plasmatic membrane ↓ Mitochondrial and ATP levels	[45]
Antispasmodic	Aqueous and-methanol extract	Isolated rabbit jejunum preparations	0.3–3 mg/ml	Antispasmodic activity mediated through K + channels activation along with weak Ca2+ antagonist effect	[46]
Antispasmodic	Hydro-alcoholic extract	Isolated tissue from rabbit jejunum	$3 \times 10^{-3} - 1.3 \times 10^{-2}$ mg/ml	Spasmolytic effect mediated through NO-activated cholinergic and histaminergic receptors	[47]
Cytoprotection	Bisabololoxide A	Thymus glands from rats	1–10 µM	Inhibition of calcium cell death	[48]
Follicle	development and function	Hydroalcoholic extract	Isolated preantral follicles from immature mouse ovaries	25 µg/ml, 50 µg/ml	↓ survival rate ↓ mean
diameter follicles ↑ progesterone and dehydroepiandrosterone hormones levels ↓ ROS production	[49]				
Immuno modulatory	Hydroalcoholic extract	J774 cell line (macrophages)	-	↓ MAPK pathway activation	[50]
Proliferative	Aqueous and ethanol extracts	Immortalized Human Keratinocytes cell line	1–50 µg/mL	↑ keratinocytes growth	[51]
Vascular protection	Chamazulene	Bovine aortic endothelial cells-1	10–250 µg/ml, 3 h	↓ ROS production	[52]
Wound healing and antiwrinkle agent	Camphor	Primary dermal fibroblast	32.5, 65, 130, and 260-µM camphor	↑ Proliferation via the PI3K/AKT and ERK signaling pathways ↓ Elastase activity ↑ collagen ↑ collagen IA, collagen IIIA, collagen IVA, and elastin expression	[53]

Table 2. *In vivo* pharmacological studies for *Matricaria chamomilla.*

Administration methods	Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Gavage gastric	Hepatoprotection	Aqueous extract	Male Wistar rats Paraquat model	Group 1: control group (saline solution) Group 2: Paraquat-treated group (5 mg/ kg/day) Group 3: Extract (50 mg/kg/day) Group 4: Paraquat (5 mg/kg) + Extract (50 mg/ kg)	↑ Total antioxidant capacity ↑ Total thiol molecules	[54]

(*Continued*)

Table 2. (Continued).

Administration methods	Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Intraperitoneal	Antidepressant	Ethanol extract	Albino Balb/C female mice Adult non-reserpinized and reserpinized mice	Group 1: normal saline Group 2: ethanol extract (25 mg/kg) Group 3: ethanol extract (50 mg/kg) Group 4: imipramine (15 mg/kg) Group 5: reserpine (5 mg/kg) Group 6: imipramine (intraperitoneal injection)	↓ Immobility duration (ethanol extract at a dose of 50 mg/kg)	[55]
	Neuroprotection	Ethanol extract	Adult Wistar rats scopolamine	Group 1: Control group (distilled water) Group 2: scopolamine (1 mg/kg) Groups 3: chamomile extract (200 mg/kg) + scopolamine simultaneously Group 4: chamomile extract (500 mg/kg) + scopolamine Groups 5: chamomile extract (200 mg/kg) Group 6: chamomile extract (500 mg/kg) 20 days	↑ Time spent on rotarod Motor coordination protection	[56]
	Neuroprotection	Ethanol extract	Male Wistar rats Formaldehyde-induced hippocampal damage	Group 1: Control (10 mg/kg normal saline) Group 2: extract (200 and 500 mg/kg) Group 3: formaldehyde (10 mg/kg) Group 4: extract (200 mg/kg) + formaldehyde (10 mg/kg) Group 5: extract (500 mg/kg) + formaldehyde (10 mg/kg)	↓ Cell death ↓ Malondialdehyde levesl ↓ Free radicals ↑ Total antioxidant capacity	[57]
	Neuroprotection	Ethanol extract	Male Wistar rats Alzheimer's disease model	Group 1: vehicle Group 2: scopolamine (1 mg/kg, intraperitoneally, 15 days) Group 3: scopolamine (1 mg/kg) and ethanol extract 200 mg/kg, 15 days) Group 4: scopolamine (1 mg/kg) and ethanol extract (500 mg/kg), 15 days Group 5: ethanol extract (200 mg/kg), 15 days Group 6: ethanol extract (200 mg/kg), 15 days	↑ Memory activity Antioxidant activity	[58]

(Continued)

Table 2. (Continued).

Administration methods	Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Administration methods	**Activity**	**Extract/essential oil/isolated compound**	**Experimental model**	**Treatments**	**Major findings**	**References**
Intraperitoneal	Neuroprotection	Hydroalcohol extract	Male Wistar rats Scopolamine-induced model of amnesia	Extract (25 and 75 mg/kg) intraperitoneally, once daily for 7 days) + scopolamine (0.7 mg/kg, 30 min)	Acetylcholinesterase activity restauration Oxidant-antioxidant balance ↑ Spontaneous alternation in the Y-maze test ↑ BDNF expression and IL1β expression	[59]
	Ovary protection	Hydroalcoholic extract	Female Wistar rats Torsion/Detorsion ovarian model	Group 1: sham operated Group 2: ovarian torsion/detorsion Group 3: rats with ovarian torsion/detorsion extracts (200 mg/kg, 30 minutes before detorsion) Group 4: healthy rats (extracts 200 mg/kg)	↑ SOD, GPx and estrogen ↓ Malondialdehyde levels	[60]
	Testis tissue protection	Hydroalcoholic extract	Male Wistar rats Torsion/Detorsion testis model	Group 1: sham operated Group 2: testicular torsion/detorsion Group 3: rats with testicular torsion/detorsion extracts (300 mg/kg, 30 minutes before detorsion) Group 4: healthy rats (extracts 300 mg/kg)	↑ SOD, GPx and testosterone hormone ↓ Malondialdehyde levels	[60]

(*Continued*)

Table 2. (Continued).

Administration methods	Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Oral	Anesthetic	Essential oil	Electric Blue Hap (*Sciaenochromis fryeri*) Yellow Princess (*Labidochromis caeruleus*)	Concentrations of anesthetic from 0.1 to 1.0 ml/L Oral	Optimal concentration for deep anesthesia: 0.6 ml/L. Minimal sedative concentration: 0.3 ml/L.	[61]
	Antihyperalgesic Antiedematous	essential oil	Male Wistar rats Inflammation model: carrageenan, dextran and histamine	Matricaria oil (25, 50 and 100 mg/kg) Carrageenan (0.1 ml/paw, 1% w/v), dextran (0.1 ml/paw, 1% w/v) or histamine (0.05 ml/paw, 1% w/v)	↓ Hyperalgesia and edema	[62]
	Antihypertensive	Total alcohol extract Oil extracted Water lifted after oil extraction	Swiss albino mice of both sex and male Wister rats	100 and 200 mg/kg Positive control: Captopril (20 mg/kg)	↓ Systolic and diastolic blood pressure ↓ Heart rate ↑ Glutathione levels ↑ SOD activity	[63]
	Anti-inflammatory	Ethanol extract	Male Wistar rats aged 7–9 weeks Carrageenan-induced paw inflammation and gastric injury	Diclofenac (483.7 mg/kg) + ethanol extract Indomethacin (212.6 mg/kg) + ethanol extract	Synergistic anti-inflammatory effect	[64]

Administration methods **Activity** **Extract/essential oil/isolated compound** **Experimental model** **Treatments** **Major findings** **References**

(*Continued*)

Table 2. (Continued).

Administration methods	Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Oral	Cardiovascular protection	Hydroalcoholic extract	Male Wistar rats	Group 1: normal dietary Group 2: control; high cholesterol diet (2%) Group 3: high cholesterol diet plus 55 mg/kg of chamomile hydroalcoholic extract Group 4: high cholesterol diet plus 110 mg/kg of chamomile hydroalcoholic extract Group 5: high cholesterol diet plus 10 mg/kg of lovastatin	↓ Serum cholesterol level, TG and LDL-c levels	[65]
	Cholesterol protection	Hydroalcoholic extract	Wistar rats Cholesterol model	Group 1: control group (common diet) Group 2: sham group (high cholesterol diet 2%) Group 3: high cholesterol diet (2%) + hydroalcoholic extract (0.55 mg/ml) Group 4: high cholesterol diet (2%) + hydroalcoholic extract (1.1 mg/ml) Group 5: high cholesterol diet (2%) and lovastatin (10 mg/kg)	Antioxidant activity (0.55 mg/ml dose)	[66]
	Hematoprotection	Chamomile decoction extract	Neutrophils from venous blood of healthy adult patients Healthy adult male Wistar rats Model stimulated neutrophils and erythrocytes	Group 1: Control group Group 2: Chamomile decoction extract (25, 50, and 100 mg/kg) Group 3: Ascorbic acid group (250 mg/kg)	↓ Neutrophil ROS production ↓ Ethanol induced hematological parameters changes ↓ Erythrocytes oxidative stress	[67]
	Ulcer protection	Aqueous residue, chloroform, ethyl acetate, petroleum ether	Indomethacin-induced ulcer models in rats	Group 1: extracts (500 mg/kg) 1 h prior to indomethacin (25 mg/kg oral) Group 2: indomethacin (25 mg/kg oral)	↓ Ulcer: ethyl acetate and chloroform fractions > petroleum ether and aqueous ethanol residue	[68]

(*Continued*)

Table 2. (Continued).

Administration methods	Activity	Extract/essential oil/isolated compound	Experimental model	Treatments	Major findings	References
Topical	Atopic dermatitis	Aqueous extract (7%)	Male seven-week old BALB/c mice Dinitrochlorobenzene-induced atopic dermatitis	Group 1: Control Group 2: Control negative, liquid petrolatum Group 3: liquid petrolatum with aqueous extract	Atopic dermatitis lesions improvement	[69]
	Oral ulcer protection	Chamomile extract	Wistar male rats Diabetes mellitus alloxan-induced	Group 1: negative control group without diabetes Group 2: positive control group with diabetes mellitus Group 3: extract groups, normoglycemic and diabetic rats Group 4: triamcinolone group	↓ Apoptosis ↓ TNF-D expression ↓ Oral ulcer	[70]
Administration methods	**Activity**	**Extract/essential oil/isolated compound**	**Experimental model**	**Treatments**	**Major findings**	**References**
Topical	Wound healing Antiwrinkle agent	Camphor	Hairless mice (SKH-1) UV-exposed mouse model	Group 1: SKH-1 mice without UV exposure Group 2: UV exposure and camphor in ethanol Group 3: UV exposure and camphor 26 mM Group 3: UV exposure and camphor 52 mM	↑ elastin, collagen IA and collagen IIIA	[53]
Transfer	Anti-angiogenic	Methanol extract	Chick chorioallantoic membrane assay Fertilized chicken eggs	Methanol extract 500 mg/ml for 72 hours	↓ Blood vessels growth	[71]

Table 3. Clinical trials for *Matricaria chamomilla*.

Activity	Reference country	Study design	Sample size	Population	Type of plant	Intervention	Duration of treatment	Results
	ORAL ADMINISTRATION							
Anxiety	Mao et al.[72] United States	Open-label study Double-blinded, placebo, randomized controlled trial	179	Adult patients diagnosed generalized anxiety disorder	Chamomile extract	*Open label study* Intervention group: chamomile extract 1500 mg (500 mg capsule 3 times daily) extract 4:1 powder standardized to a content of 1.2% apigenin *Placebo study* Placebo group; Pharmaceutical-grade lactose monohydrate	12 weeks (open-label study) 26 weeks (placebo study)	↓ generalized anxiety disorder symptoms No rate of relapse reduction
	Keefe et al.[73] United States	Open-label, placebo, two-phase randomized controlled trial	179	Adult patients diagnosed moderate and severe generalized anxiety disorder	Chamomile extract	Intervention group: chamomile extract 1500 mg (500 mg dry extract capsule 3 times daily) Placebo group: pharmaceutical-grade chamomile extract	8 weeks	↓ generalized anxiety disorder symptoms

(*Continued*)

Table 3. (Continued).

Activity	Reference country	Study design	Sample size	Population	Type of plant	Intervention	Duration of treatment	Results
Diabetes Mellitus	Zemestani et al.[74] Iran	Single-blind randomized controlled clinical trial	64	Patients with type 2 diabetes	Chamomile tea bags	Intervention group: chamomile tea (3 g/150 ml hot water/ daily) Placebo group: warm water	8 weeks	↓ glycosylated hemoglobin ↓ serum insulin levels ↓ serum malondialdehyde ↑ total antioxidant capacity ↑ antioxidant enzymes activity
	Rafraf et al.[75] Iran	Single-blind randomized controlled clinical trial	64	Adults with type 2 diabetes	Chamomile herbal tea	Intervention group: chamomile tea (3 g/150 mL)/3 times day Control group: water	8 weeks	↓ HbA1C ↓ serum insulin levels ↓ homeostatic model assessment for insulin resistance ↓ total cholesterol ↓ triglyceride ↓ low-density lipoprotein cholesterol
Activity **Results**	**Reference country**	**Study design**	**Sample size**	**Population**	**Type of plant**	**Intervention**	**Duration of**	**treatment**
ORAL ADMINISTRATION (continued)								
Hypertension	Awaad et al.[76] Kingdom of Saudi Arabia	Open-Label,		uncontrolled clinical trial	400	Normotensive [systolic blood pressure (SBP) of 120–124 mmHg and/or diastolic blood pressure (DBP) of 70–86 mmHg] or mildly hypertensive (SBP of 139–159 mmHg and/or DBP of 86–99 mmHg)	Chamomile tea	Intervention: single cup of chamomile beverage (250 ml) (made with one, two, or three teaspoonful of plant powder in 250 ml of water)

(*Continued*)

Table 3. (Continued).

Activity	Reference country	Study design	Sample size	Population	Type of plant	Intervention	Duration of treatment	Results
2 h	↓ SBP ↓ DBP ↓ HR (heart rate)							
Pain	Zafar et al.[77] Pakistan	Double blind randomized controlled trial	99	Pregnant women	Chamomile extract	Intervention group: 1 ml of saline injection and oral chamomile (3 drops) (homeophatic medicine in 30, 200 and 1 M dilutions). Placebo group: oral placebo and 1 ml of saline injection Control group: single intramuscular injection of 1 ml of Pentazocine (30 mg) and oral placebo	2–3 hours	No significant analgesia
Polycystic ovary syndrome	Heidary et al.[78] Iran	Randomized clinical trial, single-blind design	80	Women of childbearing age with polycystic ovary syndrome	Chamomile extract	Intervention group: chamomile capsule 370 mg / 3 times a day Control group: starch capsule	3 months	↓ level of testosterone
Premenstrual syndrome	Saghafi et al.[79] Iran	Double-blind randomized controlled clinical trial	60	Premenopausal women with mastalgia	Chamomile extract	Intervention group: chamomile extract 5 drops/3 times a day Placebo group	2 months	↓ pain
	Sharifi et al.[63] Iran	Prospective, randomized, double blind trial	90	Young women with regular menstrual cycle and diagnosed premenstrual syndrome	Chamomile extract	Intervention group: chamomile capsule 100 mg / 3 times a day Control group: mefenamic acid capsule 250 mg / 3 times a day	6 months	↓ intensity of premenstrual syndrome associated symptomatic psychological pains
Activity **Results**	**Reference country**	**Study design**	**Sample size**	**Population**	**Type of plant**	**Intervention**	**Duration of**	**treatment**
ORAL ADMINISTRATION (continued)								

(*Continued*)

Table 3. (Continued).

Activity	Reference country	Study design	Sample size	Population	Type of plant	Intervention	Duration of treatment	Results
Sleep	Abdullahzadeh et al.[80] Iran	Randomized, controlled quasi-experimental clinical trial research	77	Elderly hospitalized in nursing homes	Extract	Intervention group: 400 mg oral capsules/twice daily Control group: No intervention	4 weeks	↑ Sleep quality
	Adib-Hajbaghery and Mousavi[81] Iran	Single-blind randomized controlled trial	195	Elderly people	Chamomille extract	Intervention group: 200 mg chamomile extract capsules/twice daily Control group: 200 mg wheat flour capsules	28 days	↑ Sleep quality
TOPICAL ADMINISTRATION								
Carpal tunnel syndrome	Hashempur et al.[82] Iran	A randomized double-blind placebo-controlled clinical trial with parallel design	86	Adult patients with mild and moderate carpal tunnel syndrome	Chamomile oil	Intervention group: topical chamomile oil Control group: Placebo 5 drops/twice a day	4 weeks	Dynamometry, functionality, and symptom severity scores were improved
	Hashempur et al.[83] Iran	A pilot randomized placebo-controlled trial with parallel design	26	Adult patients with severe carpal tunnel syndrome	Chamomile oil	Intervention group: topical chamomile oil Control group: Placebo 5 drops/twice a day	4 weeks	Symptomatic and functional status were improved
Enuresis	Sharifi et al.[63] Iran	Double blind randomized, placebo controlled trial, parallel design	80	Children diagnosed as monosymptomatic nocturnal or daytime eneuresis	Chamomile oil	Intervention group: chamomile oil topically (6 drops/daily) Placebo group: 6 almond oil (6 drops/daily)	6 weeks	↓ frequency nocturia
Intestinal activity	Khadem et al.[84] Iran	A randomized controlled trial	142	Pregnant women aged 18–35 years undergoing cesarean section, gestational age 38–42 weeks, starvation at least 8 h before surgery	Chamomile oil	Intervention group: chamomile oil/20 drops/ every 1 h in the first 4 h and then every 8 h until fecal defecation Placebo group: placebo oil Control group: No intervention	-	↓ postoperative ileus duration (↓ time to first flatus, bowel sound, and defecation)
Activity	**Reference country**	**Study design**	**Sample size**	**Population**	**Type of plant**	**Intervention**	**Duration of**	**treatment**
Results								
TOPICAL ADMINISTRATION (continued)								

(*Continued*)

Table 3. (Continued).

Activity	Reference country	Study design	Sample size	Population	Type of plant	Intervention	Duration of treatment	Results
Migraine	Zargaran et al[85] Iran	A crossover double-blind controlled, randomized clinical trial	100	Patients with migraine (first attack under 50 years)	Oleogel	preparation of reformulated traditional chamomile oil	Intervention group: 2 ml topical	chamomile oil/ twice a day Placebo group
14 days	↓ pain ↓ nausea ↓ vomiting ↓ photophobia ↓	phonophobia						
Osteoarthritis	Shoara et al.[86] Iran	Three arm, blinded, randomized, placebo controlled clinical trial, parallel design	84	Patients with knee osteoarthritis	Chamomile oil	Intervention group: chamomile oil topically (3 times/daily) Placebo group: Paraffin Control group: Diclofenac gel	3 weeks	↓ analgesic demand Benficial effects on pain, physical function and stiffness
TOPICAL-BUCAL ADMINISTRATION								
Oral diseases	Braga et al.[87] Brazil	Randomized, controlled, phase II clinical trial with parallel groups	40	Adult patients with oral mucositis undergoing allogenic hematopoietic stem cell transplantation	Extract	Intervention group: 0.5%, 1%, or 2% (%p/p) extract/ daily Control group: No intervention	-	↓ incidence oral mucositis (1% extract) ↓ intensity oral mucositis (1% extract) ↓ duration oral mucositis (1% extract)
	Goes et al.[88] Brazil	Randomized, double blind, placebo-controlled pilot study	30	Patients with gingivitis concurrent with fixed orthodontic appliances and associated with visible plaque	Aqueous extract 1%	Intervention group: 1% extract Control group: 0.12% chlorhexidine Placebo group All mouthwash for 1 min after brushing in the morning and evening	15 days	↓ biofilm accumulation and gingival bleeding (1% *Matricaria chamomilla* extract)
Activity	**Reference country**	**Study design**	**Sample size**	**Population**	**Type of plant**	**Intervention**	**Duration of**	**treatment**
Results								
TOPICAL-BUCAL ADMINISTRATION (continued)								

(*Continued*)

Table 3. (Continued).

Activity	Reference country	Study design	Sample size	Population	Type of plant	Intervention	Duration of treatment	Results
Oral diseases	Seyyedi et al.[89] Iran	A randomized, triple-blind, placebo-controlled trial	36	Patients with recurrent aphthous stomatitis	Chamomilla tincture	Intervention group: Chamomilla tincture/10 drops/3 times day Control group: Placebo	6 days	Controlling the pain and burning sensation
	Milani et al.[90] Brazil	Case reports	2	Burning mouth syndrome Female, Caucasian and 59 and 68 years	Chamomile tea	Tea kept in mouth three minutes four times a day	One year	↓ burning sensation Improved well-being
	Tadbir et al.[91] Iran	Randomized, double-blind, placebo-controlled clinical trial	45	Burning mouth syndrome Aged 18–60 Recurrent aphthous stomatitis	Chamomile in orabase	Placebo group Control group: triamcinolone in Orabase Intervention group: chamomile in Orabase	6 days	↓ Ulcer size, pain Patient satisfaction

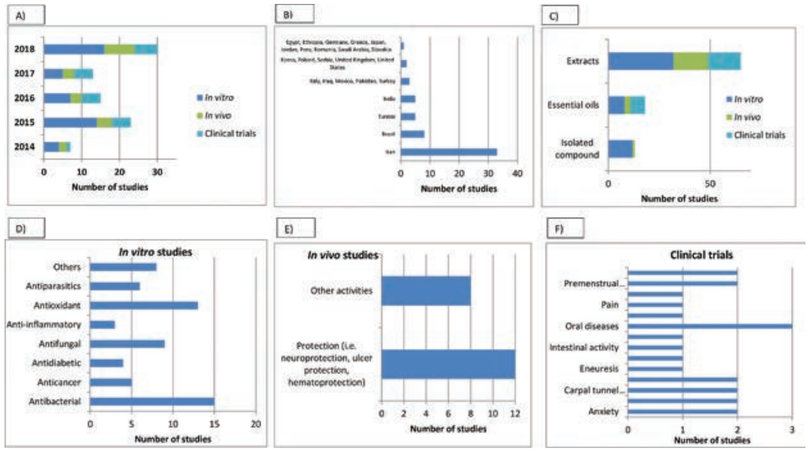

Figure 2. Main characteristics of papers published on pharmacological activity of *Matricaria chamomilla*. A) Year of publication. B) Research group country. C) Part of the plant used for research. D) Activities evaluated *in vitro* studies. E) Activities evaluated *in vivo* studies. F) Activities evaluated clinical trials.

rigid membrane rich in lipopolysaccharide which limits the access of antimicrobial molecules.[94] Hence, the highest antibacterial activity of *M. chamomilla* essential oils (1.0 μg/mL) has been found against *Bacillus cereus* (36 mm of inhibition zone for chamomile *versus* 22 mm of inhibition for streptomycin), *Bacillus subtilis* (32 mm of inhibition zone for chamomile *versus* 22 mm of inhibition for streptomycin) and *Staphylococcus aureus* (30 mm of inhibition zone for chamomile *versus* 22 mm of inhibition for streptomycin). Moreover, Gram negative bacteria have also shown to be susceptible to essential oils action, mainly *Shigella shiga* (inhibition zone of 25 mm for chamomile *versus* 17 mm of inhibition for streptomycin). The antibacterial activity of essential oils of *M. chamomilla* was attributed to alfa-bisabolol oxide (38% of concentration).[9] However, these results differ from those obtained by Mekonnen et al.[16] and Sakkas et al.[10] for the same bacterial species such as *Staphylococcus aureus and Shigella* species. These authors did not find antibacterial action for *M. chamomilla* essential oils. The bioactive compounds present in essential oils can vary qualitatively and quantitatively depends on seasonal sampling period, cultivation conditions, environmental factors and geographical location, among others.[95]

Regarding extracts, there has been investigated the antibacterial properties of aqueous, ethanol, ethyl acetate and methanol extracts from *M. chamomilla*. In general, the aqueous extracts have demonstrated to be a less potent antibacterial than the other assayed extracts.[12–14] However, methanol extracts were most active especially against *Proteus mirabilis* (maximum zone formation 6.01, no concentration was specified), *Escherichia coli* (1.2 cm inhibitory zone for 12 μg/ml of chamomile), *Staphylococcus aureus* (1.36 cm inhibitory zone for 12 μg/ml of chamomile), *Streptococcus mutans* (19.8 mm of inhibition zone for 100 μg/ml of chamomile) and *Propionibacterium acnes* (MIC value 6 mm for 10 mg/ml of chamomile).[17,20,22,23] Moreover, the ethyl acetate extracts from chamomile flowers were the most active against *Helicobacter pylori* (MIC = 31.3 μg/ml), with an MBC/MIC ratio of 4 (bactericidal activity).[11] Furthermore, there has been demonstrated that bacteria inhibitory activity is higher when chamomile extracts are incorporated to silver nanoparticles due to synergistic effects, high localized concentration of *M. chamomilla* extracts and an increase of the active surface area.[15,18]

As with antibiotics, antifungal resistance is increasing, specially with *Candida* and *Aspergillus* infections.[96] The antifungal activity of *M. chamomilla* has been investigated in essential oils and extracts (aqueous and methanol). Particularly, *M. chamomilla* essential oils (from 1 mg/mL to 250 mg/mL) have shown no antifungal activity against the fungal species *Aspergillus* spp. and *Trichophyton* spp.[16] and low activity against *Candida albicans* (MIC value of 1700 μg/ml for fluconazole (FLU)-resistant *C. albicans* strains and MIC value of 1550 μg/ml for FLU-susceptible *C. albicans* strains)[31] Dimethyl sulfoxide (DMSO) was employed as negative control. As far as the extracts, the aqueous extract is less active as an antifungal agent than the methanol extracts. The fungi *Aspergillus terreus* is especially susceptible to methanol extracts (maximum zone formation 5.89).[12,17] Furthermore, the incorporation of chamomile extracts to silver nanoparticles increases its antifungal action.[15,18]

Antiparasitics activities

Extracts, essential oils and isolated compounds from *M. chamomilla* have been also investigated for the treatment of parasitic diseases caused by amoeba (*Acanthamoeba castellan*i), protozoa (*Leishmania amazonensis, Leishmania infantum*) and nematodes (*Haemonchus contortus)*. The methanol extract of *M. chamomilla* has shown a potent anti-acanthamoeba action (IC_{50} value of 66.2 μg/ml) which is attributed to a coumarin mixture.[41] Moreover, the compound α-bisabolol, isolated from essential oil, presents amoebicidal activity (IC_{50} value of 20.8) by inducing apoptosis, decreasing ATP levels and increasing the plasmatic membrane permeability.[45] Regarding leishmanicidal activity, *M. Chamomilla* essential oils showed good activity on the promastigotes forms of *Leishmania amazonensis* (IC_{50} value of 60.1 μg/mL for 24 h and IC_{50} value of 10.8 μg/mL for 48 h) and *Leishmania infantum* (IC_{50} value of 10.4 μg/mL) as well as its isolated compound α-bisabolol (*L. amazonensis* with IC_{50} value of 16 μg/mL and *L. infantum* with IC_{50} value of 9.5 μg/mL). This sesquiterpene alcohol has also been found to be active in amastigotes forms (IC_{50} value of 5.9 for *L. amazonensis* and IC_{50} value of 4.8 μg/mL for *L. infantum*). Apoptotic effects are also implicated in the anti-Leishmania activity of *M. chamomilla.* [,43,44] Finally, extracts from *M. chamomilla* have been investigated for its ovicidal and larvicidal activity against *Haemonchus contortus* which is a gastrointestinal parasite of small ruminants. Methanol extract (1024 μg/ml) was the most active as an antihelmintic (37.5% for egg hatch test and 84% for larval development test).[40,42]

Antioxidant activity

Overproduction of reactive oxygen species (ROS) can cause damage to cellular structures (lipids, proteins and DNA) leading to substantial structural and functional damage that contributes to the pathogenesis of many chronic diseases (i.e. neurodegenerative disorders, diabetes, cancer and cardiovascular disease). Antioxidants are molecules that inhibit free radicals action through enzyme regulation, scavenging and metal chelation, and consequently avoid or delay the oxidative cellular damage.[97] The antioxidant activity of *M. chamomilla* has been investigated in extracts, essential oils and isolated compounds, with the DPPH assay being the most widely used antioxidant test. In addition to DPPH, other *in vitro* methods such as ABTS, ORAC and FRAP have been also used to evaluate its antioxidant activity. The differences among these methods lie in the mechanism of action. DPPH, FRAP and ABTS are based on the ability of antioxidants to transfer an electron to free radicals (SET method), while ORAC assay measures the capacity of antioxidants to donate a hydrogen atom (HAT method).[98] The results obtained for the different *in vitro* antioxidant models suggest that methanol extract has higher antioxidant properties than other extracts and essential oils. For example, for DPPH assay, methanol extract at 20 μg/ml (82.8% inhibition) and methanol extract of chamomile collected at low hill (238 μmol TE/100 g dw) whereas moderate activity for essential oils.[22,37] Moreover, antioxidant activity is also influenced by the extraction method. Cvetanovic et al.[19] evaluated the antioxidant activity of extracts of chamomile ligulate flowers obtained by different methods including Soxhlet, microwave-assisted, ultrasound-assisted and subcritical water. The subcritical water extracts showed the highest antioxidant activity as evidenced in DPPH (IC_{50} value of 0.021 mg/mL) and Reducing Power Test assays (EC_{50} value of 0.57 mg/mL). Regarding isolated compounds, the chamazulene isolated from essential oils of *M. chamomilla* has shown better antioxidant activity than the reference compounds butylated hydroxytoluene (BHT) and α-tocopherol in the ABTS assay (IC50 = 3.7 μg/mL for chamazulene, IC50 = 6.2 μg/mL for BHT and IC50 = 11.5 μg/mL for α-tocopherol).[34] Moreover, the compounds luteolin-7-O-β-D-glucuronide and luteolin, isolated from chamomile methanol extracts, have shown potent effect on DPPH free radical scavenging activity with IC_{50} values of 7.24 and 8.92 μM, respectively.[39]

Anticancer activity

Cancer is the second leading cause of death in the world (9.6 million of deaths in 2018).[99] The cancer pharmacological treatments have successfully evolved in recent years towards better tolerated, more effective and safe treatments.[100] However, current cancer research is focused on finding new therapeutic approaches, among other sources, in medicinal plants.[101] The anticancer properties of *M. chamomilla* were investigated in extracts from different cancer cell lines.[19,24,26,27] Hence, the hydroalcoholic extracts of aerial parts and root of *M. chamomilla* showed time and concentration anticancer activity on human breast cancer cells. The IC_{50} value after 72 h of treatment for the highest assayed concentration (1300 μg/mL for aerial parts extracts and 2000 μg/ml for root extracts) was 785 μg/mL against MDA-MB-468 and 921 μg/mL against MCF-7 for extracts from aerial parts and 1560 μg/ml against MCF-7 for extracts from root.[24,27] Moreover, as in the case of antioxidant activity, it has been observed that the method of obtaining extracts influences its anticancer efficacy; the subcritical water extracts had the most potent cytotoxic activity against the cell lines human rhabdomyosarcoma cells, cell line derived from human cervix carcinoma Hep2c and cell line derived from murine fibroblast (IC_{50} values of 30.54, 20.54 and 19.65 μg/ml, respectively).[19] Furthermore, the combination of aqueous extracts of *M. chamomilla* with silver increase the anticancer efficacy against lung cancer; the half maximal inhibitory concentration values were at 62.82 μg/mL for 24 h and 42.44 μg/mL for 48 h against human lung adenocarcinoma A549 cell line.[28] The anticancer action of *M. chamomilla* seems to be related to apoptotic and necrotic events as well as to a reduction of cell invasion and migration of cancer cells.[24,28]

Anti-inflammatory activity

Inflammation (acute and chronic inflammation) is an immune response to infection, injury or trauma that is accompanied by pain, redness, heat, swelling and loss of function. Chronic inflammation is related to many diseases such as cardiovascular events, cancer, arthritis and atherosclerosis.[102] The apigenin-7-glucoside, the major compound of *M. chamomilla*, has shown anti-inflammatory activity in bone marrow-derived macrophages (BMDMs) from C57BL/6 mice through TNF-α inhibition (over 30% of inhibition for 30 μg/mL and over 95% of inhibition for 300 μg/mL compared to cell treated with LPS).[32] Moreover, the proazulene matricine demonstrated to decrease TNF-α (52.7% of control at 75 μM) and LPS-induced (20.4% of control at 75 μM) expression of the adhesion molecule ICAM-1 as well as to reduce NF-κB signaling effect (32.3% of control at 100 μM) on human microvascular endothelial cells (HMEC-1).[33]

Antidiabetic activity

Diabetes affects around 422 million people worldwide, especially in low- and middle-income countries. It is estimated that 1.6 million people die from diabetes and 2.2 million people die from high blood glucose.[99] Villa-Rodriguez[29,30] demonstrated that chamomile extract and the major polyphenols apigenin, apigenin-7-O-glucoside, cis and trans-ferulic acid hexoside and (Z) and (E) −2-hydroxy-4-methoxycinnamic acid glucosides were effective on postprandial hyperglycemia due to the concentration-dependent salivary human α-amylase and maltase inhibition. Particularly, apigenin showed the highest effect on salivary human α-amylase (IC_{50} of 28 μM compared to control chamomile extracts with 55% of α-amylase inhibition) and apigenin-7-O-glucoside contributed to the highest maltase inhibition (33% compared to control acarbose with 50% of maltase inhibition at 0.27 μM). Moreover, apigenin-7-O-glucoside and apigenin were able to inhibit glucose and sucrose transports and regulating sugar absorption[29,30] Furthermore, methanol extract and its constituents, mainly luteolin-7-O-β-D-glucuronide and 3,5-O-di-caffeoylquinic acid suppressed rat lens aldose reductase activity with IC_{50} values of 4.61, 0.85 and 0.72 μM, respectively, as well as inhibited the accumulation of sorbitol in rat lens under high-glucose conditions. Moreover, the isolated compounds luteolin-7-O-β-D-glucuronide and luteolin demonstrated to inhibit advanced glycation end products (AGEs) formation (IC_{50} values 3.39 and 6.01 μM, respectively).[39] Finally, *M. chamomilla* ethanol extracts showed anti-glycation properties as evidenced by lipase activity inhibition with IC_{50} value of 264.2 μg/mL.[38]

In vivo studies

Rodents are the most widely used human disease experimental model for evaluating chamomile activity. Of the 20 *in vivo* studies included in this review, 18 used rats or mice as the animal model (Wistar rats, Swiss mice, BALB/c mice and SKH-1 mice). Mice and rat share many biochemical and biological similarities with humans which make them valuable experimental models. Moreover, they have short generational time and they are very prolific. Furthermore, especially mice, they have been extensively studied in genetic and there have been defined a large number of genetic lines.[103,104] On the other hand, the freshwater aquarium fish species (*Sciaenochromis fryeri* and *Labidochromis caeruleus*) have been also used in one study focused on evaluating the activity of chamomile as an anesthetic for aquaculture.[61] Moreover, the anti-angiogenesis activity of *Matricaria chamomilla* was studied on the chick chorioallantoic membrane of fertilized chicken eggs. This experimental model is commonly used in vascular-related and angiogenesis experiments for its ease of manipulation and rapid growth.[71,105]

Protection

The intraperitoneal mode of administration has been chosen to evaluate the effect of chamomile in neuroprotection and protection against torsion and detorsion in the testis and ovaries. The neuroprotective effect of extracts of *M. chamomilla* has been investigated in formaldehyde and scopolamine-induced rat models. Hence, the ethanolic extracts of *M. chamomilla* (200 mg/kg and 500 mg/kg, intraperitoneal) have shown memory protection based on antioxidant properties in the hippocampus of formaldehyde-treated rats (intraperitoneal injection of 10 mg/kg) as evidenced on malondialdehyde levels reduction (over 30% and 50% of reduction for 200 mg/kg and 500 mg/kg, respectively, compared to formaldehyde) and total antioxidant capacity increase (over 150% and 200% of increase for 200 mg/kg and 500 mg/kg, respectively, compared to formaldehyde).[57] Moreover, studies have reported that *M. chamomilla* hydroalcoholic extracts (doses of 25 mg/kg, 75 mg/kg, 200 mg/kg and 500 mg/kg, intraperitoneal) increased memory activity, restored acetylcholinesterase activity and oxidant-antioxidant imbalance, augmented BDNF expression and improved motor coordination in scopolamine-induced amnesic rats which could be therapeutically beneficial for Alzheimer's disease. Particularly, chamomile extracts at 25 mg/kg and 75 mg/kg inhibited cholinesterase activity around 45% compared to scopolamine treatment in the rat hippocampal. Moreover, chamomile extracts (25 mg/kg and 75 mg/kg) increased enzymatic antioxidant activity, especially that of the catalase enzyme (over 90% of increase at both doses). Furthermore, chamomile extract at a dose of 200 mg/kg increased over 75% latency to fall off on the carrousel of rotarod apparatus compared to scopolamine group.[56,58,59]

Soltani et al.[60,106] studied the protective effect of hydroalcoholic extracts of *M. chamomilla* on histological damage and oxidative stress situation in testis and ovarian of rats subjected to torsion and detorsion. These studies have shown that hydroalcoholic extracts of this medicinal plant (200 mg/kg and 300 mg/kg, intraperitoneal) increased antioxidant enzymatic levels (superoxide dismutase, SOD; glutathione peroxidase, GPx) and decreased malondialdehyde (MDA) levels compared to those rats suffered from an ovarian and testicular torsion followed by detorsion.

The oral administration has been the chosen route for cardiovascular function and hematoprotective works. Hence, the cardiovascular protection has been also investigated for hydroalcoholic extracts on rats fed a high cholesterol diet. Nargesi et al.[65] reported that chamomile extracts (110 mg/kg, oral) decreased serum cholesterol (88.98 mg/dl in treated rats *versus* 141.78 mg/dl in control rats), triglycerides (71.41 mg/dl in treated rats *versus* 107.85 mg/dl in control rats) and low-density lipoprotein cholesterol levels (46.90 mg/dl in treated rats *versus* 63.31 mg/dl in control rats) compared to those rats with high cholesterol diet (2%). Moreover, Amraei et al.[66] demonstrated that chamomile extracts (0.55 mg/ml, orally) have good antioxidant properties since no modifications in antioxidant system (SOD and MDA) are observed in rats after high cholesterol diet 2%.

Moreover, the potential tissue protection in blood has been investigated for chamomile extracts. Jabri et al.[67] investigated the hematoprotective effect of chamomile decoction extract (25, 50, and 100 mg/kg, oral) in a rat model of stimulated neutrophils ROS production and ethanol induced. The chamomile decoction extract reversed all oxidative changes occurred in erythrocyte and plasma including alteration in antioxidant enzyme activities, levels of calcium and free iron and content in hydrogen peroxide.[67]

The chamomile extracts have also shown to protect against ulcers. Hence, the ethyl acetate and chloroform fractions of *M. chamomilla* flower head extracts (300 mg/kg, oral) followed by petroleum ether and aqueous ethanol residue had an effective antiulcer effect on indomethacin-induced rat model as evidenced in the decrease in the number of ulcers (mean number of ulcers were 26 for indomethacin, 4 for ethylacetate fraction and 5 for chloroform extract). This protective effect may be attributed to the major compounds apigenin 7-O-glucoside and apigenin −7-O- (6- acetyl glucoside).[68] Moreover, Oliveira et al.[70] reported traumatic oral ulcers in diabetic rats alloxan-induced (45 mg/kg, topical) which may arise with extracts of *M. chamomilla*. The protective effect is related to the decrease in apoptosis and TNF-α expression.

Finally, the paraquat is a common herbicide which can cause fatal liver injury by redox alteration. Male rats were treated only with paraquat (5 mg/kg/day) or in combination with aqueous extract of *M. chamomilla* (50 mg/kg/day, gavage gastric) for 7 days to evaluate their effect on liver tissue. Results of this study showed that chamomile extract increased total antioxidant capacity (4.45 µmol/mg for chamomile *versus* 1.53 µmol/mg for paraquat) and total thiol molecules (0.28 µmol/mg for chamomile *versus* 0.11 µmol/mg for paraquat), being effective to treat paraquat poisoning.[54]

Other activities

In addition to the protective effect on organs and tissues, other activities have been investigated in relation to extracts, essential oils and isolated compounds of *M. chamomilla.*

To evaluate the activity of essential oils, the routes of administration have been oral and topical. The oral administration has been chosen for anesthetic and antipyretic activities. Hence, the essential oils of this medicinal plant have been shown to be effective as anaesthetics at an oral dose of 0.6 ml/L for deep anesthesia and an oral dose of 0.3 ml/L for minimal sedation in the freshwater aquarium fish species Electric Blue Hap and Yellow Princess.[61] Moreover, oral prophylactic treatments with essential oils of *M. chamomilla* rich in bisabolol-oxides in rat models carrageenan-induced exhibited antihyperalgesic activity (38.4%, 52.6% and 59.1% for 25, 50 and 100 mg/kg, respectively, compared to 72.8% for the reference compound ibuprofen 100 mg/kg, p.o.) and antiedematous effects (34.5%, 54.7% and 75.5% for 25, 50 and 100 mg/kg, respectively, compared to 70.6% for the reference compound indomethacin 8 mg/kg, p.o.). These activities support the traditional uses of chamomile for inflammatory conditions.[107] On the other hand, topical administration was used to investigate the activity of essential oils on wound healing. The monoterpene camphor, one major component of chamomile essential oils, has cosmetic interest in as wound healing and anti-wrinkle agent (26 and 52 mM, topical, for 2 weeks) by increasing collagen IA, IIIA and elastin expression in mouse skin exposed to UV for 4 weeks.[53]

Regarding the activity of the extracts, this has been evaluated using different modes of administration including transfer, intraperitoneal and oral. Al-Zubaidy et al.[71] demonstrated that the methanol extract of *M. chamomilla* flowers (500 mg/ml, transfer, for 72 h) had antiangiogenic activity (zone of inhibition more than 10 mm) in fertilized chicken eggs. This effect is attributed to compounds such as α-bisabolol, coumarin and apigenin which inhibit vascular endothelial growth factor production. Moreover, the intraperitoneal route of administration was selected to investigate antidepressant activity. The ethanol extract of *M. chamomilla* at an intraperitoneal dose of 50 mg/kg has antidepressant effects as evidenced by the decreased duration of immobility in adult reserpinized mice (142 s for chamomile *versus* 208.60 s for reserpine 5 mg/kg). The antidepressant activity of chamomile was even better than that of the positive control drug imipramine (15 mg/kg) (immobility duration of 182.67 s).[55] Finally, anti-inflammatory and antihypertensive activities have been investigated, the mode of administration being the oral route. There has been demonstrated that the oral ethanol extract of *M. chamomilla* coadministered with non-steroidal anti-inflammatory drugs such as diclofenac and indomethacin synergistically potentiates its anti-inflammatory activity on carrageenan-induced paw inflammation and gastric injury in rats (ED values of 483.7 mg/kg for diclofenac and chamomile and, 212.6 mg/kg for indomethacin and chamomile).[64] Finally, the water lifted after oil extraction (100 and 200 mg/kg, oral) showed a significant antihypertensive effect in Wistar rats salt-sucrose solution induced as evidenced by systolic and diastolic values and heart rate. Moreover, the water lifted after oil extraction significantly reduced the biochemical parameters aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) as well as the serum lipid profile (total cholesterol, triglycerides, and low-density lipoprotein). These effects were similar to that of the reference compound captopril.[76]

Clinical trials

Several of the clinical trials conducted during the years included in this systematic review (2014–2018) clinically validate the pharmacological activities demonstrated in *in vitro* and *in vivo* studies. This is the case of diabetes mellitus, hypertension and pain (sign of inflammation). The oral administration was the selected route to investigate the effect of chamomile on diabetes mellitus and hypertension. Hence, in a single-blind randomized controlled clinical trial, the oral administration of a chamomile tea (3 g/150 ml), three times day, during 8 weeks to male and female patients with type 2 diabetes mellitus and ages 30 to 60 (n = 32 intervention group and n = 32 placebo) was effective on glycemic control (180.50 mg/dL baseline glucose value and 160.09 mg/dL glucose value after intervention). Moreover, this chamomile tea resulted in a decrease of oxidative stress (cause and result of diabetes) as evidenced in an increase of total antioxidant capacity (1.23 mmol/L baseline value and 1.41 mmol/L value after intervention) and antioxidant enzymes activity (SOD: 1215.04 U/mg Hb baseline value and 1559 U/mg Hb value after intervention; CAT: 61.42 U/g Hb baseline value and 76.71 U/g Hb value after intervention) and a decrease of serum malondialdehyde (4.29 nmol/mL baseline value and 2.27 nmol/mL value after intervention). Furthermore, this chamomile tea improved serum lipid profile of patients with diabetes mellitus as shown in a reduction of total cholesterol (182.21 mg/dL baseline value and 164.96 mg/dL value after intervention), triglycerides (203 mg/dL baseline value and 164.37 mg/dL value after intervention) and low-density lipoprotein cholesterol (100.61 mg/dL baseline value and 91.70 mg/dL value after intervention).[74,75]

Awaad et al.[76] evaluated the antihypertensive properties of a chamomile beverage (250 ml, oral) in 200 women and 200 male nonsmoking normotensive (Systolic blood pressure, SBP, of 120–124 mmHg and/or Diastolic blood pressure, DBP, of 70–86 mmHg) or mildly hypertensive (SBP of 139–159 mmHg and/or DBP of 86–99 mmHg). Results of this clinical study showed that after two hours after the administration of tea significantly dose-dependently reduced SBP, DBP and heart rate compared to basal values in both normotensive (110.10 mmHg for chamomile *versus* 120.10 mmHg for SBP, 65.20 mmHg for chamomile *versus* 80.10 mmHg for DBP and 70 beats/min for chamomile *versus* 75 beats/min) and mildly hypertensive patients (120.10 mmHg for chamomile *versus* 150.1 mmHg for SBP, 80.10 mmHg for chamomile *versus* 95.30 mmHg for DBP and 75 beats/min for chamomile *versus* 84 beats/min).

Inflammation (acute and chronic inflammation) is an immune response to infection, injury or trauma which is accompanied by pain, redness, heat, swelling and loss of function. There are several diseases of pathological conditions that occur with pain due to an inflammatory process. *M. chamomilla* has been widely used in traditional medicine for its anti-inflammatory properties.[1]

Therefore, oral and topical administrations were selected for anti-inflammatory studies. The premenstrual syndrome consists of emotional and physical symptoms, including pain, that occur in the week or two weeks before the menstrual period.[108] Sharifi et al.[63] and Saghafi et al.[79] demonstrated in two different placebo controlled clinical trials conducted in premenopausal women that the administration of chamomile extract as drops (5 drops, topical) or capsule (100 mg, oral), 3 times a day, during several months reduced premenstrual pain. Moreover, osteoarthritis is a degenerative joint pain. The topical administration of chamomile oil three times/day for 3 weeks in patients with knee osteoarthritis reduced the analgesia demand of acetaminophen and it also brought beneficial effects on physical function and stiffness.[86] Furthermore, in another clinical study to evaluate the efficacy of chamomile on pain, oral drops were administered to pregnant women for labor pain relief. However, based on the Visual Analogue Scale for pain intensity, chamomile drops did not offer significant analgesia compared to placebo group (p-value 0.15 for pain before treatment, p-value 0.88 for pain after 30 minutes of treatment, p-value 0.26 for pain after an hour of treatment, p-value 0.52 for pain after 2 h treatment and p-value 0.33 for pain in second stage of labor).[77]

Apart from these traditional uses, *M. chamomilla* has been investigated for other potential biological activities. This section has been differentiated between oral chamomile administration and next topical chamomile administration. Hence, the herbal remedy of chamomile in patients who suffered from polycystic ovary syndrome was evaluated in a randomized clinical trial. Those women of reproductive age who received capsules of chamomile (370 mg, oral) three times/day for 3 months reported a reduction in total testosterone levels ($p = .017$). However, no changes were observed for the ratio luteinizing hormone/follicle stimulating hormone (LH/FSH) ($p = .420$).[78] Moreover, the increase in the sleep quality has been also observed in two different controlled trials performed in elderly people who suffered from sleep disorders. According to the Pittsburgh Sleep Quality Index to measure sleep quality, the oral administration of 200 mg and 400 mg of chamomile capsules twice daily for 28 days possessed sedative properties, favouring sleep in elderly people ($p < .05$ for 200 mg chamomile intervention *versus* control; $p < .001$ for 400 mg chamomile intervention *versus* control).[80,81] In addition, chamomile extract (500 mg capsule 3 times daily, extract 4:1 powder standardized to a content of 1.2% apigenin, oral) has shown to reduce generalized anxiety disorder symptoms in adult patients (55% relapse rate on placebo *versus* 26% relapse rate on chamomile).[72,73]

The effect of chamomile oil in parturient was also observed on postoperative ileus which is a common complication of pregnant women undergoing caesarean. The chamomile oil (20 drops, topical) improved gastrointestinal mobility and reduced postoperative ileus duration as seen in a decrease of time to first flatus (20.9 h intervention *versus* 27.7 h placebo), bowel sound (11.1 h intervention *versus* 18.2 h placebo) and defecation (27.6 h intervention *versus* 30.2 h placebo).[84] Moreover, the topical administration of chamomile has resulted to be effective in different oral diseases including oral mucositis, gingivitis, aphthous stomatitis and burning mouth syndrome to treat symptomatology and reduce incidence.[89–91] Finally, the chamomile oil administered topically (six drops daily) for 6 weeks reduced the nocturia frequency in children with monosymptomatic nocturnal or daytime enuresis ($p < .001$ for chamomile intervention *versus* control).[63]

Clinically, it is interesting to know the bioavailability of chamomile depending on the route of administration. Figure 3 shows the different modes of administration in both *in vivo* studies and clinical trials. However, these studies are very limited. In all clinical trials, chamomile is administered orally and topically. Dong et al.[109] investigated the pharmacokinetics properties of quercetin, luteolin and apigenin in rats after oral chamomile administration (Cmax and Tmax, respectively, 0.29 μg/ml and 0.79 h for quercetin, 3.04 μg/ml and 0.42 h for luteolin and 0.42 μg/ml and 0.51 h for apigenin). Moreover, there have been described potential CYP450 interactions of chamomile extracts in post-kidney transplant patients who are treated with high doses for long periods. No interactions neither for chamomile essential oils nor for extracts in topical use have been reported.[110]

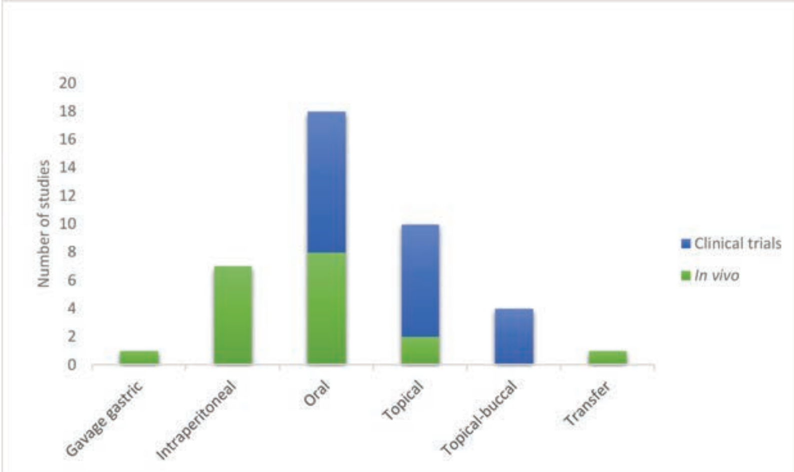

Figure 3. Administration methods of extracts, essential oils and active compounds isolated from *M. chamomilla* for *in vivo* and clinical studies.

Conclusion and future perspectives

The present update systematic review shows the effectiveness of *M. chamomilla* to improving health. *In vitro* studies have evaluated a wide range of activities including antimicrobial activity (antibacterial and antifungal) for extracts and essential oils, antiparasitics (extracts, essential oils and isolated compounds), antioxidant (extracts, essential oils and isolated compounds), cytotoxic (essential oils), antidiabetic (extracts and isolated compounds), anti-inflammatory (extracts and isolated compounds) and wound healing (isolated compound), among others. Regarding *in vivo* studies, carried out mainly with rats and mice as experimental models, the protective activity of the extracts has been evaluated (neuroprotection, cardiovascular protection, hepatoprotection, hematoprotection, ulcer protection and ovary and testis protection). In addition to the protective activity, other activities have been investigated such as wound healing (for camphor), anesthetic and antihyperalgesic (for essential oil) and antidepressant (for extracts). The vast majority of these *in vivo* tests have been performed with extracts, fewer studies with essential oil and just one work with a pure compound. Finally, all clinical trials have evaluated the activity of extracts and essential oil. Several of these clinical trials validate the pharmacological activities (diabetes mellitus, hypertension and pain) demonstrated in these *in vitro* and *in vivo* studies and others clinically evaluate other activities such as the effect on eneuresis. No discordant findings have been detected among different types of studies. Future *in vitro* and *in vivo* research should deepen into the mechanism of action and bioactive compounds responsible for the demonstrated activities for extracts and essential oils. Furthermore, in the years of this review, no clinical trial studies have been conducted with an isolated compound, this being another of the future research lines.

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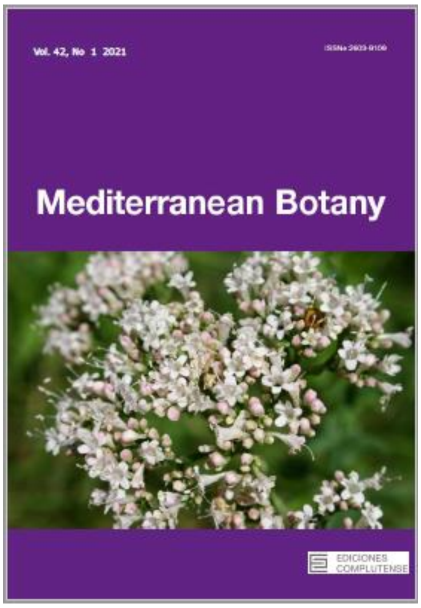
ARTÍCULO V



Updating the biological interest of *Valeriana officinalis*

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Updating the biological interest of *Valeriana officinalis*

[en] Contribution to the biological interest of *Valeriana officinalis:* an update

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Abstract. *Valeriana officinalis* L. (Caprifoliaceae) has been traditionally used to treat mild nervous tension and sleep problems. The basis of these activities are mainly attributed to valerenic acid through the modulation of the GABA receptor. Moreover, *V. officinalis* is claimed to have other biological activities such as cardiovascular benefits, anticancer, antimicrobial, and spasmolytic. The current review aims to update the biological and pharmacological studies (*in vitro*, *in vivo*, and clinical trials) of *V. officinalis* and its major secondary metabolites to guide future research.
Databases PubMed, Science Direct, and Scopus were used for literature search, including original papers written in English and published between 2014 and 2020.
There have been identified 33 articles that met the inclusion criteria. Most of these works were performed with *V. officinalis* extracts, and only a few papers (*in vitro* and *in vivo* studies) evaluated the activity of isolated compounds (valerenic acid and volvalerenal acid K). *In vitro* studies focused on studying antioxidant and neuroprotective activity. *In vivo* studies and clinical trials mainly investigated the nervous system activity (anticonvulsant activity, antidepressant, cognitive problems, anxiety, and sleep disorders). Just a few studies were focused on other different activities, highlight effects on symptoms of premenstrual and postmenopausal syndromes.
Valeriana officinalis continues to be one of the medicinal plants most used by today's society for its therapeutic properties and whose biological and pharmacological activities continue to arouse great scientific interest, as evidenced in recent publications. This review shows scientific evidence on the traditional uses of *V. officinalis* on the nervous system.
Keywords: *Valeriana officinalis*; bioactive compounds; biological activity; pharmacology activity; preclinical studies; clinical trials.

Introduction

Valeriana officinalis L. (Caprifoliaceae family), commonly known as "All-heal" (English), "Herbe aux chats" (French) and "Baldrian" (German), is an herbaceous perennial plant that grows extensively in temperate regions in Europe, Asia and North America (Hamaidia *et al*., 2016; Sundaresan *et al*., 2018) (Figure 1). The name of "*Valeriana*" derives from the Latin word "*valere*" which means to be healthy, strong, or powerful and the Latin word "*officinalis*" ("*officina*"), which refers to monastery pharmacy and apothecaries' shops (Hamaidia *et al*., 2016).

Addressing its chemical composition, there have been identified more than 150 different compounds in *Valeriana officinalis*. The qualitative and quantitative composition varies with the growing environment, climate, plant age, harvest phase and subspecies. The principal chemical constituents are alkaloids (i.e. chatinine, valerine, valerianine and actinidine), organic acids and terpenes (i.e. valerenic acid, isovaleric acid, valeric acid and acetoxyvalerenic) and iridoids (i.e. valepotriate, isovalepotriate), lignanoids (i.e. pinoresinol-4-O-D-glucoside, 8'-hydro-xypinoresinol), flavonoids (i.e. apigenin, luteolin, quercetin) (Patpcka & Jakl, 2010; Wang *et al*., 2010; Chen *et al*., 2013; Wang *et al*., 2013; Chen *et al*., 2015) (Figure 2).

The root of *V. officinalis* has been used since ancient Greece and Rome in traditional medicine until today in modern medicine to improve in the nervous state and to contribute to sleep promotion. The sesquiterpene valerenic acid is the main compound responsible for these activities through GABA receptor modulation (Felgentreff *et al*., 2012). The European Medicine Agency (EMA) based on validated scientific data, reported that the well-established use for dry ethanol (40-70%) extracts of *V. officinalis* in solid oral dosage forms is the relief of mild nervous tension and sleep disorders. Moreover, EMA includes other herbal preparations in *V. officinalis* monography, which are based on traditional use (more than 30 years of use in therapeutics) for relief of mild symptoms of mental stress and to aid sleep (Anon., 2016). Moreover, *V. officinalis* is claimed to have other biological activities such as cardiovascular benefits (reduction in blood pressure and heart rate, antiarrhythmic and regulation of blood lipid levels), anticancer, antimicrobial and spasmolytic (Letchamo *et al*., 2004; Occhiuto *et al*., 2009; Chen *et al*., 2015).

The current review aims to update the biological and pharmacological studies (*in vitro*, *in vivo*, and clinical trials) of *V. officinalis* and its major secondary metabolites to guide future research.

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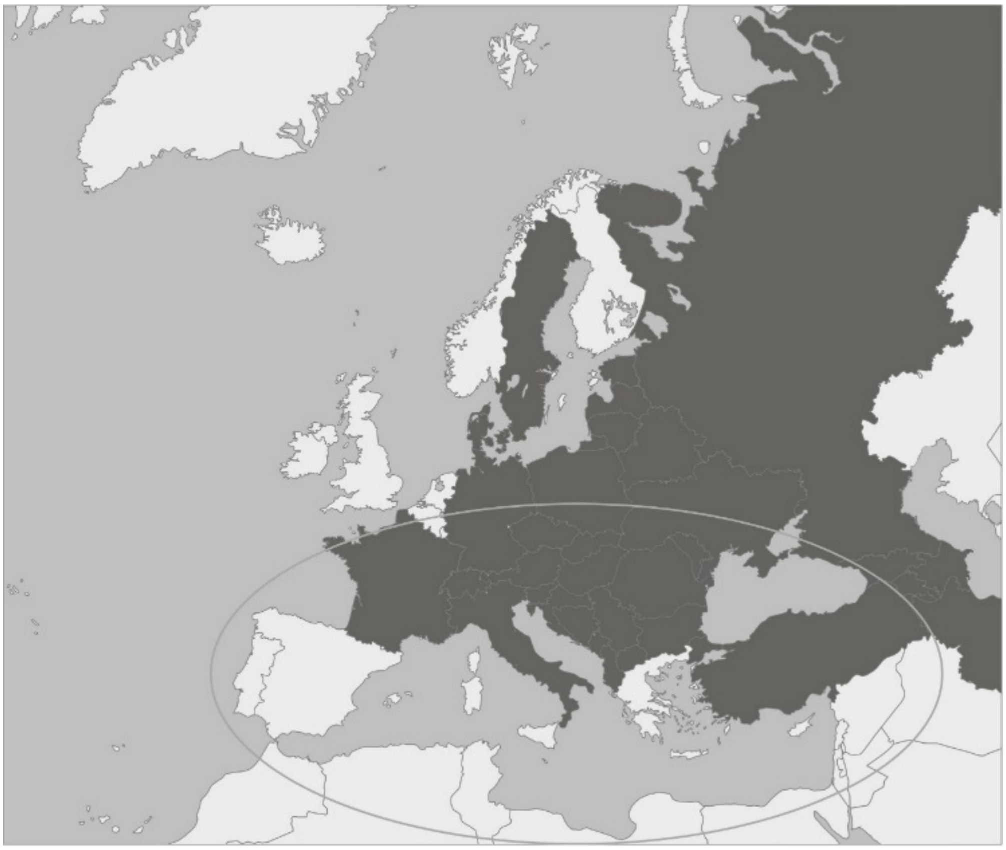

Figure 1. Native distribution of *Valeriana officinalis*.

Methods

This review focused on preclinical and clinical trials on the biological and pharmacological activity of *V. officinalis* and its major bioactive compounds. A literature search was performed in databases PubMed, Science Direct, and Scopus using the keywords *V. officinalis*, valerian, *in vitro*, *in vivo*, clinical trials, biological, and pharmacology. There were only selected original papers (excluding reviews, case reports, proceedings, editorial/letters, and conferences), written in English and published between 2014 and 2020. In addition, all works on the biological and pharmacological activity of other valerian species different than *V. officinalis* and studies on *V. officinalis* activity combined with other medicinal plant species were excluded.

Manuscripts were selected by two independent researchers who first identified all potential studies in the three cited databases to exclude then duplicates and those papers which did not meet inclusion criteria based on title and abstract analysis and full-text analysis.

Results and Discussion

Initially, we identified 556 studies (259 in PubMed, 37 in Science Direct, and 260 in Scopus). Of these reports, 91 works were excluded because they were duplicated in two or more databases. Of the 465 possible papers, 419 were excluded after analyzing title and abstract for not meeting inclusion criteria, and 13 articles were then excluded after full analysis. Finally, 33 items were included in this review. Figure 3 shows the flowchart of the literature process for the biological and pharmacology activity of *V. officinalis.*

This review consisted of 6 *in vitro* studies, 14 *in vivo* studies (1 of these reports presented *in vitro* and *in vivo* outcomes), and 13 clinical trials. Most of these works were performed with *V. officinalis* extracts, and just 5 (*in vitro* and *in vivo* studies*)* evaluated the biological and pharmacological activity of isolated compounds (valerenic acid, valeric acid, and volvalerenal acid K).

In vitro studies

Table 1 lists six articles with valerian extracts and their isolated compounds. All these works focused on studying antioxidant and neuroprotective activity, except one which aims to evaluate its antidiabetic properties. Four of these studies used chemical *in vitro* assays to measure antioxidant capacity and acetylcholinesterase inhibitory activity (Pilerood & Prakash, 2014; Li *et al*., 2015; Chen *et al*., 2016; Katsarova *et al*., 2018). In the other two *in vitro* studies, cell lines have been employed, particularly, the human neuroblastoma SH-SY5Y cells and the mouse 3T3-L1 preadipocytes (Gonulalan *et al*., 2018; Harada *et al*., 2020).

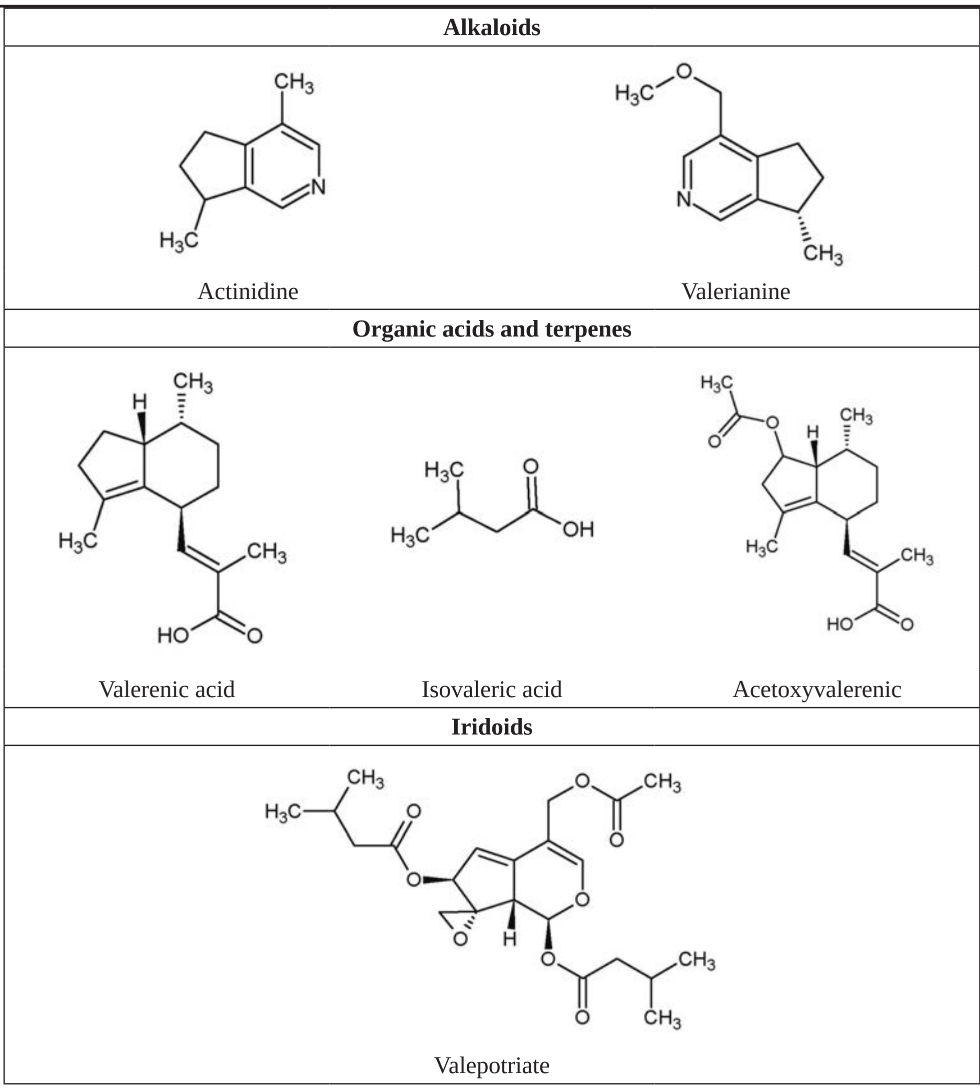

Figure 2. Chemical structures of some principal constituents identified in *Valeriana officinalis*.

Table 1. *In vitro* biological studies for *Valeriana officinalis*

Activity	Extract / isolated compounds	Experimental model	Treatments	Major findings	References
Antidiabetic	*Valeriana officinalis* root methanol extracts	Mouse 3T3-L1 preadipocytes	1, 10, and 100 µg/mL	↑ mRNA levels (PPARγ, CCAAT/enhancer-binding protein α, and adipocyte protein 2)	Harada *et al*., 2020
Antioxidant	*Valeriana officinalis* root extracts	DPPH model Reducing power Total antioxidant method	-	↑ Reducing power (80% methanolic extract) Free radical scavenging (80% methanolic extract)	Pilerood &Prakash, 2014
Antioxidant	*Valeriana officinalis* root extracts	ORAC method HORAC method	-	Low antioxidant activity (ORAC 820.5 µmol TE/g and HORAC 381.6 µmol GAE/g)	Katsarova *et al*., 2017
Antioxidant	*Valeriana officinalis* root ethanolic extracts	DPPH model FRAP method ABTS assay	-	Antioxidant activity (0.2579 mmol Trolox/g)	Li *et al*., 2015
Neuroprotection	*Valeriana officinalis* root extract Methanolic extracts of: • Valerenic acid • Acetoxy valerenic acid • Valerenic acid-free • Acetoxy valerenic acid-free	SH-SY5Y human neuroblastoma cell line	25 µg/mL	↑ BDNF expression	Gonulalan *et al*., 2018
Neuroprotection	Sesquiterpenoids Monoterpenoid	Acetylcholinesterase inhibitory activity	-	Volvalerenal acid K (IC50 0.161 µM)	Chen *et al*., 2016

Antioxidants are compounds that prevent free radical harmful effects by scavenging and metal chelating. There are different *in vitro* tests to evaluate antioxidant activity based on hydrogen atom transfer (i.e. ORAC method), electron transfer reactions (i.e. FRAP assay) and both hydrogen and electrons transfer capacities (i.e. DPPH assay) (Neha *et al*., 2019). Methanolic (80%) and ethanolic (80%) extracts of *V. officinalis* root exhibited highest reducing power activity. Moreover, 80% methanolic extract showed the highest DPPH radical scavenging activity. In another study, *V. officinalis* ethanolic extracts (95%) was effective to scavenge DPPH radical (Li *et al*., 2015). This antioxidant activity was positively correlated with flavonoids and tannin content (Pilerood &Prakash, 2014; Li *et al*., 2015). On the other hand, *V. officinalis* ethanol extracts (40%) showed low antioxidant activity in ORAC and HORAC assays (Katsarova *et al*., 2018).

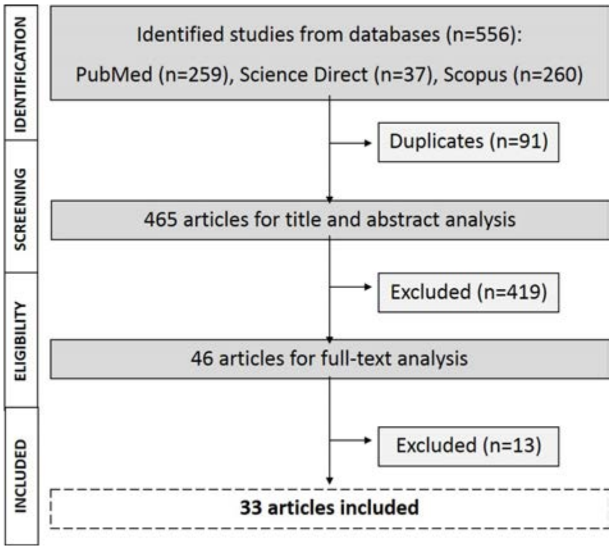

Figure 3. Flowchart of literature review process for biological and pharmacological activity of *Valeriana officinalis.*

In the last six years, the neuroprotective activity of *V. officinalis* extracts and the sesquiterpene volvalerenal acid K has been also studied using *in vitro* techniques. Low levels of Brain-Derived Neurotrophic Factor (BDNF) have been linked to depression. Hence, *V. officinalis* methanolic extract at a concentration of 25 µg/mL increased BDNF level in SH-SY5Y human neuroblastoma cell line. This activity is attributed to valerenic acid (Gonulalan *et al*., 2018). On the other hand, postmortem Alzheimer's disease brains revealed low levels in the neurotransmitter acetylcholine which lead to cognitive impairment and decline. Acetylcholinesterase hydrolyzes acetylcholine, thus targeting this enzyme will be clinically effective in slowing Alzheimer's disease progression (Anand & Singh, 2013). The compound volvalerenal acid K isolated from *V. officinalis* root demonstrated to inhibit acetylcholinesterase activity (IC_{50} value of 0.161 µM), being of interest as anti-Alzheimer agent (Chen *et al*., 2016).

Finally, the methanolic extract of *Valeriana officinalis* root demonstrated to be beneficial against type 2 diabetes by promoting dose-dependently 3T3-L1 adipocytes differentiation; this is related to direct binding to peroxisome proliferator-activated receptor γ (PPARγ) (Harada *et al*., 2020).

In vivo studies

Table 2 summarizes the main results for the fourteen *in vivo* studies. The main investigated activities were analgesic, anticonvulsant, antidepressant, anxiolytic and protective role in neurodegenerative diseases. Regarding animal models, rats and mice (mainly, Wistar and Sprague dawley rats and ICR mice) were the most common to evaluate the activity of valerian. Moreover, gerbils and zebrafish were selected as an experimental model in the other three *in vivo* studies (Torres-Hernández *et al*., 2015; Yoo *et al*., 2015; Torres-Hernández *et al*., 2016). The effect of isolated active compounds (valeric acid, valerenic acid, and volvalerenal acid K) has been investigated in six of the *in vivo* works, whereas the extracts of the root were evaluated in the other nine studies. The doses for both extracts and bioactive compounds were different in all studies.

Acute pain results from activation of nociceptors due to trauma (thermal, mechanical and chemical stimulus) or to biochemical mediators (serotonin, histamine, prostaglandins and arachidonic acid) (Johnson *et al*., 2013). The alcoholic extract of *V. officinalis* root (200 mg/kg and 400 mg/kg) reduced pain score in the acute phase and pain sensitivity in formalin induced pain in Wistar rats and Sprague Dawley rats (Taherianfard & Karamifard, 2018; Zare *et al*., 2018).

Epilepsy is a chronic and severe neurological disorder that consists of having at least two seizures caused by abnormal neuronal activity. Epilepsy affects more than 50 million people worldwide (Quintans *et al*., 2008; GBD 2016 Epilepsy Collaborators, 2019). Valerenic acid and *V. officinalis* extracts (ethanolic and aqueous) increased the latency period to the onset of a seizure and reversed altered swimming behaviors on pentylene-tetrazole-induced in zebrafish larval model epileptic seizures. This anticonvulsant activity seems to be related to *V. officinalis* ability to regulate neural activity (c-fos, npas4a, and bdnf) genes (Torres-Hernández *et al*., 2015; Torres-Hernández *et al*., 2016).

Depression is a common mental disorder that affects more than 264 million people worldwide (GBD 2017 Disease and Injury Incidence and prevalence Collaborators, 2018). Valerenic acid (0.5 mg/kg) and *V. officinalis* root extract alleviated physical and psychological stress in ICR mice by reducing 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate levels in the hippocampus-amygdala region (Jung *et al*., 2014; Jung *et al*., 2015). These activities are beneficial to treat depression and anxiety. The antidepressant effect has also been demonstrated for *V. officinalis* hydroalcoholic root extract in ovalbumin sensitized Wistar rats as evidenced in an increase in central and peripheral crossing numbers and a decrease in immobility times (Neamati *et al*., 2014).

Alzheimer's disease is the most common neurodegenerative disease. It is estimated that 43.8 million people have dementia (GBD 2016 Dementia Collaborators, 2019). The sesquiterpene volvalerenal acid K improved learning and memory abilities in SPF APPswe/PS1E9 double-transgenic dementia mice by increasing acetylcholine content and acetylcholine transferase and by reducing acetylcholinesterase activity (Chen *et al*., 2016). The hippocampus is related to short-term memory. *V. officinalis* hydroalcoholic extract has been shown to protect against morphology changes (size and number) of cerebral hippocampus astrocytes in rats (Heidarian *et al*., 2020). Parkinson's disease, the second most common degenerative neurological disorder, affects 6.1 million people globally (GBD 2016 Parkinson's Disease Collaborators, 2018). Valerenic acid alleviated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in C57BL/6 J and CD-1 mice models of Parkinson's disease by reducing pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ) and glial fibrillary acid protein (GFAP) (Rodriguez-Cruz *et al*., 2019).

In addition to all these effects, *V. officinalis* extracts have been investigated as myorelaxant agents and protect against cerebral ischemia. Hence, *V. officinalis* hydroethanolic root extract (2 and 5 g/kg doses) reduced skeletal muscle strength in Swiss mice without the side effect of other myorelaxant agents (muscle tone alteration and endurance decrease) (Caudal *et al*., 2017). Moreover, *V. officinalis* (25 and 100 mg/kg doses) protects against transient cerebral ischemia by decreasing microglial activation and lipid peroxidation in male gerbils (Yoo *et al*., 2015). Furthermore, valeric acid (0.15 mmol/kg) showed a hypotensive effect in Wistar rats, as evidenced by decreased arterial blood pressure and heart rate (Onyszkiewicz *et al*., 2020).

Clinical trials

Most clinical trials conducted in recent years with *V. officinalis* are with extracts and to evaluate different activities on the nervous system. A total of thirteen clinical trials, conducted with 747 patients (average of sample patients was 57, from 20 to 100 individuals) met the inclusion criteria of this review. They included clinical trials mainly originated from Iran (n=8), Brazil, and the USA (n=2). The overall clinical trials were randomized double-blind (except one study) (Jenabi *et al*., 2018) and placebo-controlled trials. The population included in these clinical trials has been varied from healthy volunteers to patients with different base pathologies (i.e., HIV-positive patients, infertile women, patients with acute coronary syndrome). The route of administration of valerian was oral in all studies except for one in which drops of oil (2.5%) (Bagheri-Nesami *et al*., 2015) were administered as massage. The oral doses of valerian ranged from 100 mg/daily to 1,600 mg/daily, being 530 mg the most common dose. Table 3 lists all the studies that meet the inclusion criteria. These studies are arranged alphabetically according to the authors of the investigations.

Anxiety disorders are common prevalent mental disorders (0.9%-28.3%) (Baxter *et al*., 2013). Farah *et al*. (2019) investigated the anti-anxiety effect of *V. officinalis* (100 mg in capsules, 60 minutes before the surgical procedure) and compared its effectiveness with midazolam (15 mg in tablets) in patients with anxiety due to bilateral extraction of mandibular third molars. This study revealed that midazolam is more potent as an anxiolytic drug, but *V. officinalis* causes less adverse effects (sedation and somnolence). Similar results of anti-anxiety properties of *V. officinalis* were observed in patients submitted to impacted lower third molar surgery and treated with valerian (100 mg, 1 hour before the surgical procedure) (Pinheiro *et al*., 2014). Valerian capsules (1,500 mg) has also been shown to significantly reduce anxiety in infertile women who undergo hysterosalpingography (Gharib *et al*., 2015). Moreover, a recent clinical trial revealed that valerian root extract (100 mg, thrice daily for four weeks) has anxiolytic properties by altering functional brain connectivity, as shown in an increase in frontal brain region alpha coherence and a reduction in theta coherence. Coherence in the electrical activity of the brain measures synchrony degree between two or more brain regions to frequency values in a unit of time (Roh *et al*., 2019).

Cognitive problems are more significant in patients with hemodialysis than in the general population. Valerian extract is an agonist of adenosine A1 receptors. This medicinal plant has sedative effects, and it also inhibits cholinergic transmission and increases delta frequency strength in the frontal cortex (Samaei *et al*., 2018). Valerian (capsules, 530 mg, 60 minutes before bed, for one month) could be effective and significantly improve cognitive status; however, no significant changes were observed in the EGG of the hemodialysis patients (Samaei et al., 2018). Moreover, it was demonstrated that *V. officinalis* root extract (1,060 mg/daily each 12 h for eight weeks) reduced odds of cognitive dysfunction in patients scheduled for elective coronary artery bypass graft surgery using cardiopulmonary bypass (Hassani *et al*., 2015). The cognitive status was evaluated in both cited works using the Mini-Mental State Examination (MMSE) questionnaire (11 questions regarding memory and orientation, attention and concentration, language and understanding abilities and visual-spatial abilities; a score of less than nine indicates that the patient has severe cognitive impairment) (Hassani *et al*., 2015; Samaei et al., 2018).

Table 2. *In vivo* biological studies for *Valeriana officinalis*

Activity	Extract / isolated compound	Experimental model	Doses	Major findings	References
Analgesic	Valerian root	Wistar rats Formalin-induced	200 mg/kg of alcoholic extract of valerian root	↓ Pain score in acute phase	Zare *et al*., 2018
Analgesic	*Valeriana officinalis* rhizome extract	Sprague Dawley rats	400 mg/kg	↓ Pain sensitivity	Taherianfard & Karamifard, 2018
Anticonvulsant	Valerenic acid *Valeriana officinalis* extracts (aqueous and ethanolic)	Zebrafish (*Danio rerio*) PTZ-Induced seizures	Valerenic acid (37 µg/ml) Ethanolic valerian extract (0.5 and 1 mg/ml) Aqueous valerian extract (5 mg/ml)	↑ Latency period to the onset of seizure	Torres-Hernández *et al*., 2015
Anticonvulsant	*Valeriana officinalis* aqueous root extract	Zebrafish (*Danio rerio*)	1, 2.5, 5, and 7 mg/ml	Reversion of PTZ-altered swimming behaviors ↑ Neural-activity genes (npas4a and bdnf)	Torres-Hernández *et al*., 2016
Antidepressant	*Valeriana officinalis* hydroalcoholic root extract	Wistar rats	50, 100 and 200 mg/kg	↑ Central and peripheral crossing number ↓ Immobility times	Neamati *et al*., 2014
Antidepressant	*Valeriana officinalis* root extracts	ICR mice	100 mg/kg/0.5 ml	↓ Physical and psychological stress ↓ MHPG-SO4 and 5-HIAA levels	Jung *et al*., 2014
Anxiolytic	Valerenic acid	ICR mice	0.2, 0.5, and 1.0 mg/kg/0.3 mL	↓ Immobility time ↓ Corticosterone levels ↓ Physical and psychological stress response ↓ 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate levels	Jung *et al*., 2015
Anxiolytic	Valerenic acid	CD-1 mice	0,5 mg/kg	Anxiolytic effect	Becker *et al*., 2014
Cardioprotective	*Valeriana officinalis* root extract	Male gerbils	25 and 100 mg/kg	Cerebral ischemia protection ↓ Microglial activation ↓ Lipid peroxidation	Yoo *et al*., 2015
Cardioprotective	Valeric acid	Wistar rats	0.15 mmol/kg	↓ Arterial blood pressure ↓ Heart rate	Onyszkiewicz *et al*., 2020
Myorelaxant	*Valeriana officinalis* hydroethanolic root extract	Swiss mice	2 or 5 g/kg	↓ skeletal muscle strength	Caudal *et al*., 2018
Neuroprotection	Valerenic acid	Parkinson's disease model: MPTP-induced mouse C57BL/6 J mice and CD-1 mice	2 mg/kg body weight, *i.p*	↓ Pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ) ↓ GFAP proteins	Rodríguez-Cruz *et al*., 2019
Neuroprotection	Volvalerenal acid K	APPswe/PSΔE9 double-transgenic mice	0.65, 1.30 and 2.60 mg/kg/day	↑ Learning and memory abilities	Chen *et al*., 2016
Neuroprotection	*Valeriana officinalis* hydroalcoholic extract	Sprague dawley rats	300, 400, 600 mg extract daily	↑ Number of astrocytes ↓ Large diameter of astrocytes	Heidarian *et al*., 2020

Insomnia causing absenteeism and social disability affects around one-third of adult people (Bent *et al*., 2006). There are consistent evidence that valerian optimizes the quality of sleep and induces sleep. *V. officinalis* (530 mg, 1 hour before bed, four weeks) improved sleep and anxiety in HIV-positive patients treated with efavirenz (Ahmadi *et al*., 2017). Moreover, the combination of valerian oil 2.5% (2 drops, three nights) improved sleep quality and reduced waking during the night in patients who suffered from acute coronary syndrome (Bagheri-Nesami *et al*., 2015). The sleep status (time and quality) was measured using different techniques such as St. Mary's Hospital Sleep Questionnaire (SMHSQ) (survey with 14 Likert-scale questions and a fill-in-the-blank response) and a validated Persian version of the Pittsburgh Sleep Quality Index (PSQI) (survey with 7 components; a score greater than five indicates that the patient does not sleep long enough and the sleep is not of quality) (Bagheri-Nesami *et al*., 2015; Ahmadi *et al*., 2017). Furthermore, Mineo *et al*. (2017) investigated the effect of a single dose of *V. officinalis* (900 mg; valerenic acid 0.8%) at the cortical level in healthy volunteers using transcranial magnetic stimulation. This study revealed that *V. officinalis* reduced intracortical facilitatory circuits. On the other hand, a dose of 1,600 mg of valerian does not affect drive stimulator performance in healthy volunteers compared to the placebo group (Thomas *et al*., 2016).

Furthermore, a dose of 530 mg of valerian root extraction (2 capsules for one month) resulted in decreasing daily livings, disability, and severity of the tension-type headache. The effect of valerian on headache impact was measured using surveys [headache impact test questionnaire (HIT-6), headache disability inventory (HDI), and Visual Analogue Scale (VAS)] in baseline and one month after the intervention (Azizi *et al*., 2020).

In addition to all these studies, other activities have been studied in humans. Hence, valerian (530 mg, twice per day, two months) reduced the severity and frequency of hot flashes in menopausal women (Jenabi *et al*., 2018). Moreover, valerian (2 capsules daily for three months) reduced emotional, physical, and behavioral symptoms of premenstrual syndrome in women university students (Behboodi Moghadam *et al*., 2016).

Table 3. Clinical trials for *Valeriana officinalis*. Abbreviations are: S. size, sample size.

Study (author, year, country)	Study design	S. size	Population	Intervention	Results
Ahmadi *et al*., 2017 Iran	Randomized, double-blind, placebo	51	HIV-positive patients treated with efavirenz	Intervention group: valerian (530 mg, 1 hour before bed, 4 weeks) Placebo group	↑ Sleep ↓ Anxiety
Azizi *et al*., 2020 Iran	Randomized, double-blind, placebo	88	Tension-type headache	Intervention group: 530 mg of valerian root extraction Placebo group	↓ Disability ↓ Severity score ↓ Daily livings
Bagheri-Nesami *et al*., 2015 Iran	Randomized, double-blind, controlled	90	Patients with acute coronary syndrome	Intervention group: acupressure with valerian oil 2.5% (2 drops, 3 nights) Control group: massage	↑ Sleep quality ↓ Waking
Behboodi Moghadam *et al*., 2016 Iran	Double-blind, placebo	100	Premenstrual syndrome women	Intervention group: valerian (2 capsules daily, 3 months) Placebo group	↓ Emotional, physical and behavioral symptoms
Farah *et al*., 2019 Brazil	Randomized, double-blind, crossover and prospective	20	Anxious patients with an indication for bilateral extraction of mandibular third molars	Intervention group: Valerian (100 mg in capsules, 60 minutes before surgical procedure) Control group: Midazolam (15 mg in tablets, 60 minutes before surgical procedure)	↓ physiological parameters No sedation and less somnolence than midzolam
Gharib *et al*., 2015 Iran	Randomized, double-blind, placebo	64	Infertile women undergoing hysterosalpingography	Intervention group: valeric capsules (1500 mg) Placebo group	↓ Anxiety
Hassani *et al*., 2015 Iran	Randomized, double-blind, placebo	61	Patients scheduled for elective CABG surgery using CPB	Intervention group: valerian capsule (1,060 mg/daily) Placebo group	↓ Odds of cognitive dysfunction
Jenabi *et al*., 2018 Iran	Randomized, triple-blind, placebo	60	Postmenopausal women	Intervention group: valerian (530 mg, twice per day, two months) Placebo group	↓ Hot flashes (severity and frequency)
Mineo *et al*., 2017 USA	Randomized, double-blind, placebo, crossover	50	Healthy volunteers	Intervention group: *Valeriana officinalis* extract (900 mg with valerenic acid 0.8%) Placebo group	↓Intracortical facilitation
Pinheiro *et al*., 2014 Brazil	Randomized, double-blind, placebo	20	Patients submitted to impacted lower third molar surgery	Intervention group: valerian capsule (100 mg) Placebo group	↓ Anxiety
Roh *et al*., 2017 Korea	Randomized, double-blind, placebo, crossover	64	Volunteers suffering psychological stress	Intervention group: valerian root extract (100 mg/thrice daily for 4 weeks) Placebo group	↑ Frontal brain region alpha coherence ↓ Theta coherence
Samaei *et al*., 2018 Iran	Randomized, double-blind, placebo, crossover	39	Hemodialysis patients	Intervention group: valerian capsules (530 mg, 60 min before bed, 1 month) Placebo group	↑ Cognitive status
Thomas *et al*. 2016 USA	Randomized, double-blind, placebo, crossover	40	Healthy adult	Intervention group: valerian (1600 mg) Placebo group	No effect on drive stimulator performance

Regarding adverse effects, only two of the thirteen clinical trials have reported adverse drug reactions (Ahmadi *et al*., 2017; Farah *et al*. (2019). Remarkably, these adverse events were dizziness, somnolence, and nausea. These adverse drug reactions were typed A related to the mechanism of action, dose-related toxicities, and predictable (Iasella et al., 2017).

Finally, no clinically relevant interactions have been reported for valerian root and other drugs that are metabolized by cytochrome P450 isoforms (CYP3A4, CYP2D6, CYP1A2, and CYP2E1) (Anon., 2003; Kelber *et al*., 2014; Anon., 2016).

Conclusions

Valeriana officinalis continues to be one of the medicinal plants most used by today's society for its therapeutic properties and whose biological and pharmacological activities continue to arouse great scientific interest, as evidenced in recent publications. This review shows scientific evidence on the traditional uses of *V. officinalis* on the nervous system. The *in vitro* studies revealed the potential antioxidant activities of *V. officinalis,* which could therapeutically contribute to prevent and protect against oxidative stress-related diseases. Moreover, *in vivo* studies explored its effective activity on different nervous system diseases such as depression, epilepsy, and neurodegenerative disorders. Based on clinical trials, there is consistent evidence of the efficacy of *V. officinalis* in anxiety, cognitive problems, and insomnia without causing side effects. This report highlights the potential biological properties of the compounds volvalerenal acid K and valerenic acid *in vitro* and *in vivo* studies. However, there is a lack of efficacy and safety of these major bioactive compounds in clinical trials. Therefore, future research should be focus on studying the clinical activity of these secondary metabolites as well as investigating new and different biological activities of *V. officinalis*.

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ARTÍCULO VI

DNA-Based Authentication and Metabolomics Analysis of Medicinal Plants Samples by DNA Barcoding and Ultra-High-Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry (UHPLC-MS)

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Article

DNA-Based Authentication and Metabolomics Analysis of Medicinal Plants Samples by DNA Barcoding and Ultra-High-Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry (UHPLC-MS)

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Abstract: There is growing interest for medicinal plants in the world drug market. Particularly, *Matricaria recutita* L., *Valeriana officinalis* L., *Tilia* spp., and *Camellia sinensis* (L.) Kuntze are some of the most consumed medicinal plants for treatment of minor health problems. Medicinal plants are seen as natural and safe; however, they can cause interactions and produce adverse reactions. Moreover, there is lack of consensus in medicinal plants regulation worldwide. DNA barcoding and UHPLC-MS technique are increasingly used to correctly identify medicinal plants and guarantee their quality and therapeutic safety. We analyzed 33 samples of valerian, linden, tea, and chamomile acquired in pharmacies, supermarkets, and herbal shops by DNA barcoding and UHPLC-MS. DNA barcoding, using *matk* as a barcode marker, revealed that CH1 sold as *Camellia sinensis* was *Blepharocalyx tweediei*, and sample TS2 sold as linden belong to Malvales. On the other hand, UHPLC-MS analysis revealed the presence of bioactive compounds (apigenin-7-glucoside, acetoxy valerenic acid, valerenic acid, epigallocatechin, and tiliroside). However, none of samples met minimum content of these active principles (except for valerenic acid in VF3) according to the European Medicines Agency (EMA) and Real Spanish Pharmacopeia. In conclusion, this study revealed the need to incorporate DNA barcoding and HPLC-MS techniques in quality controls of medicinal plants.

Keywords: medicinal plants; DNA barcoding; *matk*; HPLC-MS method

1. Introduction

Medicinal plants constitute the basis of traditional and modern primary healthcare. Over 80% of the population, mainly of developing countries, depend on traditional and herbal medicine. Moreover, at least 25% of drugs in the modern pharmacopeia are derived from plants [1]. Furthermore, it is estimated that pharmacological activity has been evaluated in only 15% of all 300,000 plant species identified [2]. The consumption of medicinal plants for disease prevention and health promotion has increased significantly in the last two decades [3]. The reasons that explain the rise in therapeutic use of medicinal plants are several. They include natural tendency in population, erroneous perception of its safety, lower economic cost compared to conventional medicines, and polypharmacy [4,5]. The high demand for medicinal plants is reflected in the economic data of world market. The global trade in herbs was over USD 83 billion in 2012, being especially high in India, China, and Germany [6,7]. These medicinal plants are available in pharmacies, supermarkets, and herbal shops [4,8].

These medicinal plants must meet standards of quality, safety, and efficacy. In this context, pharmacopeia monographs include tests and acceptance criteria ranging from botanical identification, pharmacognostic evaluation, and chemical characterization with chromatographic methods to evaluate the quality of herbal medicines [9]. However, one of the biggest difficulties related to quality is that commercial medicinal plants are crushed or powdered, being problematic to identify phenotype or part of the plant [10]. The DNA barcoding technique is postulated as an effective tool to overcome limitations in the quality controls of commercial medicinal plants. DNA-based techniques consist of using short DNA sequences from standardized gene regions (rbcL, matK, and ITS2) [11–13]. One of the great advantages of DNA barcoding technique is that the result is not influenced by harvesting period, growth condition, environmental factors, and sample age, among other factors [14–16]. Moreover, high-performance liquid chromatography (HPLC) and mass chromatography (MS) techniques are widely useful to identify and quantify bioactive compounds found in medicinal plants [14]. Therefore, the correct botanical identification by DNA barcoding and the precise bioactive compounds determination by HPLC-MS constitute an integrated approach to guarantee quality and safety of market medicinal plants [17–19].

Matricaria recutita L. (chamomile), *Valeriana officinalis* L. (valerian), *Tilia* spp. (linden), and *Camellia sinensis* (L.) Kuntze (tea) are found among the most consumed medicinal plants. They are commonly acquired in pharmacies, herbal shops, and supermarkets. Chamomile flowers as infusions have a beneficial effect on digestion; valerian root in capsules are used for reducing anxiety and a nervous state and improving sleep; linden leaves as infusions reduce anxiety symptoms; tea leaves as capsule or infusion bring relief from digestive problems [20].

The aim of the present work is to apply DNA barcoding and UHPLC-MS methods as a tool to evaluate the quality of market samples of *Matricaria recutita*, *Valeriana officinalis*, *Tilia* spp., and *Camellia sinensis.*

2. Results and Discussion

2.1. DNA Barcoding Analysis

Commercial herbal products can be adulterer, replaced, or suffer some kind of contamination [20,21]. Herbal products are sold as ground or powdered form of a raw herb, which makes correct botanical identification difficult [22]. DNA barcoding constitutes a very useful tool for quality control and, consequently, for clinical safety [23,24]. Molecular analyses is crucial for accurate and fast identification of medicinal plants, since the plant fragments sold in the market is difficult to identify using traditional methods especially due to lack of morphological features. Moreover, they often mixed with other plant materials, and in such cases, molecular analysis is one of the best approaches for accurate sample identification [25,26].

In this study, the DNA barcode marker *matk* was used to identify four of the most consumed medicinal plants, which were *M. recutita, V. officinalis, Tilia* spp., and *C. sinensis* [27–29]. A total of 33 market samples (pharmacies, herbal shops, and supermarkets) were investigated (nine samples from *M. recutita* flowers, seven from *V. officinalis* root, nine from *Tilia* spp. Leaves, and eight from *C. sinensis* leaves). DNA was successfully extracted from 23 of 33 markets samples. Raw material conditions (i.e., very dry, storage quality) could explain why DNA could not be extracted in 10 of the samples. A total of eighteen new *matk* sequences were generated for 23 samples isolated from chamomile, linden, tea, and valeria acquired in different distribution channels. These *matk* sequences were aligned with 38 sequences downloaded from GenBank. A total of 10 sequences were in *C. sinensis* data matrix, 20 in *M. recutita*, nine in *V. officinalis*, and 17 in *Tilia* spp. The length of all newly generated *matk* sequences was above 700 bp. All new sequences generated for this study have been deposited in GenBank.

The results of the molecular phylogenetic and DNA barcoding analyses were largely congruent. In the phylogenetic analysis, all samples were grouped together forming monophyletic clades except for CH1, TS2, and VH2 samples (Figures S1–S4). The authenticity of these samples was confirmed with DNA barcoding analysis using the Barcode of Life Data (BOLD) system. The results of the BOLD system blast are depicted in Figures S5–S8. The sample codes MH1 to MH3, MF2, and MS1 to MS3 were identified as *M. recutita*; CH2 and CF3 as *C. sinensis*; VH1, VS1, and VF2 as *Valeriana hirtella* Kunth.; TH1 to TH3 resulted *Tilia cordata* Mill. (Table 1). The sample CH1 sold as *C. sinensis* did not correspond to this species but to *Blepharocalyx tweediei* [30] Berg. *Blepharocalyx tweediei* is a tree native to Argentina and Uruguay that is traditionally used as infusion for cough, bronchospasm, diarrhea, and other intestinal disorders. Further, TS2 sample sold as Linden matched with Malvales; no closely related species was detected. Remarkably, samples sold as *V. officinalis* and *Tilia platyphyllos/europea* Scop. were identified as *Valeriana hirtella* Kunth. and *T. cordata*, respectively. On the other hand, all samples sold as chamomile were correctly identified as *Matricaria recutita*. Therefore, our study has identified alterations in *Camellia sinensis* and *Tilia* spp. in one of the three and one of the four samples, respectively, of these two genera belonged to entirely different species/order. Figure 1 shows the DNA final barcode identification of the analyzed marked medicinal plants. A percentage of 36.4 of plant samples were identified as right species, 48.5% were incomplete samples, and 15.2% were consider as replacements. This work reveals the need for a correct botanical identification for valerian and linden to ensure the precise labeling. DNA-based sample authentication is necessary for market medicinal plants for correct identification [13,31,32].

Table 1. Sample identification using the Barcode of Life Data (BOLD) systems.

Sample Code	Labelled	BOLD System Identification
MH1	Matricaria	*Matricaria recutita*
MH2	Chamomile	*Matricaria recutita*
MH3	*Matricaria chamomilla*	*Matricaria recutita*
MF2	Chamomile	*Matricaria recutita*
MS1	Chamomile	*Matricaria recutita*
MS2	*Matricaria recutita*	*Matricaria recutita*
MS3	Chamomile	*Matricaria recutita*
CH1	*Camellia sinensis*	*Blepharocalyx tweediei*
CH2	Thea	*Camellia sinensis*
CF3	Thea	*Camellia sinensis*
VH1	*Valeriana officinalis*	*Valeriana hirtella*
VS1	*Valeriana officinalis*	*Valeriana hirtella*
VF2	*Valeriana officinalis*	*Valeriana hirtella*
TH1	*Tilia platyphyllos*	*Tilia cordata*
TH2	Linden	*Tilia cordata*
TH3	Tilia Europea	*Tilia cordata*
TS2	Linden	*Malvales*

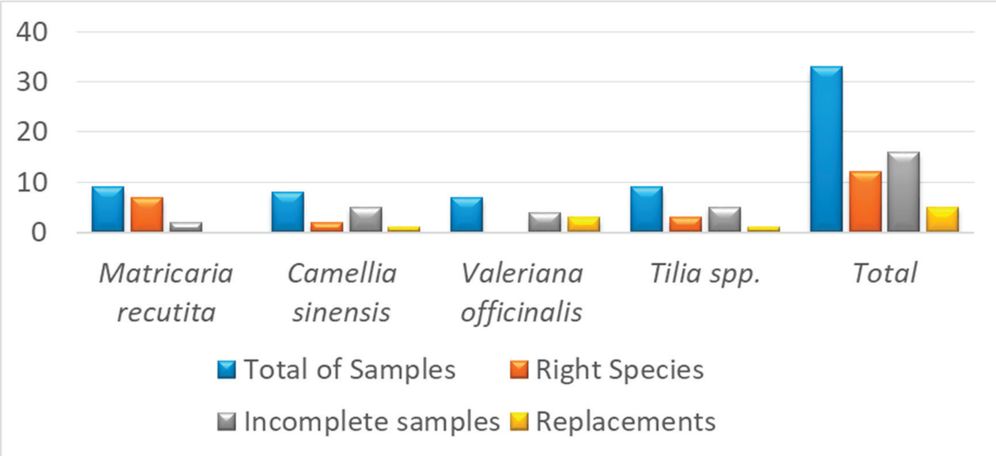


Figure 1. DNA-sequence-based identification of the analyzed medicinal plants marked samples.

2.2. UHPLC/MS Analysis

UHPLC-MS based metabolomics approach allows to qualitatively and quantitatively analyze all metabolites in medicinal plant species with high sensitivity and precision [33]. The first of these techniques (molecular assays) can only authenticate the medicinal plant, while the second assesses (chromatographic assays) its quality and can provide information on the presence and concentration of compounds with pharmacological activity, but can never determine the identity of the species in question (providing no direct evidence of fraud). The simultaneous use of both techniques is therefore an additional advantage for the evaluation of the quality of medicinal plants and thus for their efficacy and safety. The 33 market samples of the medicinal plants *M. recutita*, *V. officinalis*, *Tilia* spp. and *C. sinensis* were analyzed by HPLC-MS to identify and quantify the main bioactive compounds responsible for their pharmacological activity [34] (Tables 2–5, Figure 2). Particularly, the compound apigenin-7-glucoside was identified in *M. recutita* samples. The concentration of apigenin-7-glucoside ranged from 0.001 to 0.035%. Most of the samples acquired in the different commercial establishments had an average content of this active principle of 0.003%. Only two commercial samples of chamomile had a higher content in apigenin-7-glucoside, specifically MH1 sample acquired in an herbal shop (0.035%) and MS3 sample acquired in a supermarket (0.016%). The European Medicines Agency and the Real Spanish Pharmacopeia establish that apigenin-7-glucoside content should be at least 0.25% of dried drug [35,36], thus being the content in this bioactive compound lower in all analyzed samples. Moreover, previous studies have identified that the content for apigenin-7-glucoside in dry material of *Matricaria chamomilla* varied from 210 to 1110 mg/100 g using UPLC-UV method [37].

Table 2. Content of apigenin-7-glucoside in *Matricaria recutita* in samples labeled as acquired in pharmacies, herbal shops, and pharmacies. Data are expressed as means ± standard deviations of triplicate independent analyses.

Matricaria recutita L.	
Sample	**Apigenin-7-glucoside (mg/g) (mean ± SD)**
MH1	0.35 ± 0.015
MH2	0.02 ± 0.004
MH3	0.05 ± 0.005
MS1	0.07 ± 0.004
MS2	0.06 ± 0.003
MS3	0.16 ± 0.009
MF1	0.03 ± 0.002
MF2	0.01 ± 0.004
MF3	0.03 ± 0.005

Table 3. Content of valerenic acid and acetoxyvalerenic acid in *Valeriana* in samples labeled as acquired in pharmacies, herbal shops, and pharmacies. Data are expressed as means ± standard deviations of triplicate independent analyses.

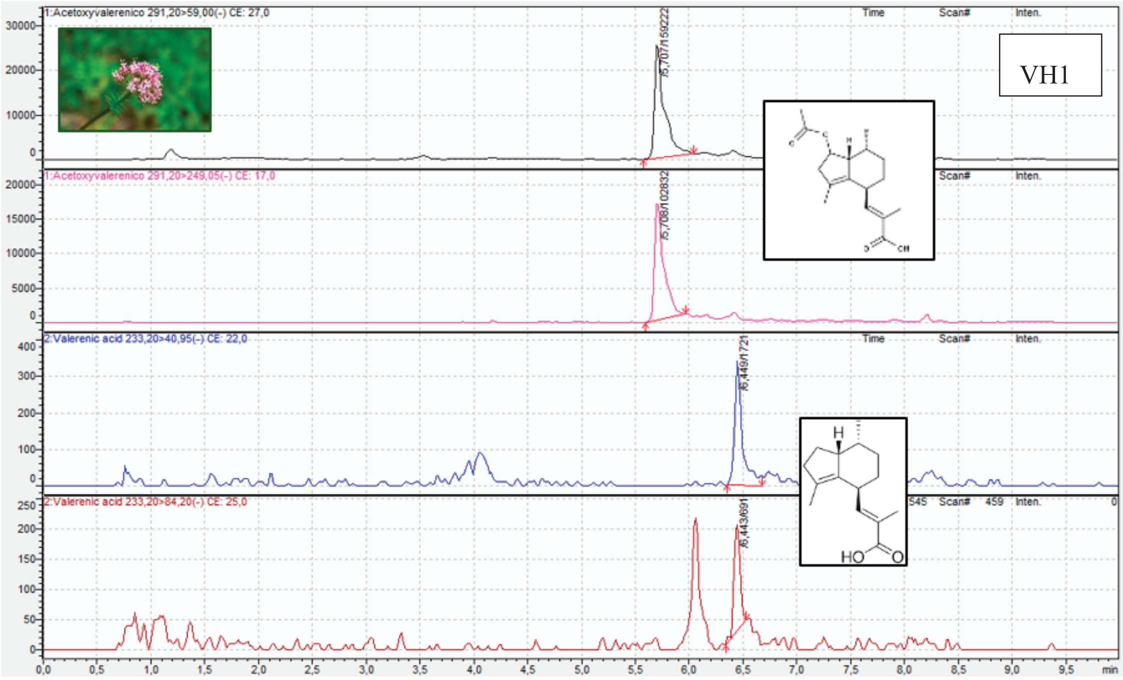
Valeriana Officinalis L.		
Sample	**Acetoxyvalerenic Acid (mg/g) (mean ± SD)**	**Valerenic Acid (mg/g) (mean ± SD)**
VH1	0.26 ± 0.030	1.09 ± 0.017
VH2	0.24 ± 0.011	1.15 ± 0.017
VH3	0.24 ± 0.014	1.01 ± 0.020
VF1	0.20 ± 0.020	0.48 ± 0.015
VF2	0.29 ± 0.017	0.78 ± 0.026
VF3	0.53 ± 0.014	1.67 ± 0.030
VS1	0.25 ± 0.055	0.84 ± 0.015

Table 4. Content of epigallocatechin in *Camellia sinensis* in samples labeled as acquired in pharmacies, herbal shops, and pharmacies. Data are expressed as means ± standard deviations of triplicate independent analyses.

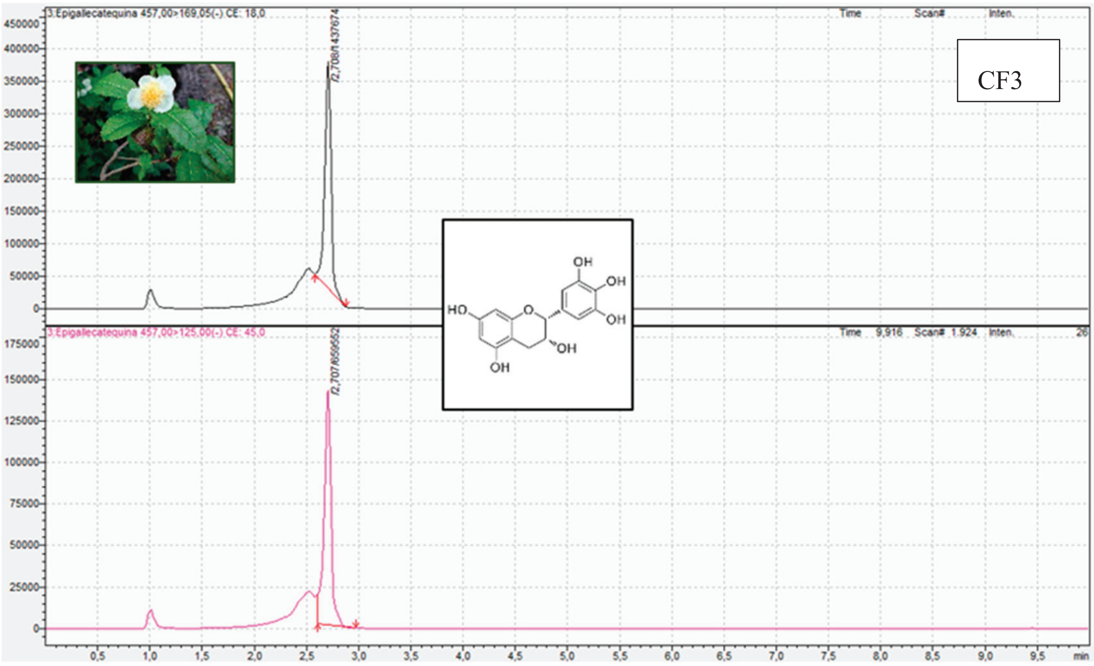
Camellia Sinensis (L.) Kuntze	
Sample	**Epigallocatechin (mg/g) (mean ± SD)**
CH1	21.2 ± 0.025
CH2	47.2 ± 0.015
CH3	23.2 ± 0.060
CS1	32.9 ± 0.025
CS2	37.1 ± 0.011
CF1	15.1 ± 0.020
CF2	25.9 ± 0.011
CF3	25.5 ± 0.030

Table 5. Content of tiliroside in *Tilia* in samples labeled as acquired in pharmacies, herbal shops, and pharmacies. Data are expressed as means ± standard deviations of triplicate independent analyses.

Tilia spp.	
Sample	**Tiliroside (mg/g) (mean ± SD)**
TH1	0.32 ± 0.056
TH2	0.39 ± 0.045
TH3	0.08 ± 0.020
TS1	0.27 ± 0.026
TS2	0.42 ± 0.032
TS3	0.43 ± 0.025
TF1	0.23 ± 0.020
TF2	0.36 ± 0.010
TF3	0.35 ± 0.011

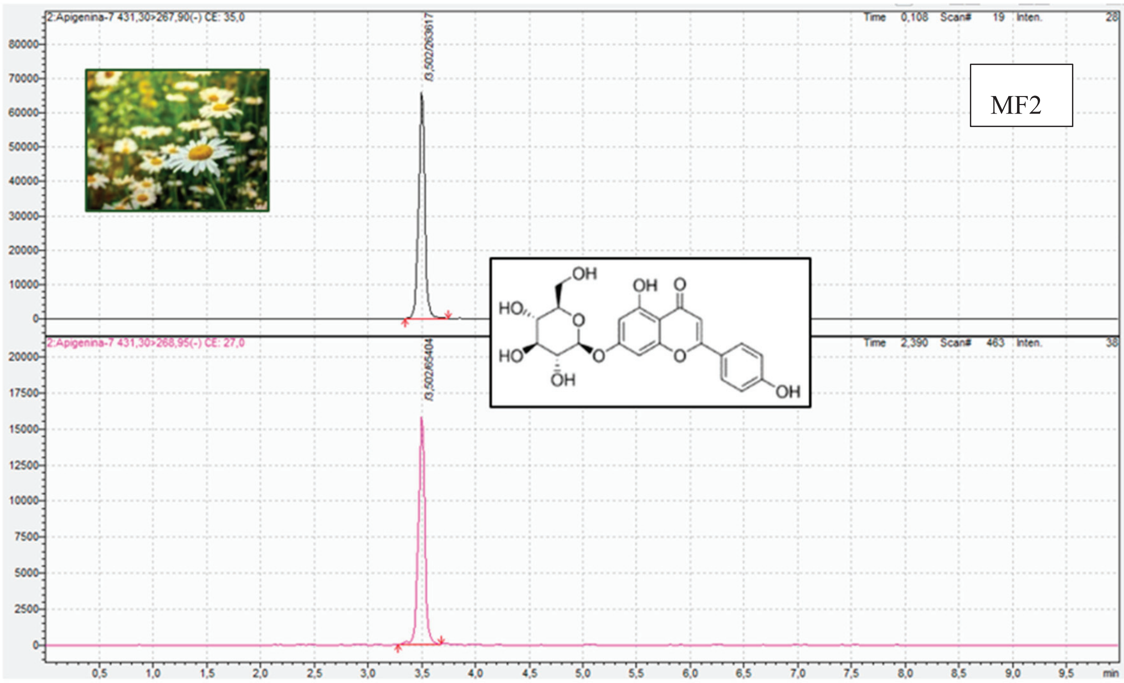


(**A**)

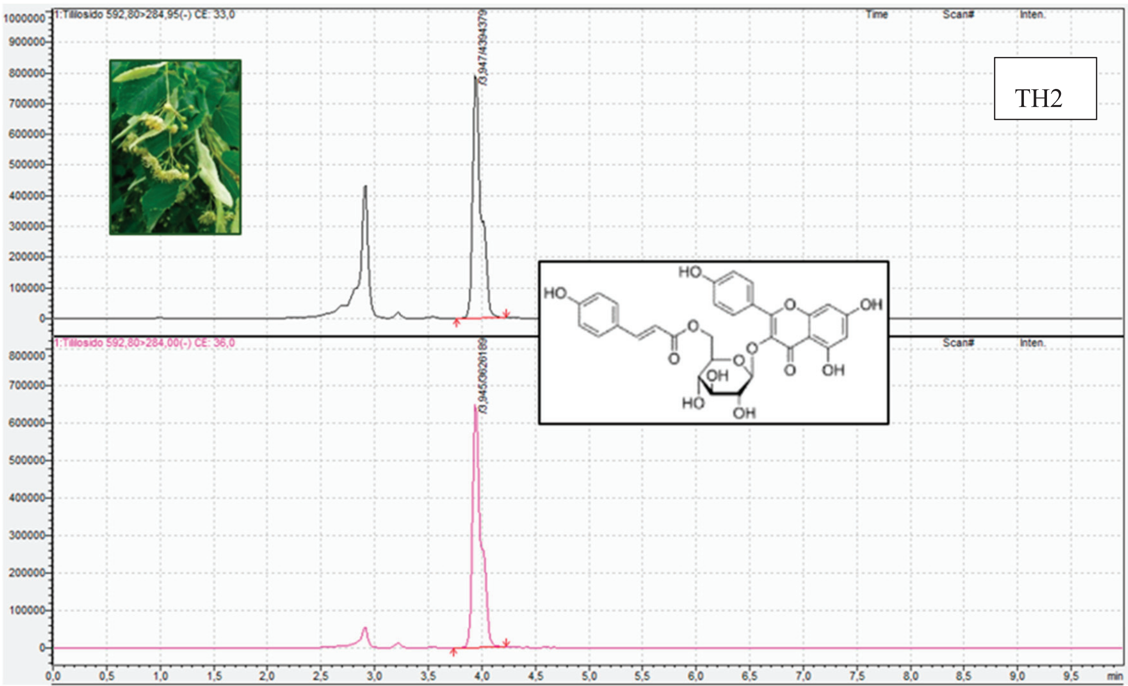


(**B**)

Figure 2. *Cont.*



(**C**)



(**D**)

Figure 2. Representative UHPLC-ESI-QqQ-MS/MS chromatograms for (**A**) acetoxyvalerenic and valerenic acids presented in valerian samples, (**B**) epigallocatechin presented in tea samples, (**C**) apigenin presented in chamomile, and (**D**) tiliroside presented in linden.

For *V. officinalis* samples, the bioactive compounds identified were acetoxy valerenic acid and valerenic acid. Acetoxy valerenic acid concentration ranged from 0.020 to 0.053%. The average content for this compound for all samples (except for VF3) was 0.025%. The content in acetoxy valerenic acid for samples VF3 acquired in pharmacy was 0.053%. On the other hand, a variable content of valerenic acid was identified among the different valerian samples (ranging from 0.048 to 0.167%). Hence, the content of this bioactive compound was similar in those samples from herbal shops (from 0.101 to 0.115%) and very different among pharmacy samples (0.048, 0.078, and 0.167%). On the other hand, the content of valerenic acid in the valerian sample from supermarket was 0.084%. According to the European Medicines Agency and the Real Spanish Pharmacopeia, the content in sesquiterpeneic acids should be not less than 0.17% expressed as valerenic acid [36,38]. The only sample that meets this requirement is VF3; this sample acquired in pharmacy could not be identified in the DNA barcoding study. Moreover, Navarrete et al. (2013) analyzed valerenic acid content in valerian species using liquid chromatography with ultraviolet detection. Particularly, the content of valerenic acid was 0.88% [39].

The compound epigallocatechin was identified and quantified in *C. sinensis* samples. Its content varied between 1.51 and 4.72%. The lowest content corresponded to a pharmacy sample and the highest content to an herbal shop sample. The most common average content identified in four of six samples from pharmacies and supermarkets was 2.39%. On the other hand, the epigallocatechin content of samples from supermarkets was very similar (3.29 and 3.71%). The content in flavan-3-ols including epigallocatechin should range from 10–25% of dried drug according to European Medicines Agency [40,41]. Previous works reported that the content in epigallocatechin was 4.62% in *Camellia sinensis* samples [42].

Finally, tiliroside was identified in tea samples with a content variation between 0.008 and 0.043%. The low content of 0.008% was quantified in an herbal shop sample. The rest of linden samples had an average of 0.034%. There are no data on tiliroside content neither in the European Medicines Agency nor in the Real Spanish Pharmacopeia [43]. The content of tiliroside has been identified to be higher in inflorescences that in leaves in *Tilia cordata* (49.2 μg/g versus 16.1 μg/g) [44].

The analysis of the secondary metabolites revealed that all the plant species analyzed, regardless of whether or not they had been correctly identified botanically, contained to a greater or lesser extent the bioactive compounds that were sought. This shows that these compounds are presented in other species of the same genus (i.e., *Valeriana hirtella*) and of other orders (i.e., Malvales). Specifically, the tiliroside has been identified in many different families including Malvaceae and Tiliaceae [44].

3. Materials and Methods

3.1. Reagents

Methanol HPLC grade was obtained from Panreac Química (Barcelona, Spain). Acetonitrile HPLC grade, formic acid HPLC grade, SYBR safe DNA gel stain, and the primers MatK-1RKIM-f and MatK-3FKIM-r were from Thermo Fisher Scientific (Runcorn, Cheshire, UK). Agarose MB 250 was purchased from Biotools Biotechnological and Medical Laboratories SA (Madrid, Spain). Apigenin-7-glucoside, epigallocatechin, and tiliroside were acquired from Extrasynthese (Genay Cedex, France). Acetoxy acid was from Sigma-Aldrich (St. Louis, MO, USA), and valerenic acid was from Chromadex (Irvine, CA, USA).

3.2. Herbal Products

The 33 samples of the four medicinal plants *Matricaria recutita* L., *Valeriana officinalis* L., *Tilia* spp., and *Camellia sinensis* (L.) Kuntze were acquired from supermarkets, herbal shops, and pharmacies located in the Autonomous Community of Madrid (Spain). Particularly, nine of these samples were from *M. recutita* flowers (3 samples from pharmacies, 3 samples from herbal shops, and 3 samples from supermarkets), seven from *V. officinalis* root (3 samples from pharmacies, 3 samples from herbal shops, and 1 sample from supermarkets), nine from *Tilia* spp. leaves (3 samples from pharmacies, 3 samples from herbal shops, and 3 samples from supermarkets), and eight from *C. sinensis* leaves (3 samples from pharmacies, 3 samples from herbal shops, and 2 samples from supermarkets) (Table 6). They were stored in conditions of temperature and ambient humidity.

Table 6. Samples of the medicinal plants chamomile, valerian, tea, and linden acquired from supermarkets, herbal shops, and pharmacies located in the Autonomous Community of Madrid.

	Chamomile			**Valerian**	
Pharmacies	**Herbal Shops**	**Supermarkets**	**Pharmacies**	**Herbal Shops**	**Supermarkets**
MF1	MH1	MS1	VF1	VH1	VS1
MF2	MH2	MS2	VF2	VH2	
MF3	MH3	MS3	VF3	VH3	
	Linden			**Tea**	
Pharmacies	**Herbal Shops**	**Supermarkets**	**Pharmacies**	**Herbal Shops**	**Supermarkets**
TF1	TH1	TS1	CF1	CH1	CS1
TF2	TH2	TS2	CF2	CH2	CS2
TF3	TH3	TS3	CF3	CH3	

3.3. DNA Barcoding Analysis

3.3.1. DNA Extraction

DNA was extracted from market medicinal plants samples of roots (*V. officinalis*), leaves (*Tilia* spp. and *C. sinensis*), and flowers (*M. recutita*) using the Speed Tools tissue DNA extraction kit Biotools Biotechnological and Medical Laboratories following manufacturer's instructions. Approximately, 100 mg of samples were pulverized with a sterile mortar in liquid nitrogen at room temperature and secondary metabolites were eliminated with methanol before starting DNA extraction. Then, samples were soaked in methanol for 2 h to remove potential secondary metabolites and dried overnight. Later, samples were incubated into lysis buffer initially at 65 °C for 30 min. In addition, root material samples were kept at room temperature overnight. After DNA extraction, samples were revealed in a 1% agarose gel stained with SYBR safe to check DNA quality [18].

3.3.2. PCR and Sequencing

Matk was chosen in this study, since it is one of the universal DNA barcode markers for land plants [25]. The matK gene of chloroplast is 1500 bp long, located within the intron of the trnK. Since this gene is larger in length, a fragment of this is used for DNA barcoding analysis. Further, the gene contains high substitution rates within the species and is a potential candidate for DNA barcoding studies [11]. PCR amplifications of *matk* were performed using specific primers MatK-1RKIM-f and MatK-3FKIM-r (Ki-Joong Kim, pers. comm.). The reaction mixture (25 µL final volume) contained 5 µL of DNA (1:10), 4.5 µL sterile water, 12.5 µL REDTaq ReadyMix PCR Reaction M, and 1.5 µL of each primer (forward and reverse) at 10 µM. PCR amplifications were carried out in a Techne R TC-3000 thermal cycler with the following conditions: one initial heating step of 45 s at 98 °C, followed by 35 cycles of 10 s at 98 °C, 30 s at 54 °C, and 40 s at 72 °C. A final extension step of 10 min at 72 °C was added, after which samples were kept at 4 °C.

Once PCR amplification is completed, a 1% agarose gel stained with SYBR safe was used to visualize DNA. PCR products were purified by Speed-Tools PCR Clean Up (Biotools Biotechnological and Medical Laboratories SA, Madrid, Spain) kit following the manufacturer's instructions, and sequencing was performed with labelling using BigDye Terminator v. 3.1 Kit (Applied Biosystems, Madrid, Spain) as follows: 35 cycles of 20 sec at 94 °C, 20 sec at 48 °C, and 4 min at 60 °C. Sequences were obtained in an ABI PRISM 3130 Genetic Analyzer (Life Technologies, Alcobendas, Madrid, Spain). This is a standard approach to analyze PCR products.

3.3.3. Data Analysis

First, DNA sequences were assembled and manually adjusted in BioEdit sequence alignment editor software (v 7.2). Then, a second edition and assembly of the sequence fragments was made with the program SeqMan v.7 (Lasergene R, DNASTAR, Madison, WI, USA). Sequence identity was assessed using the mega-BLAST search function in GenBank [45,46]. Sequences with equal and above 95% similarity were downloaded from the GenBank and aligned with the newly generated sequences for this study. Separate dataset for each taxa viz. *M. recutita*, *V. officinalis*, *Tilia* spp., and *C. sinensis* were prepared for phylogenetic analysis. Each dataset was aligned using MAFFT v.7 [47] implementing the G-INS-I alignment algorithm, "1PAM/K = 2" scoring matrix with an offset value of 0.0, and the remaining parameters set to default values. The ambiguous regions in the *matk* alignment were assessed and removed using the least stringent option in Gblocks v.0.91b [48]. The alignments were analyzed using the maximum likelihood approach with RAxML v.8.2.6 program [49] as implemented on the CIPRES Web Portal, with the GTRGAMMA model. Nodal support was evaluated using 1000 bootstrap pseudoreplicates. The maximum likelihood (ML) trees were mid-point rooted. Phylogenetic trees were drawn using FigTree v.1.4.2 [50]. Moreover, sample identification was also assessed using a genetic distance-based blast option on the Barcode of Life Data System (BOLD Systems v3) [51]. The default setting was used for BLAST algorithm of the standard BOLD identification engine for *matK* sequences.

3.4. UHPLC-MS/MS Analysis

Samples were previously pulverized with a mortar and pestle sterile. Thirty milligrams of each medicinal plant samples were dissolved in 1 mL of ethanol/water 70/30% (*v/v*). Then, chamomile, linden, and tea samples were diluted 1:5 or 1:500, and valerian samples were diluted 1:4. HPLC standards were prepared at a concentration of 20 mg/L in methanol HPLC grade. Dilutions were prepared in a range of 0.05 to 1 mg/L in ethanol/water 70/30% (*v/v*) [52,53].

An ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry technique (UHPLC-QqQ-MS/MS) was developed using a LC-QQQ 8030 equipment (Shimazdu, Tokyo, Japan). The column was Phenomenex Gemini 5u C18 110A, 150 × 2 mm (Phenomenex, Alcobendas, Spain). The gradient mode was 7 min 5–95% Phase B; 8 min 95% Phase B; 8.5 min 5% Phase B using acetonitrile; in Phase A 0.1% formic acid in water. The flow rate was 0.5 mL/min and the infection volume was 10 µl for all medicinal plant samples, except for valerian, which was an injection volume of 20 µL.

Regarding LC-MS analysis, the mass spectrometer electrospray capillary voltage was maintained at 4.0 kV and the drying gas temperature at 250 °C with a flow rate of 15 mL/min. Nebulizer working flow was set at 1.5 mL/min. Nitrogen was used as both nebulizing and drying gas. Detection was carried out in Multiple reaction monitoring (MRM) mode, with a dwell time of 100 ms by monitoring three selective transitions for each parent compound. MRM transitions and their collision energy (CE) are shown in Table 7. The sample injection volume was 10 µL.

Data are expressed as means ± standard deviations of triplicate independent analyses.

Table 7. Multiple reaction monitoring (MRM) transitions and t collision energy conditions.

Active Compound	Transition	CE	Dwell (msec)
Acetoxyalerenic acid	291.3 > 59.00	27 V	100
	291.3 > 249.05	17 V	100
Valerenic acid	233.2 > 40.92	22 V	100
	233.2 > 84.20	25 V	100
Epigallocatechin	457 > 169.05	18 V	100
	457 > 125.00	45 V	100
Tiliroside	592.8 > 284.95	33 V	100
	592.8 > 254.95	55V	100
Apigenin-7-*O*-glucoside	431.3 > 267.90	35 V	100
	431.3 > 150.95	52 V	100

4. Conclusions

DNA barcoding is revealed as an effective and necessary tool in the identification and authentication of plant species that are used in therapeutics. Since DNA barcoding does not provide qualitative or quantitative information on the metabolites of the plant raw material, its use together with chromatographic techniques such as HPLC-MS allows us to determine the precise identification of the species and the metabolic profile with great sensitivity and precision. The HPLC-MS technique combines the high resolution of ultra-high-performance liquid chromatography with high-resolution MS for molecular quantification. This technique has shown a high efficiency and resolution, a short time analysis, and increased sensitivity. The current work showed a high success rate in obtaining PCR amplification and sequencing of *matk* locus for the accurate sample identification of the market samples of medicinal plants. DNA barcoding demonstrated that the labeling of some medicinal plants acquired in different distribution channels is incorrect, demonstrating the need to apply DNA barcoding methods in the quality control of herbal products to ensure correct botanical identification and therefore ensure product quality. Moreover, the UHPLC-MS analysis displayed that all the analyzed samples presented the bioactive compounds (apigenin-7-glucoside, acetoxy valerenic acid, valerenic acid, epigallocatechin, and tiliroside) responsible for the pharmacological activity. This content was very variable for some plant species, as is the case of valerenic acid in valerian. In addition, none of the analyzed samples (except for valerenic acid in VF3) met the minimum content of these active principles according to the European Medicines Agency and the Real Spanish Pharmacopeia. This study shows the need to incorporate leading-edge molecular and analytical techniques for the quality control of plant species that are used in therapeutics to guarantee patient safety.

Future trends in the field of medicinal plants should be aimed at conducting more quality control studies in other widely consumed species and incorporating the combination of these molecular and analytical techniques in the plant industry for therapeutic purposes and even incorporating them into official documents (Pharmacopoeia, EMA).

Supplementary Materials: The following are available online at http://www.mdpi.com/2223-7747/9/11/1601/s1, Figure S1: Phylogenetic tress for *M. recutita.* Figure S2: Phylogenetic tress for *V. officinalis.* Figure S3: Phylogenetic tress for *Tilia* spp. Figure S4: Phylogenetic tress for *C. sinensis.* Figure S5: BOLD System for *M. recutita.* Figure S6: BOLD System for *V. officinalis.* Figure S7: BOLD System for *Tilia* spp. Figure S8: BOLD System for *C. sinensis.*

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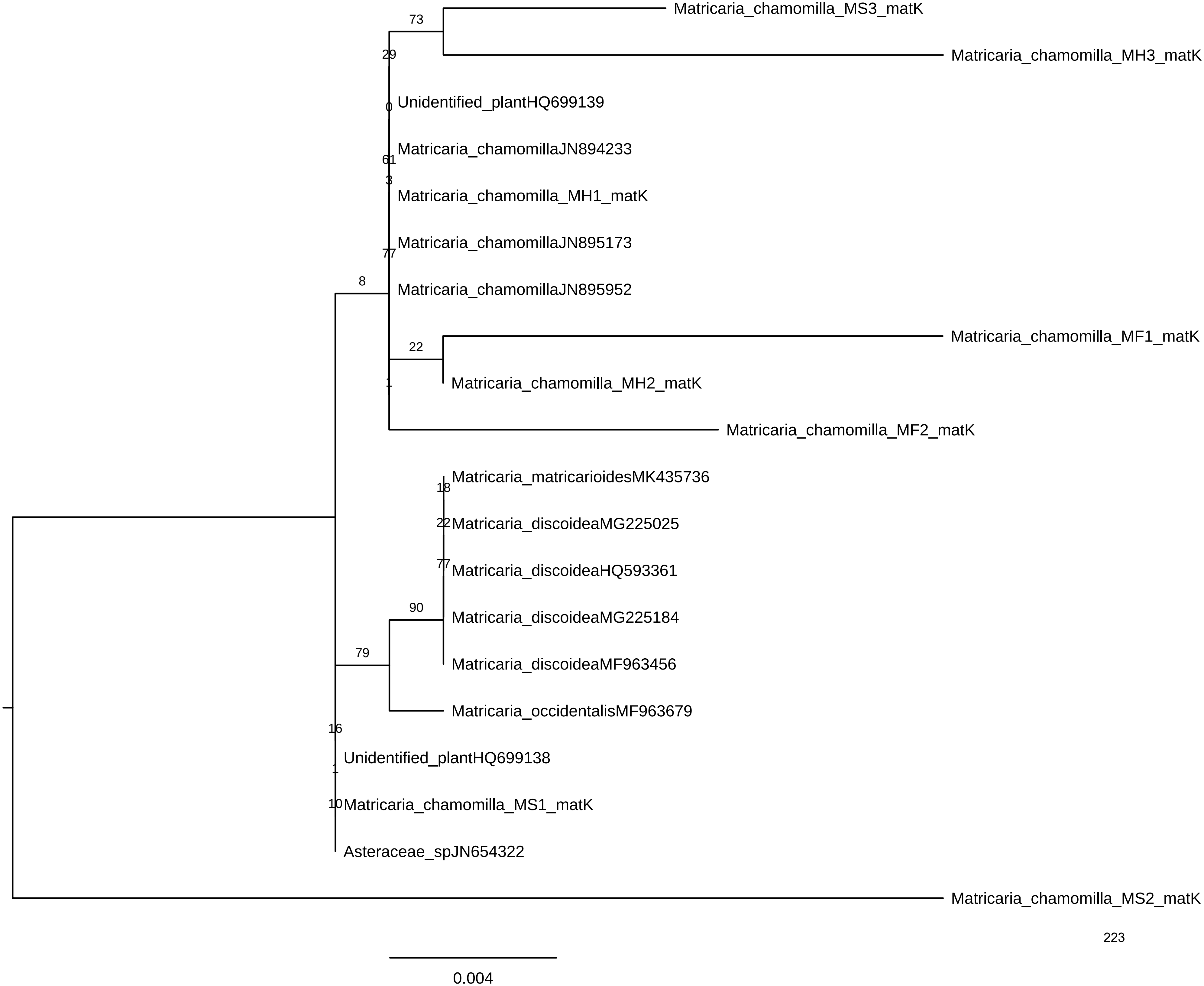
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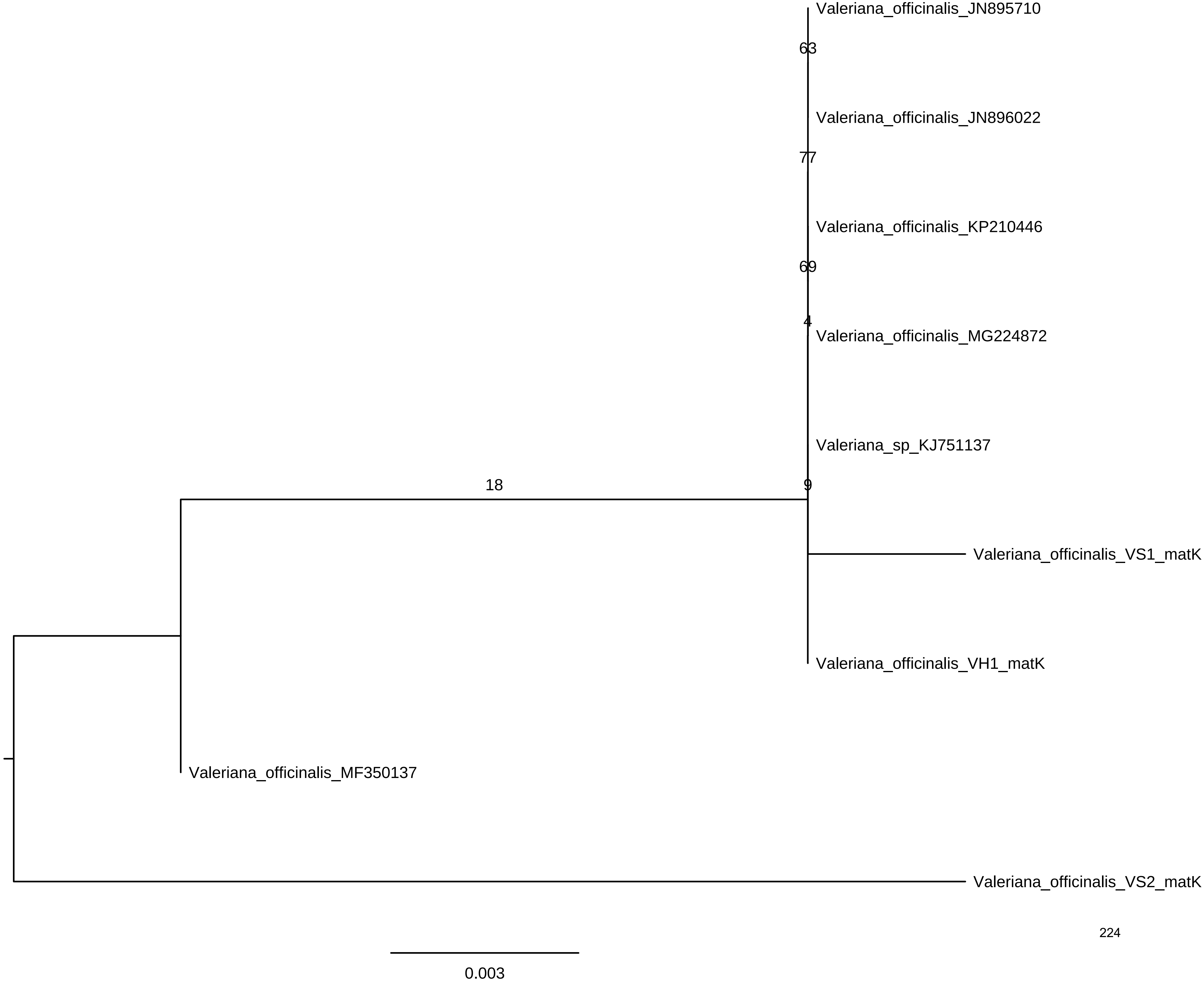
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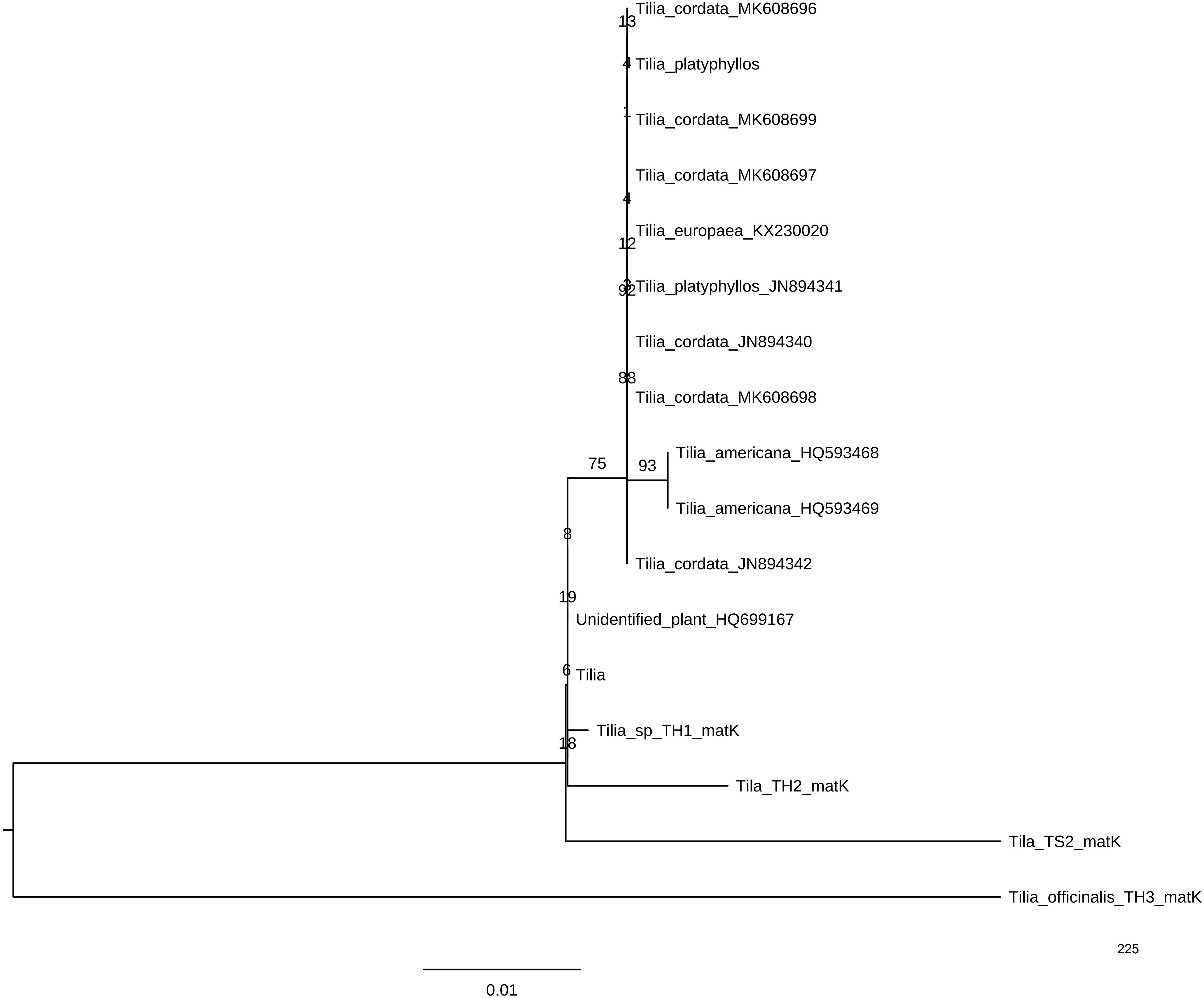
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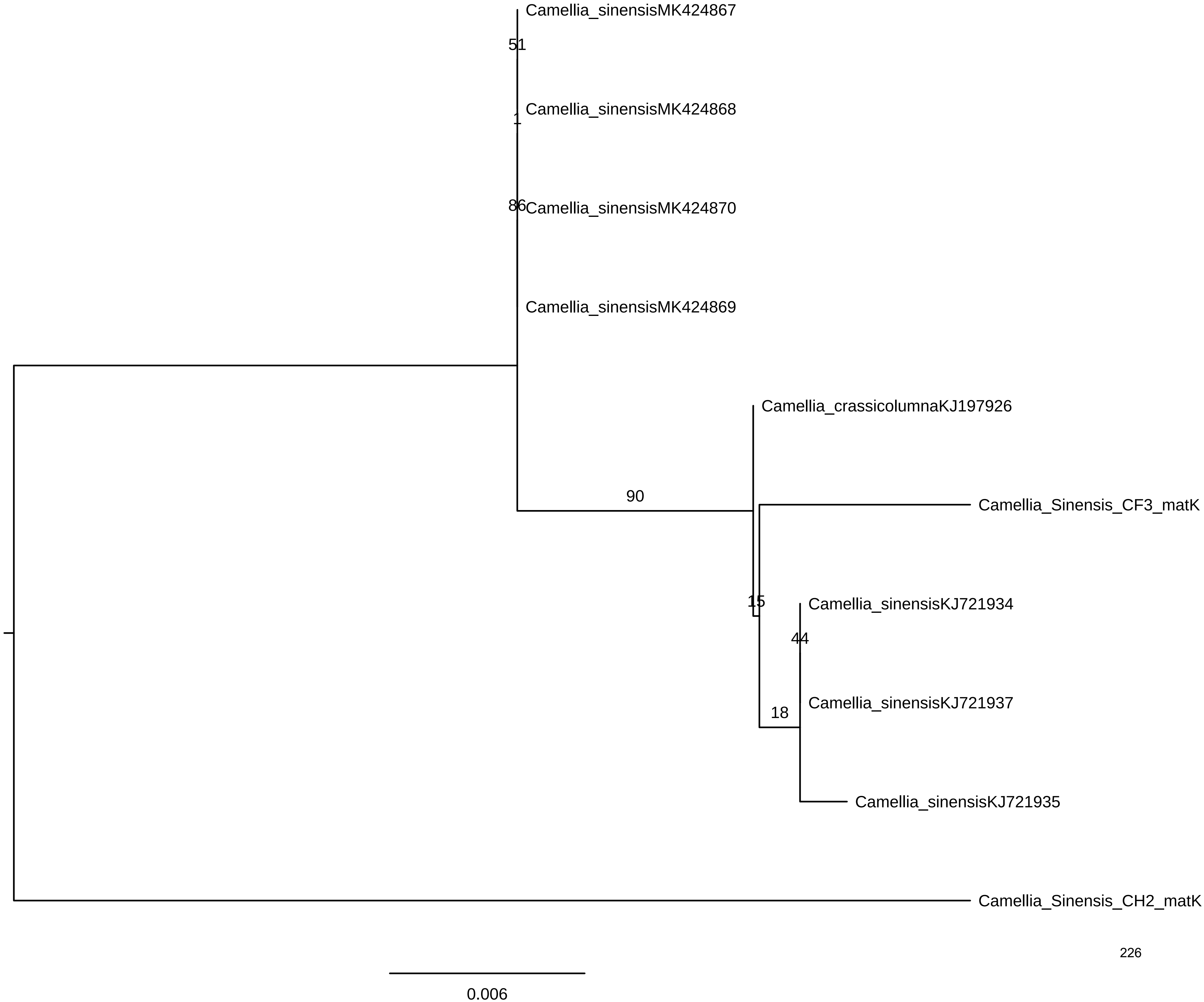
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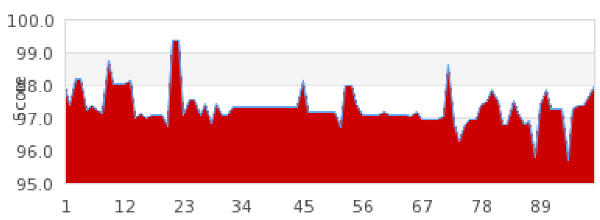
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

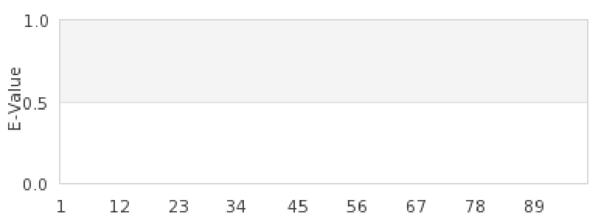
Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

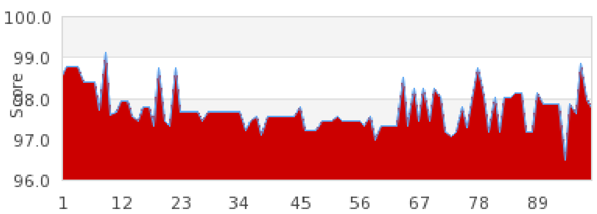
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

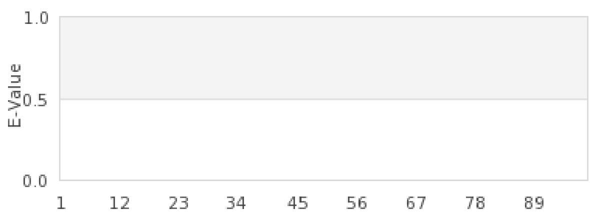
Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



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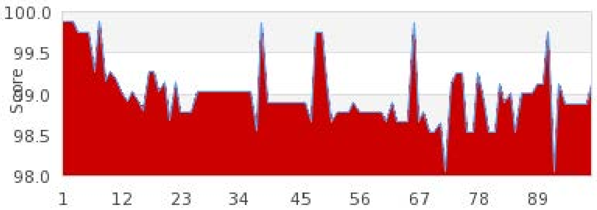
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	**Best ID**	**Search DB**
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



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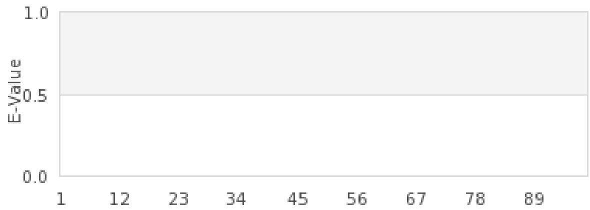
E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

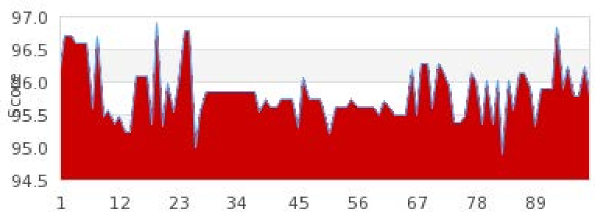
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

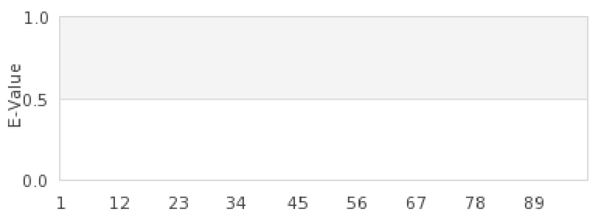
Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

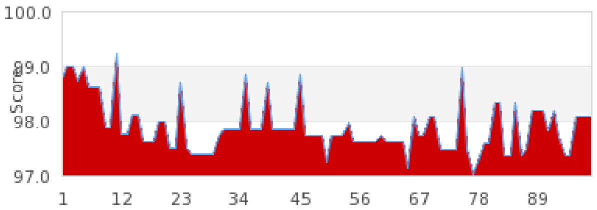
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



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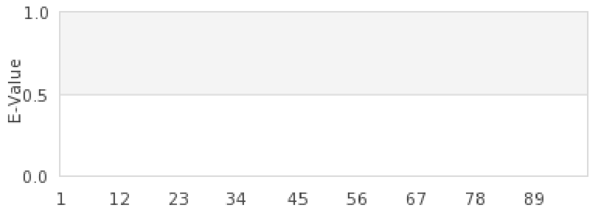

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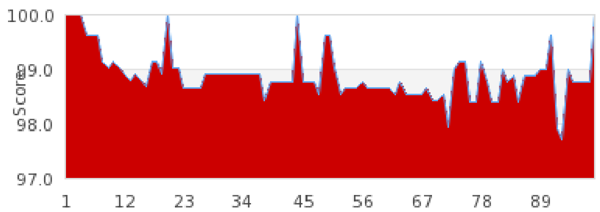
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

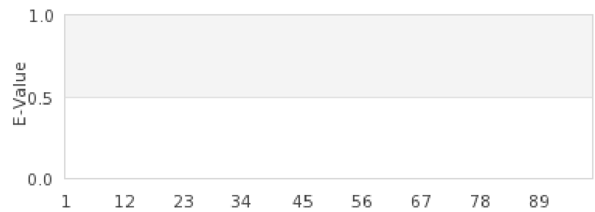
Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

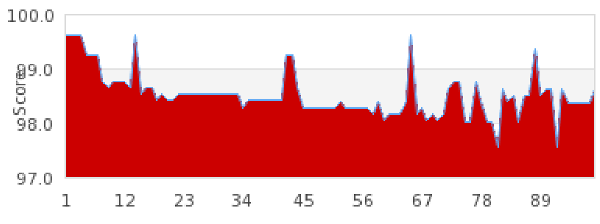
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



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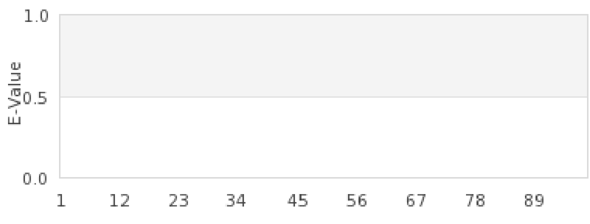


E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

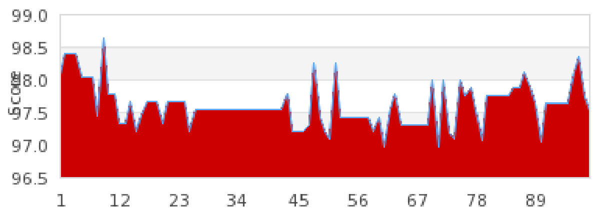
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Matricaria recutita*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Asterales - *Matricaria recutita*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

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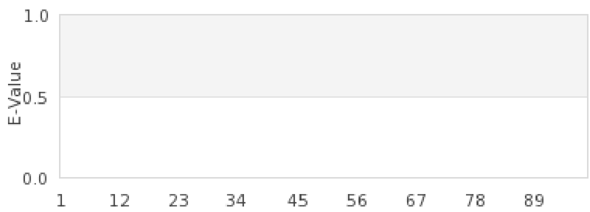


E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

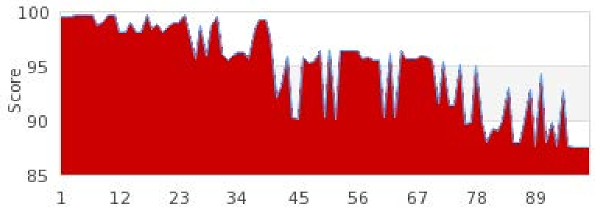
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	**Best ID**	**Search DB**
unlabeled_sequence	*Valeriana hirtella*	MATK_RBCL

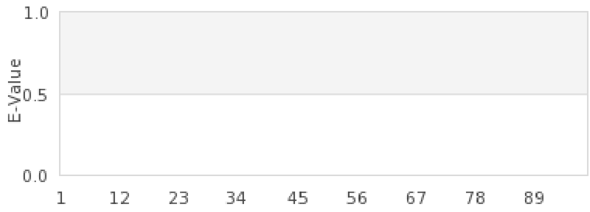
Query: unlabeled_sequence
Top Hit: Dipsacales - *Valeriana hirtella*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

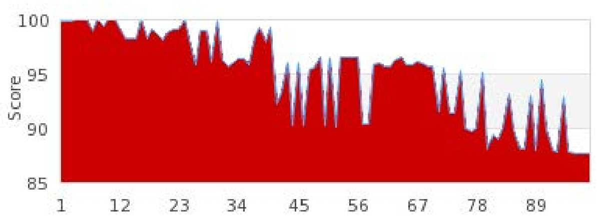
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Valeriana hirtella*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Dipsacales - *Valeriana hirtella*

Score Summary



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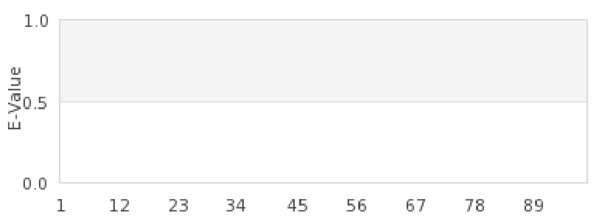
E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

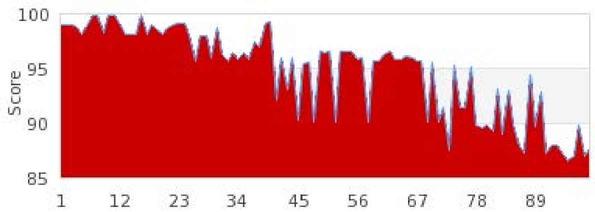
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Valeriana hirtella*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Dipsacales - *Valeriana hirtella*

Score Summary



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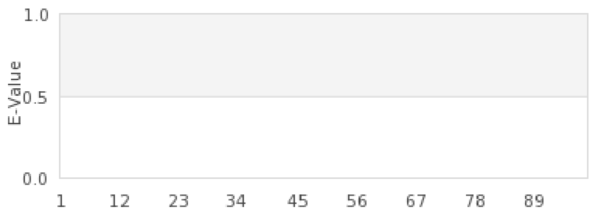


E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

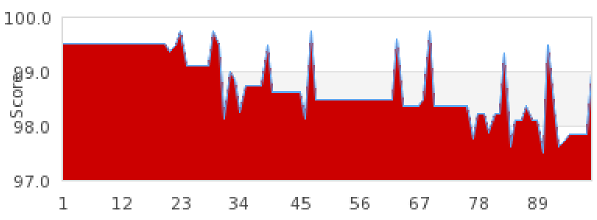
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	**Best ID**	**Search DB**
unlabeled_sequence	*Tilia cordata*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Malvales - *Tilia cordata*

Score Summary



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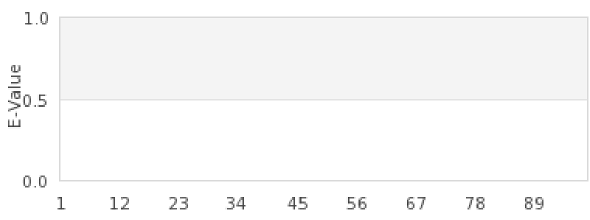
E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

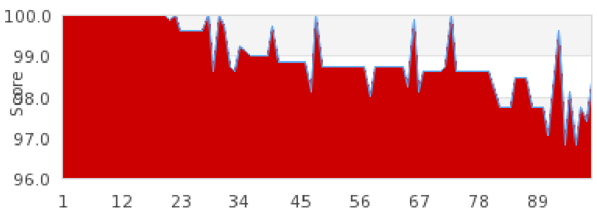
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Tilia cordata*	MATK_RBCL

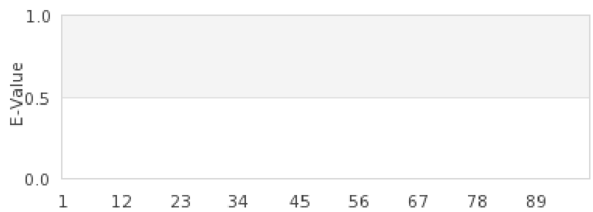
Query: unlabeled_sequence
Top Hit: Malvales - *Tilia cordata*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

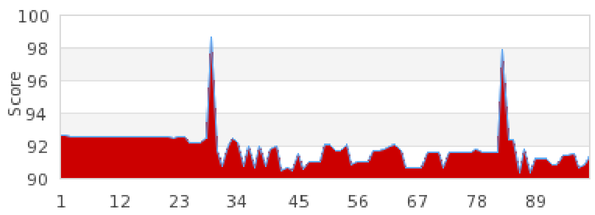
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Tilia cordata*	MATK_RBCL

Query: unlabeled_sequence
Top Hit: Malvales - *Tilia cordata*

Score Summary



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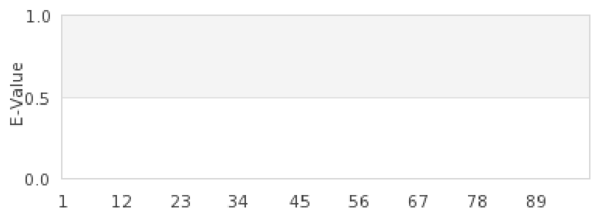
E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

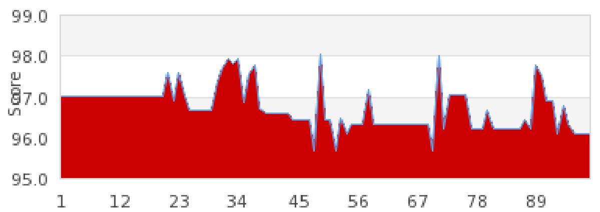
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Malvales*	MATK_RBCL

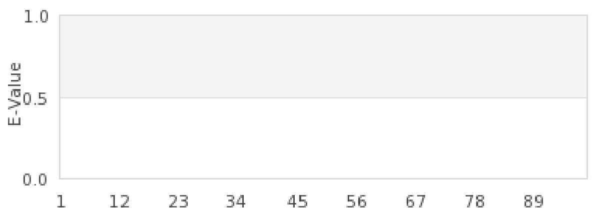
Query: unlabeled_sequence
Top Hit: Malvales

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

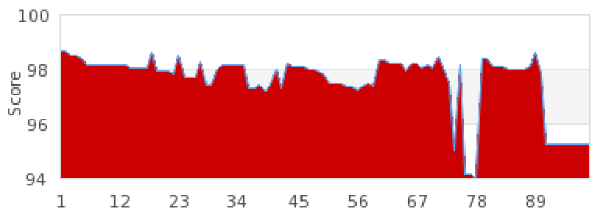
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Camellia sinensis*	MATK_RBCL

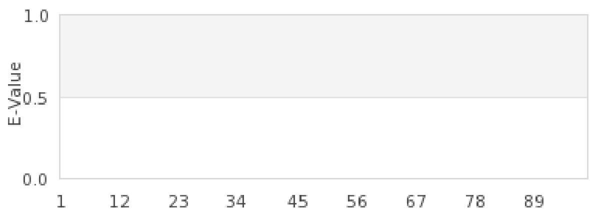
Query: unlabeled_sequence
Top Hit: Ericales - *Camellia sinensis*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

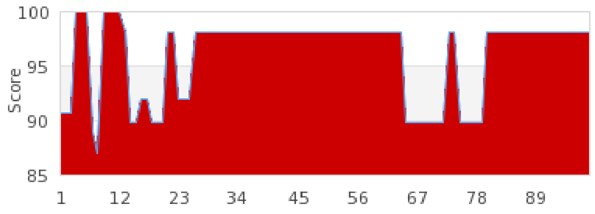
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	**Best ID**	**Search DB**
unlabeled_sequence	*Blepharocalyx tweediei*	MATK_RBCL

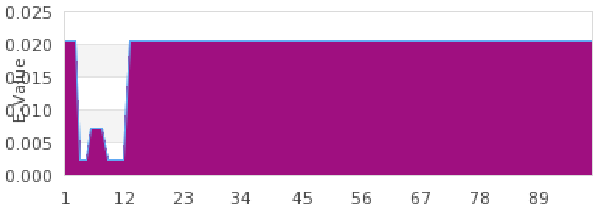
Query: unlabeled_sequence
Top Hit: Myrtales - *Blepharocalyx tweediei*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

243

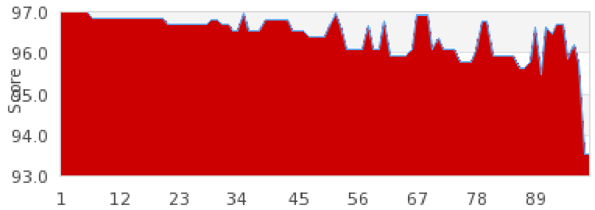
IDENTIFICATION ENGINE: RESULTS

Results Summary

Query ID	Best ID	Search DB
unlabeled_sequence	*Camellia sinensis*	MATK_RBCL

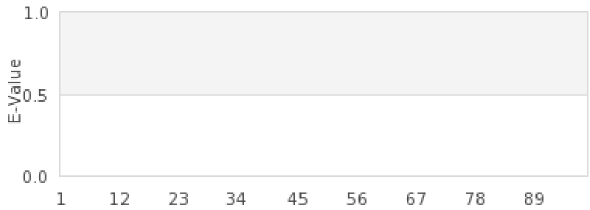
Query: unlabeled_sequence
Top Hit: Ericales - *Camellia sinensis*

Score Summary



Scores indicate the degree of similarity between the query sequence and hits. Higher is better.

E-Value Summary



E-Values are an indicator of the likelihood that a given match was generated randomly. Lower is better.

IX. DISCUSIÓN GENERAL

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Las plantas medicinales se utilizan en diferentes culturas del mundo como medicamentos fitoterapéuticos o como productos fitoterápicos en base al saber popular o al conocimiento científico. Desde la década de los 70, la Organización Mundial de la Salud (OMS) reconoce el valor de las plantas medicinales como importante recurso terapéutico, y ha venido alentando, principalmente a los países ricos en biodiversidad a que elaboren políticas que permitan el desarrollo de productos de calidad para su uso en la salud pública internacional. Las plantas medicinales han demostrado ser esenciales para la salud humana como fuente de moléculas con potencial terapéutico y base para la obtención de nuevos medicamentos. En el año 1978, la OMS declaró que las plantas medicinales son "fuente inagotable de nuevos fármacos". Cobran así especial relevancia por contener diversidad de compuestos activos con diferentes propiedades farmacológicas, lo que ha promovido e incentivado la realización de estudios para identificar y validar cada vez más especies medicinales (World Health Organization, 2013).

La Organización Mundial de la Salud, en su documento "Estrategia de la OMS sobre medicina tradicional. 2014-2023", establece la forma en que se utilizan las medicinas tradicionales (World Health Organization, 2013):

1) *Utilización en países en los que la medicina tradicional es una de las principales prácticas de atención de salud*. Poseen acceso limitado a los servicios de salud y medicina convencional; el acceso a la medicina tradicional es muy asequible. Por ejemplo, en África existe 1 curandero por cada 500 habitantes mientras que la ratio de médicos por habitante es de 1:40.000. Por tanto, los curanderos son fuente directa de atención sanitaria para millones de personas que habitan en zonas rurales.

2) *Utilización de medicina tradicional por influencias culturales e históricas*. En este contexto se incluyen países que, a pesar de disponer de un buen sistema convencional de atención de salud, siguen recurriendo a la medicina tradicional como Singapur (76% de la población) y República de Corea (86% de la población).

3) *Utilización de la medicina tradicional como terapia complementaria*. En esta situación se incluyen a la mayoría de los países europeos y Estados Unidos (países desarrollados con un sistema de salud afianzado).

En este sentido, el informe anual de la OMS publicado en 2019, en el seno de la 72ª Asamblea Mundial de la Salud, analiza la situación de la aplicación de la "Estrategia sobre la medicina tradicional 2014-2023" en diferentes países del mundo. Cabe destacar que el número de países que disponen de marco jurídico y reglamentación de la medicina tradicional y complementaria ha aumentado de 79 (año 2012) a 109 (año 2018), y continúa en aumento de forma gradual. En este informe destaca también, como logro importante, la inclusión de un capítulo sobre medicina tradicional en la Undécima Revisión de la Clasificación Estadística Internacional de Enfermedades y Problemas de Salud Conexos (CIE-11. Clasificación Internacional de Enfermedades de la OMS). Además, en la edición de 2018 de la Lista Mundial de Referencia se incluyen también 100 Indicadores Sanitarios Básicos y Objetivos de Desarrollo Sostenible (ODS) relacionados con la salud. El informe concluye que la OMS seguirá fomentando el uso seguro y eficaz de la medicina tradicional, complementaria e integral a través de la reglamentación, la investigación y la integración de los productos, prácticas y profesionales en los sistemas de salud existentes. Todo ello permitirá sentar las bases para incluir el uso de plantas medicinales en las estadísticas de salud (OMS, 2019).

Los datos de consumo de plantas medicinales a nivel mundial muestran que, en China, el 30%-50% de los medicamentos consumidos derivan de plantas. En Europa, Alemania es el país donde más se emplean las medicinas naturales (90% de la población *versus* el 50% de la población en otros países del continente europeo). En Estados unidos, el 17,9% de los adultos manifestaron consumir suplementos de plantas y en Canadá, el 70% de la población los ha utilizado al menos una vez. Si nos referimos a países en vías de desarrollo, alrededor del 80% de la población depende de las plantas para su atención primaria de salud (Lazarou & Heinrich, 2019; Coutinho Moraes et al., 2015; Bardia et al., 2007).

Hoy en día los consumidores perciben las plantas medicinales como un adyuvante o una forma más suave y holística de hacer frente a problemas de salud crónicos y otras enfermedades autolimitantes. Esta percepción es paralela a los avances en los enfoques ómicos y la farmacología de red, que han permitido que las propiedades sinérgicas de los productos a base de plantas se liberen de la etiqueta de "curandero", adquiriendo evidencias científicas firmes. El resultado es que el uso de las plantas medicinales está aumentando en todo el mundo, principalmente en América del Norte y Europa, y su mercado crece debido, entre otros factores, al interés de los consumidores por su salud y bienestar (Vargas-Murga et al., 2011). Además, el aumento de la migración en todo el mundo ha ayudado a difundir los conocimientos tradicionales, dando lugar a la transferencia de medicinas e información médica de una cultura a otra (por ejemplo, de la medicina ayurvédica y la medicina tradicional china a países occidentales) (Lazarou & Heinrich, 2019; Coulter & Willis, 2004).

El potencial valor de las plantas medicinales en la búsqueda de nuevos medicamentos es una de las principales razones que justifican su investigación. Se estima que menos del 10% de las especies de angiospermas existentes en el mundo han sido evaluadas para determinar su composición química y sus propiedades farmacológicas. En cuanto a los constituyentes químicos con utilidad en terapéutica, la Administración de Medicamentos y Alimentos (*Food and Drug Administration, FDA*) aprobó 1562 nuevas moléculas, entre los años 1981 y 2014, de las cuáles más de la mitad derivaban de compuestos naturales (Newman & Cragg, 2016).

En algunos países se han desarrollado programas de prospección para investigar la actividad farmacológica de las especies vegetales y sus componentes activos, tales como el Convenio Merck-INBio en Costa Rica para especies tropicales, el programa de búsqueda de compuestos activos contra el Cáncer y el SIDA del Instituto Nacional del Cáncer en Estados Unidos y, el proyecto de prospección bioquímica del bosque tropical de Yutajé, en Venezuela, entre otros (Burton & Evans-Illidge, 2014, Cragg et al., 2013).

En los programas de descubrimiento de fármacos a partir de productos naturales es necesario tener en cuenta aspectos relacionados con la recolección; en muchos casos se trata de especies silvestres, siendo necesario proteger los lugares de recolección para evitar su desaparición. También hay que considerar que la composición química puede variar por distintos factores como el estadio de crecimiento del vegetal en el momento de la recolección y otras cuestiones relacionadas con aspectos climáticos y edáficos. Además, por cuestiones legales relacionadas con las patentes se debe demostrar que existe una notable diferencia entre el material o fenómeno natural conocido y la realización de ensayos clínicos que deben ser rigurosos (David et al., 2015).

Por último, destacar la existencia de importante información etnofarmacológica sobre uso tradicional de plantas medicinales por distintas poblaciones a través de los años que puede ayudar en la búsqueda de compuestos con actividad farmacológica, facilitando el descubrimiento de nuevos compuestos, que serán posteriormente estudiados (Sammons et al., 2016; AlBedah et al., 2013; Abdullahi, 2011).

El creciente interés en los productos naturales en los últimos años se refleja en indicadores de investigación. Así, si se revisan bases de datos científicas se observa un significativo aumento en el número de estudios de investigación. Por ejemplo, desde comienzos de los años 80, el número de publicaciones sobre plantas medicinales ha aumentado hasta en 10^4. Las investigaciones más recientes sobre uso de plantas medicinales se centran en estudiar, analizar y dar respuesta a preguntas como ¿cuándo y por qué las personas recurren al empleo de plantas medicinales como opción terapéutica? y ¿cuáles son sus resultados y/o beneficios?

CONSUMO DE PLANTAS MEDICINALES

Las encuestas sobre consumo de plantas medicinales en Europa y Estados Unidos son escasas y, en muchos casos, presentan limitaciones metodológicas. Los datos disponibles muestran patrones de uso y consumo variables entre países, y dentro de ellos, en función de diversos factores como cultura, importancia histórica y legislación o reglamentos existentes (García-Álvarez et al., 2014; Baulies Romero et al., 2011).

La escasez de datos se ve aún más limitada por el hecho de que las plantas medicinales se presentan en diferentes formas como alimentos (tés y zumos), suplementos alimenticios y productos medicinales a base de plantas, entre otros. Estas categorías de productos, en función del uso, están reguladas por una legislación específica que difiere entre los distintos países de la Unión Europea. Así, algunos países regulan el uso de productos a base de plantas (incluyendo listas negativas y positivas), otros aplican condiciones específicas de uso (incluidos niveles máximos de uso o advertencias para el consumidor) y, en otros existen requisitos menos específicos. Una complejidad añadida radica en la aplicación del "principio básico europeo de reconocimiento mutuo", en virtud del cual cualquier producto que se comercialice legalmente en un Estado Miembro puede venderse en los 27 Estados Miembros.

Actualmente, el estudio más amplio realizado sobre consumo de plantas es la encuesta de consumidores PlantLIBRA que recoge información sobre pautas de uso de los consumidores de productos y suplementos a base de plantas en seis países europeos. En este estudio se han identificado un total de 941 plantas medicinales que forman parte de complementos alimenticios a base de plantas como ginkgo, aloe, valeriana, alcachofa y melisa, entre otros. La encuesta pone de relieve la complejidad de la medición del consumo de estos productos, en particular a nivel europeo, indicando que la incorporación de parámetros de medida del consumo de plantas medicinales en las encuestas nacionales sobre alimentación podría proporcionar datos de interés para realizar evaluaciones exhaustivas de los riesgos y beneficios de este consumo (García-Álvarez et al., 2014)

Como ya hemos comentado anteriormente, hay un aumento en el empleo de especies vegetales para tratar problemas de salud en países occidentales. Estos estudios sobre consumo de plantas se centran en segmentos o grupos concretos de la sociedad, quedando patente la siguiente cuestión ¿Cómo utilizan estos recursos la población en general?

Las razones por las que la población tiende a utilizar las plantas medicinales son comunes en la mayoría de los casos; sin embargo, se han identificado diferencias entre países y regiones. Así, los motivos por los que la población recurre al empleo de plantas medicinales incluyen mayor demanda de los servicios de salud, deseo de obtener más información para aumentar los conocimientos sobre opciones disponibles en el tratamiento de sus dolencias, creciente insatisfacción con los existentes servicios de atención de salud, y renovado interés por la atención integral de la persona y la prevención de enfermedades (Craft et al., 2015; Ritchie, 2007). Así, por ejemplo, un estudio realizado en el *Royal London Hospital for Integrated Medicine* sobre causas por las que los pacientes recurren al empleo de plantas medicinales reveló que se debía principalmente a que otros tratamientos no habían sido eficaces, por preferencia personal o cultural o bien porque habían experimentado efectos adversos con otros tratamientos. En Australia, los principales motivos de empleo de medicina tradicional o complementaria fueron el fracaso de tratamientos de medicina convencional y el deseo de llevar un modo de vida sano (Oliver, 2013).

Entre los estudios más recientes sobre uso de plantas medicinales destaca el realizado en Reino Unido en el que participaron un total de 157 personas (87% entre 45 y 64 años, y 13% >65 años). El 80% de los participantes manifestaron utilizar plantas medicinales para tratar problemas de salud (74% como protección de la salud, 38% para prevenir enfermedades y 49% para tratar alguna patología). Además, el 95% de los encuestados confiaba en las propiedades medicinales de las plantas. En cuanto a las fuentes de información sobre plantas medicinales destacaban los libros (57%) seguido de Internet (53%), amigos, compañeros de trabajo o vecinos (51%) y profesionales de la salud (42%). Asimismo, más de la mitad de los participantes consideraban que las plantas eran seguras (51%) y que tenían menos efectos secundarios que las medicinas convencionales (55%). Por último, el 24% de los usuarios de plantas medicinales informaron a su médico, y la mayoría de los profesionales médicos informados aceptaron su uso (47%). Esta encuesta piloto proporciona información actualizada sobre uso de medicinas a base de plantas medicinales por parte de la población general y las actitudes de los profesionales de la salud en el Reino Unido (Zahn et al., 2019).

En España, la mayoría de los estudios sobre plantas medicinales se basan principalmente en su uso tradicional. Estos trabajos se centran en la relación planta-ser humano profundizando en los remedios a base de plantas medicinales que se utilizan tradicionalmente para tratar alteraciones del estado de salud. Por el contrario, y en comparación con otros países europeos y Estados Unidos, la información disponible en España sobre perspectivas y usos actuales de las plantas medicinales es muy limitada (McBride et al., 2020; Vinagre et al., 2019; Rivera el al., 2019; Calvo & Cavero, 2016; Devesa et al., 2004). Destaca el estudio realizado por el Centro de Investigación sobre Fitoterapia (INFITO), en el año 2015, que recoge que 7 de cada 10 españoles utilizan preparados de plantas medicinales para prevenir o tratar afecciones, siendo las más utilizadas las que se emplean para tratar el insomnio como valeriana, pasiflora y amapola de California (Infito, 2015).

Sobre la base de lo anteriormente expuesto, el primer objetivo de esta Tesis Doctoral ha sido estudiar el consumo y empleo de plantas medicinales por la población de la Comunidad de Madrid, analizando el conocimiento, percepciones y actitudes e incluyendo información relativa a la identificación de riesgos y precauciones asociadas a su consumo concomitante con medicamentos convencionales.

La Comunidad Autónoma de Madrid es la más densamente poblada de España (676 habitantes por km^2). La mayor parte de la población se concentra en la ciudad de Madrid y en las zonas metropolitanas circundantes. Incluso las zonas rurales tienen su referencia en la vida urbana. La población es muy diversa en su origen, cultura y nivel socioeconómico. En el estudio realizado en esta Tesis Doctoral se ha intentado representar localidades con diferentes entornos sociales. Para determinar si la muestra encuestada era representativa, se analizaron los últimos datos estadísticos relacionados con sexo, edad y ocupación que se encuentran disponibles en la página web del Instituto de Estadística/Ciencias Sociales (http://www.madrid.org/iestadis/).

En nuestro trabajo, el consumo de plantas medicinales fue mayor que el estimado para otras ciudades españolas (Batanero-Hernán et al., 2017). Los encuestados usan plantas medicinales con fines terapéuticos principalmente por su fácil acceso y la percepción de eficacia y seguridad. Los resultados del estudio muestran que el perfil más común de consumidor de plantas medicinales es el de una mujer joven, entre 18-44 años, con estudios superiores. Esta alta prevalencia en la preferencia de las plantas medicinales por usuarios con niveles educativos altos y, por el género femenino también se ha confirmado en trabajos anteriores (Hitl et al., 2019; Bhamra et al., 2017; Ceutericket al., 2008). Además, el hecho de que las encuestas se hayan realizado principalmente en establecimientos sanitarios explica que el número de encuestados sea predominantemente mujeres ya que en España la visita a estos lugares es más frecuente por parte del género femenino. En cuanto a los rangos de edad, los consumidores más habituales son aquellos con edades entre los 18 y los 44 años, seguidos de los de 45 a 64 años y, por último, los mayores de 65 años. Esta tendencia creciente de utilizar productos naturales para lograr un estilo de vida saludable, especialmente entre la población más joven y con un nivel de educación más elevado, se ha puesto ya de manifiesto en estudios previos (Vinagre et al., 2019; Zahn et al., 2019;). Por el contrario, este patrón contrasta con otros estudios realizados en otras partes de Europa, donde la frecuencia de uso es mayor en personas mayores que en jóvenes (Zahn et al., 2019; Raal et al., 2013) o con estudios de Estados Unidos en los que el consumo de plantas medicinales es más frecuente en personas de edad intermedia (Živković et al., 2020; Bent, 2008).

Entre las limitaciones identificadas en trabajos publicados sobre prevalencia del consumo de plantas medicinales se incluye que el concepto de planta medicinal no está bien definido, y que se suele proporcionar una lista cerrada de plantas medicinales, limitando el conocimiento de sus usos y la posibilidad de ampliar a aquellas referidas por el consumidor (Živković et al., 2020; Lazarou & Heinrich, 2019). En esta Tesis Doctoral, estas limitaciones han sido solventadas al incluir en la encuesta la definición de planta medicinal y, al preguntar de forma abierta sobre qué plantas medicinales empleaban los entrevistados. Así, los resultados de nuestra encuesta identificaron un total de 78 plantas medicinales, de las cuales 72 eran usadas por mujeres y 49 por hombres. No hubo diferencias significativas en el consumo de plantas medicinales entre mujeres y hombres, pero sí se encontraron preferencias por alguna de ellas entre sexos. Así, las plantas *Melissa officinalis* L., *Cynara scolymus* L., *Echinacea angustifolia* DC*, Equisetum arvense* L. y *Mentha piperita* L. son utilizadas preferentemente por mujeres, mientras que *Vitis vinifera* L. y *Tribulus terrestris* L. lo son por hombres. En términos generales, las especies más utilizadas por la población son *Matricaria recutita* L.*, Valeriana officinalis* L.*, Tilia spp., Aloe vera* (L.) Burm.f. y, *Camellia sinensis* (L.) Kuntze. Además, se ha detectado que la población encuestada hace un uso correcto de las plantas medicinales en relación con las enfermedades para las que son eficaces (Sánchez et al., 2020a).

Para las especies de plantas medicinales más consumidas se ha determinado el nivel de Fidelidad (FL) que refiere al porcentaje de informantes que utilizan una cierta planta medicinal para tratar una afección específica, el Valor de Uso (VU) que mide la importancia relativa de una planta medicinal para los encuestados y, el Factor de consenso de los informantes (FCI) que estima la variabilidad de uso de cada planta medicinal. Así, los resultados del Nivel de Fidelidad (FL) de las 10 plantas medicinales más usadas mostraron que los valores más altos fueron para *Eucalyptus* spp. (FL 90,47%, problemas respiratorios), seguido de *Matricaria* *recutita* L. y *Mentha pulegium* L. (85,55% y 84,09%, respectivamente para el tratamiento de problemas digestivos) y, *Valeriana officinalis* L. (76,38%, para el tratamiento del insomnio). Los datos relativos al Valor de uso (VU) revelaron que el valor más alto correspondía a *Mentha pulegium* (0,130) seguido de *Aloe vera* (0,097), *Vaccinium macrocarpon* Aiton (0,080), *Camellia sinensis* L. Kuntze (0,072) y *Eucalyptus* spp. (0,071). Por último, el valor más alto del Factor de consenso de los informantes (FCI) fue para el empleo de las plantas medicinales frente a alteraciones del metabolismo y depresión (FCI = 1,) seguido de dolor (FCI = 0,97), insomnio (FCI = 0,96) y ansiedad (FCI = 0,95) (Sánchez et al., 2020a).

Entre las formas de consumo, el principal método utilizado es la infusión seguido de las cápsulas. La población adquiere las plantas medicinales principalmente en farmacias comunitarias, herbolarios y supermercados. La mayoría de las plantas medicinales pueden adquirirse de forma libre en supermercados como la manzanilla, el té y el poleo, mientras que algunas especies vegetales sólo están disponibles en farmacias comunitarias y herbolarios como el verbasco (*Verbascum thapsus* L.) y el pinillo (*Ajuga chamaepitys* Schrb.). Los encuestados perciben que las plantas medicinales dispensadas en farmacias comunitarias son de mejor calidad y eficacia que las de otros lugares de adquisición. Esta pauta de adquisición de productos de fitoterapia con fines terapéuticos también se ha observado en otros países como Reino Unido o Serbia (Živković et al., 2020; Lazarou & Heinrich, 2019).

En cuanto a la seguridad, más de la mitad de los encuestados perciben el uso de las plantas medicinales como seguras por considerarlas "naturales" e "inofensivas". Sin embargo, las plantas medicinales, al igual que los medicamentos convencionales pueden producir reacciones adversas y/o interacciones. En este sentido, sería de interés incluir el consumo de plantas medicinales en la historia clínica habitual del paciente a fin de poder identificar posibles reacciones adversas e interacciones con otros medicamentos. Esto garantizaría la seguridad, eficacia y calidad en el uso de las plantas medicinales, constituyendo así un sistema de salud integral. Además, un factor añadido a la seguridad farmacológica es la escasa formación académica de los profesionales sanitarios sobre plantas medicinales durante los estudios de grado. En España, sólo los farmacéuticos reciben esta formación, limitándose así el conocimiento y la capacidad de identificación de reacciones adversas e interacciones por parte de otros profesionales sanitarios.

En relación a las interacciones entre plantas medicinales y medicamentos convencionales, los datos avalados científicamente son escasos (Kahraman et al., 2020; Tres, 2016; Alissa et al., 2014). El conocimiento de las interacciones y, la comprensión de los mecanismos moleculares que intervienen en sus procesos es una forma de garantizar el uso seguro de las plantas medicinales y, ayudar en la elección de la mejor estrategia terapéutica. Hay evidencias de interacciones entre *Hyperycum perforatum* L. con digoxina, indinavir y ciclosporinas (Martins, 2018) y, *Ginkgo biloba* L. Mant. Pl. con omeprazol (Kharchoufa et al., 2018; Rogozea, 2018). En nuestro estudio hemos identificado potenciales interacciones de tipo farmacodinámico con las especies empleadas más frecuentemente como *M. recutita* y lormetazepam, *M. officinalis* y alprazolam, y *V. officinalis* y lormetazepam, aumentando en este último caso, el efecto hipnótico de las benzodiacepinas (Orellana-Paucar & Vintimilla-Rojas, 2020; Sánchez et al., 2020a; Sys et al., 2020). Además, en este trabajo también se han reportado reacciones adversas asociadas al uso de las plantas medicinales como ansiedad, taquicardia, mareos y síntomas gastrointestinales. Varios de los encuestados manifestaron no informar del consumo de plantas medicinales al profesional sanitario, lo que conlleva la ya comentada falta de notificación de reacciones adversas e interacciones sobre plantas medicinales (Sánchez et al., 2020a).

Este trabajo, realizado en diferentes zonas de la Comunidad Autónoma de Madrid, aporta nuevas perspectivas y un mayor conocimiento sobre las razones actuales y el modo de consumo de las plantas medicinales por la población. El farmacéutico se consolida como el profesional sanitario y experto en plantas medicinales y, las farmacias comunitarias como referencia en la dispensación de plantas medicinales que ofrecen garantías de calidad.

VALIDACIÓN FARMACOLÓGICA DE LOS USOS TRADICIONALES

Una vez obtenida la información sobre las plantas medicinales más consumidas por la población en la Comunidad Autónoma de Madrid, el siguiente paso ha sido el de realizar una extensa revisión bibliográfica sobre las especies más utilizadas *Aloe vera, Camellia sinensis, Matricaria recutita, Tilia* spp y *Valeriana officinalis* con el objetivo de realizar una actual evaluación y validación farmacológica. La especie medicinal *Tilia* spp. se descartó posteriormente ya que no había estudios farmacológicos recientes.

Camellia sinensis (L.) Kuntze

Camellia sinensis (L.) Kuntze (Familia Theaceae) es un árbol que crece principalmente en climas tropicales y subtropicales. El té elaborado con las hojas de *Camelia sinensis* es una de las bebidas más consumidas en el mundo. Puede clasificarse, dependiendo del grado de fermentación, como té verde (no fermentado), té blanco y amarillo (ligeramente fermentado), té oolong (semifermentado), té negro (fermentado) y té rojo o *pu-erh* (post-fermentado). El té negro es el más producido y consumido en todo el mundo (78% del total, especialmente en los países occidentales), seguido del té verde (20%, especialmente en China, la India y el Japón) y el té oolong (<2%) (Luo et al., 2019). Los principales compuestos bioactivos identificados en las hojas de *Camellia sinensis* son flavanoles (i.e. epigalocatequina-3-galato), flavonoles (i.e. apigenina, miricetina, quercetina y rutina), teaflavinas y arubiginas. El tipo y cantidad de estos compuestos viene determinado por el grado de fermentación de las hojas. Así, la epigalocatequina-3-galato es el principal compuesto presente en el té verde, mientras que las teaflavinas se encuentran en grandes cantidades en el té negro (Valduga et al., 2019; Konieczynski et al., 2017). Los beneficios para la salud de *Camellia sinensis* incluyen propiedades antioxidantes, antiinflamatorias, anticancerígenas, reductoras del colesterol y protectoras del sistema cardiovascular, entre otras (Bedrood et al., 2018; Naveed et al., 2018; Li et al., 2015).

En esta Tesis Doctoral, nos hemos centrado en actualizar la actividad farmacológica de *Camellia sinensis* en relación con los trastornos metabólicos y endocrinos (osteoporosis, hipertensión, diabetes, síndrome metabólico, hipercolesterolemia y obesidad). La revisión sistemática incluyó estudios en fase preclínica y clínica publicados entre los años 2014-2019. La búsqueda se realizó en las bases de datos científicas de Pubmed, Science Direct y Scopus empleando combinación de las siguientes palabras clave "*Camellia sinensis*", "osteoporosis", "hipertensión", "diabetes", "síndrome metabólico", "hipercolesterolemia" y "obesidad". Se excluyeron todos aquellos trabajos publicados que fueran artículos de revisión, cartas a editores y conferencias, así como aquellos estudios que evaluaran la actividad de otras especies de *Camellia* y mezclas de plantas medicinales.

Inicialmente, se identificaron un total de 1.384 estudios (Pubmed n=170, Science Direct n=1.177 y Scopus n=37). De ellos, 40 estudios aparecían en dos o más bases de datos. Además, se excluyeron un total de 1.264 artículos después del análisis de títulos y resúmenes (n=1.237) y tras el análisis de texto completo (n=27) por no cumplir los criterios de inclusión, siendo finalmente un total de 80 artículos los que se han incluido en esta revisión sistemática para la validación farmacológica (Sánchez et al., 2020b).

El té verde ha sido el más estudiado y, de los compuestos aislados destacan los trabajos que evalúan los efectos de la epigalocatequina-3-galato. La mayoría de los estudios farmacológicos fueron realizados *in vitro* e *in vivo* y, las principales patologías investigadas fueron diabetes y obesidad. Aunque los resultados de los estudios *in vitro* e *in vivo* para las diferentes patologías metabólicas y endocrinas estudiadas son numerosos y prometedores, los ensayos clínicos realizados en los últimos años con el té y sus compuestos bioactivos son limitados.

Aloe vera (L.) Burm.F.

El *Aloe vera* (*Aloe barbadensis* Miller, Famililа Xanthorrhoeaceae) es una planta perenne con flores tubulares de color amarillo brillante que está ampliamente distribuida en zonas cálidas y secas de África septentrional, Oriente Medio y África meridional, Este de Asia, sur del Mediterráneo e Islas Canarias. El término de *Aloe vera* deriva de "Allaeh" (palabra árabe que significa "sustancias amargas brillantes") y "Vera" (palabra latina que significa "verdadero") (Maan et al., 2018).

El gel mucilaginoso incoloro de las hojas de *Aloe vera* ha sido ampliamente utilizado con fines farmacológicos y cosméticos. Tradicionalmente, esta planta medicinal se ha empleado para tratar problemas de la piel como quemaduras, eccemas y heridas y, problemas digestivos por sus propiedades antiinflamatorias, antimicrobianas y cicatrizantes. Además, el *Aloe vera* ha demostrado otras propiedades terapéuticas como anticancerígeno, antioxidante, antidiabético y antihiperlipidémico (Malik et al., 2003).

El *Aloe vera* contiene más de 75 compuestos diferentes, entre ellos vitaminas (A, C, E y B12), enzimas (amilasa, catalasa y peroxidasa), minerales (zinc, cobre, selenio y calcio), azúcares (monosacáridos como manosa-6-fosfato y polisacáridos como glucomananos), antraquinonas (aloína y emodina), ácidos grasos (lupeol y campesterol), hormonas (auxinas y giberelinas), y otros componentes como ácido salicílico, lignina y saponinas (Maan et al., 2018).

Para esta Tesis Doctoral se ha realizado una actualización de las actividades farmacológicas (*in vitro*, *in vivo* y ensayos clínicos) de *Aloe vera* publicadas en los últimos años (2014-2019) en la base de datos de Pubmed. Se excluyeron todos aquellos trabajos que incluían *Aloe vera* combinado con otras plantas medicinales, así como los estudios sobre otras especies de *Aloe*.

Esta revisión ha permitido concluir la validación de los usos tradicionales y profundizar en el mecanismo de acción, identificando compuestos responsables de estas actividades. Asimismo, estudios recientes han investigado nuevas acciones para *Aloe vera* y sus compuestos activos, destacando especialmente su prometedor papel como agente citotóxico, antitumoral, anticancerígeno y antidiabético. En los estudios *in vitro*, las actividades antimicrobianas, antiinflamatorias, citotóxicas, y de protección de la piel han sido las más estudiadas. Entre estos trabajos *in vitro* destacan especialmente aquellos que evalúan la acción protectora del *Aloe vera* en alteraciones óseas como la osteoporosis; los resultados son prometedores por lo que estudios futuros deberán encaminarse a validar dicha actividad en animales de experimentación y en ensayos clínicos. Los estudios *in vivo* se han centrado principalmente en evaluar su papel como agente cardioprotector, antitumoral, y protector de la piel. En estos estudios *in vitro* e *in vivo* se han investigado actividades farmacológicas tanto de extractos de aloe como de compuestos aislados, especialmente, aloe-emodina y aloína. En concreto, la aloe-emodina ha mostrado interesantes propiedades antimicrobianas, antidiabéticas, citotóxicas, cardioprotectoras y protectoras óseas (estudios *in vitro*), así como antiinflamatorio y compuesto protector de la piel (estudios *in vivo*). La aloína fue efectiva en procesos inflamatorios y alteraciones óseas (estudios *in vitro*) así como en algunos tipos de cáncer y enfermedades cardiovasculares (estudios *in vivo*). Por último, los ensayos clínicos son limitados y se centran en estudiar los efectos protectores de extractos de aloe en el aparato digestivo y en la piel (Sánchez et al., 2020c).

***Valeriana officinalis* L.**

Valeriana officinalis L. (familia Caprifoliaceae), conocida comúnmente como "All-heal" (inglés), "Herbe aux chats" (francés) y "Baldrian" (alemán), es una planta herbácea perenne que crece extensamente en regiones templadas de Europa, Asia y América del Norte (Sundaresan et al., 2018). El nombre de "Valeriana" deriva de la palabra latina "valere", que significa estar sano, fuerte o poderoso, y de la palabra latina "officinalis" que hace referencia a las farmacias y boticas de los monasterios (Hamaidia et al., 2016).

Su composición es muy variable dependiendo de la época de recolección y almacenamiento. Los principales compuestos identificados son iridoides (i.e. valepotriatos, isovalepotriatos), aceites esenciales (terpenos como ácido valerénico, ácido isovalérico, ácido valérico y acetoxivalerénico), trazas de alcaloides piridínicos (i.e. chatinina, valerina, valerianina y actinidina) y, aminoácidos libres, ácido γ-aminobutírico (GABA), tirosina, arginina y glutamina (Castillo & Martínez, 2015; Patocka & Jakl, 2010).

Tradicionalmente, la raíz de *V. officinalis* se ha utilizado para mejorar controlar los nervios y facilitar el sueño, reduciendo la frecuencia de los despertares. El ácido valerénico es el principal compuesto responsable de estas actividades a través de la modulación del receptor

GABA. La Agencia Europea de Medicamentos (EMA), basándose en datos científicos validados, informó que los extractos secos de etanol (40-70%) de *V. officinalis* en formas posológicas orales sólidas son útiles en el alivio de la tensión nerviosa leve y en los trastornos del sueño. Además, *V. officinalis* tiene otras actividades biológicas a nivel cardiovascular (reducción de presión arterial y frecuencia cardíaca, antiarrítmica y regulación de niveles de lípidos en la sangre), anticancerosa, antimicrobiana y espasmolítica (Harada et al., 2020; Chen et al., 2015; Occhiuto et al., 2009).

En esta Tesis Doctoral se ha realizado una actualización farmacológica (*in vitro, in vivo* y ensayos clínicos) de *Valeriana officinalis* L. en los últimos años (2014-2020) empleando las bases de datos Pubmed, Science Direct y Scopus. Las palabras clave para la búsqueda bibliográfica fueron "*V. officinalis*", "valerian", "*in vitro*"," *in vivo*", "clinical trials", "biological" y "pharmacology". Se excluyeron trabajos sobre la actividad de *V. officinalis* combinada con otras especies vegetales y, aquellos que evaluaban la actividad de especies diferentes de valeriana.

Se identificaron un total de 556 estudios (259 en PubMed, 37 en Science Direct y 260 en Scopus). De éstos, 33 artículos cumplían los criterios de inclusión establecidos y fueron los incluidos en esta revisión sistemática.

La revisión realizada para este trabajo de Tesis Doctoral muestra evidencias científicas sobre los usos tradicionales de *V. officinalis* en el sistema nervioso, validando así su uso farmacológico. Los estudios *in vitro* revelaron potenciales actividades antioxidantes de *V. officinalis* que podrían contribuir a prevenir y proteger frente a enfermedades relacionadas con el estrés oxidativo. Además, en los estudios *in vivo* se ha explorado su actividad en diferentes alteraciones del sistema nervioso como depresión, epilepsia y trastornos neurodegenerativos. Sobre la base de los ensayos clínicos, hay pruebas consistentes de la eficacia de *V. officinalis* en el tratamiento de la ansiedad, los problemas cognitivos y el insomnio. En esta revisión sistemática se ha puesto también de manifiesto las potenciales propiedades farmacológicas de compuestos como ácido volvalerenal K y ácido valeriánico en estudios *in vitro* e in *vivo*. Las investigaciones futuras deben centrarse en la realización de ensayos clínicos que permitan poner de manifiesto la actividad de estos compuestos bioactivos así como en la investigación de nuevas y diferentes actividades biológicas de *V. officinalis* (Sánchez et al., 2020d).

Matricaria recutita L.

La manzanilla (*Matricaria chamomilla* L., *Matricaria recutita* L.), perteneciente a la familia de las Asteráceas, es una planta herbácea anual nativa de Europa y Asia occidental, que también se ha extendido en los continentes americano y oceánico. Esta planta común tiene numerosos usos terapéuticos y cosméticos y, un alto valor nutritivo. *M. chamomilla* se ha utilizado ampliamente en la medicina tradicional por sus acciones relajantes, digestivas, antiinflamatorias y cicatrizantes. En cosmética, los aceites esenciales y las infusiones de las partes aéreas de *M. chamomilla* se han utilizado como ingredientes de productos capilares, jabones y perfumes por sus propiedades colorantes y aromáticas. Además, *M. chamomilla* es una de las infusiones más empleadas popularmente. Estas propiedades se atribuyen a sus compuestos activos, entre los que se encuentran flavonoides (i.e. apigenina, quercetina, luteolina), cumarinas (i.e. herniarina) y sesquiterpenos (i.e. α-bisabolol, óxido de bisabolol, camazuleno) (Singh et al., 2011; McKay & Blumberg, 2006.)

En la revisión sistemática realizada para esta Tesis Doctoral se han evaluado las propiedades farmacológicas (*in vitro* e *in vivo*) y los ensayos clínicos de los extractos, aceite esencial y compuestos aislados de la manzanilla. Las palabras claves usadas para esta búsqueda fueron "*Matricaria recutita*", "*Matricaria chamomilla*" "*in vivo*", "*in vitro*", "clinical trials" y "pharmacology". La búsqueda se realizó en las bases de datos de Scopus, Science Direct y Pubmed. Se incluyeron trabajos publicados entre 2014 y 2018. Los estudios sobre actividad de *Matricaria recutita*/*Matricaria chamomilla* combinados con otras plantas medicinales o bien aquellos centrados en otras especies de manzanilla fueron excluidos de esta revisión sistemática.

Los resultados obtenidos permiten concluir la eficacia de la *M. chamomilla* frente a diferentes alteraciones de la salud. Los estudios *in vitro* revelaron que la manzanilla tenía propiedades antimicrobianas (extractos y aceite esencial), antiparasitaria (extractos, aceite esencial y compuestos aislados), antioxidante (extractos, aceite esencial y compuestos aislados), citotóxica (aceite esencial), antidiabética (extractos y compuestos aislados), antiinflamatoria (extractos y compuestos aislados) y cicatrizante (compuestos aislados). En cuanto a los estudios *in vivo*, realizados principalmente con ratas y ratones como modelos experimentales, se ha evaluado la actividad de los extractos en neuroprotección, protección cardiovascular, hepatoprotección, hematoprotección y, protección frente a úlceras. Además de la actividad protectora frente a diversas patologías, se han investigado otras actividades como cicatrizante (alcanfor), anestésica y antihiperalgésica (aceite esencial) y antidepresiva (extractos). La mayoría de estas pruebas *in vivo* se han realizado con extractos, seguido de los estudios con aceite esencial y en menor medida con compuesto aislado (Sánchez et al., 2020e).

Por último, los ensayos clínicos han investigado la actividad farmacológica de los extractos y el aceite esencial de la manzanilla. Estos ensayos clínicos bien validan actividades farmacológicas demostradas en estos estudios *in vitro* e *in vivo* como su efecto en la diabetes mellitus, hipertensión y dolor mientras otros evalúan otras nuevas actividades como el efecto sobre la eneuresis.

Futuras investigaciones deberían profundizar el estudio de los mecanismos de acción y determinar los compuestos responsables de las actividades demostradas en los extractos y aceite esencial. Además, en los ensayos clínicos se debería evaluar el efecto de los compuestos aislados como una interesante y potencial línea de investigación.

IDENTIFICACIÓN MOLECULAR POR ADN-BARCODING

Los productos a base de plantas medicinales, ya sean suplementos dietéticos o fitomedicamentos, generaron 107.000 millones de dólares en el año 2017, lo que supone un aumento significativo respecto al año 2000 (60.000 millones de dólares). Los fitomedicamentos representan una gran parte del mercado farmacéutico en Estados Unidos y Europa. A nivel mundial, Europa, Asia-Pacífico y Japón son los mayores mercados para suplementos y remedios a base de plantas medicinales (Global Industry Analyst, Inc. 2020). El aumento comercial de las plantas medicinales ha traído consigo un aumento de los incidentes relacionados con contaminación (insecticidas, pesticidas, drogas sintéticas y metales pesados) y adulteración (sustitución de una planta por otra de forma intencionada o por identificación errónea), lo que genera una mayor preocupación por la seguridad, eficacia y calidad de los productos a base de plantas. Además, la seguridad y eficacia de estos productos están directamente vinculadas al control de calidad de las materias primas de las que parten todos los medicamentos y/o preparados a base de plantas medicinales (Govindaraghavan et al., 2012; Betz et al., 2011).

Hasta la fecha, los métodos empleados para la identificación del material vegetal se basan en técnicas morfológicas y micrográficas, si bien no todos los materiales de partida se someten a pruebas rutinarias de autenticidad o adulteración. En los últimos años se han producido avances en la secuenciación de ADN y estos han impulsado el empleo del código de barras de ADN (*DNA barcoding*) como método para la identificación y autentificación de plantas medicinales. Esta técnica es una herramienta eficaz para superar las limitaciones existentes en los controles de calidad actuales de las plantas medicinales. El *DNA barcoding* es un método genómico en el que se utiliza una pequeña región de un gen denominada *DNA barcode* (entre 400 y 800 pares de bases) para identificar molecularmente, con gran precisión y fiabilidad, una especie vegetal concreta. Son secuencias relativamente cortas que se obtienen mediante amplificación empleando la técnica de reacción en cadena de la Polimerasa (PCR). Los códigos de barras genéticos en plantas incluyen la enzima Ribulosa-1,5-bisfosfato carboxilasa oxigenasa (RuBisCo, *rbcl*) y la maturasa K (*matK*) que se localizan en el genoma del cloroplasto (Ichim, 2019; CBOL plant working group, 2009; Chase et al., 2007; Kress et al., 2005). Las bases de datos *Barcode of Life Database* (BOLD) y la *International Nucleotide Sequence Database Collaborative*, recogen secuencias de marcadores de diferentes especies para poder analizar y comparar los datos de códigos de barras de ADN.

La técnica del *DNA Barcoding* tiene diversidad de aplicaciones incluidas la identificación de nuevas especies, especies crípticas (sin diferencias morfológicas) y especies comercializadas (Khairil et al., 2020; Hausmann et al. 2011). Esta técnica ha permitido detectar sustituciones en diferentes especies vegetales adquiridas en supermercados, tiendas minoristas e Internet [por ejemplo, productos comercializados de *Ginkgo biloba* L. (16%), suplementos dietéticos de cimicífuga (25%), productos de *Cassia angustifolia* M. Vahl (7%), y productos de ginseng coreano (50%)] (Little, 2014; Wallace et al, 2012). Además, el *DNA barcoding* permite resolver cuestiones relacionadas con taxonomía, filogenia molecular, genética de poblaciones y biogeografía (Janjua et al., 2016). Cabe destacar también que el método del *DNA barcoding* ha sido incluido por primera vez para identificar la especie *Ocimum tenuiflorum* L. (Familia Lamiaceae) en la Farmacopea Británica.

En este trabajo de Tesis Doctoral se ha aplicado el método de *DNA barcoding* como herramienta para evaluar la calidad de muestras de la raíz de valeriana (7), de las hojas del té (8), de las hojas de la tila (9) y de la inflorescencia de la manzanilla (9) adquiridas en herbolarios, farmacias comunitarias y supermercados. Estas son las plantas medicinales más consumidas en la Comunidad Autónoma de Madrid según nuestro estudio poblacional.

El DNA se logró aislar en 23 de las 33 muestras adquiridas. Además, se generaron 18 nuevas secuencias *matk* que se alinearon con 38 secuencias disponibles en GenBank. De las 33 muestras de especies vegetales adquiridas, el 36,4% fueron identificadas como correctas, 48,5% como muestras incompletas y, el 15,2% como sustituciones. En este último caso destaca la muestra CH1 etiquetada como *C. sinensis* pero molecularmente identificada como *Blepharocalyx tweediei* Berg y la muestra TS2 vendida como tilo pero analizada dentro del orden de las Malvales. Además, muestras etiquetadas como *V. officinalis* (VH1, VS1, VF2) y *Tilia platyphyllos/europea* Scop. (TH1, TH2, TH3) se identificaron como *Valeriana hirtella* Kunth. y *T. cordata*, respectivamente.

Los resultados obtenidos en este trabajo evidencian la importancia del método de *DNA Barcoding* como herramienta clave de control de calidad para la identificación y autenticación de especies vegetales comercializadas con fines terapéuticos.

ESTUDIO ANALÍTICO POR HPLC

En el reino vegetal existe una gran variedad de metabolitos. La base de datos *Dictionary of Natural Products* recoge un total de 200.000 metabolitos secundarios de plantas con 170.000 estructuras diferentes.

La cromatografía de líquidos (LC-MS) junto con la cromatografía de gases (GC-MS) y la electroforesis capilar (CE-MS), acopladas a técnicas como la espectrometría de masas y espectroscopia de resonancia magnética nuclear (NMR) son las técnicas analíticas más comúnmente empleadas para identificar y cuantificar metabolitos en las especies vegetales (Salem et al., 2020; Beyoglu & Idle, 2020; Pieczykolan et al., 2019; Guillaume & Veuthey, 2017). Así, estos métodos se han utilizado como control de calidad para detectar adulteraciones en muestras comerciales como por ejemplo en medicamentos antimaláricos que contenían *Artemisia afra* Jacq, que carece de artemisinina en lugar de *Artemisia annua* L. (Castilho et al., 2008).

La metodología del *DNA barcoding* no proporciona información sobre los metabolitos contenidos en las especies vegetales. Por ello, como control de calidad, hemos realizado un análisis metabolómico mediante Cromatografía liquida de ultra alto rendimiento acoplada a espectrometría de masas en tándem (UHPLC-MS/MS) en las 33 muestras adquiridas en diferentes canales de distribución con el fin de determinar la composición cualitativa y cuantitativa de los compuestos bioactivos responsables de la actividad farmacológica. Los compuestos identificados en las muestras comercializadas de las plantas medicinales más consumidas en la Comunidad Autónoma de Madrid son apigenina-7-glucosido (*Matricaria recutita*), ácido valerénico y ácido acetoxivalerénico (*Valeriana officinalis),* epigalocatequina (*Camellia sinensis*) y tilirósido (*Tilia* spp.).

Para la cuantificación de las muestras se desarrolló un método por cromatografía líquida de ultra alto rendimiento junto con la técnica de espectrometría de masas de triple cuadrupolo (UHPLC-QqQ-MS/MS), utilizando un equipo LC-QQQ 8030 (Shimazdu, Tokio, Japón).

Para la manzanilla, el contenido medio de apigenina-7-glucósido fue de 0,003%, siendo las muestras MH1 (0,035%, adquirida en herbolario) y MS3 (0,016%, adquirida en supermercado) las que tenían mayor cantidad de este compuesto. Según la Agencia Europea del Medicamento (EMA) y la Real Farmacopea Española, el contenido de apigenina-7-glucósido debe ser al menos 0,25% de la droga seca (EMA 2020; Real Farmacopea Española, 2020).

En las muestras de valeriana se identificaron los compuestos ácido valerénico y ácido acetoxi valerénico. El contenido de ácido valerénico fue muy variable desde 0,048% a 0,167%. Según la EMA y la Real Farmacopea Española, el contenido en ácidos sesquiterpénicos no debe ser inferior al 0,17% expresado como ácido valerénico, siendo la muestra VF3 adquirida en farmacia comunitaria la única que cumplía este criterio (EMA 2020; Real Farmacopea Española, 2020). Por otro lado, la concentración media del compuesto ácido acetoxi valerénico era de 0,025% a excepción de la muestra VF3 (0,053%).

En las muestras de té, el contenido medio de la epigalocatequina fue de 2,85%, siendo las muestras adquiridas en farmacias comunitarias las que presentaban menor cantidad de este principio activo. Según la EMA, el contenido en flavan-3-oles, incluyendo la epigalocatequina, debería ser entre 10 y 25% de la droga seca (EMA, 2020).

Por último, el contenido en tilirósido en las muestras de tila, varió entre 0,008% a 0,043%. No hay datos ni en la EMA ni en la Real Farmacopea Española sobre contenido de referencia entilirósido.

Este estudio demuestra la necesidad de incorporar técnicas moleculares y analíticas en el control de calidad de especies vegetales comercializadas con fines terapéuticos para garantizar la calidad, efectividad y seguridad al paciente.

X. CONCLUSIONES

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El trabajo de Tesis Doctoral titulado *"Plantas medicinales en Farmacia comunitaria: de la validación farmacológica al ADN Barcoding"* se ha desarrollado con el objetivo aportar nuevos conocimientos sobre el empleo de las plantas medicinales para tratar problemas de salud, abordando todos aquellos aspectos que garantizan su calidad, seguridad y eficacia. Tras la realización de encuestas sobre el consumo de plantas medicinales, el trabajo se ha iniciado realizando una extensa validación bibliográfica sobre las actividades farmacológicas de las especies vegetales más usadas por la población en la Comunidad de Madrid, como base fundamental de la medicina basada en la evidencia. La segunda parte del trabajo se ha centrado en aplicar metodologías innovadoras que permitan evaluar la calidad y, por tanto, eficacia y seguridad, de las especies vegetales: el código de barras de ADN (DNA *Barcoding*) ha permitido la identificación y autentificación inequívoca de las especies, cuantificándose posteriormente los compuestos bioactivos empleando la Cromatografía liquida de ultra alta eficacia acoplada a espectrometría de masas (UHPLC-MS).

Los resultados obtenidos permiten extraer las siguientes conclusiones:

1. Se ha realizado un estudio poblacional sobre el consumo y empleo de plantas medicinales por la población en la Comunidad Autónoma de Madrid, analizando el conocimiento, percepciones y actitudes, así como la información relativa a la identificación de riesgos y precauciones asociadas a su consumo.

   Esta encuesta mostró:

   a) Un 89,6% de los encuestados manifiesta emplear plantas medicinales, de forma ocasional o frecuente, con fines terapéuticos. En términos generales se puede afirmar que la población encuestada hace un uso correcto de las plantas medicinales en relación con las enfermedades para las que son eficaces.

   b) El perfil más habitual del consumidor son mujeres con edad entre 18-44 años, nivel superior de estudios y en activo.

   c) Se identificaron un total de 78 plantas medicinales siendo las más consumidas manzanilla *(Matricaria recutita* L.), valeriana *(Valeriana officinalis* L.), tila *(Tilia* spp.), aloe (*Aloe vera* L.) y te (*Camellia sinensis* (L.) Kuntze).

   d) El principal uso terapéutico de las plantas medicinales en la población encuestada es para tratar problemas digestivos, seguido de trastornos del sueño y ansiedad.

   e) Se ha determinado el nivel de Fidelidad, el Valor de Uso y el Factor de consenso de los informantes. Los valores más elevados fueron, en cuanto al Nivel de Fidelidad, para *Eucalyptus* spp. (90,47%, problemas respiratorios) y *Valeriana officinalis* L. (76,38%, para el tratamiento del insomnio). Los datos relativos al Valor de uso (VU) revelaron que el valor más alto correspondía a *Mentha pulegium* L. (0,130). El valor más alto del Factor de consenso de los informantes (FCI) fue para el empleo de las plantas medicinales frente a alteraciones del metabolismo y depresión (FCI = 1) seguido de dolor (FCI = 0,97), insomnio (FCI = 0,96) y ansiedad (FCI = 0,95).

a) Las farmacias comunitarias son el principal lugar de adquisición de las plantas medicinales y la infusión, la forma de consumo más habitual.

b) Aproximadamente la mitad de la población encuestada piensa que las plantas medicinales pueden producir efectos secundarios. Un 3,5% manifiesta haber sufrido alguna reacción adversa, como ansiedad, taquicardia y mareos.

c) El 21,1% de la población encuestada ha consumido plantas medicinales junto con medicamentos convencionales, identificándose interacciones entre valeriana y benzodiacepinas.

2. Se ha realizado la revisión y el análisis de los estudios sobre actividad farmacológica, como fuente de validación, de las especies más consumidas. Así:

a) La revisión y análisis de los usos farmacológicos actuales de *Camellia sinensis* reveló que las patologías más investigadas fueron diabetes y obesidad. El té verde ha sido el más estudiado y, de los compuestos aislados destacan los trabajos que evalúan los efectos de la epigalocatequina-3-galato.

b) El extracto de *Aloe vera* y sus compuestos aislados aloe-emodina y aloína muestran un prometedor papel como agentes citotóxicos, antitumorales, anticancerígenos y antidiabéticos. La mayoría de los estudios publicados son estudios *in vivo* e *in vitro*. Los ensayos clínicos son limitados y están focalizados en la actividad digestiva y protectora de la piel.

c) La valeriana ha sido investigada recientemente en estudios *in vitro*, *in vivo* y ensayos clínicos por sus propiedades beneficiosas a nivel del sistema nervioso central. Esta actividad se atribuye principalmente a los compuestos ácido volvalerenal K y ácido valerénico.

d) Los estudios recientes sobre *Matricaria recutita* (aceite esencial, extractos y compuestos aislados) permiten concluir que es eficaz frente a diferentes alteraciones de la salud, entre las que cabe destacar la hipertensión, la diabetes mellitus tipo II y el tratamiento del dolor.

e) No se encontraron estudios recientes sobre actividad farmacológica de *Tilia* spp.

3. Se ha evaluado la calidad de muestras comerciales de las especies más utilizadas por la población, empleando el código de barras de ADN (DNA *Barcoding*) como método para la identificación y autentificación de las especies vegetales y la Cromatografía liquida de ultra alta eficacia acoplada a espectrometría de masas (UHPLC-MS) como método de cuantificación de los compuestos bioactivos.
4. En relación con el empleo de ADN Barcoding:
   a) Se logró aislar ADN con calidad para ser secuenciado en el 69,7 % de las muestras comerciales de las especies en estudio. Las secuencias generadas se alinearon con las secuencias disponibles en GenBank.
   b) El empleo de maturasa k (*matk)* como marcador para la identificación molecular permitió la identificación con asignación correcta respecto a su etiquetado en el 36,4 % de las muestras estudiadas, mientras que se han detectado sustituciones en el 15,2 %.
   c) Las sustituciones más significativas corresponden a *Camellia sinensis* y *Tilia cordata*.
5. El análisis cualitativo y cuantitativo de los compuestos responsables de la actividad farmacológica por UHPLC/MS ha puesto de manifiesto:
   a) El contenido medio de apigenina-7-glucósido en las muestras de manzanilla se encuentra en rango de 0,001 a 0,035 %, correspondiendo el mayor contenido a una muestra adquirida en supermercado.
   b) El contenido de ácido valerénico fue muy variable en las muestras analizadas, encontrándose en valores comprendidos entre 0,048% a 0,167%, siendo la de mayor contenido una muestra adquirida en farmacia. La concentración media de ácido acetoxi valerénico ha sido de 0,025%.
   c) En las muestras de té, el contenido medio de la epigalocatequina fue de 2,85%, siendo las muestras adquiridas en farmacias las que presentaban menor cantidad de este principio activo.
   d) El contenido en tilirósido presente en las muestras de tila varió entre 0,008% a 0,043%.
6. El empleo conjunto de técnicas moleculares, como ADN *Barcoding* y técnicas analíticas de alta precisión, como la UHPLC/MAS, en el control de calidad de especies vegetales comercializadas con fines terapéuticos, permite garantizar la calidad, efectividad y seguridad del paciente.

A la vista de todos los resultados obtenidos, podemos concluir que se aporta una base científica sólida para el estudio de las plantas medicinales utilizadas por la población con fines terapéuticos.

XI. REFERENCIAS BIBLIOGRÁFICAS

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