UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: An overview of the biological activities of *Aristeguietia glutinosa*, *Lepechinia rufocampii*, and *Croton elegans* (endemic plants of Ecuador) and its potential application in drug discovery

Trabajo de integración curricular presentado como requisito para la obtención del título de Bióloga

Autor:

Selena Leonor Tinoco Valencia

Tutor:

PhD, Santiago Vispo Nelson

Urcuquí, noviembre 2020.

Urcuquí, 18 de noviembre de 2020

SECRETARÍA GENERAL
(Vicerrectorado Académico/Cancillería)
ESCUELA DE CIENCIAS BIOLÓGICAS E INGENIERÍA
CARRERA DE BIOLOGÍA
ACTA DE DEFENSA No. UITEY-BIO-2020-00037-AD

A los 18 días del mes de noviembre de 2020, a las 14:30 horas, de manera virtual mediante videoconferencia, y ante el Tribunal Calificador, integrado por los docentes:

Presidente Tribunal de Defensa	Dra. SPENCER VALERO, LILIAN MARITZA , Ph.D.
Miembro No Tutor	Dra. RODRIGUEZ CABRERA, HORTENSIA MARIA , Ph.D.
Tutor	Dr. SANTIAGO VISPO, NELSON FRANCISCO , Ph.D.

El(la) señor(ita) estudiante **TINOCO VALENCIA, SELENA LEONOR**, con cédula de identidad No. **1724761794**, de la **ESCUELA DE CIENCIAS BIOLÓGICAS E INGENIERÍA**, de la Carrera de **BIOLOGÍA**, aprobada por el Consejo de Educación Superior (CES), mediante Resolución **RPC-SO-37-No.438-2014**, realiza a través de videoconferencia, la sustentación de su trabajo de titulación denominado: **AN OVERVIEW OF THE BIOLOGICAL ACTIVITIES OF ARISTEGUIETIA GLUTINOSA, LEPECHINIA RUFOCAMPII, AND CROTON ELEGANS (ENDEMIC PLANTS OF ECUADOR) AND ITS POTENTIAL APPLICATION IN DRUG DISCOVERY**, previa a la obtención del título de **BIÓLOGO/A**.

El citado trabajo de titulación, fue debidamente aprobado por el(los) docente(s):

Tutor	Dr. SANTIAGO VISPO, NELSON FRANCISCO , Ph.D.

Y recibió las observaciones de los otros miembros del Tribunal Calificador, las mismas que han sido incorporadas por el(la) estudiante.

Previamente cumplidos los requisitos legales y reglamentarios, el trabajo de titulación fue sustentado por el(la) estudiante y examinado por los miembros del Tribunal Calificador. Escuchada la sustentación del trabajo de titulación a través de videoconferencia, que integró la exposición de el(la) estudiante sobre el contenido de la misma y las preguntas formuladas por los miembros del Tribunal, se califica la sustentación del trabajo de titulación con las siguientes calificaciones:

Tipo	Docente	Calificación
Presidente Tribunal De Defensa	Dra. SPENCER VALERO, LILIAN MARITZA , Ph.D.	10,0
Tutor	Dr. SANTIAGO VISPO, NELSON FRANCISCO , Ph.D.	10,0
Miembro Tribunal De Defensa	Dra. RODRIGUEZ CABRERA, HORTENSIA MARIA , Ph.D.	10,0

Lo que da un promedio de: **10 (Diez punto Cero)**, sobre 10 (diez), equivalente a: **APROBADO**

Para constancia de lo actuado, firman los miembros del Tribunal Calificador, el/la estudiante y el/la secretario ad-hoc.

Certifico que *en cumplimiento del Decreto Ejecutivo 1017 de 16 de marzo de 2020, la defensa de trabajo de titulación (o examen de grado modalidad teórico práctica) se realizó vía virtual, por lo que las firmas de los miembros del Tribunal de Defensa de Grado, constan en forma digital.*

TINOCO VALENCIA, SELENA LEONOR
Estudiante

LILIAN MARITZA SPENCER VALERO
Firmado digitalmente por LILIAN MARITZA SPENCER VALERO
Fecha: 2020.11.18 15:50:09 -05'00'

Dra. SPENCER VALERO, LILIAN MARITZA , Ph.D.
Presidente Tribunal de Defensa

Firmado electrónicamente por:
NELSON
FRANCISCO
SANTIAGO VISPO

Dr. SANTIAGO VISPO, NELSON FRANCISCO , Ph.D.
Tutor

HORTENSIA MARIA RODRIGUEZ CABRERA
Firmado digitalmente por HORTENSIA MARIA RODRIGUEZ CABRERA
Fecha: 2020.12.07 10:28:04 -05'00'

Dra. RODRIGUEZ CABRERA, HORTENSIA MARIA , Ph.D.
Miembro No Tutor

Firmado electrónicamente por:
KARLA
ESTEFANIA
ALARCON FELIX

ALARCON FELIX, KARLA ESTEFANIA
Secretario Ad-hoc

AUTORÍA

Yo, **Selena Leonor Tinoco Valencia**, con cédula de identidad 1724761794, declaro que las ideas, juicios, valoraciones, interpretaciones, consultas bibliográficas, definiciones y conceptualizaciones expuestas en el presente trabajo; así cómo, los procedimientos y herramientas utilizadas en la investigación, son de absoluta responsabilidad de el/la autora (a) del trabajo de integración curricular. Así mismo, me acojo a los reglamentos internos de la Universidad de Investigación de Tecnología Experimental Yachay.

Urcuquí, agosto 2020.

Selena Leonor Tinoco Valencia

CI: 1724761794

AUTORIZACIÓN DE PUBLICACIÓN

Yo, **Selena Leonor Tinoco Valencia**, con cédula de identidad 1724761794, cedo a la Universidad de Tecnología Experimental Yachay, los derechos de publicación de la presente obra, sin que deba haber un reconocimiento económico por este concepto. Declaro además que el texto del presente trabajo de titulación no podrá ser cedido a ninguna empresa editorial para su publicación u otros fines, sin contar previamente con la autorización escrita de la Universidad.

Asimismo, autorizo a la Universidad que realice la digitalización y publicación de este trabajo de integración curricular en el repositorio virtual, de conformidad a lo dispuesto en el Art. 144 de la Ley Orgánica de Educación Superior.

Urcuquí, agosto 2020.

Selena Leonor Tinoco Valencia

CI: 1724761794

Dedicatoria

Con mucho cariño,

Este logro se lo dedico a mi padre, Pablo Tinoco, quien con su esfuerzo, consejos y optimismo, me ha motivado siempre a cumplir con mis objetivos y metas.

A mi madre, Sandra Valencia, quien con su amor, valentía y tiempo me ha apoyado de manera incondicional en cada etapa de mi vida.

A mi familia, por ser un ejemplo de dedicación y trabajo que he reflejado en mi desarrollo personal y profesional.

Selena Leonor Tinoco Valencia

Agradecimientos

A Dios, por guiarme y darme la sabiduría necesaria para poder completar esta etapa de mi vida satisfactoriamente.

A la Universidad Yachay Tech y a mis profesores, por brindarme diversas oportunidades académicas que me han llenado de conocimientos y valores personales.

A mi tutor, Nelson Vispo Ph.D., por darme la confianza para alcanzar mis objetivos, por apoyarme y darme alientos para culminar este trabajo.

A mis padres, Pablo y Sandra, por ser el soporte fundamental de mi formación personal y profesional.

A mi abuelita Maggie, por ser la persona más generosa, cariñosa y por creer siempre en mí y en mis capacidades.

A mi familia, por alegrar mi vida y ser un pilar importante en el desarrollo de la persona que soy ahora.

A mis amigos, por ser los mejores acompañantes de aventuras y mi apoyo incondicional durante toda esta etapa.

Selena Leonor Tinoco Valencia

Resumen

La resistencia antimicrobiana ha generado un riesgo para la salud pública, dificultando el tratamiento médico y necesitando con urgencia la creación de nuevos medicamentos que puedan contrarestar este problema. Las plantas poseen una extensa diversidad de metabolitos secundarios (phytochemicals) con propiedades biológicas capaces de tratar varias enfermedades, por lo que son consideradas como potenciales candidatos en el desarrollo de fármacos. En el Ecuador, la medicina tradicional atribuye a las plantas endémicas *Aristeguietia glutinosa*, *Lepechinia rufocampii*, and *Croton elegans* una gran variedad de actividades terapéuticas. Sin embargo, los estudios fitoquímicos y biológicos en estas especies son limitados, por lo que su propiedad como agente terapéutico no ha sido definido en su totalidad. Además, las familias a las que pertenecen estas especies tienen un enriquecedor historial de plantas medicinales, lo que destaca la importancia de estudiar estas plantas. En esta revisión bibliográfica, se describe brevemente la historia de las plantas medicinales del Ecuador con un enfoque principal en la reciente evidencia científica sobre las actividades biológicas de éstas tres especies endémicas. También, se describe la familia taxonómica a la que pertenecen, destacando la actividad farmacológica de algunas de sus más importantes especies. Además, se detalla el proceso para el descubrimiento de medicamentos desde que se obtiene el material vegetal hasta que se comprueba su actividad biológica a través de test de sensibilidad *in vitro*, incluyendo diferentes métodos de extracción, aislamiento y caracterización del extracto de la planta.

Palabras clave: antimicrobiano, planta, actividad biológica, fármaco, producto natural

Abstract

Antimicrobial resistance has generated a risk to public health, making medical treatment difficult and urgently requiring the creation of new drugs that can counteract this problem. Plants have a wide variety of secondary metabolites (phytochemicals) with biological properties capable of treating several diseases, which is why they are considered as potential candidates in drug development. In Ecuador, traditional medicine attributes to the endemic plants *Aristeguietia glutinosa*, *Lepechinia rufocampii*, and *Croton elegans* a great variety of therapeutic activities. However, the phytochemical and biological studies in these species are reduced, so its property as a therapeutic agent has not been fully defined. Furthermore, the families to which these species belong have an enriching history of medicinal plants, which highlights the importance of studying these plants. In this bibliographic review, the history of medicinal plants of Ecuador is briefly described with a primary focus on the recent scientific evidence on the biological activities of these three endemic species. Also, the taxonomic family to which they belong is described, highlighting the pharmacological activity of some of its most important species. Additionally, the drug discovery process is described from when the plant material is obtained until its biological activity is verified through *in vitro* sensitivity tests, including different methods of extraction, isolation, and characterization of the plant extract.

Keywords: antimicrobial, plant, biological activity, drug, natural product

Contents

List of Figures

List of Tables

Chapter I

1. Introduction

Ecuador is considered one of the 17 most mega-diverse countries in the world for its high biological diversity in fauna and flora with 0.2% of the entire land surface of the planet, sharing this category with countries such as Colombia, Peru, Venezuela, Mexico and Brazil (1). In 1999 the first "*Catálogo de Plantas Vasculares del Ecuador*" was published, which registers a total of 15,306 native species present in the country. For 2012, this number increased 2,443 species after several published studies of new species and also based on botanical collections, representing a total of 17,748 vascular plants confirmed 13 years later. However, according to the National Institute of Biodiversity (INABIO), until January 2019, any new plants have been registered, but it is estimated that this number may increase to 25,000 species with more studies and expeditions (2). According to the "*Enciclopedia de Plantas Útiles del Ecuador*," 16,216 species have been registered as useful plants, where around 60% of them are used for medicinal purposes, being 75% native plants, 11% introduced and 5% endemic of the country (3).

Among the plant species sold by the markets of Ecuador, there are 3 central endemic plants that, based on ancestral knowledge, are used to treat a wide variety of ailments and diseases. Among these endemic species is *Aristeguietia glutinosa* Lam. commonly known as "matico", *Lepechinia rufocampii* or "salvereal", and *Croton elegans* Kunth or "mosquera" (4). These plants correspond to some of the most diverse families in the country, such as Asteraceae (matico), Euphorbiaceae (mosquera) and Lamiaceae (salvereal) (5). Knowing the medicinal properties of some of the most important species of the families to which these endemic plants belong, gives us an idea of the relevance of carrying out phytochemical studies to know their potential biological activity (3). However, there is minimal research and development on the antimicrobial and other medicinal properties that these plants possess, being of vital importance to the scientific support of their therapeutic activities to allow researchers to discover new natural products that can be used to create new medicines (6,7).

The main problem that has led scientists to use natural sources for the creation of new drugs is antimicrobial resistance (AMR). AMR is considered a global threat to public health and urgently requires the attention and action of the governments and society. It occurs naturally over time, mainly due to genetic changes (8). However, due to several factors such as inadequate medical prescriptions, reduced administration, low education of the population, inappropriate use of antimicrobials in livestock production, and lack of control and prevention, they have allowed this

problem to accelerate considerably (8,9). The rapid spread of resistance to currently available medications has limited antimicrobial therapies, increasing mortality rates, treatment costs, and their effectiveness, which leads to finding new alternatives that are potentially effective for the treatment of different infectious diseases (10).

Due to the significant increase in AMR, the use of plant extracts has become very important in the health system, since these natural products have often been used as the primary source for the elaboration of traditional and modern medicine (10). In the case of antibiotics, which have undoubtedly revolutionized the practice in medicine due to their role in advancing surgical procedures, childbirth, and chemotherapy, resistance to this type of antimicrobial has not only prevented their use in these procedures and the treatment of chronic diseases but have also delayed the progress achieved (11). For this reason, many efforts have been made to obtain new drugs from plants, looking for chemical substances with antimicrobial properties in them that allow combating AMR and other essential diseases, thus improving current treatment methods (12).

Since ancient times, traditional medicine has used plant extracts as therapeutic agents due to the presence of phytochemicals, which are secondary metabolites present in plants, known to have several biological activities that serve to combat human pathogens and other diseases. These include activities such as antibacterial, antiviral, antifungal, antioxidant, and even anti-cancer properties (13,14). In this regard, researchers have tried to isolate, purify, and identify secondary metabolites that may be useful for antimicrobial therapies in a quest to discover new drugs (13). The phytochemical screening assays that are carried out to characterize the plant extracts indicate the presence of secondary metabolites (phytochemicals) in the sample and the biological tests that are carried out later, help to indicate their potential antimicrobial activity against certain microorganisms (15). For this reason, The World Health Organization (WHO) suggests that, for the implementation of traditional medicine in the health system, it must first be verified through studies its efficacy and safety in the treatment of several diseases (10).

In this bibliographic review, the history of the medicinal plants of Ecuador is described with a focus on three endemic plants found in the local markets of the country (*Aristeguietia glutinosa*, *Croton elegans*, *Lepechinia rufocampii*), a recent insight is provided on the experimental evidence of their therapeutic activity, also, to emphasize the importance of the medicinal properties of the families to which these species belong. Furthermore, a general analysis of the different extraction, isolation, and characterization methods implemented in plant extracts is also described, followed by the different antimicrobial biological tests that are carried out to know if the plant material chosen has therapeutic properties that can contribute to the discovery of new drugs.

Chapter II

2. Problem Statement

The rapid development of antimicrobial resistance has created a risk to public health worldwide. This global threat is generated when microorganisms such as fungi, bacteria, viruses, and parasites undergo changes that make medications such as fungicides, antibiotics, antivirals, and antiparasitics, ineffective (8). The main reasons why antimicrobial resistance is facilitated are the inappropriate use of medications, weak medical prescriptions, and deficiency in the prevention and control of different infections (9). This problem has caused that the medicines that are currently available do not fulfill their function as an antimicrobial in an efficient way, suggesting new alternatives such as the use of medicinal plants to treat these infections (10).

Since Ecuador is a country with high biodiversity in flora, it has a great study potential related to its medicinal plants, putting a significant interest on the endemic plants of the region of which the research field based on its therapeutic properties is minimal.

2.1 Objective

To prepare a review document with relevant and current information on the different studies and methods used for the development of new drugs from natural resources, focusing on the implementation of these techniques in some endemic medicinal plants of Ecuador.

2.2 Specific Objectives

i. To analyze generally the vascular plants of Ecuador focusing on the useful medicinal plants of the region.
ii. To describe geographically and therapeutically each endemic medicinal plant (*Aristeguietia glutinosa, Lepechinia rufocampii*, and *Croton elegans*) based on the ancestral knowledge and currently biological activity studies.
iii. To describe the biological activities of the families to which these endemic plants belong to highlight their importance in the medical field.
iv. To report general details of the methods used to extract, isolate, and characterize the chemical compounds of interest and the biological activity tests to verify their antimicrobial properties.

Chapter III

3. History of vascular plants of Ecuador

Since the man came to the Andean region of Ecuador approximately 10,000 years ago, plants have played a fundamental role in our culture. Natural resources were then used for food purposes, as construction materials, fuel, and the primary medicinal source, uses that continue to this day (16). After the Spanish conquest in the 16th century and its influence on the different Ecuadorian cultures, in addition to the entry and exit of people, the number of medicinal species used in the country has increased, enriching indigenous ancestral knowledge (4,17). The importance of the use of traditional medicine focuses mainly on rural communities since they are the ones that depend exclusively on natural resources to treat their diseases and ailments as a result of a weak health system, the lack of medicines, and mostly because the ancestral medicinal knowledge is very abundant. However, popular medicine is also used by the country's urban communities since they cover absolutely all social classes (17).

The Andean region has around 25% of the world's biological diversity (18). In Ecuador, it occupies around 70.000 km2, where it houses 64% of the total plant species, thus representing the Andean region almost a quarter of the country (16). The Andes Mountain Range forms an essential role in this ecological variety since it defines an altitude gradient and also because it crosses different countries. In the case of Ecuador, it divides it into 3 regions: Coast, Andes, and Amazon, which give them a great diversity of species (16,18).

A group of specialists from around the world, together with the Swedish scientist Gunnar Harling, promoted the publication of the first part of Flora of Ecuador in 1973 (18). However, it was not until 1999 that all knowledge of Ecuadorian flora was recorded for the first time in the "*Catálogo de Plantas Vasculares del Ecuador"* published by Jørgensen & León-Yánez. This summarized the apparent increase in plants discovered in Ecuador from 4,000 species in 1830, 10,000 in 1910, 14,000 in 1970, and a total of 15,306 native plant species by 1999. Among the 15,306 species, 1,298 were pteridophytes, 17 gymnosperms, 13,991 angiosperms, and from all 4,176 were registered as endemic. For 2005, a Catalog supplement was published by Ulloa and Neill, which registered 820 new species and 337 new vascular plant registrations, and for a second supplement in 2011, 719 new species and 421 registries were registered for Ecuador. In 2012, a total of 17,748 plant species were recorded, including 1,422 pteridophytes, 18 gymnosperms, 16,308 angiosperms, and of which approximately 5,500 are endemic to the region. (1). To date, no new

species have been registered, but it is estimated that in the future, this number will rise to 25.000 species of vascular plants present in Ecuador (**Figure 1**) (2).

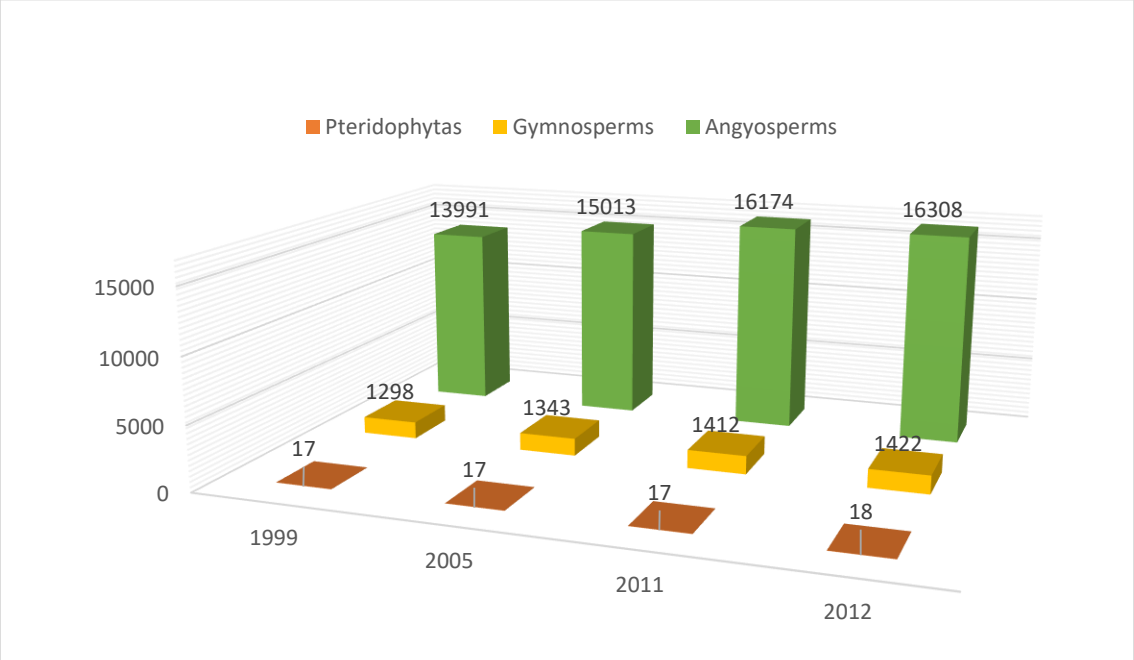

Figure 1. Number of registered vascular plants of Ecuador from 1999 (first report) to 2012 (last report).

3.1 Useful plants and ethnobotany

Ethnobotany is the study of the direct relationship that exists between humans and plants and how they have influenced the different customs and cultural traditions of a wide variety of countries (16,18). Ethnobotany has become a scientific discipline with interdisciplinary and multidisciplinary characteristics, showing a wide range of applications (18). Plants with medicinal properties are the most abundant, followed by those for food, ornamental, and construction uses (16).

Since 1995, studies of collection of plant specimens and ethnobotanical surveys have been carried out in different natural areas of Ecuador (4). Of the more than 17,000 reported vascular plants, around 30% (n = 5,172) have been classified as useful species in the country by the "*Enciclopedia de Plantas Útiles del Ecuador*" published by the QCA Herbarium of the Pontificia Universidad Católica of Quito in 2008 (3,18). This means that, of every 10 plants, 3 are used for different purposes by the population. Of the total useful plants, 60% represent medicinal species, 55% those that are raw materials for construction, 30% with food uses, and 20% are those that have common

uses such as rituals and similar practices. The reason why it exceeds 100% is that many of these plant species have more than one use (3).

The studies carried out on the ethnobotany of Ecuador have focused mainly on indigenous communities in the Amazon, where the uses that communities such as the Shuar, Secoya, Wao, Zápara, and Achaur give to plants in the region have been analyzed and described (18). But if the use of medicinal plants of different ethnic groups is taken into account, the Kichwa of the East have 26%, the Kichwa of the Sierra 18% and the mestizos 14%, while in 38% of the medicinal plant species there is no record of any ethnic group using them (19).

Research on the uses of plants in the country is minimal (18). An analysis of the phytochemical properties of some Asteraceae as medicinal plants was carried out in Chimborazo by Abdo et al. (1995), and in 2003, Padilla carried out a similar investigation in Asteraceae of the Andean region (16). In 2010 a publication by Gerique analyzed the ethnobotany of the Saraguros, Mestizos, and Shuar. In the same year, Ansaloni, along with other researchers, analyzed medicinal plants used to alleviate digestive problems by communities in the provinces of Cañar, Azuay, and Loja (17). One of the most recent studies was in 2017, a project led by the Pontificia Universidad Católica del Ecuador (PUCE) published the book "*Los kichwas del alto Napo y sus plantas medicinales*", which describes 30 plants that treat 49 diseases, symptoms and affections of the Kichwa community and that in this way seeks to preserve the ancestral knowledge of plant resources and their therapeutic development in the country (20).

The Kichwa of the Andes, the Tsa chi of the Coast and the mestizos, are the ethnic groups that use the most significant number of medicinal plants followed by the Achuar, Secoya and Siona (**Figure 2**), even though it is estimated that the Kichwa and the mestizos possess an even greater number of species since in their majority, the medicinal plants that have not been reported come from the provinces of the inter-Andean region (19). In the case of the Andes Kichwa, they use different species to treat neurological problems and to calm ailments during pregnancy, childbirth, and postpartum, while the Eastern Kichwa and the Wao use traditional medicine to treat mainly problems of the skin (3).

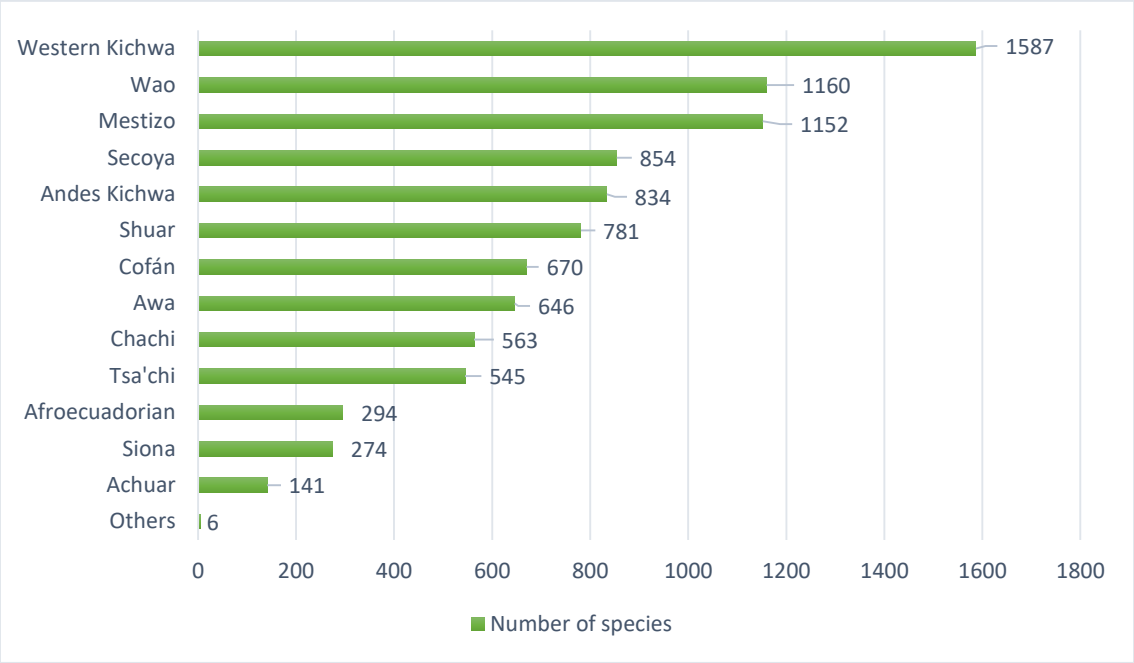

Figure 2. Number of useful plant species used by some ethnic groups of Ecuador.

3.1.1. Medicinal plants of Ecuador

Ethnobotanists have focused in recent years on the study of plants sold by local markets since they are considered places where there is a cultural exchange through the trade in natural resources and their derivatives (21,22). Vendors sell medicinal plants and provide information on what part of the plant should be used, the dosage necessary to calm the condition, and the method used for its administration (23). Traditional markets thus manage to provide a deeper and more enriching insight on ancestral knowledge and the use that is given to different plant species in the region, in addition to allowing the development of rural communities and biological conservation (22).

According to the World Health Organization (WHO), the health and well-being of 80% of the population of developing countries depend mainly on traditional medicine, based on the use of a diverse range of plants used for healing purposes when there is no access to better health care or when it is the only medical resource (21,23). The importance of knowing traditional medicine and studying ancestral knowledge lies throughout the world, especially in Latin America, where studies of ethnobotany and its history are necessary to meet the high demand (21). In Ecuador, the plant species that are traded between the local markets of the capitals of the Andes provinces correspond to a large number of ailments (4).

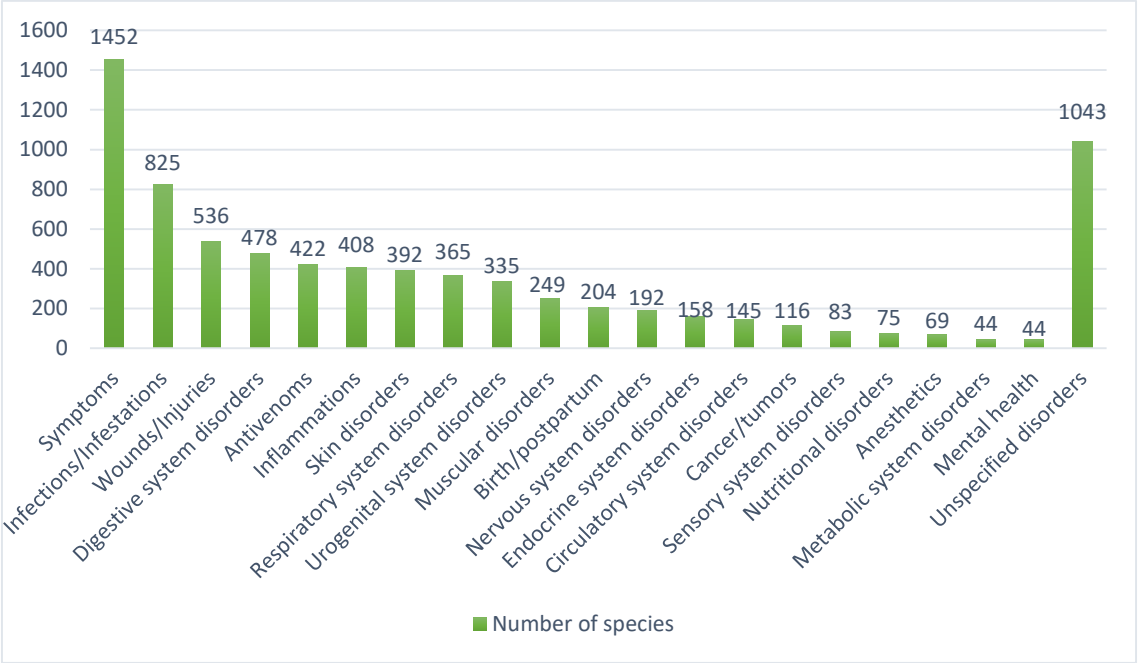

Figure 3. Number of plant species used for treating different ailments in Ecuador.

The "*Enciclopedia de Plantas Útiles del Ecuador*" has documented the use for 5,172 plant species and has grouped medicinal plants (60% = 3,118 species) in 24 disease categories (3,18) (**Figure 3**). Among the categories that present the most use of medicinal species are symptoms (47%), which is based on the treatment of pathological perceptions of the patient (fever, stomachache, diarrhea) (3). Followed by infections (26%), which is the category that focuses on treating diseases caused by bacteria, viruses, fungi, and parasites. However, bacterial infections are the main responsible for a large number of deaths globally (3,24). Wounds and injuries treatment represent 17% of all medicinal plants, while digestive system disorders represent 15%. The use of medicinal plants as antivenoms (14%) are used mostly to treat snake bites. However, plant resources are also widely used to treat inflammations (13%), dermatological problems (13%), and disorders of the respiratory system (12%). The rest of the categories represent a percentage of less than 8% of the total medicinal plants, although there are 30% of useful plants that have not been reported with any therapeutic use (3). A brief summary is shown in **Table 1.**

Table 1. Disease categories according to "*Enciclopedia de Plantas Útiles del Ecuador*" and some of the plant species used by local communities to treat these ailments.

Disease category	Ailments treated	Common plant species	Ref

Symptoms (47%)	Pathological perceptions: fever, stomachaches, headaches, cough, diarrhea	Chamomile (*Matricaria recutita*), lemon verbena (*Cymbopogon citratus*), verbena (*Verbena litoralis*), and ginger (*Zingiber officinale*)	(3)
Infections (26%)	Infections caused by bacteria, fungi, parasites or viruses such as bacterial food poisoning, dermatomycosis, malaria	Chigger (*Margyricarpus pinnatus*), paico (*Chenopodium ambrosioides*), matico (*Aristeguietia glutinosa*), blackberry (*Solanum nigrescens*), python (*Grias neuberthii*)	(3)
Wounds and injuries (17%)	Wounds and injuries caused by cuts, accidents	Matico (*Aristeguietia glutinosa*), dragon's blood (*Croton lechleri*)	(3)
Digestive system disorders (15%)	Flatulence, constipation, diarrhea, hangover	Dandelion (*Taraxacum officinale*)	(3,21)
Antivenom (14%)	Snake, scorpion, ant, manta rays bites		(3)
Inflammations (13%)	Internal inflammations (liver, kidney) and external inflammations (any part of the body)	Matico (*Aristeguietia glutinosa*), blackberry (*Solanum nigrescens*)	(3,4)
Dermatological disorders (13%)	Acne, gangrene, hair loss, dandruff		(3,21)
Respiratory system disorders (12%)	Flu, colds, coughing, asthma	*Borago officinalis*, *Verbena litoralis*	(3,22)
Urogenital system (8%)	Kidney, prostate, diuretic problems	Giant horsetail (*Equisetum giganteum*)	(3)
Skeletal system (8%)	Postpartum and childbirth ailments and recovery treatnents		(3)
Nervous system (6%)	Lack of coordination, memory loss, anxiety	Lemon balm (*Melissa officinalis*), valerian species (*Valeriana decusata*, *Valeriana officinalis*, *Valeriana plantaginea*)	(3)
Endocrine system (5%)	Menstrual cycle pain and irregularities	Moradilla (*Alternanthera porrigens*)	(3)
Circulatory system (5%)	Body pressure, chest tightness, cholesterol	Marco (*Ambrosia arborescens*)	(3,4)

Others (< 3%)	Visual problems, anemia, depression, allergies	Allergies: *Mollinedia ovata*, *Witheringia solanacea*	(3,21)

Phytotherapy studies the use of medicinal plants and their derivatives to prevent or treat diseases of all kinds. Most traditionally, herbal medicine uses the entire plant to treat conditions mainly through infusions or teas (25). However, it also uses different parts of the plant, such as the root, bark, leaves, flowers, seeds, and essential oil (26). In most parts of the plant, there are a large number of active compounds that possess biological properties that provide therapeutic effects in living organisms (27). In Ecuador, the majority of medicinal plants used for healing purposes are herbs (35%), shrubs (29%), trees (20%), and the one with the least uses are vines (4%) which can be shown in **Figure 4** (3).

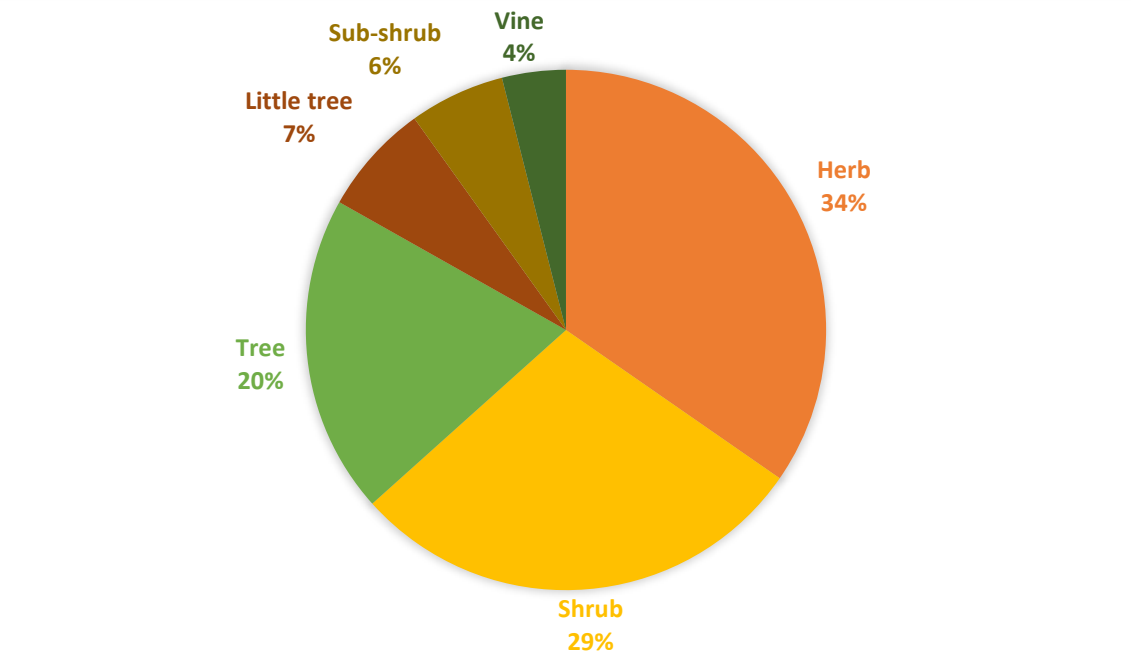

Figure 4. Plant habits used with medical purposes in Ecuador.

Of these, the morphological structures of the plant such as the leaves (30%) are used most abundantly, followed by the use of the entire plant (10%) and flowers (6%) according to the records of useful medicinal plants (9).

Chapter IV

4. Endemic plants of Ecuador

Among the medicinal species that are recorded in the "*Botánica Económica de Los Andes Centrales"* book are 5 endemic plants: matico (*Aristeguietia glutinosa*), mosquera (*Croton elegans*), salvereal (*Lepechinia rufocampii*), pumamaqui (*Oreopanax ecuadorensis*) and floripondio (*Brugmansia aurea*). Based on ethnobotanical surveys and studies, a high number of medicinal uses have been recorded for these endemic plants (**Table 2**). Among the most common conditions are inflammations, bacterial and viral diseases, as well as uses to alleviate small discomforts such as wounds and muscle pain (4). However, only matico, mosquera and salvereal are the species that are mostly sold by the local markets of the different provinces of Ecuador. These plants correspond to some of the most diverse families in the country, such as Asteraceae (matico), Euphorbiaceae (mosquera) and Lamiaceae (salvereal) (3).

Table 2. Endemic plants of Ecuador along with their medicinal use

Scientific name	Common name	Treating condition
Aristeguietia glutinosa (Lam.) R.M King & H. Rob.	Matico	Inflammation, cold, vaginal bath, postpartum bath, healing, itch, hot bath, ulcers, cancer
Brugmansia aurea Lagerh	Floripondio	Inflammation, hot bath, postpartum bath
Croton elegans Kunth	Mosquera	Inflammation, molar pain, healing, warts, tonsillitis, vaginal bath
Lepechinia rufocampii Epling & Mathias	Salvereal	Inflammation, hot bath
Oreopanax ecuadorensis Seem.	Pumamaqui	Cold, hot bath, postpartum bath

Medicinal plants and the different extracts obtained from the leaves, inflorescences, stems, fruits, and roots, are of major importance in traditional medicine and currently occupy an essential place

in the development of new medicines in the pharmaceutical industry (28). According to ESPOL Technological Magazine (2010), around 80% of the population of Ecuador uses traditional medicine as the first resource to treat any type of ailment (29). For this reason, Ecuador, as a megadiverse country, has a critical study potential in the discovery of various biological activities of the plants present in the region, especially those that only grow in the country (endemic). However, most of these species have not been scientifically evaluated, and the information on them is very reduced (28). In the following chapter, the taxonomy (**Table 3**), a geographical and therapeutic description of the three endemic species that are most used and that are widely distributed in the local markets of the country is presented. In addition to a list of the phytochemical and biological studies that have been developed to date.

Table 3. Taxonomy of endemic plantas of Ecuador: *Aristeguietia glutinosa, Croton Elegans, and Lepechinia rufocampii.*

	Aristeguietia glutinosa	***Croton Elegans***	***Lepechinia rufocampii***
Kingdom	Plantae	Plantae	Plantae
Phylum	Magnoliphyta	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Equisetopsida	Magnoliophyta
Order	Asterales	Malpighiales	Lamiales
Famly	Astereaceae	Euphorbiaceae	Lamiaceae
Genus	Aristeguietia	Croton L.	Lepechinia
Common names	Matico, yerba del soldado, matigo, migla, chuzalongo	Mosquera, cucharilla, mosquero, purga	Salvereal, Salvia gateada

4.1. *Aristeguietia glutinosa* Lam.

Ariesteguietia glutinosa is a shrub that belongs to the Astereaceae family and is located at 2000-4000m at sea level. This species has been described among the endemic plants of the Ecuadorian Andean region, and its location has been reported in some provinces of the Sierra such as Azuay, Cañar, Tungurahua, Cotopaxi, Chimborazo, Pichincha and Imbabura and the East, in Napo (29). The most popular name by which *A. glutinosa* is known as matico, although depending on the place it has other names such as yerba del soldado, matigo, migla y chuzalongo (30). In traditional medicine, the parts of the plant that are most used for medicinal purposes are the leaves, flowers

and the stem, and therapeutic properties such as antimicrobial, antiviral, antirheumatic, healing, antiseptic, antioxidant, anti-inflammatory (respiratory tract conditions) and reliever of gastrointestinal problems have been attributed (31). Phytochemical studies have described the presence of flavonoids, steroids, coumarins, alkaloids, triterpenes, saponins, and phenolic compounds (32). In the latter ones are tannins, one of the most important active compounds in this species, to which the healing activity is mainly attributed (31). Several studies are based on obtaining different matico extracts to test their possible biological activities. Among them, its antifungal activity has been proven against organisms that cause dermatomycosis such as *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, *Candida albicans*, from the essential oil with a concentration of 2-5% (6,28). Another paper described the hydro-ethanolic extract obtained from the aerial parts (leaves, inflorescences, and twigs) of the plant as a potential anti-*trypanosoma cruzi* agent, the microorganism that causes Chagas disease, obtaining two important compounds (15-hydroxy -labd-7-en-17-al and (+) - 13,14,15,16-tetranor-labd-7-en-17,12-olide), to which this therapeutic property is attributed (30). A similar study was carried out in vivo in BALB / c mice inoculated with *Trypanosoma cruzi* trypomastigotes, where favorable results were obtained in the same way (33). The anti-inflammatory effect of *A. glutinosa* was tested in two types of in vivo studies, the first was performed in *Mus musculus* mice from the ethanolic extract obtained from matico leaves and the second was performed in zebrafish according to the *Danio rerio* model from hydro-ethanolic and choloformic extracts. Both studies demonstrated the anti-inflammatory activity of *A. glutinosa*. (31,34). The phytochemistry of matico has not been widely explored, although only one study has been carried out to analyze all the compounds present in the plant from its leaves. However, it was not possible to find complete information (35). Despite the wide distribution of *A. glutinosa* in the Andean region of Ecuador, it faces risks in its species due to deforestation, caused mainly by the need for agricultural land, which is why the "Libro Rojo" of endemic plants of Ecuador classifies it as a species with least concern (36). A summary of these properties is shown in **Table 4.**

Table 4. Biological studies of *Aristeguietia glutinosa* Lam.

Study	Type	Organism	Extract	Method	Plant part	Phytohemicals	Ref
Antifungal	*In vitro*	*Trichophyton mentagrophytes*ATCC 9533, *Trichophyton rubrum* ATCC 28188, *Microsporum canis* ATCC 36299, *Candida albicans* ATCC 10231	Essential oil	Steam distillation	-	-	(28)
Anti-*Trypanosoma cruzi*	*In vitro*	*Trypanosoma cruzi* epimastigotes	Hydro-ethanolic	TLC	Aereal parts (leaves, inflorescences, and twigs)	diterpenoids, (+)-15-hydroxy-labd-7-en-17-al / (+)-13,14,15,16-tetranor-labd-7-en-17,12-olide	(30)
Anti-inflammatory	*In vivo*	*Mus musculus* (mice)	Ethanolic	Maceration	Leaves	Alkaloids, triterpenes, steroids, tannins, coumarins	(34)
			Hexanic			Alkaloids, triterpenes y steroides	
			Dichloromethane-methanol			Alkaloids, triterpenes, steroids, tannins	
Antifungal	*In vitro*	*Trichopython mentagrophytes, Trichophyton rubrum,*	Essential oil	Steam distillation	-	-	(6)

		Microsporum canis, Candida albicans					
Anti-*Trypanosoma cruzi*	*In vivo*	*Trypanosoma cruzi* trypomastigotes strain was carried out in BALB/c mice inoculated	Hydro-ethanolic	-	Aerial parts	-	(33)
Anti-inflammatory	*In vivo*	Zebrafish model (*Danio rerio*)	Hydro-ethanolic, Chloroform	Maceration	-	-	(31)

4.2. *Croton Elegans* Kunth

The *Croton Elegans* shrub is an endemic plant of Ecuador belonging to the Euphorbiaceae family. It grows at 1500-3500 m at sea level and is distributed throughout the inter-Andean zone between the province of Imbabura and Loja, including Pichincha, Tungurahua, and Carchi (37,38), especially on the roads and edges of the roads (39). It is mostly known as a mosquera, although it can also be found as a cucharilla, mosquero and purga. Among the pharmacological properties attributed to this plant are antimicrobial, antifungal, anti-inflammatory (40), antitumor, antiallergic, healing (38), antioxidant, antipasmodic, anti-hypertensive (41), antiviral, also used for treatment of ulcers, tonsillitis, angina and to relieve digestive problems (42). In traditional medicine, the resin of the mosquera is one of the most used since it treats molar pain, gum infection, remove warts, relieve pain in the throat, among other conditions.

While latex plays an important role also when used as external healing and as a reliever in insect bites (37). There is not much research on the chemical composition of this plant, so up to now, only one chemical report on *C. elegans* has been published. Due to the richness of compounds with biological activities that the genus and the family of this species present, the study focused on the hexane extract obtained from the leaves of moquera and through chromatographic methods, compounds such as flavonoids, phenolic compounds, tannins, sterols, triterpenoids (friedelin and cycloeucalenol) and alkaloids (pallidine and O-methylpallidine) were found. The last two being one of the most important discoveries as they are different enantiomers in this genus (43). The various therapeutic properties of *C. elegans* have been proven in vitro and in vivo studies. One of them verified the healing activity in *Mus musculus* mice from ethanolic, aqueous, and ethereal extracts (42), while another study analyzed the same property with ethanolic extracts on canine females undergoing ovary hysterectomy (OVH) (40). A third study focused on microorganisms

causing dermatomycosis such as *Trichophyton mentagrophytes*, Trichophyton rubrum, *Microsporum canis* and *Candida albicans* to determine the antifungal activity of the ethanolic extracts of the mosquera leaves, obtaining an evident inhibition in all the microorganisms except for *Candida albicans* (41). However, the antibacterial activity against the organisms *Staphyloccocus aureus, Streptoccocus mutans, Streptoccocuspneumoniae, Streptoccocus pyogenes* through extracts of chloroform and hexane, was the only study where no favorable results were obtained (38). Given that it is a very abundant species and that the threats it faces are deforestation and human-caused fires, it is considered by the "Libro Rojo" of Endemic Species of Ecuador as a species of least concern (39). A summary of these properties is shown in **Table 5.**

Table 5. Biological studies of *Croton Elegans* Kunth.

Study	**Type**	**Organism**	**Extract**	**Method**	**Plant part**	**Chemical compounds**	**Ref**
Healing cream	*In vivo*	*Mus musculus* mice	ethanolic, aqueous, ethereal	Maceration	-	Alkaloids, Flavonoids, coumarins, lactones	(42)
Post-surgical healing	*In vivo*	Canine female subjected to OVH	Ethanolic	Maceration	Leaves	-	(40)
Antifungal	*In vitro*	*Trichophyton mentagrophytes* ATCC 9533, *Trichophyton rubrum* ATCC 28188, *Microsporum canis* ATCC 36299, *Candida albicans*	Ethanolic	Maceration and perlocation	Leaves	Catechins, resins, lactones, quinones, flavonoids, alkaloids, priterpenes and steroids	(41)
Healing cream	-	-	Ethanolic	Maceration	Leaves	-	(37)
Antibacterial	In vitro	*Staphyloccocus aureus* ATCC: 25923,*Streptoccocus mutans* ATCC: 25175, *Streptoccocus*	Chloroform-hexane	Soxhlet	Leaves	Quinones, alkaloidal, flavonoids, catechins, amino acids, esins	(38)

		pneumonia ATCC: 49619,*Streptoccocus pyogenes* ATCC: 19615					

4.3. *Lepechinia rufocampii* Epling & Mathias

Lepechinia rufocampii, commonly called salvereal or salvia gateada, is an endemic species of the inter-Andean zone of Ecuador that has been reported mainly in the province of Azuay. It belongs to the Lepechinia genus and the Lamiaceae family and is found in Andean forests at 2000-4000 m at sea level (44). It is a greenish shrub that has a pleasant aroma and its application in medicine lies in the use of its leaves, flowers and stem for therapeutic purposes (45). The use of salvereal in conditions of the respiratory tract (cough and tuberculosis) has been described in traditional medicine, as well as an antispasmodic, diuretic, stimulant and to control the menstrual cycle. Externally it is used for facial treatments, molar pain, angia, and as an antifungal. Phytochemical and biological activity studies on *L. rufocampii* are scarce. So far, only one study published in 2013 has described the possible antibacterial activity of salvereal essential oil against *Escherichia coli* and *Salmonella thyphimurium*. However, the identified compounds (β-pinene, α-pinene, limonene, linalol, menthone) did not show significant inhibition against these microorganisms (46). Being a species that is threatened by civilization and the deforestation of forests in the area, it is considered vulnerable by the "Libro Rojo" of Endemic Plants of Ecuador (45). A summary of these properties is shown in **Table 6.**

Table 6. Biological studies of *Lepechinia rufocampii* Epling & Mathias.

Study	**Type**	**Organism**	**Extract**	**Method**	**Plant part**	**Phytochemicals**	**Ref**
Antibacterial	In vitro	*Escherichia coli* and *Salmonella thyphimurium*	Essential oil	Steam distillation	Leaves	β-pineno, α-pineno, limoneno, linalol, mentona	(46)

4.4. Biological properties of Astereaceae, Euphorbiaceae and Lamiaceae families

4.4.1. Astereaceae

The family Astereaceae (Compositae), is one of the largest and most diverse families comprising around 1,600 genera and more than 23,000 species in the world (47,48) . It is also known as "daisy" or "sunflower" since most species belonging to this family have star-shaped inflorescences that open their petals during the day and close at night (49). It is the family of angiosperm plants that registers the highest abundance of useful plants in Ecuador (50). For being so diverse, especially in the Andean regions (51), a large number of investigations have been led in the last 25 years to learn more about these species, focusing on aspects such as their morphology, ecology, anatomy, and ontogeny as well as phytochemical and toxicological analyzes (52). Plants belonging to this family have been characterized by extensive studies on their compounds and biological activities summarized in **Table 7** (53). They are mainly known for their therapeutic properties as an antibacterial, anti-inflammatory, anti-tumor, insecticides (52), antifungals (54), and antioxidant agents. Being species that are often subjected to extreme conditions and environmental stress factors (such as microbial infection and UV light (47), the compounds responsible for pharmacological activities can vary, presenting a high diversity of secondary metabolites (51). Among the most abundant compounds in this family are phenoles, flavonoids, and dipernoids (54). The Astereaceae family includes some of the oldest and most studied medicinal species on the planet (48). Its most representative species for being widely used, studied, and distributed by the markets are *Baccharis trimera* (Less), *Matricaria chamomilla* L. (camomile), *Cynara scolimus* L. (artichoke), *Vernonia condensata* Baker (necroton) and *Arnica montana* L. (true arnica) (52).

Table 7. Description of the biological activities and chemical composition of some Astereaceae species.

Species	**Common name**	**Biological activity**	**Ref**	**Phytochemicals**	**Ref**
Baccharis trimera	Carqueja	Antioxidants, anti-inflammatory, antispasmodic, antiparasitic, antibacterial, antiviral	(55)	Phenolic compounds, sesquiterpenes, saponins, flavonoids, and diterpenic lactones	(56)

		Liver and gastrointestinal disorders	(57)		
***Matricaria Chamomilla* L.**	Chamomile	analgesic, antioxidant, anti-inflammatory, antimutagenic, antispasmodic	(58)	phenols, flavonoids, coumarines, polyacetylenes, sesquiterpenes	(57)
		antibacterial, antifungal	(59)		
		inflamaciones en la piel	(60)		
Cynara Scolymus	Artichoke	antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, anticarcinogenic, hepatoprotective	(61)	Polyphenols, inulin, flavonoids, apigenin, luteolin, cynarin	(61)
		digestive system disorders	(62)		
***Vernonia condensata* Baker**	Fitagirl, necroton	antioxidant, analgesic, anti-inflammatory, anti-tumor	(63)	saponins, alkaloids, phenols, flavonoids, tannins	(63)
		hepatoprotective, antivenom	(64)		
		gastrointestinal diseases	(65)		
		Antibacterial, antiulcerogenic	(66)		
***Arnica Montana* L.**	Arnica	antibacterial, anti-inflammatory, anti-rheumatic, analgesic	(49)	flavonoids, sesquiterpenes, terpenoids, lactons, phenolic acids	(67)
		antifungal, antioxidant and antisclerotic	(67)		
Silybum marianum	milk thistle	kidney, spleen, liver, and gallbladder diseases Hepatoprotective, anticarcinogenic	(68)	Sylimarin active componente - flavonolignan (flavonoids and lignans) such as silybin, isosilybin(A and B), silydianin, and silychristin	(68)
Echinacea spp.	Echinacea	Antioxidant, antibacterial, digestive disorders, respiratory tract infections	(69)	Terpenes, phenolic compounds, alkaloids, caffeic acid derivatives, alkylamides	(69)

***Matricaria chamomilla* L.**	German *chamomile*	anti-inflammatory, analgesic, sedative, antimicrobial, anti-allergic, anti-hyperglycemia and antispasmodic	(70)	Phenolic compounds, flavonoids, coumarins	(70)
Chamaemelum nobile	Roman chamomile	antibacterial, antifungal, insecticidal, hypotensive, intestinal problems, anti-inflammatory, antioxidant, antispasmodic, and sedative.	(57)	Sesquiterpenes, flavonoids, coumarins, polyacetylenes, phenolic acids, triterpenes and steroids	(57)
Taraxacum officinale	Dandelion	diuretic, antioxidant, anti-rheumatic, anti-allergic, anti-inflammatory, analgesic, anticoagulant, antimicrobial, anti-carcinogen	(71)	Terpenes, phenolic compounds, flavonoids	(71)

4.4.2. Euphorbiaceae

Euphorbiaceae (Spurge family) is one of the most extended and most complex families of flowering angiosperm plants in the world and is found mainly in the tropics and subtropics. It is considered of great importance because it is the source of the development of medicinal therapies, toxins, and research material for many phytochemical studies (72). The Euphorbiaceae family is made up of more than 300 genera and 8,000 species, of which it registers a high number of medicinal species that are widely distributed throughout the world (73). The biological activities provided by several members of Euphorbiaceae is extensive, since in traditional medicine these plants have been used as antibacterial, antiviral, antifungal, anticancer, wound-healing, hepatoprotective, antispasmodic, anti-diarrheic, anti-inflammatory, insecticidal, to relieve problems related to the nervous and circulatory system (74), and as an antioxidant (72). Studies on their chemical composition revealed that most of them have alkaloids, steroids, phenolic compounds, flavonoids, saponins (73), tannins, and peptides as secondary metabolites (75). Because these plant resources are widely distributed and exposed to different environments, they have been in need of developing an extensive range of defensive secondary metabolites, which would explain its great therapeutic variety (76). Due to their high medicinal value and economic importance, the species *Acalypha indica* L., *Euphorbia hirta* L., *Croton bonplandianum* baill, and *Jatropha gossypifolia* L. are considered one of the plants with great international trade and full of great medicinal properties (73). The biological activities of these plants, among others, are described in **Table 8**.

Table 8. Description of the biological activities and chemical composition of some Euphorbiaceae species.

Species	Common name	Biological activity	Ref	Phytochemicals	Ref
Acalypha Indica L.	Indian acalypha	antimicrobial, antidiabetic, antioxidant, antivenom	(77)	saponins, flavonoids, tannins, phenolic compounds	(78)
		hepatoprotective, anti-inflammatory, anticancer and analgesic	(75)		
		skin disorders, asthma, rheumatism, pneumoniae	(79)		
Euphorbia hirta L.	Garden spurge/ asthma plant	antimicrobial, antifungal, anti-inflammatory, anticancer, antioxidant, antidiabetic	(80)	tannins, saponins, alkaloids, flavonoids	(81)
		hepatoprotective, antiparasitic	(82)	terpenoids, steroids, proteins, oils, coumarins and phenolic compounds	(82)
		sedative, analgesic, wound healing, treating gastrointestinal and respiratory disorders	(83)		
Croton bonplandianum Baill	Ban tulsi	analgesic, antimicrobial, antioxidant, anticancer, anticoagulant, wound healing	(84)	terpenoids, flavonoids	(84)
		hepatoprotective, insecticide, antifungal, antivenom, gastrointestinal disorders, respiratory diseases	(85)	steroids, phenolic compounds, alkaloids, and carotinoids	(85)
	Cotton-leaf physic nut	Skin disorders, cancer and diabetes treatment,	(86)	Phenolic compounds, flavonoids and alkaloids	(86)

Jatropha gossypifolia L.		Antibacterial, antioxidant			
		Digestive system disorders, as purgative, diuretic	(87)		
Alchornea glandulosa	Tapiá	anti-inflammatory, control of inflammatory disorders, antitumoral, antimicrobial, rheumatism and muscular pain	(88)	Alkaloids, terpenes and steroids, phenolic acid, saponins	(88)
Croton draco	Sangre de drago	hemostatic, antidiarrheal, antiulcer, antiviral, healing, anti-tumor, anti-inflammatory, antioxidant and antimicrobial	(89)	Calcaids, tannins, diterpenes and volatile oils	(89)

4.4.3. Lamiaceae

The Lamiaceae family constitutes around 236 genera and a little more than 6,900 species, representing one of the largest groups of medicinal herb plants (90). The plants of this family have extensive use in traditional medicine, modern medicine, and also in complementary medicine (91,92). The most abundant plant species of Lamiaceae are popular aromatic plants that are found in many parts of the world such as oregano (*Origanun vulgare*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*) and sage (*Salvia officinalis*), important species especially in the traditional Spanish remedies (91,93). These plants have been used for healing purposes of treating ailments such as gastritis, skin problems (dermatitis), respiratory system disorders, as an antioxidant, anti-inflammatory, antimicrobial (91), antifungal, anti-tumor, antidepressive, anticancer, antiangiogenic and antihepatotoxic (94). The essential oil extracted from these plants is the most studied by scientists to test their different therapeutic properties. However, different parts and extracts of the plant are also used to know its pharmacological effects (91,94). The biological activities described for the Lamiaceae family are mainly because these herbs are rich

in phenolic compounds, in addition to possessing compounds such as eugenol, carvacrol, and thymol in their essential oil with a role of potentially therapeutic agent (93). The pharmacological properties and phytochemical composition of somo Lamiaceae plants are described in **Table 9.**

Table 9. Description of the biological activities and chemical composition of some Lamiaceae species.

Species	**Common name**	**Biological activity**	**Ref**	**Phytochemicals**	**Ref**
Rosmarinus officinalis L.	Rosemary	antioxidant, antidiuretic, hepatopretective, antinociceptive, antithrombotic, antiulcerogenic	(95)	Phenolic compounds (caffeic acid, rosmrinic and carnosic acid, carnosol, hesperidin), flavonoids, diterpenoids	(96)
		anti-inflammatory, antiproliferative, antimicrobial, metabolic and neurological disorders	(97)		
		antifungal and insecticide	(96)		
Thymus vulgaris L.	Thyme	antimicrobial, disinfectant, antiseptic, carminative, astringent, tonic, anti-inflammatory	(98)	Thymol (active component), terpenes, sesquiterpenes	(98)
		antioxidant, anti-carcinogenesis, antispasmodic, antiparasitic, antifungal, wound healing, sedative, skin disorders, circulatory, respiratory, digestive and nervous system disorders	(99)		
Salvia officinalis L.	Sage	anthelmintic, antiseptic, antibacterial, anti-tumor,	(100)	alkaloids, flavonoids, saponins, phenolic compounds, terpenoids,	(101)

		antimutagenic, antifungal and antioxidant		steroids, and polyacetylenes	
Origanum vulgare L.	Oregano	antimicrobial, anticancer, anti-inflammatory, antiseptic	(102)	Carvacrol, thymol, flavonoids and phenolic acids, sterols	(103)
		antiviral, antispasmodic, antiproliferative	(103)	Tannins, terpenoids	(102)
Ocimum basilicum L.	Basil	anti-osteoporotic, antianxiolytic, sedative, antibacterial activity, cytotoxic activity, cardiovascular disease, anti-hepatotoxicity effect, antioxidant capacity,hypoglycemic effect, anti-inflammatory effect, insecticidal	(104)	Alkaloids, Saponins, Flavonoids, Tannins, Glycosides, Steroids, Terpenoids and Resins	(105)

Chapter V

5. Drug discovery of natural plant products

Natural products are defined as substances or compounds obtained from living organisms such as plants, animals, mushrooms, and even from a wide variety of microorganisms. It can also refer to the organism itself (plant, animal) or different parts of it, such as leaves, stems, roots, organs of animals, or glands (106). The discovery of new drugs is linked to a challenging task for scientists since it requires looking for a candidate that is viable and solid enough to be considered as a possible therapeutic agent (107). In this way, natural products and their derivatives have been one of the primary sources and significant importance in the development of new drugs because they are the main active ingredient (108). It is estimated that around 40% of the drugs in the world correspond to products of natural or semi-synthetic origin of their derivatives (107). When we refer to the natural products of plants, these correspond to the secondary metabolites produced by all these organisms as a result of their adaptation to different environments (abiotic and biotic factors) or as a defense mechanism against predators. For these reasons, there is a wide variety of secondary metabolites in the plant structure and thus represent a potential candidate in drug discovery (109).

According to the Food and Drug Administration (FDA) and the European Medical Agency (EMA), around a quarter of the drugs that have been approved have plant origin (110). In Figure 5 is shown that between 1981-2019, the FDA has approved 1,881 drugs, of which 19% were derivatives of natural products ("ND"), 7.4% were a mixture of botanical drugs ("NB"), 3.8% belonged to natural products that have not been altered ("N"), another 3.5% corresponded to drugs synthesized from natural pharmacophore ("NS") products and the rest corresponded to synthetic drugs ("S") (111). However, of the total plant species on Earth, only 10% have been registered with uses in folk medicine, and of these, 5% have been subjected to phytochemical studies proving their medicinal properties (15). Until 2015, the number of known drugs that were developed from plant screenings was 135, and it is estimated that 700 new drugs will be discovered with phytochemical studies of the remaining plant species (109). This means that there is a wide field of pharmacological research on terrestrial plants leading to a possible herb-drug development (15).

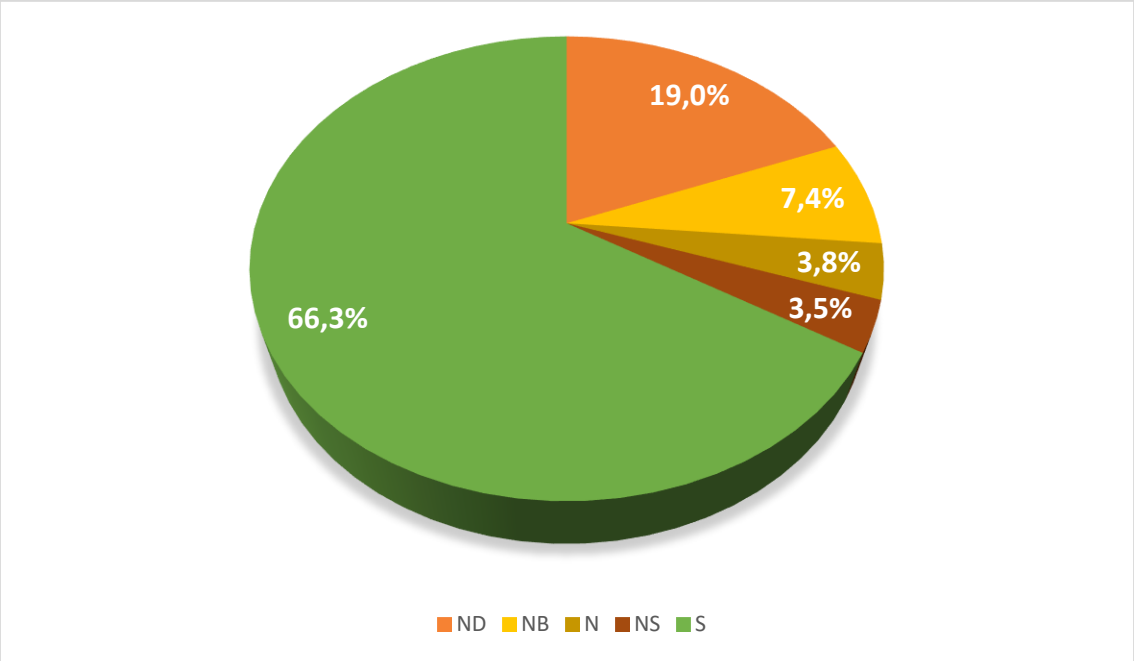

Figure 5. FDA approved drugs between the years 1981 to 2019 (n= 1881).

"ND": Natural product derivative, "N": Natural product which is unmodified in structure, "NB": Botanical drug as it is a mixture of natural products, "S": Totally synthetic drug, "NS": Made by total synthesis, but the pharmacophore is from a natural product

The bioactive compounds of drugs interact with macromolecules of the human body, such as proteins or nucleic acids, to produce a therapeutic effect against a type of ailment or disease. This process has led scientists to think about the importance of identifying chemical compounds from plants, using isolated components as the main ingredient in drugs, thus promising better medical treatment (109). However, in traditional medicine, plant extracts are used to treat any type of ailment in a combination of chemical compounds, since the isolated compounds may not have a specific biological activity, so a synergistic activity is required to fulfill this function on some occasions. Due to the complexity of the diseases, the drugs that exist must be capable of treating the ailments effectively and safely. This is why some of the drugs that are based on a single compound have failed in their process to be an effective drug to treat diseases (110). Since it can be a very complicated and time-consuming process, looking for a mixture of chemical compounds in plant extracts, instead of single compounds, is one of the new methodologies used in order to show high biological activity (108).

5.1. Advantages and disadvantages

If we compare with synthetic drugs, medicines with natural active ingredients have managed to moderate the side effects of diseases and severe treatments such as chemotherapy (109). The main problem with synthetic drugs is that they produce a large number of adverse effects that are dangerous to people's health and that they are used as a last resort to treat terminal diseases such as cancer. The secondary metabolites of plants have been shown to be harmless and less toxic than synthetic drugs, as they are considered compounds that are made within living organisms, so they would be less aggressive to the human body than synthetic ones (15,107). So far, it is estimated that 40% of existing phytochemicals are not registered in current medications, suggesting that these compounds may be complementary to molecules that are produced synthetically. Another benefit of natural products in the pharmaceutical industry is that their large chemical composition has been shown to be capable of interacting with other molecules to produce a biological effect, which corresponds to one of the essential requirements in the development of safe and effective drugs (107).

According to several studies, the interest in researching the natural products of plants decreased due to the difficulty in obtaining the necessary raw material due to problems of legislation and permits to have access to natural resources, the high costs of the procedures to identify the compounds, the difficulty to isolate the chemical components (112), the redundancy of the molecules found, the high toxicity that some chemicals presented and above all, the little investment by governments in the development of new drugs from plant resources. Despite all the disadvantages shown above, chemical synthesis failed to meet expectations to reach the market by the pharmaceutical industry (109). The popularity of synthetic products lay in their available cost of production, optimal time, easy quality control, quick effects, and a better regulation system, but their safety and efficacy were still in question, thus encouraging the use of natural products (113). An evident decrease in the number of synthetic drugs was found between 1981-2010, of which the 1,135 new drugs approved by the FDA, only 36% were synthetic while the rest had at least one active compound of plant origin or derivatives. In addition to the fact that the number of drugs approved by these organizations is decreasing as the years go by. Thus, in 2010, of the 45 new drugs proposed by pharmaceutical companies, only 21 were approved. For this reason, the scientific community regained research interest in the use of chemical substances obtained through plants for the development of new drugs that could treat a large number of diseases (109).

One of the disadvantages and advantages of extracting bioactive compounds in plant species is their complex chemical structure (107). Elements such as high molecular weight, a high number of rings, a large number of chiral centers, free rotatable bonds, among others, are responsible for increasing the complexity of the molecular structure in these plants (109). This does not only make their study and synthesis difficult due to costs and time, but also by having this great

diversity there is a greater probability of finding new compounds with therapeutic properties. These complex structures allow natural products to serve as precursors for the production of semi-synthetic drugs, medicines that can also be produced by total synthesis, as well as allowing the creation of analog drugs based on these molecules as templates (107).

5.2. Drugs of natural origin on the market

Currently, there are many plant-derived drugs that you can be found on the market today. The main development of drugs with natural products is based on the production of plant extracts, which, based on ethnobotany and phytochemical studies, can determine these molecules as enhancers for the production of drugs or as dietary supplements (109). One of the most common semi-synthetic drugs used as an analgesic and anti-inflammatory in the world is aspirin. Bayer discovered it in 1899, and its active principle is acetylsalicylic acid that comes from salicin from the bark of the Salix alba tree (109,113). Another drug used for the treatment of several types of cancer, such as lung, ovarian, and especially breast cancer, is Taxol. This drug originates from the bark of *Taxus brevifolia* tree, from which the active compound paclitaxel was extracted k, giving it this therapeutic function (15,109,113). Due to the low yield of extraction from the bark and the high demand, it is currently produced synthetically (109). Hypericin or pseudohypericin are active principles extracted from the flowers of the *Hypericum perforatum* (St. John's wort) plant and are used mainly as antidepressants and retroviral by preventing encapsulated viruses such as HIV from being transcribed (114). A drug like Veregen, its trade name, is used to treat genital wounds caused by sexually transmitted diseases such as human papillomavirus, and its main compound is the sinecatechins extracted from the *Camellia sinensis* plant (109,115). Another widespread drug known as one of the opioid pain relievers is morphine. This alkaloid was extracted from the *Papaverum sonniferum* (opium poppy) plant by Merck in 1826, and it helps to relieve pain by being able to control different signals from the brain and nervous system in response to any pain (116).

After several studies were carried out by the National Cancer Institute (NCI), it was possible to isolate the prostatin compound from the bark of a Samoan plant called *Homalanthus nutans*, which, through the activation of the kinase C protein pathway, inhibits infections by HIV-1 and reduces its latency in the body as well (109,117). The bioactive compound camptothecin is an alkaloid used as a drug for the treatment of cancer, isolated from the stem and bark of the *Camptotheca acuminata* tree, important in traditional Chinese medicine. This metabolite has antitumor activity by acting as an antiproliferative against malignant tumors in the colon, lung, and breasts (118). *Catharanthus roseus* is the plant from which the compound vinblastine is

extracted, an anticancer agent used to treat especially Hodgkin's disease, testicular tumors, breast carcinoma, and even leukemia (110). Artemisinin is a secondary metabolite (sesquiterpene lactone) obtained from the wormwood of *Artemisia annua*, an ancient Chinese plant used in different therapeutic therapies. Its main function is as an antimalarial when treating multi-drug resistant strains of falciparum malaria (113,119). A second drug to prevent and treat malaria is quinine, approved by the FDA in 2004 (12). This secondary metabolite (alkaloid) is obtained through the bark of the cinchona tree. Its ancestral use is centered as an antimalarial drug, although it also has antiarrhythmic activities (120). Among other drugs with pharmacological use is curare (3). This medicine is isolated from the stem and bark of the *Chondrodendron tomentosum* plant and was used since ancient times as anesthesia because its main active component, D-tubocurarine, can cause muscle relaxation, which facilitates the procedure during surgical operations. However, an inappropriate dose can cause muscle paralysis and be fatal (121). In **Table 10** there is a small summary of the most common drugs on the market that are developed from natural products.

Table 10. Brief summary of the origin and biological activity of some of the most common drugs derived from natural products.

Commercial name	**Plant source**	**Active compound**	**Biological activity**	**Ref**
Aspirin	*Salix alba*	acetylsalicylic acid	Analgesic, anti-inflammatory	(15)
Taxol	*Taxus brevifolia*	paclitaxel	Cancer treatment	(15)
Hypericin	*Hypericum perforatum*	Hypericin / pseudohypericin	Retroviruses, antidepressant	(114)
Veregen	*Camellia sinensis*	sinecatechins	Genital wounds	(115)
Morphine	*Papaverum sonniferum*	Morphine	Analgesic	(116)
Prostatin	*Homalanthus nutans*	Prostatin	HIV-1 treatment	(117)
Artemisinin	*Artemisia annuato*	Artemisinin	Malaria treatment	(119)
Silymarin	*Silybum marianum*	Silibinin	Antioxidant, antineoplastic, hepatoprotective	(113,122)
Atropine	*Atropa belladonna*	atropine	Antimuscarinic, anticholinergenic	(123)
Curare	*Chondrodendron tomentosum.*	D-tubocurarina	Anesthesia	(121)

Galantamine	*Galanthus nivalis*	Galantamine	Alzhemer's treatment	(124)
Digitoxin	*Digitalis purpurea*	Digitoxin	Heart deficiency	(125)
Quinine	*Cinchona tree*	Quinine	Antimalarial	(125)
Pilocarpine	*Pilocarpus jaborandi*	Pilocarpine	Cholinergic agonist	(126)
Vinblastine	*Catharanthus roseus*	vinblastine	Anticancer	(110)
Aescin	*Aesculus hippocastanum*	Aescin	Anti-inflammatory	(107)
Caffeine	*Camellia Sinensis*	Caffeine	CNS stimulant	(107)
Vincamine	*Vinca minor*	Vincamine	Cerebral stimulant	(107)
Vumon	*Podophyllum peltatum*	Teniposide	Anticancer	(107)
Camptothecin	*Camptotheca acuminata*	Camptothecin	Anticancer	(118)

These and other drugs of natural origin have revolutionized current medicine. Taking into account that almost 80% of the botanical diversity in the world has not been studied for the analysis of possible biological activities, designing drugs by taking advantage of the plant resources seems to promise a successful future in the medical field, allowing to combat a large number of diseases worldwide. To develop it, different methodologies are used to define the chemical composition of plants and to identify secondary metabolites in their extracts that may have a possible therapeutic effect and thus be the protagonists for drug discovery (109).

5.3. Process for testing the biological activity of natural products

Plants have formed a fundamental role as part of modern medicine and the development of the pharmaceutical industry, mainly for the fourth critical reason. The first is because plants are used in traditional medicine as direct therapeutic agents. The second is that they are used as raw material for the production of other semi-synthetic chemical components. The third is based on its complex and diverse chemical structure that has been used as the basis for creating new synthetic compounds, and the fourth is that they serve as guides for the discovery of new medicinal agents according to the knowledge that exists about their taxonomy (127). The research that leads to drug discovery is based on the study of phytochemistry. Phytochemistry or phytoanalysis is a scientific discipline that aims to define the chemical composition of plant resources and study these components to discover potential therapeutic agents (15). The secondary metabolism of plants produces biologically active compounds that are formed as a defense mechanism and adaptation to the environment. These chemical compounds are also

known as secondary metabolites or phytochemicals (128). Among the most common phytochemicals are phenols, alkaloids, terpenes, flavonoids, saponins, steroids, and others (129). According to the literature, it has been shown that phenolic compounds are the most abundant among plants (45%) and produce the most biological activities (128). It is estimated that approximately 50k secondary metabolites have been described in plants, and with those for which a phytochemical screening is not yet available, it could reach more than 200k (129). Among the properties assigned to these active components are antimicrobial, anticancer, antioxidant, antifungal, antiviral, and some others (15). **Table 11** describes the several biological properties attributed to the different secondary metabolites based on the information of previous studies in the literature.

Table 11. Biological properties of some essential secondary metabolites isolated from plants.

Secondary metabolite	**Biological activity**	**Ref**
Alkaloids	Anticancer, antibacterial, antiviral, antioxidant, antifungal	(130)
Phenolic compounds	Antibacterial, antifungal, antioxidant, anticancer, antiviral, antitumoral, anti-inflammatory	(128)
Terpenoids	Antibacterial, antifungal, anti-inflammatory, anti-hypertensive	(128,131)
Flavonoids	Antioxidant, anti-inflammatory, antiulcer, antiviral, anti-cancer	(132)
Tanins	Antibacterial, antiviral, antifungal, antioxidant	(133)
Saponins	analgesic, antifungal, antimicrobial, antiviral, antioxidant	(134)
Coumarins	Anti-inflammatory, anticancer, anticoagulant, antioxidant, antiviral, antibacterial	(135)

To achieve a successful process in the development of new drugs, it is first necessary to extract, isolate, purify and characterize the phytochemicals and then perform *in vitro* tests to know their biological activity (15). After these studies are completed and if its medicinal properties can be verified, it enters the clinical testing phase to develop and launch the drug on the market (136). The following section describes the procedure to collect, extract and isolate the active principles of the plants, in addition to the different techniques that are most used today to identify them and then to prove their biological activity (**Figure 6**)

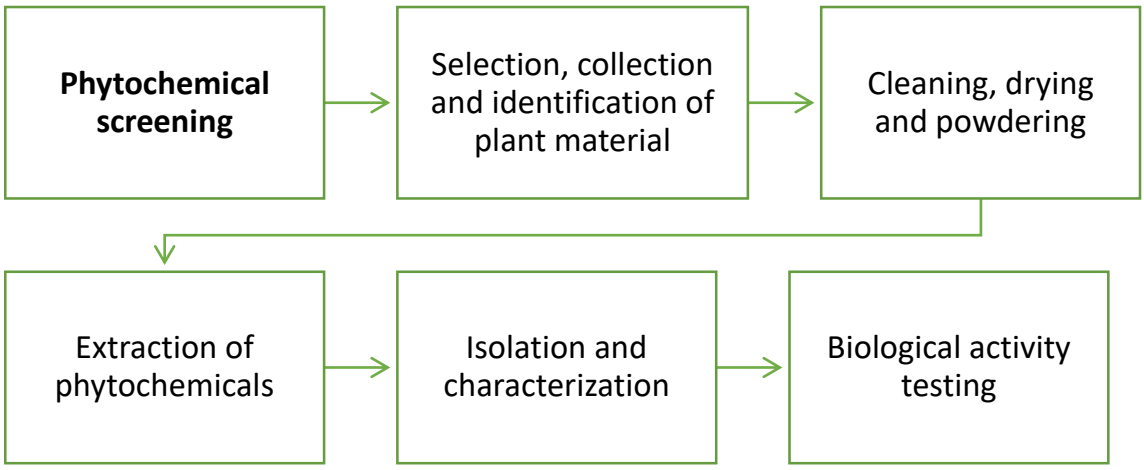

Figure 6. Procedure for testing the biological activity of natural products.

5.3.1. Selection, collection, and identification of plants

The development of new drugs has a fundamental basis on the previous knowledge that is known about the plant of interest. Selecting the plant material is a crucial step since the scientific value given to the research depends on it (129). For this reason, in-depth bibliographic research must be carried out to know and understand its medicinal uses based on ethnobotany, ethnogeography, its chemical composition, the importance and uses of taxonomy, toxicology, and other areas, which will allow us to understand better the plant species and its possible use as a pharmacological agent (137).

Wild forests have a great diversity of medicinal plants, so most of the plant materials are collected in these places. However, by having so much variety, a taxonomic identification error is widespread, and there may even be traces of pesticides in the samples. For this reason, herbariums also play an important gathering place for vegetal species where there is better control of resources and where plant species have already been identified previously (127,138). Once the plant has been collected and identified, it is placed in a dry plastic bag to keep the humidity and in good condition for subsequent analysis (139).

5.3.2. Cleaning, drying, and powdering

When the plant species have been collected, it is necessary to enter a cleaning and drying process to prevent the chemical composition of the plant from being affected (138). First, you must define which part of the plant is the one to be studied and then proceed to remove those parts that are damaged or eaten by insects, as in the case of leaves, for example. Once this is done, the material can be washed with water or 1% sodium hypochlorite, as recorded in some studies, to remove traces of soil or other microorganisms (41). It is generally better to wash the plant material with your hands to obtain better results and avoid damaging the sample (127).

Once the study material has been cleaned, it is dried. It is essential to dry the plant as soon as possible after it has been harvested to avoid damage and to be able to dehydrate it to be stored without a problem in case it is needed later (138). Besides, it is recommended to avoid the plant being in contact with sunlight because the volatile components can undergo chemical reactions as a result of exposure to UV light. The appropriate temperature is between 40 - 70 ^{o}C depending on the method used, although the most common are ventilated ovens (140). However, it should not exceed 300 ^{o}C since thermolabile compounds (compounds that are destroyed when they reach their boiling point) can break down (129). The drying of the plant material can be done through sunlight, although it is a method that requires a lot of space and time depending on the amount of material and the humidity of the plant, in addition to the fact that it can undergo chemical changes that can affect the study as mentioned earlier. Another method is to use artificial dryers (warm-drying is the most common) such as chambers or ovens, although space is smaller and can be a bit more expensive, this equipment is faster because they reduce the drying time to a few hours or minutes, they are efficient and less laborious (127).

When the material is dehydrated, it must be ground or reduced to powder to increase the surface area of the plant, and thus when different solvents are used to obtain the phytochemicals (secondary metabolites), they can quickly enter the cells and extract them (41). Powdering the plant material allows us to obtain small particles and a homogeneous sample, in such a way that it improves the extraction process. According to several studies, the ideal particle size should be 0.5mm so that it can make contact with the solvent successfully (141). In the case that the particle is tiny, it can cause problems since, by being so fine, it is capable of absorbing the solute excessively and affecting the filtration process (136).

5.3.3. Extraction

Extraction is the first step for the phytochemical analysis of the plant, and it is performed through various standard procedures. It is mainly based on the extraction of chemical compounds (phytochemicals) from the plant material that must then be isolated and characterized (142). To

obtain the bioactive components of the plant, different solvents are used (127). Before starting the extraction process, it is necessary to know if there are phytochemical studies previously published in the literature. This is important since if the target components and the extraction method are known as well as the solvents used to obtain them, they will simplify the study. But if the target components are not known, then it is better to use different solvents and repeated extractions to be able to remove each chemical component from the plant material as much as possible (143). How solvent acts on the raw material is by penetrating the solid matrix (cells), then the solute (metabolite) manages to dissolve in the solvent, then the solute diffuses out of the cell, and finally, the solutes are extracted and collected (136). One type of extract can contain analytes or compounds that are different from those obtained in other extracts. This means that depending on the type of solvent used, different secondary metabolites can be obtained. For this reason, for the choice of solvents, it is recommended to start from the least polar (petroleum ether, hexane, ethyl acetate or chloroform) to those with increasing polarity (ethanol and water) (**Figure 7**) (140,143).

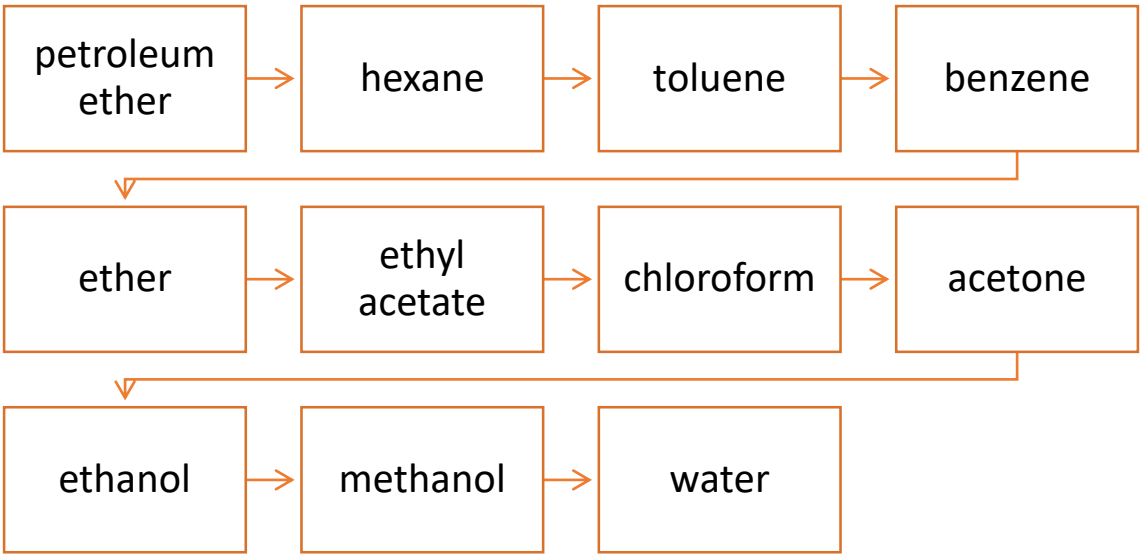

Figure 7. Principal solvents ordered from least to higher polarity.

However, the solvent is chosen according to the nature of the bioactive compound to be extracted, since solvents with a polarity similar to that of the compound, improve the extraction results (15,142). Ethanol (EtOH) and methanol (MeOH) are considered the universal solvents for extracting most phytochemicals (136). In **Table 12**, you can see some of the most abundant secondary metabolites in plants and the solvents used to extract them.

Table 12. Principal solvents used to extract a wide variety of active chemical compounds (secondary metabolites) from plants.

Solvents	**Secondary metabolites**
Aqueous (H2O)	Tannins, terpenoids, saponins, lectines
Ethanol (EtOH)	Terpenoids, polyphenols, alkaloids, flavonoids, sterols
Methanol (MeOH)	Saponins, tannins, lactones, flavonoids, polyphenols, anthocyanins, terpenoids, alkaloids
Chloroform	Flavonoids, terpenoids
Ether	Terpenoids, coumarins, alkaloids
Acetone	Phenols, flavonoids

Increasing the extraction time does not affect the efficiency of the applied method at all, although it does improve it. As the solvent-solute ratio is higher, the extraction efficiency will increase as the equilibrium of the solute is reached inside and outside the solid material. But if the solvent-solute ratio is too high, then excess extraction will occur, and the concentration of the solutes will be lower, thus requiring more time to reach an adequate concentration for phytochemical analysis (136). Among the most used extraction methods are: 1) the simplest and most conventional ones such as maceration, infusion, percolation, decoction, Soxhlet extraction, and steam distillation; and 2) those a little more complicated and modern such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFC), ultrasound-assisted extraction or sonication (UAE) and enzyme-assisted extraction (EAE) (15).

5.3.3.1. *Methods of extraction*

5.3.3.1.1. Maceration

This method is based on taking the powder or parts of the plant and placing them in a container with a lid together with the solvent. The material is kept submerged at room temperature and with constant agitation for at least 3 days, and then with a strainer or filter paper, the solvent is separated from the plant residues. The type of solvent chosen determines the compounds obtained in the extract (141,144).

5.3.3.1.2. Decoction

This method is often used for the harder structures of the plant, such as roots and barks. It is based on putting the crude extract in water until boils and leaves it for 15 minutes. After this time, the volume of water will be reduced, it is cooled, and the concentrated extract is filtered. This process

is applicable for thermosetting and water-soluble compounds and does not apply to the extraction of thermolabile components (136,137).

5.3.3.1.3. Percolation

It is a procedure where a percolator is used, in which the crude material and a certain amount of solvent are introduced, closed, and left to rest for about 4 hours. As time passes and the solvent becomes saturated, more solvent is added to form a shallow layer on the plant material (adding fresh solvent allows the herb to be continuously extracted), and it is left to macerate in the percolator for 24 hours. Then more solvent is added until the percolate reaches the required volume and finally is proceeded to the filtration or decantation process (136,138).

5.3.3.1.4. Soxhlet extraction

The method consists of placing the sample in the Soxhlet equipment and boiling the solvent in the flask. The solvent vapors rise through the extractor and condense in the refrigerant, falling little by little onto where the crude extract is. The advantage of using this method is that it performs several extractions continuously with the same solvent, which means it recycles it, making it a more profitable method. Thermolabile compounds are not optimal to extract with this method since, due to the high temperature, it can degrade them (137).

5.3.3.1.5. Microwave-assisted extraction (MAE)

MAE uses the energy emitted by the microwave to facilitate the extraction of analytes. A quantity of solvent is used, and it is mixed together with the sample while it is heated in the microwave. At that moment, the energy of the microwave increases the pressure, breaking the cells of the plant, freeing the chemical compounds (137). The radiation and dipole interaction of the molecules induced by electromagnetic waves allows for better and rapid penetration of the solvent into the particles of the plant material (141). However, MAE favors polar compounds and solvents with a high dielectric constant since they capture microwave radiation in a better way (140,141).

5.3.3.1.6. Supercritical fluid extraction (SFC)

The SFC technique uses a substance (supercritical fluid) capable of standing a temperature and pressure higher than its thermodynamic critical point, which allows it to diffuse through solids like a gas and to dissolve materials like a liquid (145). This means that a supercritical fluid (SF) behaves more like a gas, but has a solubility similar to a liquid, allowing the extraction of a wide variety of phytochemicals (136,141). CO2 is generally used as an extraction fluid due to its low cost and toxicity, high abundance, and efficiency. CO2 can become SF when the temperature exceeds 31.1 ° C and 7380 kPa and has higher solubility with nonpolar analytes (141,144).

5.3.3.1.7. Ultrasound-assisted extraction (UAE)

This method uses ultrasound to facilitate the extraction process. Ultrasounds are waves that have frequencies ranging from 16kHz to 1Ghz, imperceptible to the human ear (140). These vibrations serve as a source of energy to cause acoustic cavitation, which facilitates the release of metabolites from the sample by increasing the permeability of cell walls and allowing the solvent to diffuse rapidly (138,140). The advantage of this technique is that it is carried out at room temperature and that by subjecting the sample to an ultrasonic bath for a specific time (usually 30-70 min) many samples can be extracted at the same time (140).

5.3.3.1.8. Enzyme-assisted extraction (EAE)

The EAE procedure is based on the use of specific enzymes (usually cellulases, hemicellulases, and pectinases) to disrupt, through hydraulic actions, the complex structure of the cell wall so that the extraction and release of chemical compounds through solvent is possible (146).

5.3.3.1.9. Steam distillation

Steam distillation is a technique used to extract mainly temperature-sensitive compounds such as aromatic (volatile) components present in the essential oil of plants. Water is introduced into the distillation apparatus, and as the temperature rises, the water vapor transports those compounds insoluble in water and carries them to the condensing flask. At the end of the process, it is filled with distilled water, and by decantation, it is possible to separate the essential oil from the liquid phase (140).

5.3.4. Isolation and characterization techniques

Once the plant extract has been obtained with any of the previous methods, it has a complex mixture of natural products (secondary metabolites) that need to be isolated and purified to obtain the purely natural product. Compounds have different polarities and physical characteristics that must be taken into account since they can make the identification and characterization process difficult (142). Two types of techniques are used to isolate and detect secondary metabolites: 1) Chromatographic techniques such as thin-layer chromatography (TLC), column chromatography (CC), High-pressure liquid chromatography (HPLC) and High-performance thin-layer chromatography (HPTLC) and 2) non-chromatographic techniques such as Fourier Transform Infrared Spectroscopy (FTIR), (NMR), and (GC-MS) (15,142).

5.3.4.1. *Chromatographic techniques*

They are methods that separate the different chemical compounds according to their polarity, shape, size, and charge (15). There are 2 essential phases in these techniques: a) stationary phase is the one that has a substance or adsorbent with different affinities to the compounds that the sample has, it can be a reliable, gel or liquid (if it is liquid, it is attached to a solid). Silica gel, cellulose powder, aluminum, starch, etc., are usually used as adherents of the stationary phase. And we also have the b) mobile phase, which is a liquid or gas that moves in one direction along with the stationary phase (solvents). Its principal focuses on the application of the sample (extract) on a surface or a solid where the stationary phase allows the separation of natural products helped by the mobile phase (147).

5.3.4.1.1. Thin Layer Chromatography (TLC)

The TLC technique is applied mainly for compounds with low molecular weight and uses an adsorbent adhered to a plate as the stationary phase (15). The literature reports that around 90% of phytochemical analyzes are performed with silica gel (136). In this method, the mobile phase (solvent) travels upward through the stationary phase by capillary action, thus achieving that the analytes separate according to their polarity concerning the chosen solvent (148). In the case that the molecules are colorless, it is necessary to use inflorescence, radioactivity, or any chemical substance that allows them to emit a color that indicates their position on the plate. The reaction can be observed visually or under UV light. TLC also allows us to calculate the position of the analyte using the relationship of the distance traveled by the compound and the mobile phase (147). This value is the retention factor (Rf) and how to calculate it is shown in **Figure 8.**

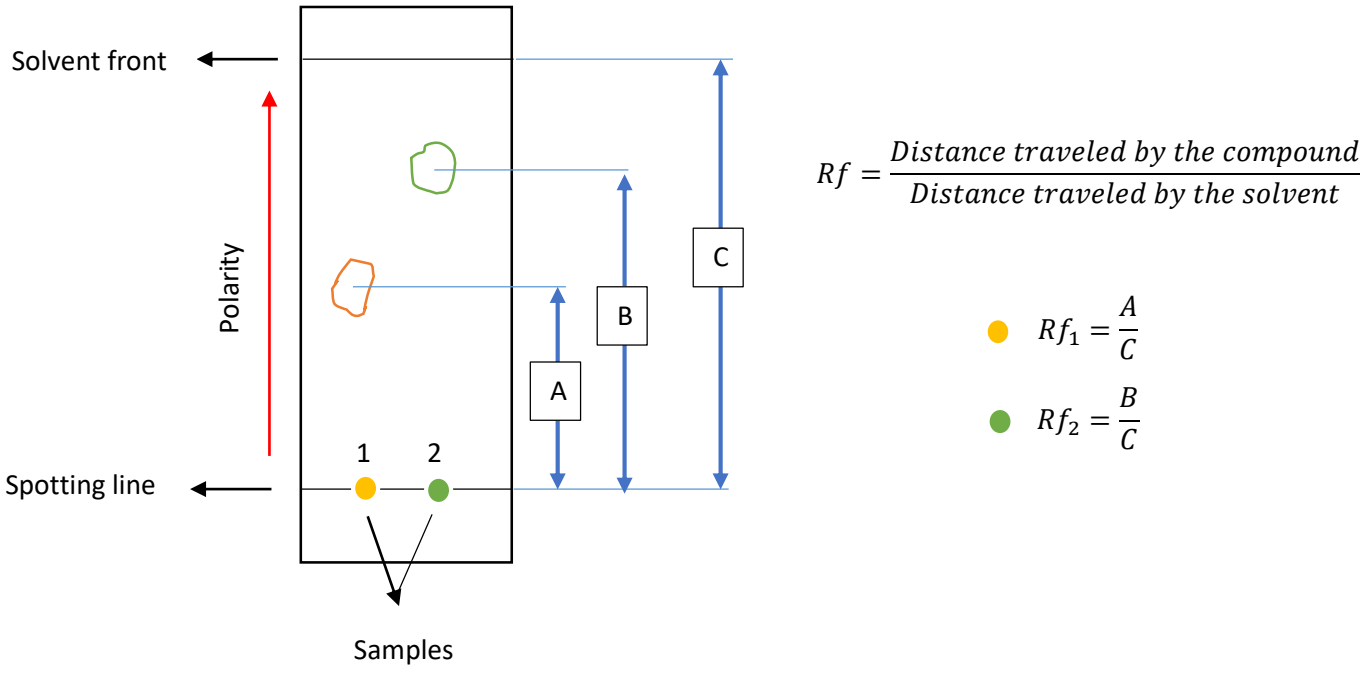

Figure 8. TLC retention factor (Rf) calculation.

5.3.4.1.2. Column Chromatography (CC)

Column chromatography is applied to separate compounds with more complex characteristics such as having a higher molecular weight and concentration, as well as differences between their structure and net charge. The column is covered by the stationary phase (any type of adsorbent) and the extract is deposited at the top of the column to be absorbed in the stationary phase. Then, the mobile phase is added and allowed to pass through the stationary phase, carrying the analytes with it at different speeds and thereby separating the chemical compounds from the sample. The speed at which the mobile phase runs depends on the polarity and affinity of the natural products towards the solvent (149).

5.3.4.1.3. High-pressure liquid chromatography (HPLC)

HPLC is a technique that efficiently separates molecules in biological, environmental, and pharmaceutical samples (137). The main characteristic of HPLC is that it uses pressure to increase the speed at which the sample compounds travel through the column (stationary phase) carried by the mobile phase, increasing the separation power of this method. The applied pressure must be high and lies between 250-400 bar, so it is best applied for samples containing thermolabile compounds (150). In this technique, a detector is used (usually UV light due to its high sensitivity), which allows detecting those components capable of absorbing UV light at a wide range of wavelengths, even when they are found in minimal quantities in the sample. A recorder is also needed and allows the chemical composition of the sample to be graphically represented through a chromatogram (15).

5.3.4.1.4. High-performance thin-layer chromatography (HPTLC)

High-performance thin-layer chromatography is an improved technique based on the same principles of TLC. This method uses much smaller plates and thicker coating layers than in TLC, improving the performance of analyte separation. The advantage of HPTLC is the presence of a densitometer, which records the chemical composition of the sample according to its UV light absorbance, showing quantitative analysis of the different metabolites found in a chromatogram (151). It is an effective, efficient, fast chromatographic method capable of analyzing a wide variety of samples (136).

5.3.4.1.5. Gas Chromatography (GC)

The GC uses a column where the stationary phase is located, and unlike other chromatography techniques, the mobile phase (inert gas) only helps to transport the compounds through the column but does not interact with them. Due to the affinity of the chemical compounds in the sample with the stationary phase, the molecules separate and then exit at different speeds (152).

5.3.4.2. *Non-Chromatographic Techniques*

5.3.4.2.1. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR is a technique that uses light in the infrared range of the electromagnetic spectrum so that components of an unknown sample can capture and measure them based on a wavelength. The chemical bonds of the molecules are the main functional groups identified by this method and are so specific that they are known as a molecular "fingerprint" (129). Being a computerized technique, unlike others, it is more sensitive and faster in its detection and identification of compounds. Its main advantage is to record all the information of the sample in a spectrum simultaneously. Samples for FT-IR can be prepared depending on whether it is solid or liquid. In the case of solid samples, they must be ground until the particles are uniform and mixed with potassium bromide (KBr) to form a homogeneous granule that will be used for analysis. On the other hand, if you have a liquid sample, a small drop is placed between two plates of sodium chloride (NaCl), forming a film that will allow you to perform the analysis (137).

5.3.4.2.2. Nuclear magnetic resonance spectroscopy (NMR)

NMR was first reported by Shuker in 1996 when it succeeded in describing and identifying drug molecules using this technique, and its use has been increasing rapidly since then (136). It is a method used to determine the structural frameworks concerning the position and type of different carbon isotopes (C-13 NMR) or the hydrogens (H-NMR) present in the molecules of the sample to be studied (128). For NMR analysis, the amount of sample required is small, and the advantage is that it can be reused for further studies (15).

5.3.4.2.3. Mass Spectrometry (MS)

The MS captures the separated compounds and breaks them into ionized fragments, which makes it possible to detect and identify the molecules taking into account the mass/charge ratio. By using both techniques together, the performance and detection sensitivity increases and is even perfect for those samples with volatile compounds since it is a specific requirement of GC (152).

5.3.5. Preclinical *in vitro* biological activity tests

Antimicrobial susceptibility tests are used to test the biological activity of extracts or pure compounds against different microorganisms such as bacteria and fungi (153). The chemical diversity of natural products provides the opportunity to verify the antimicrobial activity in this type of test, allowing the discovery and development of new drugs, in addition to helping to predict therapeutic results (154). There are two types of methods, diffusion, and dilution. The

diffusion technique involves the spread of the therapeutic agent on the agar plate inoculated with a specific microorganism. In comparison, the dilution technique facilitated the calculation of the minimal inhibitory concentration (MIC) values of any type of tested antimicrobial agent in an agar or broth medium. MIC (expressed in µg / mL) refers to the lowest concentration of an extract or active compound that is required to inhibit the growth of the microorganism (155). Both diffusion and dilution methods are techniques where factors such as the extraction method used, the culture medium or the microorganisms tested, can affect the results obtained (156). A microorganism is considered susceptible to an antimicrobial substance when its growth is inhibited with a minimum concentration of one quarter to one-eighth of the standard dose found in serum when administered to a human (157). According to several studies, the most prominent diffusion method is disk-diffusion and agar well diffusion, while broth and agar dilution stand out in dilution methods. These techniques are described in this section as they are the most well-known and essential in vitro tests to evaluate the antimicrobial activity of pure components or extracts (153).

5.3.5.1. *Diffusion methods*

5.3.5.1.1. Disk-Diffusion Method

Also known as "the Kirby-Bauer method," it is an efficient method to know the antimicrobial effect of a previously isolated compound. First, on a petri dish with Muller-Hinton agar (MHA), the microorganism to be tested must be inoculated; usually, new subcultures are used (157). Then, small filter paper disks (about 6 mm in diameter) with a specific concentration of the tested antimicrobial agent (extract, natural products, drugs) are placed on the inoculated agar, and it is incubated overnight for 16-24 h at 35-37 ºC (158). If the agent is an active compound, a zone of inhibition will form around the disk, and its size will depend on the antimicrobial potency and the rate of diffusion of the sample through the agar medium (Figure No. 9) (158,159). This technique has been improved and standardized by different institutes around the world, such as The Clinical & Laboratory Standards Institute (CLSI) in the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in Europe (**Figure 9**) (155).

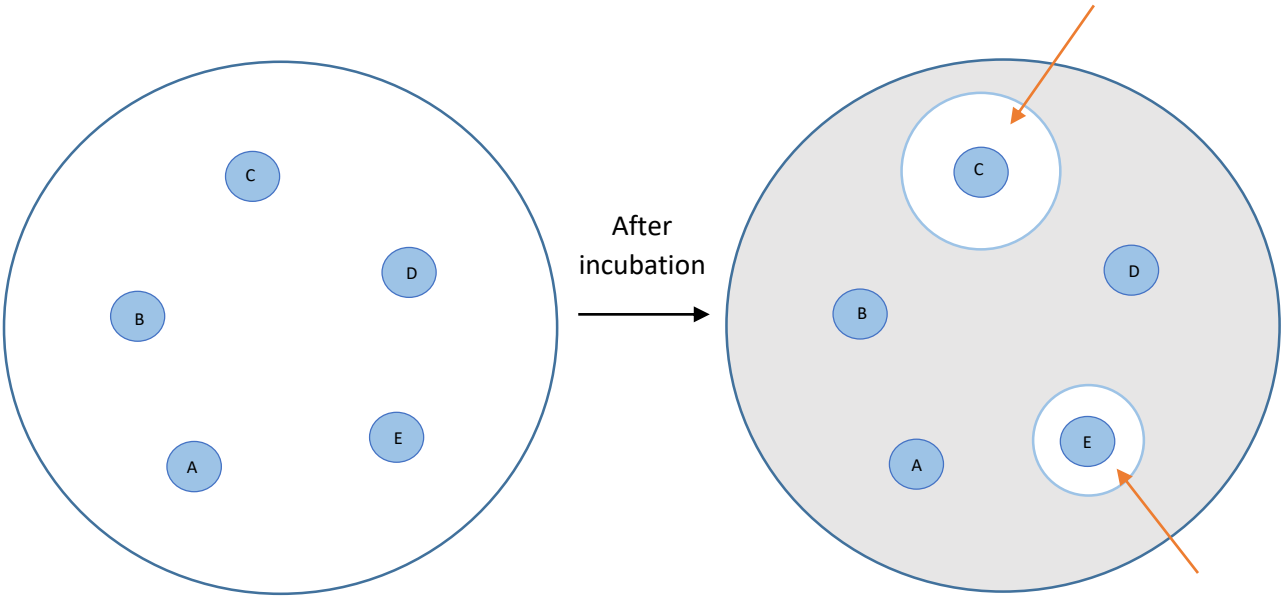

Figure 9. Diagram illustrating the Kirby-Bauer disk-diffusion method using 5 different antibiotics (A, B, C, D, E). Arrows indicate zone of inhibition.

5.3.5.1.2. Well Diffusion Method Agar

This technique is very similar to the Disk-diffusion method. The microorganism of interest is inoculated on an agar culture medium, and small holes (diameter 6-8 mm) are made with a sterile tip or any other instrument. A volume of 20 to 100 µL of the antimicrobial agent to be tested is placed in these wells and the plate is incubated overnight (153,159). The presence of halos means that the sample diffused from the hole into the agar medium, forming zones of inhibition where the microorganism did not grow, which provides information on the minimum inhibitory concentration (MIC) (159).

5.3.5.2. *Dilution Methods*

5.3.5.2.1. Broth dilution method

It is the most basic method for evaluating the biological activity of a possible therapeutic component and has been recommended by the CSLI as a standard method for antimicrobial susceptibility testing (160). This procedure involves growing the organism in a liquid medium (usually Muller-Hinton) containing concentrations of the antimicrobial agent is generally double dilutions (1,2,4,6,16 mg/ml), into which a known quantity of the microorganism is inoculated. Depending on whether it is inoculated in test tubes with a volume of 2 ml (161), it is called a microdilution, and if it is inoculated in the volume of 96-well plates, it is called a microdilution

(153). The tubes or plates are then incubated overnight at appropriate conditions depending on the microorganism, and the results can be analyzed with the naked eye. If it shows a turbidity, it means that there was microbial growth, and if not, there was an inhibition of growth by the action of the antimicrobial agent. In this way, the MIC value can be known by taking into account the minimum concentration at which no growth of the microorganism was seen (158). According to the CSLI, the broth dilution method is standardized to analyze bacteria, fungi, and yeasts against different samples for therapeutic purposes (153).

5.3.5.2.2. Agar dilution method

The agar dilution method consists of preparing agar plates (Muller-Hinton) with different concentrations of the antimicrobial agent to be studied, usually in double dilutions, as in the broth dilution method. Then, a defined number of the microorganism (1-5 ml) is inoculated on the agar plate and incubated overnight at conditions dependent on the organism that was chosen. Colonies that are not capable of growing at the different concentrations of the antimicrobial agent will show a zone of inhibition that can be calculated to determine the MIC (155,161). This technique is beneficial for testing active compounds with possible antibacterial and antifungal activity (153).

When the compound has been isolated, preclinical *in vitro* tests are carried out to test the efficiency of the natural product as an antimicrobial. Once positive results are obtained in these tests, *in vivo* tests are performed and then regulatory authorities such as the US Food and Drug Administration (FDA) authorize the study of the compound in clinical trials (136). Clinical research refers to studies that are done to see how the drug interacts with the human body and if it works as it should (162). It is estimated that the average time it takes to develop a drug is a minimum of 10 years, and the investment cost exceeds 800 million dollars (163). The main idea of developing new drugs is that the active compounds discovered can reach the market as therapeutic agents for the treatment of several diseases. However, of all the drugs that enter clinical trials, only 10% are approved and make it to market (162).

Chapter VI

6. Conclusion and Outlooks

Currently, due to the rapid development of antimicrobial resistance, infections mainly caused by bacteria, fungi, and viruses have become a threat to public health around the world. Traditional medicine plays an essential role in the discovery of new plant species with therapeutic activities, especially in the Andean region of Ecuador due to its great diversity in flora. Given the reduced information on the pharmacological properties of endemic plants of Ecuador and their taxonomic importance in the medical field, they are considered as potential study material for the development of new drugs. Furthermore, the vast and diverse chemical composition of plants promises to be the primary source for the discovery of new bioactive compounds as therapeutic agents. For this reason, different extraction methods, characterization, and sensitivity tests are part of the drug discovery process and are considered necessary tests to find candidates of natural origin that do not cause side effects in the human body, which is an advantage over synthetic drugs currently available.

Even when the use of these compounds sounds promising, their extraction remains a challenge that must be overcome, since both the solvent and the method used can affect the final compounds extracted. In addition to using correct extraction methods, it is also needed to keep a clear record of the plants of interest and their medicinal uses. The information collected on many occasions is often confusing and unclear, which only slows the investigation process. So far, it is known that this is the first report that highlights the importance of the use of the ancestral knowledge of these endemic plants as potential therapeutic agents, thus providing a new vision for the development of new drugs in the country in a future.

References

1. Neill D. ¿Cuantas especies nativas de plantas vasculares hay en Ecuador? Rev Amaz Cienc y Tecnol [Internet]. 2015;1. Available from: https://revistas.proeditio.com/REVISTAMAZONICA/article/view/151/129
2. INABIO. Información, investigación y resultados asociados, sumado a los métodos necesarios para implementar la Estrategia desarrollada y compartida [Internet]. 2019 [cited 2020 Aug 25]. Available from: http://inabio.biodiversidad.gob.ec/2019/01/30/3-informacion-investigacion-y-resultados-asociados-sumado-a-los-metodos-necesarios-para-implementar-la-estrategia-desarrollada-y-compartida/
3. Torre, Lucía de la ; Navarrete, Hugo ; Muriel M., Priscilla ; Macía Barco, Manuel Juan ; Balslev H. Plantas Medicinales del Ecuador. In: Enciclopedia de las plantas útiles del Ecuador [Internet]. 2008. Available from: https://www.researchgate.net/publication/310828407_Enciclopedia_de_las_Plantas_Utiles_del_Ecuador
4. Cerón C. Plantas Medicinales de los Andes ecuatorianos [Internet]. Botánica E. Moraes M, Øllgaard B, Kvist P, Borchsenius F, Balslev H, editors. Universidad Mayor de San Andrés, La Paz; 2006. 246–267 p. Available from: https://www.researchgate.net/publication/228584502_Etnobotanica_en_los_Andes_del_Ecuador
5. Varela J, Serna E, Torres S, Yaluff G, De Bilbao NIV, Miño P, et al. In vivo anti-trypanosoma cruzi activity of hydro-ethanolic extract and isolated active principles from aristeguietia glutinosa and mechanism of action studies. Molecules [Internet]. 2014;19(6):8488–502. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6270975/
6. Ayala Valarezo SE, Vásquez Villarreal TA. Evaluación de la actividad antifúngica in vitro del Marco (Ambrosia arborescens Mill.) y matico (Aristeguietia glutinosa Lam.) sobre hongos patógenos causantes de la dermatomicosis [Internet]. Universidad Politécnica Salesiana; 2014. Available from: http://dspace.ups.edu.ec/handle/123456789/7303
7. Lozada Fiallos MA. Estudio fotoquímico y evaluación de actividad antibacteriana sobre Staphyloccocus aureus ATCC: 25923, Streptoccocus mutans ATCC: 25175, Streptoccocus pneumoniae ATCC: 49619, Streptoccocus pyogenes ATCC: 19615de extractos apolares (Cloroformo –Hexano) de C [Internet].

Universidad Politécnica Salesiana; 2016. Available from: http://dspace.ups.edu.ec/handle/123456789/13534

8. WHO. Antimicrobial resistance [Internet]. 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
9. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrob Resist Infect Control [Internet]. 2017 May 15;6(1):47. Available from: http://aricjournal.biomedcentral.com/articles/10.1186/s13756-017-0208-x
10. Marasini BP, Baral P, Aryal P, Ghimire KR, Neupane S, Dahal N, et al. Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. Biomed Res Int [Internet]. 2015;2015. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4337259/
11. Marston HD, Dixon DM, Knisely JM, Palmore TN, Fauci AS. Antimicrobial resistance. JAMA - J Am Med Assoc [Internet]. 2016 Sep 20;316(11):1193–204. Available from: https://pubmed.ncbi.nlm.nih.gov/27654605/
12. Arulmozhi P, Vijayakumar S, Kumar T. Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms. Microb Pathog [Internet]. 2018 Oct 1;123:219–26. Available from: https://www.intechopen.com/books/pharmacognosy-medicinal-plants/natural-products-in-drug-discovery
13. Compean KL, Ynalvez RA. Antimicrobial activity of plant secondary metabolites: A review. Res J Med Plant [Internet]. 2014;8(5):204–13. Available from: https://www.researchgate.net/publication/264458521_Antimicrobial_Activity_of_Plant_Secondary_Metabolites_A_Review
14. Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, et al. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity [Internet]. Vol. 196, Microbiological Research. Elsevier GmbH; 2017. p. 44–68. Available from: https://pubmed.ncbi.nlm.nih.gov/28164790/
15. Amit Koparde A, Chandrashekar Doijad R, Shripal Magdum C. Natural Products in Drug Discovery. In: Pharmacognosy - Medicinal Plants [Internet]. IntechOpen; 2019 [cited 2020 Aug 25]. Available from: https://www.intechopen.com/books/pharmacognosy-medicinal-plants/natural-products-in-drug-discovery
16. De la Torre L, Muriel P, Balslev H. Etnobotánica en los Andes del Ecuador. In: Moraes R, Øllgaard B, Kvist P, Balslev F, editors. Botánica Económica de los Andes Centrales [Internet]. Universidad Mayor de San Andrés, La Paz; 2006. p.

246–67. Available from: https://www.researchgate.net/publication/228584502_Etnobotanica_en_los_Andes_del_Ecuador

17. Rios M, Koziol MJ, Borgtoft Pedersen H, Granda G. Plantas útiles del Ecuador: aplicaciones, retos y perspectivas [Internet]. Corporación Sociedad para la Investigación y Monitoreo de la Biodiversidad Ecuatoriana (SIMBIOE); 2007. Available from: http://biblioteca.udla.edu.ec/client/es_EC/default/search/detailnonmodal/ent:$002f$002fSD_ILS$002f0$002fSD_ILS:11795/ada?qu=ANIMACIONES&ic=true&te=ILS&ps=300
18. Minga A. Relación entre conocimiento tradicional y diversidad de plantas en el bosque protector Aguarongo, Azuay Ecuador [Internet]. Universidad Politécnica Salesiana; 2014. Available from: https://dspace.ups.edu.ec/bitstream/123456789/7087/1/UPS-CT003837.pdf
19. Jørgensen PM, Ulloa C, Maldonado C. Riqueza de plantas vasculares. In: Botánica Económica de los Andes Centrales. 2006. p. 37–50.
20. Vacas O, Medina D, Íñiguez J, Navarrete H. Los kichwas del alto Napo y sus plantas medicinales [Internet]. Pontificia Universidad Católica del Ecuador; 2017. Available from: https://edipuce.edu.ec/los-kichwas-del-alto-napo-y-sus-plantas-medicinales/
21. Tinitana F, Rios M, Romero-Benavides JC, de la Cruz Rot M, Pardo-de-Santayana M. Medicinal plants sold at traditional markets in southern Ecuador. J Ethnobiol Ethnomed [Internet]. 2016 Jul 5;12(1). Available from: https://pubmed.ncbi.nlm.nih.gov/27380631/
22. Lima PGC, Coelho–Ferreira M, da Silva Santos R. Perspectives on Medicinal Plants in Public Markets across the Amazon: A Review. Econ Bot [Internet]. 2016 Mar 1;70(1):64–78. Available from: https://link.springer.com/article/10.1007/s12231-016-9338-y
23. Nguyen TS, Xia NH, Van Chu T, Van Sam H. Ethnobotanical study on medicinal plants in traditional markets of son la province, Vietnam. For Soc [Internet]. 2019 Nov 1;3(2):171–92. Available from: https://www.semanticscholar.org/paper/Ethnobotanical-study-on-medicinal-plants-in-markets-Nguyen-Xia/21d9836ce328fe009bf9262d3fe1d5f4e99b00b8
24. Bussmann RW, Sharon D. Two decades of ethnobotanical research in Southern Ecuador and Northern Peru. Ethnobiol Conserv [Internet]. 2014;3(2014). Available from: https://www.researchgate.net/publication/263161883_Two_decades_of_ethnobo

tanical_research_in_Southern_Ecuador_and_Northern_Peru
25. Falzon CC, Balabanova A. Phytotherapy: An Introduction to Herbal Medicine. Prim Care - Clin Off Pract [Internet]. 2017 Jun 1;44(2):217–27. Available from: https://pubmed.ncbi.nlm.nih.gov/28501226/
26. Wyk B-E van, Wink M. Medicinal Plants of the World [Internet]. 2017. Available from: https://books.google.es/books?hl=es&lr=&id=UAitDwAAQBAJ&oi=fnd&pg=PA3&dq=medicinal+plants&ots=goGi13Xbom&sig=5FsFZewvldSwiCv8cmgWG4Ze8mA#v=onepage&q=medicinal plants&f=false
27. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: Past history and future perspective. J HerbMed Pharmacol [Internet]. 2018 Dec 29;7(1):1–7. Available from: http://www.herbmedpharmacol.com
28. Dávila C, Pazos L. Evaluación de la actividad antifúngica in vitro de emulsiones de Marco (Ambrosia arborescens Mill.) y matico (Aristeguietia glutinosa Lam.) sobre hongos patógenos causantes de la dermatomicosis [Internet]. Universidad Politécnica Salesiana; 2015. Available from: https://dspace.ups.edu.ec/bitstream/123456789/9232/1/UPS-QT06947.pdf
29. Tropicos. Aristeguietia glutinosa (Lam.) R.M. King & H. Rob. [Internet]. Catalogue of the Vascular Plants of Ecuador. 2009. Available from: http://legacy.tropicos.org/Name/2712521?projectid=2&langid=66
30. Varela J, Lavaggi M, Cabrera M, Rodríguez A, Miño P, Chiriboga X, et al. Bioactive-guided identification of labdane diterpenoids from aerial parts of Aristeguietia glutinosa as anti-Trypanosoma cruzi agents - PubMed. Nat Prod Commun [Internet]. 2012;7:1139–42. Available from: https://pubmed.ncbi.nlm.nih.gov/23074890/
31. Buestan A, Guaraca A. Actividad Anti-inflamatoria de los extractos de plantas medicinales empleados Austro Ecuatoriano en el modelo de Danio rerio [Internet]. [Cuenca]: Universidad de Cuenca; 2013. Available from: http://dspace.ucuenca.edu.ec/bitstream/123456789/547/1/tesis.pdf
32. López F, Tituaña K. Estudio de estabilidad de cremas faciales elaboradas con matico (Aristeguietia glutinosa) e ishpingo (Ocotea quixos) [Internet]. Universidad Politécnica Salesiana; 2017. Available from: https://dspace.ups.edu.ec/bitstream/123456789/14282/1/UPS-QT11774.pdf
33. Varela J, Serna E, Torres S, Yaluff G, De Bilbao NIV, Miño P, et al. In vivo anti-trypanosoma cruzi activity of hydro-ethanolic extract and isolated active principles from aristeguietia glutinosa and mechanism of action studies. Molecules [Internet]. 2014;19(6):8488–502. Available from:

/pmc/articles/PMC6270975/?report=abstract

34. Andrade JD, Murillo MA. Evaluación de la actividad antiinflamatoria de Aristeguietia glutinosa en ratones Mus musculus [Internet]. Escuela Superior Politécnica de Chimborazo; 2019. Available from: http://dspace.espoch.edu.ec/handle/123456789/13085
35. Rodríguez B. Estudio Fitoquímico de las Hojas de la Aristeguietia Glutinosa (lam.) [Internet]. [Riombamba]: Escuela Superior Politécnica de Chimborazo; 2014. Available from: http://bibliotecas.espoch.edu.ec/cgi-bin/koha/opac-detail.pl?biblionumber=37170
36. León-Yánez S, Valencia N, Pitman N, Endara L, Ulloa Ulloa C, Navarrete H. Aristeguietia glutinosa [Internet]. Libro Rojo de Plantas Endémicas del Ecuador. 2019. Available from: https://bioweb.bio/floraweb/librorojo/FichaEspecie/Aristeguietia glutinosa
37. Repositorio Digital: Flora de la Mitad del Mundo U. MOSQUERA: Croton elegans [Internet]. 2019. Available from: https://floradelamitaddelmundo.wordpress.com/2019/02/07/mosquera-croton-elegans/
38. Lozada MA. Estudio Fitoquímico y Evaluación de Actividad Antibacteriana Sobre Staphyloccocus aureus ATCC: 25923, Streptoccocus mutans ATCC: 25175, Streptoccocus pneumoniae ATCC: 49619, Streptoccocus pyogenes ATCC: 19615 de Extractos Apolares (Cloroformo-Hexano) de C [Internet]. [Quito]: UNIVERSIDAD POLITÉCNICA SALESIANA; 2016. Available from: https://dspace.ups.edu.ec/bitstream/123456789/13534/1/UPS-QT11203.pdf
39. León-Yánez S, Valencia N, Pitman N, Endara L, Ulloa Ulloa C, Navarrete H. Mapa de Croton elegans [Internet]. Libro Rojo de Plantas Endémicas del Ecuador. 2019. Available from: https://bioweb.bio/floraweb/librorojo/FichaEspecie/Croton elegans
40. Barrionuevo AE. Evaluación del Extracto Etanólico de Mosquera “Croton elegans”, en Concentración de 10, 20 y 30% a dosis de 2ml; en Cicatrización Post-Quirúrgica en Ovario Histerectomia en Caninas Mestizas en el Centro de Gestión Zonal Animal de Carapungo en el Distrito [Internet]. [Latacunga]: Universidad Técnica de Cotopaxi; 2011. Available from: http://repositorio.utc.edu.ec/bitstream/27000/802/1/T-UTC-1161.pdf
41. Guayasamin ME. Evaluación Antimicótica de Extracto de Mosquera (Croton elegans) frente a Trichophyton mentagrophytes ATCC 9533, Trichophyton rubrum ATCC 28188, Microsporum canis ATCC 36299, Candida albicans ATCC 10231, Patógenos de Dermatomicosis [Internet]. [Quito]: Universidad Politécnica

Salesiana; 2016. Available from: https://dspace.ups.edu.ec/bitstream/123456789/13225/1/UPS-QT10561.pdf

42. Tigse CE. COMPROBACIÓN DE LA ACTIVIDAD CICATRIZANTE DEL EXTRACTO DE HIERBA MOSQUERA (Croton elegans kunth) EN RATONES (Mus musculus) [Internet]. [Riobamba]: ESCUELA SUPERIOR POLITÈCNICA DE CHIMBORAZO; 2015. Available from: http://dspace.espoch.edu.ec/bitstream/123456789/4516/1/56T00573 UDCTFC.pdf
43. Herrera C, Perez Y, Morocho V, Armijos C, Malagón O, Brito B, et al. PRELIMINARY PHYTOCHEMICAL STUDY OF THE ECUADORIAN PLANT CROTON ELEGANS KUNTH (EUPHORBIACEAE). Chil Chem Soc [Internet]. 2018;1. Available from: https://scielo.conicyt.cl/pdf/jcchems/v63n1/0717-9324-jcchems-63-01-3875.pdf
44. León-Yánez S, Valencia N, Pitman N, Endara L, Ulloa Ulloa C, Navarrete H. Mapa de Lepechinia rufocampii [Internet]. Libro Rojo de Plantas Endémicas del Ecuador. 2019. Available from: https://bioweb.bio/floraweb/librorojo/FichaEspecie/Lepechinia rufocampii
45. Herbario Azuay. Lepechinia rufocampii Epling & Mathias [Internet]. Universidad del Azuay. 2019. Available from: https://herbario.uazuay.edu.ec/muestras/detalle/7540
46. Calderón D, Guerrero AI. Análisis del efecto antibacterial de aceites esenciales de Lepechinia rufocampii y Minthostachys tomentosa sobre cepas de Escherichia coli y Salmonella thyphimurium [Internet]. Cuenca; 2013. Available from: http://dspace.ucuenca.edu.ec/bitstream/123456789/4542/1/Tesis.pdf
47. Kenny O, Smyth TJ, Walsh D, Kelleher CT, Hewage CM, Brunton NP. Investigating the potential of under-utilised plants from the Asteraceae family as a source of natural antimicrobial and antioxidant extracts. Food Chem [Internet]. 2014 Oct 15;161:79–86. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0308814614005299
48. Tanvir R, Sajid I, Hasnain S. Biotechnological potential of endophytic actinomycetes associated with Asteraceae plants: Isolation, biodiversity and bioactivities. Biotechnol Lett [Internet]. 2014 Dec 29;36(4):767–73. Available from: https://link.springer.com/article/10.1007/s10529-013-1430-0
49. Kriplani P, Guarve K, Baghael US. Arnica montana L. - a plant of healing: review. J Pharm Pharmacol [Internet]. 2017 Aug 1;69(8):925–45. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/jphp.12724
50. Silveira Rabelo AC, Caldeira Costa D. A review of biological and

pharmacological activities of Baccharis trimera. Chem Biol Interact [Internet]. 2018 Dec 25;296:65–75. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0009279718305337

51. Carrillo-Hormaza L, Mora C, Alvarez R, Alzate F, Osorio E. Chemical composition and antibacterial activity against Enterobacter cloacae of essential oils from Asteraceae species growing in the Páramos of Colombia. Ind Crops Prod [Internet]. 2015 Dec 23;77:108–15. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0926669015303460
52. Antonio CNS, Selene M de M, Elnatan B de S, Raquel O dos SF. The genus Eupatorium L. (Asteraceae): A review of their antimicrobial activity. J Med Plants Res [Internet]. 2017 Jan 17;11(3):43–57. Available from: https://academicjournals.org/journal/JMPR/article-abstract/4F30B6962396
53. Chaib F, Allali H, Bennaceur M, Flamini G. Chemical Composition and Antimicrobial Activity of Essential Oils from the Aerial Parts of Asteriscus graveolens (Forssk .) Less . and Pulicaria incisa (Lam .) DC.: Two Asteraceae Herbs Growing Wild in the Hoggar. Chem Biodivers [Internet]. 2017 Aug 1;14(8):e1700092. Available from: http://doi.wiley.com/10.1002/cbdv.201700092
54. Koc S, Isgor BS, Isgor YG, Shomali Moghaddam N, Yildirim O. The potential medicinal value of plants from Asteraceae family with antioxidant defense enzymes as biological targets. Pharm Biol [Internet]. 2015 May 4;53(5):746–51. Available from: http://www.tandfonline.com/doi/full/10.3109/13880209.2014.942788
55. Sabir SM, Athayde ML, Boligon AA, Rocha JBT. Antioxidant activities and phenolic profile of Baccharis trimera, a commonly used medicinal plant from Brazil. South African J Bot [Internet]. 2017 Nov 1;113:318–23. Available from: https://www.sciencedirect.com/science/article/pii/S0254629917307007
56. Simões CMO, Mentz LA, Schenkel EP, Irgang BE, Stehmann JR. Plantas da medicina popular no Rio Grande do Sul [Internet]. Universidad Federal do Rio Grande do Sul. 1988. Available from: https://books.google.com.ec/books/about/Plantas_da_medicina_popular_no_Rio_Grand.html?hl=pt-BR&id=IMhfAAAAMAAJ&redir_esc=y
57. Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (Matricaria chamomilla L.): An overview. Pharmacogn Rev [Internet]. 2011 Jan;5(9):82–95. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3210003/
58. Heydari Shayesteh O, Chehregani Rad A, Reza Hosseini Zijoud S, Ranjbar A. Hepatoprotective Effect of Matricaria chamomilla.L in Paraquat Induced Rat Liver Injury Hepatoprotective Eff ect of Matricaria chamomilla.L in Paraquat

Induced Rat Liver Injury. MPS Tavakol HS al Eff ect M.chamomilla Paraquat Liver … Drug Res [Internet]. 2014;64:1–5. Available from: https://www.researchgate.net/profile/Akram_Ranjbar/publication/261328907_Hepatoprotective_Effect_of_Matricaria_chamomillaL_in_Paraquat_Induced_Rat_Liver_Injury/links/5485813b0cf283750c371c1a/Hepatoprotective-Effect-of-Matricaria-chamomillaL-in-Paraquat-In

59. Höferl M, Wanner J, Tabanca N, Ali A, Gochev V, Schmidt E, et al. Biological Activity of Matricaria chamomilla Essential Oils of Various Chemotypes. Planta Medica Int Open [Internet]. 2020 Sep 15;07(03):e114–21. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/a-1186-2400
60. Stanojevic LP, Marjanovic-Balaban ZR, Kalaba VD, Stanojevic JS, Cvetkovic DJ. Chemical Composition, Antioxidant and Antimicrobial Activity of Chamomile Flowers Essential Oil (Matricaria chamomilla L.). J Essent Oil-Bearing Plants [Internet]. 2016 Nov 16;19(8):2017–28. Available from: https://www.tandfonline.com/doi/abs/10.1080/0972060X.2016.1224689
61. Kollia E, Markaki P, Zoumpoulakis P, Proestos C. Antioxidant activity of Cynara scolymus L. and Cynara cardunculus L. extracts obtained by different extraction techniques. Nat Prod Res [Internet]. 2017;31(10):1163–7. Available from: https://www.tandfonline.com/doi/abs/10.1080/14786419.2016.1219864
62. Vamanu E, Vamanu A, Nita S, Colceriu S. Antioxidant and Antimicrobial Activities of Ethanol Extracts of Cynara Scolymus (Cynarae folium, Asteraceae Family). Trop J Pharm Res [Internet]. 2011 Dec 15;10(6):777–83. Available from: https://www.ajol.info/index.php/tjpr/article/view/72883
63. Lopes CB, da Camara CAG, de Moraes MM. Composition of Essential Oils from the Leaves, Stems, and Flowers of Vernonia condensata of Pernambuco, Brazil. Chem Nat Compd [Internet]. 2019 Jul 15;55(4):756–8. Available from: https://link.springer.com/article/10.1007/s10600-019-02802-8
64. Toyang NJ, Verpoorte R. A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). J Ethnopharmacol [Internet]. 2013 Apr 19;146(3):681–723. Available from: https://www.sciencedirect.com/science/article/abs/pii/S037887411300069X
65. Kiplimo J. A Review on the Biological Activity and the Triterpenoids from the Genus Vernonia (Asteraceae Family). Int Res J Pure Appl Chem [Internet]. 2016 Jan 10;11(3):1–14. Available from: https://www.researchgate.net/publication/300423722_A_Review_on_the_Biological_Activity_and_the_Triterpenoids_from_the_Genus_Vernonia_Asteraceae_Family

66. Da Silva JB, Temponi VDS, Gasparetto CM, Fabri RL, Aragão DMDO, Pinto NDCC, et al. Vernonia condensata Baker (Asteraceae): A promising source of antioxidants. Oxid Med Cell Longev [Internet]. 2013; Available from: https://www.hindawi.com/journals/omcl/2013/698018/
67. Sugier, Sugier, Jakubowicz-Gil, Winiarczyk, Kowalski. Essential Oil from Arnica Montana L. Achenes: Chemical Characteristics and Anticancer Activity. Molecules [Internet]. 2019 Nov 16;24(22):4158. Available from: https://www.mdpi.com/1420-3049/24/22/4158
68. Abenavoli L, Izzo AA, Milić N, Cicala C, Santini A, Capasso R. Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. Phyther Res [Internet]. 2018 Nov 1;32(11):2202–13. Available from: http://doi.wiley.com/10.1002/ptr.6171
69. Sharifi-Rad M, Mnayer D, Morais-Braga MFB, Carneiro JNP, Bezerra CF, Coutinho HDM, et al. Echinacea plants as antioxidant and antibacterial agents: From traditional medicine to biotechnological applications. Phyther Res [Internet]. 2018 Sep 1;32(9):1653–63. Available from: http://doi.wiley.com/10.1002/ptr.6101
70. Haghi G, Hatami A, Safaei A, Mehran M. Analysis of phenolic compounds in Matricaria chamomilla and its extracts by UPLC-UV. Res Pharm Sci [Internet]. 2014 Feb;9(1):31–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4292179/
71. Martinez M, Poirrier P, Chamy R, Prüfer D, Schulze-Gronover C, Jorquera L, et al. Taraxacum officinale and related species - An ethnopharmacological review and its potential as a commercial medicinal plant. Journal of Ethnopharmacology [Internet]. 2015 Apr 6;169:244–62. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0378874115002263
72. Ramalho SD, Pinto MEF, Ferreira D, Bolzani VS. Biologically Active Orbitides from the Euphorbiaceae Family. Planta Med [Internet]. 2018 Jul 1;84(9–10):558–67. Available from: https://pubmed.ncbi.nlm.nih.gov/29169187/
73. Islam MS, Ara H, Ahmad KI, Uddin MM. A Review on Medicinal Uses of Different Plants of Euphorbiaceae Family. Univers J Pharm Res [Internet]. 2019 Mar 7; Available from: http://ujpr.org/index.php/journal/article/download/236/308
74. Salatino A, Salatino MLF, Negri G. Traditional uses, chemistry and pharmacology of Croton species (Euphorbiaceae). Journal of the Brazilian Chemical Society [Internet]. 2007;18(1):11–33. Available from: https://www.scielo.br/scielo.php?pid=s0103-50532007000100002&script=sci_arttext

75. Zahidin NS, Saidin S, Zulkifli RM, Muhamad II, Ya'akob H, Nur H. A review of Acalypha indica L. (Euphorbiaceae) as traditional medicinal plant and its therapeutic potential. J Ethnopharmacol [Internet]. 2017 Jul 31;207:146–73. Available from: https://pubmed.ncbi.nlm.nih.gov/28647509/
76. Mwine JT, Damme P Van. Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features. J Med Plants Res [Internet]. 2011;5(5):652–62. Available from: https://academicjournals.org/journal/JMPR/article-full-text-pdf/E2D4CC218726
77. Pwnn T, Bgk P. The important biological activities and phytochemistry of Acalypha indica. Int J Res Pharm Sci [Internet]. 2016;6(1):30–5. Available from: http://www.ijrpsonline.com/pdf/6005.pdf
78. Batubara I, Wahyuni WT, Firdaus I. Utilization of Anting-Anting (Acalypha indica) Leaves as Antibacterial. IOP Conf Ser Earth Environ Sci [Internet]. 2016 Feb 22;31(1):12038. Available from: https://iopscience.iop.org/article/10.1088/1755-1315/31/1/012038
79. Govindarajan M, Jebanesan A, Reetha D, Amsath R, Pushpanathan T, Samidurai K. Antibacterial activity of Acalypha indica L. Eur Rev Med Pharmacol Sci [Internet]. 2008;12:299–302. Available from: https://pubmed.ncbi.nlm.nih.gov/19024213/
80. Mekam PN, Martini S, Nguefack J, Tagliazucchi D, Stefani E. Phenolic compounds profile of water and ethanol extracts of Euphorbia hirta L. leaves showing antioxidant and antifungal properties. South African J Bot [Internet]. 2019 Dec 1;127:319–32. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0254629919315133
81. Kumari I, Pandey RK. Antibacterial activity of Euphorbia hirta L. In: Applications of Biotechnology for Sustainable Development [Internet]. Springer Singapore; 2017. p. 1–5. Available from: https://www.springer.com/gp/book/9789811055379
82. Al-Snafi PDAE. Pharmacology and therapeutic potential of Euphorbia hirta (Syn: Euphorbia pilulifera)- A review. IOSR J Pharm [Internet]. 2017 Mar;07(03):07–20. Available from: https://www.researchgate.net/publication/314216010_Pharmacology_and_therapeutic_potential_of_Euphorbia_hirta_Syn_Euphorbia_pilulifera-A_review
83. Abu Bin Nyeem M, Sadul Haque M, Akramuzzaman M, Mohammad Abu Bin Nyeem C, Siddika R, Sultana S, et al. Euphorbia hirta Linn. A wonderful miracle plant of mediterranean region: A review. J Med Plants Stud [Internet]. 2017;5(3):170–5. Available from: http://www.plantsjournal.com/archives/2017/vol5issue3/PartC/5-2-50-684.pdf

84. Qaisar MN. Phytochemical and biological studies of Croton bonplandianum (Euphorbiaceae) [Internet]. [Pakistan]: BAHAUDDIN ZAKARIYA UNIVERSITY MULTAN; 2015. Available from: http://prr.hec.gov.pk/jspui/bitstream/123456789/9443/1/Muhammad_Naeem_Qaisar_Pharmacy_2017_BZU_11.01.2018.pdf
85. Dutta S, Chaudhuri TK, Tapas C, Chaudhuri K. Pharmacological aspect of Croton bonplandianus Baill: A comprehensive review. J Pharmacogn Phytochem [Internet]. 2018;7(1). Available from: http://www.indianmedicine.nic.in/
86. Okoh SO, Iweriebor BC, Okoh OO, Nwodo UU, Okoh AI. Antibacterial and Antioxidant Properties of the Leaves and Stem Essential Oils of Jatropha gossypifolia L. Biomed Res Int [Internet]. 2016;2016. Available from: https://www.hindawi.com/journals/bmri/2016/9392716/
87. Paniagua-Zambrana NY, Bussmann RW, Romero C. Jatropha curcas L. Jatropha gossypifolia L. Jatropha multifida L. Euphorbiaceae. In: Ethnobotany of the Andes [Internet]. 2020. p. 1–9. Available from: https://www.researchgate.net/publication/339453112_Jatropha_curcas_L_Jatropha_gossypifolia_L_Jatropha_multifida_L_Euphorbiaceae
88. Martínez C, Mosquera O, Niño J. Medicinal plants from the genus Alchornea (Euphorbiaceae): A review of their ethnopharmacology uses and phytochemistry [Internet]. 2017. p. 162–205. Available from: https://www.researchgate.net/publication/315471928_Medicinal_plants_from_the_genus_Alchornea_Euphorbiaceae_A_review_of_their_ethnopharmacology_uses_and_phytochemistry_Plantas_Medicinales_del_genero_Alchornea_Euphorbiaceae_-_Review_de_los_usos_etnofarm
89. Ana M-U, Jerelly H-A, Benjamín V-C, Valente V-O, Lucía D-R, Carla R-M, et al. Actividad antibacteriana del extracto hidroalcohólico Croton draco sobre bacterias de importancia sanitaria. Abanico Vet [Internet]. 2020 Jan 1;10(1). Available from: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2448-61322020000100101&lng=es&nrm=iso&tlng=es
90. Karpiński TM. Essential Oils of Lamiaceae Family Plants as Antifungals. Biomolecules [Internet]. 2020 Jan 7;10(1):103. Available from: https://www.mdpi.com/2218-273X/10/1/103
91. Nieto G. Biological Activities of Three Essential Oils of the Lamiaceae Family. Medicines [Internet]. 2017 Aug 23;4(3):63. Available from: http://www.mdpi.com/2305-6320/4/3/63
92. Nolkemper S, Reichling J, Stintzing FC, Carle R, Schnitzler P. Antiviral effect of

aqueous extracts from species of the Lamiaceae family against Herpes simplex virus type 1 and type 2 in vitro. Planta Med [Internet]. 2006 Dec 7;72(15):1378–82. Available from: https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-2006-951719

93. Mazurek AP, Bojarski J, Marcinkowski K, Furmanowa M, Gutkowska B, Kaliszan R, et al. Chemical Composition and Antibacterial Activity of Some Medicinal Plants From Lamiaceae Family. Drug Res ACTA Pol Pharm [Internet]. 2015;77(2):757–67. Available from: www.actapoloniaepharmaceutica.pl
94. Cocan I, Alexa E, Danciu C, Radulov I, Galuscan A, Obistioiu D, et al. Phytochemical screening and biological activity of lamiaceae family plant extracts. Exp Ther Med [Internet]. 2018 Feb 1;15(2):1863–70. Available from: http://www.spandidos-publications.com/10.3892/etm.2017.5640/abstract
95. Habtemariam S. The Therapeutic Potential of Rosemary (Rosmarinus officinalis) Diterpenes for Alzheimer's Disease. Evidence-based Complement Altern Med [Internet]. 2016; Available from: https://www.hindawi.com/journals/ecam/2016/2680409/
96. Nieto G, Ros G, Castillo J. Antioxidant and Antimicrobial Properties of Rosemary (Rosmarinus officinalis, L.): A Review. Medicines [Internet]. 2018 Sep 4;5(3):98. Available from: http://www.mdpi.com/2305-6320/5/3/98
97. Sánchez-Camargo A del P, Herrero M. Rosemary (Rosmarinus officinalis) as a functional ingredient: recent scientific evidence. Curr Opin Food Sci [Internet]. 2017 Apr 1;14:13–9. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2214799316301825
98. Jafarikukhdan S, Hosseini A, Armand A. The Application of Medicin-al Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. Int J Clin Med [Internet]. 2015;6:635–42. Available from: https://www.scirp.org/pdf/IJCM_2015091513262965.pdf
99. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras M del M, Segura-Carretero A, et al. Thymol, thyme, and other plant sources: Health and potential uses. Phyther Res [Internet]. 2018 Sep 1;32(9):1688–706. Available from: http://doi.wiley.com/10.1002/ptr.6109
100. Alexa E, Sumalan R, Danciu C, Obistioiu D, Negrea M, Poiana M-A, et al. Synergistic Antifungal, Allelopatic and Anti-Proliferative Potential of Salvia officinalis L., and Thymus vulgaris L. Essential Oils. Molecules [Internet]. 2018 Jan 16;23(1):185. Available from: http://www.mdpi.com/1420-3049/23/1/185
101. Ghorbani A, Esmaeilizadeh M. Pharmacological properties of Salvia officinalis and its components. J Tradit Complement Med [Internet]. 2017 Oct 1;7(4):433–

40. Available from: https://www.sciencedirect.com/science/article/pii/S2225411017300056
102. Pezzani R, Vitalini S, Iriti M. Bioactivities of Origanum vulgare L.: an update. Phytochem Rev [Internet]. 2017 Dec 1;16(6):1253–68. Available from: https://link.springer.com/article/10.1007/s11101-017-9535-z
103. Oniga I, Pușcaș C, Silaghi-Dumitrescu R, Olah N-K, Sevastre B, Marica R, et al. Origanum vulgare ssp. vulgare: Chemical Composition and Biological Studies. Molecules [Internet]. 2018 Aug 19;23(8):2077. Available from: http://www.mdpi.com/1420-3049/23/8/2077
104. Miraj S, Kiani S. Study of pharmacological effect of Ocimum basilicum: A review. 2016;8:276–80. Available from: www.scholarsresearchlibrary.com
105. Jacob JKS, Carlos RCA, Divina CC. Phytochemical composition, antibacterial and antifungal activities of sweet basil (Ocimum basilicum). Adv Environ Biol [Internet]. 2016 Jul 1;10(7):84–90. Available from: https://go.gale.com/ps/anonymous?id=GALE%7CA469314831&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=19950756&p=AONE&sw=w
106. Orhan IE. Pharmacognosy: Science of natural products in drug discovery. BioImpacts [Internet]. 2014;4(3):109–10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4204033/
107. Lahlou M. The Success of Natural Products in Drug Discovery. Pharmacol Pharm [Internet]. 2013;4:17–31. Available from: https://www.scirp.org/pdf/PP_2013062411214748.pdf
108. Harvey AL. Natural products in drug discovery. Drug Discovery Today [Internet]. 2008 Oct 1;13(19–20):894–901. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1359644608002651
109. Bernardini S, Tiezzi A, Laghezza Masci V, Ovidi E. Natural products for human health: an historical overview of the drug discovery approaches. Natural Product Research [Internet]. 2018 Aug 18;32(16):1926–50. Available from: https://www.tandfonline.com/doi/abs/10.1080/14786419.2017.1356838
110. Thomford N, Senthebane D, Rowe A, Munro D, Seele P, Maroyi A, et al. Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. Int J Mol Sci [Internet]. 2018 May 25;19(6):1578. Available from: https://www.mdpi.com/1422-0067/19/6/1578
111. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod [Internet]. 2020;83(3):770–803. Available from: https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01285

112. Calixto JB. The role of natural products in modern drug discovery. An Acad Bras Cienc [Internet]. 2019;91. Available from: https://www.scielo.br/scielo.php?script=sci_arttext&pid=S0001-37652019000600603
113. Veeresham C. Natural products derived from plants as a source of drugs. Journal of Advanced Pharmaceutical Technology and Research [Internet]. 2012 Oct;3(4):200–1. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3560124/
114. National Center for Biotechnology Information. Hypericin [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Hypericin
115. RxList. Veregen (Sinecatechins Ointment): Uses, Dosage, Side Effects, Interactions, Warning [Internet]. 2019. Available from: https://www.rxlist.com/veregen-drug.htm
116. National Center for Biotechnology Information. Morphine [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov//#query=Morphine+3
117. Miana G, Riaz M, Shahzad-ul-Hussan S, Paracha R, Paracha U. Prostratin: An Overview. Mini-Reviews Med Chem [Internet]. 2015 Sep 29;15(13):1122–30. Available from: https://pubmed.ncbi.nlm.nih.gov/25963564/
118. National Center for Biotechnology Information. Camptothecin [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Camptothecine
119. National Center for Biotechnology Information. Artemisinin [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Artemisinin#section=Structures
120. National Center for Biotechnology Information. Quinine [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Quinine
121. ABC. La verdadera historia del curare [Internet]. Sociedad. 2018. Available from: https://www.abc.es/sociedad/abci-verdadera-historia-curare-201801042129_noticia.html?ref=https:%2F%2Fwww.google.com%2F
122. National Center for Biotechnology Information. Silibinin [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Silibinin
123. Fang LH, Wang JH, Du GH. Atropine. In: Natural Small Molecule Drugs from Plants [Internet]. Springer Singapore; 2018. p. 181–6. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470551/
124. National Center for Biotechnology Information. Galantamine [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Galantamine

125. Barrueto F. Digitoxin. In: Encyclopedia of Toxicology [Internet]. Elsevier; 2005. p. 380–2. Available from: https://www.sciencedirect.com/science/article/pii/B012369400000435X
126. National Center for Biotechnology Information. Pilocarpine [Internet]. PubChem. 2020. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Pilocarpine
127. Raaman N. Phytochemical Techniques [Internet]. New Delhi: New India Publishing Agency; 2010. Available from: https://books.google.es/books?hl=es&lr=&id=6Gxp_nVK3ucC&oi=fnd&pg=PA1&dq=phytochemical+technique&ots=rKSv4J5xj_&sig=bAgjoIYSYl1UEIvTTDUVQvfrhs8#v=onepage&q=phytochemical technique&f=false
128. Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of Medicinal Plants. J Pharmacogn Phytochem [Internet]. 2013;1(6). Available from: www.phytojournal.comwww.phytojournal.com
129. Velavan S. Phytochemical Techniques - A Review. World J Sci Res [Internet]. 2015;1(2):80–91. Available from: http://www.harmanpublications.com
130. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, et al. The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins (Basel) [Internet]. 2019 Nov 11;11(11):656. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6891610/
131. Martin-Smith M, Khatoon T. Biological Activity of the Terpenoids and Their Derivatives. In: Progress in drug research [Internet]. Birkhäuser Basel; 1963. p. 279–346. Available from: https://link.springer.com/chapter/10.1007/978-3-0348-7050-4_4
132. Karak P. Biological Activities of Flavonoids: An Overview. Int J Pharm Sci Res [Internet]. 2019;10(4):1567–74. Available from: http://ijpsr.com/bft-article/biological-activities-of-flavonoids-an-overview/?view=fulltext
133. Krzyzowska M, Tomaszewska E, Ranoszek-Soliwoda K, Bien K, Orlowski P, Celichowski G, et al. Tannic acid modification of metal nanoparticles: Possibility for new antiviral applications. In: Nanostructures for Oral Medicine [Internet]. Elsevier Inc.; 2017. p. 335–63. Available from: https://www.sciencedirect.com/science/article/pii/B9780323477208000134
134. Sapna D D, Dhruv G. Desai, Harmeet Kaur. Saponins and their biological activities [Internet]. 2009. Available from: https://www.researchgate.net/publication/288126191_Saponins_and_their_biological_activities
135. Xu L, Zhao X-Y, Wu Y-L, Zhang W. The Study on Biological and Pharmacological Activity of Coumarins. 2015; Available from:

https://www.researchgate.net/publication/300617793_The_Study_on_Biological_and_Pharmacological_Activity_of_Coumarins#:~:text=The Study on Biological and Pharmacological Activity of Coumarins,-Conference Paper (PDF&text=Coumarin has drawn much attention,-H

136. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arabian Journal of Chemistry [Internet]. 2017 Feb 1;10:S1409–21. Available from: https://www.sciencedirect.com/science/article/pii/S1878535213001056
137. Mtewa A, Deyno S, Kasali F, Annu A, Sesaazi D. General Extraction, Isolation and Characterization Techniques in Drug Discovery: A Review | International Journal of Sciences: Basic and Applied Research (IJSBAR). Int J Sci Basic Appl Res [Internet]. 2018 Apr 15;38. Available from: https://www.gssrr.org/index.php/JournalOfBasicAndApplied/article/view/8856
138. Sahira Banu K, Cathrine L. General Techniques Involved in Phytochemical Analysis. Int J Adv Res Chem Sci [Internet]. 2015;2(4):25–32. Available from: www.arcjournals.org
139. Harborne JB. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis - A.J. Harborne - Google Libros [Internet]. 3rd ed. Chapman & Hall; 1998. Available from: https://books.google.es/books?hl=es&lr=&id=2yvqeRtE8CwC&oi=fnd&pg=PR7&dq=phytochemical+technique&ots=xzjmV1ViW2&sig=VHVcPjb-dWCUjBsOIIIwWcvVs-Y#v=onepage&q=phytochemical technique&f=false
140. Romanik G, Gilgenast E, Przyjazny A, Kamiński M. Techniques of preparing plant material for chromatographic separation and analysis. J Biochem Biophys Methods [Internet]. 2007 Mar 10;70(2):253–61. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0165022X06001965
141. Nn A. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants [Internet]. 2015;4(3):196. Available from: https://www.longdom.org/open-access/a-review-on-the-extraction-methods-use-in-medicinal-plants-principle-strength-and-limitation-2167-0412-1000196.pdf
142. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African J Tradit Complement Altern Med [Internet]. 2011;8(1):1–10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3218439/
143. Liu Z. Preparation of Botanical Samples for Biomedical Research. Endocrine, Metab Immune Disord Targets [Internet]. 2008 Jun 3;8(2):112–21. Available

from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3936020/

144. Swami S, Singh S, Longo G, Rakesh D. Extraction Technologies for Medicinal and Aromatic Plants [Internet]. International Centre For Science and High Technology; 2008. Available from: https://www.unido.org/sites/default/files/2009-10/Extraction_technologies_for_medicinal_and_aromatic_plants_0.pdf
145. López García Rocío de la Vega Merino Andrea Sánchez Belloso L, López Ruiz B. Extracción con fluidos supercríticos. Reduca (Recursos Educ Ser Congr Alumnos [Internet]. 2012;4(10):142. Available from: http://www.revistareduca.es/index.php/reduca/article/view/1210
146. Marathe SJ, Jadhav SB, Bankar SB, Singhal RS. Enzyme-Assisted Extraction of Bioactives. In: Food Bioactives: Extraction and Biotechnology Applications [Internet]. Springer International Publishing; 2017. p. 171–201. Available from: https://link.springer.com/chapter/10.1007/978-3-319-51639-4_8
147. Coskun O. Separation Tecniques: Chromatography. North Clin Istanbul [Internet]. 2016;3(2):156. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5206469/
148. Sherma J, Fried B. Handbook of Thin-Layer Chromatography [Internet]. Vol. 3. 1991. Available from: https://books.google.com.ec/books?hl=es&lr=&id=s2nzNmEntZAC&oi=fnd&pg=PP1&dq=Handbook+of+Thin-Layer+Chromatography&ots=MPkJgC0sL6&sig=lvZdCBk7aPnRxt60sUIvlZybBWM#v=onepage&q=Handbook of Thin-Layer Chromatography&f=false
149. Das M, Dasgupta D. Pseudo-affinity column chromatography based rapid purification procedure for T7 RNA polymerase. Prep Biochem Biotechnol [Internet]. 1998;28(4):339–48. Available from: https://pubmed.ncbi.nlm.nih.gov/9805352/
150. Grosser K, van Dam NM. A straightforward method for glucosinolate extraction and analysis with high-pressure liquid chromatography (HPLC). J Vis Exp [Internet]. 2017 Mar 15;2017(121). Available from: https://pubmed.ncbi.nlm.nih.gov/28362416/
151. Attimarad M, Mueen Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm Methods [Internet]. 2011 Apr 1;2(2):71–5. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2229470811220016
152. Sparkman OD, Penton ZE, Kitson FG. Introduction and History. In: Gas Chromatography and Mass Spectrometry: A Practical Guide [Internet]. Elsevier; 2011. p. 2–13. Available from:

https://www.sciencedirect.com/science/article/pii/B9780123736284000010
153. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal [Internet]. 2016;6(2):71. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5762448/
154. Klančnik A, Piskernik S, Jeršek B, Možina SS. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. J Microbiol Methods [Internet]. 2010 May 1;81(2):121–6. Available from: https://www.sciencedirect.com/science/article/pii/S0167701210000618
155. Tenover FC. Antibiotic Susceptibility Testing. In: Encyclopedia of Microbiology [Internet]. Elsevier Inc.; 2009. p. 67–77. Available from: https://www.sciencedirect.com/science/article/pii/B978012373944500239X
156. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol [Internet]. 2005 Aug 22;100(1–2):80–4. Available from: https://pubmed.ncbi.nlm.nih.gov/15964727/
157. Christenson JC, Korgenski EK, Relich RF. Laboratory Diagnosis of Infection Due to Bacteria, Fungi, Parasites, and Rickettsiae. In: Principles and Practice of Pediatric Infectious Diseases. Elsevier Inc.; 2018. p. 1422-1434.e3.
158. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. Clin Infect Dis [Internet]. 2009 Dec 1;49(11):1749–55. Available from: https://academic.oup.com/cid/article/49/11/1749/344384
159. Horváth G, Bencsik T, Ács K, Kocsis B. Sensitivity of ESBL-Producing Gram-Negative Bacteria to Essential Oils, Plant Extracts, and Their Isolated Compounds. In: Antibiotic Resistance: Mechanisms and New Antimicrobial Approaches [Internet]. Elsevier Inc.; 2016. p. 239–69. Available from: https://www.sciencedirect.com/science/article/pii/B9780128036426000125
160. Brown-Elliott BA, Nash KA, Wallace RJ. Antimicrobial Susceptibility Testing, Drug Resistance Mechanisms, and Therapy of Infections with Nontuberculous Mycobacteria. Clin Microbiol Rev [Internet]. 2012 Jul 1;25(3):545–82. Available from: https://cmr.asm.org/content/25/3/545
161. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 2008 32 [Internet]. 2008 Jan 17;3(2):163–75. Available from: https://www.nature.com/articles/nprot.2007.521
162. Akhondzadeh S. The Importance of Clinical Trials in Drug Development. Avicenna J Med Biotechnol [Internet]. 2016;8(4):151. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5124250/

163. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci [Internet]. 2005 Dec 22;78(5):431–41. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0024320505008799