UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO:

Characterization of the antimicrobial and antioxidant properties of four native plants of Ecuador (*Ilex guayusa*, *Aloysia citrodora*, *Clinopodium nubigenum, and Croton lechleri*).

Trabajo de Integración Curricular Presentado como Requisito para la Obtención del Título de Ingeniera Biomédica

Autor:

Johanna Lissette Sailema Guevara

Advisor:

PhD. Nelson Santiago Vispo

Urcuquí, noviembre 2020

Urcuquí, 18 de diciembre de 2020

SECRETARÍA GENERAL
(Vicerrectorado Académico/Cancillería)
ESCUELA DE CIENCIAS BIOLÓGICAS E INGENIERÍA
CARRERA DE BIOMEDICINA
ACTA DE DEFENSA No. UITEY-BIO-2020-00052-AD

A los 18 días del mes de diciembre de 2020, a las 14:00 horas, de manera virtual mediante videoconferencia, y ante el Tribunal Calificador, integrado por los docentes:

Presidente Tribunal de Defensa	Dr. TELLKAMP TIETZ, MARKUS PATRICIO , 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 **SAILEMA GUEVARA, JOHANNA LISSETTE**, con cédula de identidad No. **1804905394**, de la **ESCUELA DE CIENCIAS BIOLÓGICAS E INGENIERÍA**, de la Carrera de **BIOMEDICINA**, aprobada por el Consejo de Educación Superior (CES), mediante Resolución **RPC-SO-43-No.496-2014**, realiza a través de videoconferencia, la sustentación de su trabajo de titulación denominado: **Characterization of the antimicrobial properties of four native plants of Ecuador (Ilex guayusa, Aloysia citrodora, Clinopodium nubigenum, Croton lechleri)**, previa a la obtención del título de **INGENIERO/A BIOMÉDICO/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	Dr. TELLKAMP TIETZ, MARKUS PATRICIO , Ph.D.	9,6
Miembro Tribunal De Defensa	Dra. RODRIGUEZ CABRERA, HORTENSIA MARIA , Ph.D.	10,0
Tutor	Dr. SANTIAGO VISPO, NELSON FRANCISCO , Ph.D.	10,0

Lo que da un promedio de: **9.9 (Nueve punto Nueve)**, 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.*

NELSON
FRANCISCO
SANTIAGO VISPO

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

Tutor

HORTENSIA MARIA RODRIGUEZ CABRERA

Firmado digitalmente por HORTENSIA MARIA RODRIGUEZ CABRERA

Dra. RODRIGUEZ CABRERA, HORTENSIA MARIA , Ph.D.

Miembro No Tutor

KARLA ESTEFANIA ALARCON FELIX

ALARCON FELIX, KARLA ESTEFANIA

Secretario Ad-hoc

AUTORÍA

Yo, **JOHANNA LISSETTE SAILEMA GUEVARA**, con cédula de identidad **1804905394**, 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, noviembre, 2020.

Johanna Lissette Sailema Guevara
CI: 1804905394

AUTORIZACIÓN DE PUBLICACIÓN

Yo, **JOHANNA LISSETTE SAILEMA GUEVARA,** con cédula de identidad **1804905394**, 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, noviembre, 2021.

Johanna Lissette Sailema Guevara
CI: 1804905394

DEDICATORIA

Dedico el presente trabajo a mi familia, amigos y a cada una de las personas que de una u otra forma me han brindado su apoyo incondicional, ya que cada una ha cumplido una labor muy importante en este proceso y han contribuido para que esta nueva etapa profesional inicie.

Pero en especial está dedicada con mucho amor y cariño a mis padres, a mis hermanas y aquellos ángeles que desde el cielo me han cuidado mis abuelitos, todos ellos han sido quienes han guiado mi camino hacia la superación y han confiado plenamente en mis capacidades.

AGRADECIMIENTO

En primera instancia me permito agradecer a Dios que con su infinita bondad me ha regalado un día más de vida, y me ha ayudado a crecer como persona ya que cada día ha puesto a prueba mi perseverancia y mis ganas por salir adelante.

Agradezco de una manera muy particular a mis padres Ramiro Sailema y Lorena Guevara por ser una pieza crucial en esta etapa, ya que me han brindado su apoyo económico y moral en todo momento. También doy gracias a mis hermanas Vanessa y Camila, que a su corta edad ha estado siempre junto a mí a pesar de las adversidades, de una u otra manera han contribuido para que este trabajo llegue a su culminación de la mejor manera, ya que ellas siempre me brindaron su ayuda.

Doy gracias a mi tutor de tesis PhD. Nelson Santiago Vispo, quien ha sido mi guía en este proceso, pero sobre todo por su infinita paciencia y por sus enseñanzas.

Gracias a todos por ser parte de esta etapa tan importante en mi vida.

INDEX

Table Index

Graph Index

ABSTRACT

The use of traditional medicine in Ecuador is extensive, and many plants contain secondary metabolites that can be used to develop new drugs to fight against diseases. In recent years, various researchers have used medicinal plants to evaluate antimicrobial, antifungal, anti-allergenic, and antioxidant activity. In traditional medicine, these plants are commonly used to treat infections, inflammations, wounds or injuries, disorders of the skin, subcutaneous tissues, or disorders of the digestive system. One of the most common uses in treating renal and urinary tract pathologies. This work presents a broad bibliographic review of four native plants of Ecuador *(Ilex guayusa., Aloysia citrodora, Clinopodium nubigenum, Croton lechleri)* used traditional medicine, describing their ethnobotanical uses, natural properties, pharmacological effects, and chemical components. A great variety of publications confirm the existence of secondary metabolites that present antimicrobial and antioxidant effects. The information is summarized in a comparative table for each of the extraction methods used in each of these tests and the solvent that has been used for their extraction. Finally, several of these species are shown to be used in the pharmaceutical industry and potentially treat thousands of pathologies. One of the significant problems in which the Worldwide Health Organization (WHO) is involved in the growing antimicrobial resistance, and one of the potential solutions is the study of plant species used by indigenous people.

Key words: medicinal plants, *Ilex guayusa, Aloysia citrodora, Clinopodium nubigenum, Croton lechleri,* antimicrobial resistance, antioxidant activity.

RESUMEN

El uso de la medicina tradicional en Ecuador es extenso y las plantas contienen metabolitos secundarios que pueden usarse para producir nuevos medicamentos e incluso combatir más de una enfermedad. En los últimos años, varios investigadores han utilizado plantas medicinales para evaluar la actividad antimicrobiana, antifúngica, anti-alérgica y antioxidante. En la medicina tradicional, estas plantas se utilizan comúnmente para tratar infecciones, inflamaciones, heridas o lesiones, trastornos de la piel o tejidos subcutáneos, trastornos del sistema digestivo, entre otros. Este trabajo muestra una amplia revisión bibliográfica de cuatro plantas nativas del Ecuador (*Ilex guayusas., Aloysia citrodora*, *Clinopodium nubigenum, Croton lechleri*) usadas en la medicina tradicional, dando a conocer su etnobotánica, propiedades naturales, efectos farmacológicos y componentes químicos. Se encontraron una gran variedad de publicaciones que confirman la existencia de metabolitos secundarios que presentan resistencia antimicrobiana y capacidad antioxidante. Resumo en una tabla de comparación cada uno de los métodos de extracción usados en cada uno de estos ensayos y además el solvente que se ha usado para su extracción. Finalmente, se ha logrado evidenciar que varias de estas especies pueden ser usadas en la industria farmacéutica y llegar a tratar hasta a miles de patologías. Uno de los grandes problemas en los que se ve involucrada la Organización Mundial de la Salud (OMS) es la creciente resistencia antimicrobiana y una de las soluciones potenciales es el estudio de especies vegetales usado por los pueblos indígenas.

Palabras claves: plantas medicinales, *Ilex guayusa., Aloysia citrodora, Clinopodium nubigenum, Croton lechleri***,** actividad antioxidante, actividad antimicrobiana

INTRODUCTION

According to the World Health Organization (WHO), antimicrobial resistance has become one of the main problems for public health worldwide (1), and a leading driver of bacterial evolutionary tempo growth. Worldwide, antibiotics are used excessively and inappropriately (2)(3). The resistance of microorganisms to existing drugs and pesticides is a problem that tends to increase, which is why the search for new antimicrobials to combat diseases is hoped to overcome the issue of bacterial resistance, some available, and side effects agents continue(4).

Humans traditionally have used medicinal plants due to trial and error, their intimate relationship with nature, and the exchange of knowledge between cultures. This knowledge has been inherited from generation to generation(5). Even so, over time, new uses have been attributed. Some plants have been used for various purposes, such as food preservation, pharmaceutical products, alternative medicine, and natural therapies(6). Plants may have various active principles such as antioxidants, antibacterial, cytotoxic, antiviral, fungicidal, and nutrient agents to combat different diseases (7).

In recent years the interest in alternative medicine to treat pain and diseases that affect human beings has been increasing. Thus, research aimed at the cultivation, characterization, and processing of medicinal plants for therapeutic purposes are considered to be strategic and fundamental (8). To use antimicrobials of natural origin is an alternative under development and exploitation(9) in light of the antimicrobial resistance generated due to the excessive use of commercial drugs.

PROBLEM STATEMENT

One of the most current problems is antibiotic diseases, which has led man to seek new solutions to combat them. Several of these solutions have been based on the use of medicinal plants. Over the years, we have found that alternative medicine can be a good source for novel pharmacological agents. Some researchers have studied some of the medicinal plants' active principles and have obtained encouraging results(10). These studies suggest a high potential for secondary metabolites in the treatment of some diseases.

Ecuador has a great variety of plant species that have been recognized and are studied by several scientists to determine their pharmaceutical properties(11). The secondary metabolites that each of these medicinal plants produces can become fundamental for developing new drugs. Despite their potential, these novel compounds might not be accepted in Ecuador because studies have been carried out in other countries, and the number of secondary metabolites of these species may vary according to the environmental conditions in which they have been grown(11). For this reason, it is essential to carry out an analysis of the species that are commercialized day by day in each of the markets of the cities of Ecuador. In this way, each of their active compounds can be properly identified to contribute to the development of new weapons against pathogens. For this reason, it is essential to carry out an analysis of the species that are commercialized day by day in each of the markets of the cities of Ecuador. In this way, each of their active compounds can be properly identified to contribute to the development of new weapons against pathogens.

OBJECTIVES

1. *General*

Carry out a bibliographic review on the characterization of the antimicrobial properties of four native plants of Ecuador (*Ilex guayusa.*, *Aloysia citrodora*, *Clinopodium nubigenum*, *Croton lechleri*).

1. *Specific*

- Compile information about the characterization of the antimicrobial properties of native plants (*Ilex guayusa., Aloysia citrodora, Clinopodium, Croton lechleri*).
- Carry out a comparison of the antimicrobial properties of four native plants (*Ilex guayusa*, *Aloysia citrodora*, *Piper pubinervulum*, *Croton lechleri*).
- Analyze the extraction methods and the solvents used in the tests of plants *Ilex guayusa*, *Aloysia citrodora*, *Piper pubinervulum*, *Croton lechleri*.
- Detail the antioxidant properties of four native plants (*Ilex guayusa*, *Aloysia citrodora, Piper pubinervulum, Croton lechleri*).

CHAPTER I

1. Theoretical Background

Ecuador has a wide variety of plant species that have been used in traditional medicine (11). Furthermore, there are a large number of ethnobotanical studies conducted in this country. Most of them have documented the presence of antimicrobial and antioxidant activity. Currently, there is a growing interest in conducting in-depth studies of various native plants to learn about their healing properties.

Antimicrobial resistance is one of the most worrying phenomena because of the increase in pathologies due to infection caused by bacteria and fungi (7). Counteracting these diseases is of utmost importance in the health field; therefore, new schemes are being developed to combat them (12). One of the great approach is antimicrobial activity tests, which can be used for drug discovery. By knowing the active principles of plants, the presence of secondary metabolites that act as antimicrobial agents can be determined.

1.1. Extraction

Extraction is the first step in separating the desired natural products from the raw materials. The extraction methods include solvent extraction, distillation method, pressing, and sublimation according to the extraction principle. Solvent extraction is essential. Besides, the extraction solvent's selection is another critical factor influencing the quality of the extract (13).

The extraction methods most traditional and widely used are maceration, extraction by soxhlet, and percolating. These methods are easy. However, new techniques have been implemented over time, such as supercritical fluid extraction, microwave-assisted extraction, and ultrasound extraction. One advantage is that these reduce the extraction time and the solvent's volume compared to the previous methods (14). The extraction of solvent properties, the particle size of the raw materials, the solvent-solid ratio, the extraction temperature, and the extraction duration will affect the extraction efficiency (Table 1)(15).

Table1. A brief summary of various extraction methods for natural products (16).

Method	Solvent	Temperature	Pressure	Time	Volume of organic solvent consumed	Polarity of natural products extracted
Maceration	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Dependent on extracting solvent
Percolation	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Dependent on extracting solvent
Decoction	Water	Under heat	Atmospheric	Moderate	None	Polar compounds
Reflux extraction	Aqueous and non-aqueous solvents	Under heat	Atmospheric	Moderate	Moderate	Dependent on extracting solvent
Soxhlet extraction	Organic solvents	Under heat	Atmospheric	Long	Moderate	Dependent on extracting solvent
Pressurized liquid extraction	Water, aqueous and non-aqueous solvents	Under heat	High	Short	Small	Dependent on extracting solvent
Supercritical fluid extraction	Supercritical fluid (usually S-CO2), sometimes with modifier	Near room tempera-ture	High	Short	None or small	Nonpolar to moderate polar compounds
Ultrasound assisted extraction	Water, aqueous and	Room temperature, or under heat	Atmospheric	Short	Moderate	Dependent on extracting solvent

	non-aqueous solvents					
Microwave assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Short	None or moderate	Dependent on extracting solvent
Enzyme assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature, or heated after enzyme treatment	Atmospheric	Moderate	Moderate	Dependent on extracting solvent
Hydro distillation and steam distillation	Water	Under heat	Atmospheric	Long	None	Essential oil (usually non-polar)

1.2. Antimicrobial resistance

According to the World Health Organization (WHO), the problem of antimicrobial resistance has become one of the main problems for public health worldwide (1) due to the improper and excessive use of antibiotics in the area of medicine, agriculture, and aquaculture (17).

With the appearance of a more significant number of microorganisms that are multi-resistant to commercial antibiotics, the threat in the prevalence of infectious diseases has increased. This increasing has threatened the health system since it limits the treatment options for bacterial infections and, therefore, reduces clinical efficacy and increases treatment costs (18). Resistance, in turn, is caused by mutations or by the acquisition of genes that confer resistance through horizontal gene transfer (19).

Horizontal gene transfer is a process that affects biological and biological lineages at different scales, distorting phylogenetic signals due to the mismatch of characters that occurs between the entities involved (20).

There are three transfer mechanisms:

- Transduction is based on the exchange of genes by a bacteriophage.
- Transfer by transformation is a mechanism by which bacteria trap free DNA; some are naturally transformable is not as common as conjugation, but its role within nature is considered essential DNA.
- Conjugation uses a somewhat more complicated mechanism. It is the transfer of DNA by direct contact between two bacteria (donor to recipient) using a conjugation machinery that is generally encoded at the plasmid level. In addition to being the main mechanism for transferring antibiotic resistance genes(21).

The growing global challenge of antimicrobial resistance requires the investigation of new sources of potentially significant antimicrobial agents from natural sources, an activity of great importance for public health (22).

1.2.1. Secondary metabolites of plants

Plants produce many secondary metabolites, and this allows them to interact with the environment. Approximately many species of plants have metabolites with antimicrobial activity, and of these, they are frequently used. However, it is estimated that only 20-30% of them have been investigated (23).

Secondary metabolites can be divided into several main groups based on their chemical structures, the best-studied being terpenoids, cyanogenic glycosides, glucosinolates, alkaloids, and phenolic compounds (24).

Terpenes or terpenoids

Approximately 30,000 terpenoids have been described, which present enormous structural variability, but have their biosynthetic origin in common. This group is classified according to its carbon units. In this way, the 10-carbon terpenoid (a terpene) is designated as monoterpene, hemiterpene to the five-carbon terpenoid (half terpene), sesquiterpene to the 15-carbon terpenoid (1,5 terpene), diterpene to the 20-carbon terpenoid, and as triterpene to the 30-carbon terpenoid. The differences between the two microorganisms terpenes' biological activities can be explained by the differences in the mycobacteria wall composition, mostly of mycolic acids. This entire lipid barrier prevents the passage of solutes through it and protects the mycobacterium from environmental changes (25).

Cyanogenic glycosides

They are the secondary metabolism product in the synthesis of plant-specific compounds composed of an α-hydroxy nitrile type aglycone and sugar, mainly D-glucose. The distribution of cyanogenic glycosides (GC) in the plant kingdom is relatively wide, reaching at least 2,500 cultivars, among which a large number belongs to the Fabaceae, Rosaceae, Linaceae, Compositae, and other families. Cyanogenic glycosides are essential components of the plant's defense against general herbivores due to their bitter taste and the release of toxic HCN on the damaging tissue (26)(27).

Glucosinolates

Glucosinolates are rich in sulfur; they contain a central carbon atom linked by sulfur to sugar and nitrogen to a sulfated oxime. Also, the central carbon is attached to a side group. In the Brassicaceae family, glucosinolates are metabolites that occur abundantly and characteristically. Thanks to the action of the myrosinase enzyme, they produce isothiocyanates, nitriles, and other compounds, whose concentration varies according to the organ, age of the plant, and environmental conditions and possessing various activities (28).

Alkaloids

Alkaloids were initially defined as essential pharmacologically active nitrogenous compounds of plant origin. They are secondary metabolites of plants that are synthesized by amino acids. Therefore, an alkaloid is a chemical compound with nitrogen that comes from the metabolic process of amino acid. Many of the alkaloids discovered are not pharmacologically active in mammals, and some are neutral rather than necessary, despite the presence of a nitrogen atom in the molecule (29).

Phenolic compounds

Plant phenolic compounds vary widely in size and complexity, but all generally possess (or are derived from compounds that possessed) an aromatic arene (phenyl) ring with at least one hydroxyl group attached. The phenolic hydroxyl group is acidic compared to other hydroxyl groups because it resides on an arene ring, efficiently stabilizing a deprotonated oxygen substituent. As a result, phenolic compounds are reactive and suitable for building large polymer blocks, such as lignins or suberins, and forming a large number of compounds that play essential roles in many aspects of plant biology (30).

1.3.Antioxidant activity

Antioxidants are molecules that prevent the oxidation of other molecules. In general, there is an electron transfer during chemical reaction from an agent substance to an oxidant (31). This reaction can produce free radicals that in turn start chain reactions that might damage cells because free radicals are very reactive, scavaging electrons from stable molecules to reach electrochemical (32).

The antioxidant activity of plants is related to the presence of secondary metabolites like phenolic compounds because they can give up hydrogen atoms from an aromatic hydroxyl group to a free radical. Also, phenolic compounds perform various other functions (33). For example, they are part of plant growth and reproduction. Among the phenolic compounds are flavonoids, which, in addition to their proven antioxidant activity, they have a great diversity of therapeutic effects, such as cardiotonic, anti-inflammatory, hepatoprotective, antineoplastic, antimicrobial, and vasodilator activities (31)(34).

Flavonoids have shown intense antioxidant activity in *in vitro* systems mainly to their low oxidation potential, which gives them the ability to stabilize free radicals by donating electrons or hydrogen atoms. Despite its potent *in vitro* activity, its *in vivo* effect is limited by several factors, including low absorption, low bioavailability, and high purification rate.

Carotenoids are non-enzymatic antioxidants that play an essential biological role because they protect the photosynthetic apparatus of plants against damage from light and prevent damage due to photosensitivity in bacteria, animals, and humans (35). Besides, they reduce genetic damage and malignant transformations, inhibiting tumor induction caused by UV rays as well as chemical agents, and reduce pre-malignant lesions in humans. Its primary free radical stabilization mechanism is determined by its ability to stabilize singlet oxygen and convert it once again into a less reactive form (triplet) (31)(33).

1.4. Medicinal plants

For several years, medicinal plants have different purposes, such as food preservation, pharmaceutical products, alternative medicine, and natural therapies. Each of its properties has focused on developing new alternatives for the health field (36). Nowadays, the consumption of natural antioxidants, antibacterial, cytotoxic, antiviral, fungicidal, and nutrient agents has increased (7). Medicinal plants are composed of active principles that are absorbed, metabolized, distributed, and excreted following the same routes as drugs.

Many studies in the XXI century where the search focuses on different natural resources, mainly plants. Due to their richness in compounds with different activities, including the ability to inhibit microorganisms' growth caused by various infectious diseases (37).

In research carried out in the last decade in our country, the Encyclopedia of Useful Plants of Ecuador indicate that of the 5,172 useful species (plants of which uses have reported), 60% are medicinal, 55% are raw material, 30% are edible, and 20% were used in social uses, which include religious rites and similar practices. The sum of these percentages exceeds 100%, which amounts to more than 3,000 species approximately (38).

In a study carried out on medicinal plants used in specific communities in the provinces of Azuay, Cañar, and Loja, for gastrointestinal disorders, in which interviews were conducted in 17 communities, the plants used were collected with the respective ethnobotanical records. One hundred twenty-five drugs and botanical samples were collected from 43 families, 92 genera, and 117 species. Of these, there are 80 samples of native plants, 39 introduced, and three endemics, where it was found that the most used plant parts were the leaves, followed by the stem and flowers, to a lesser extent the roots, young shoots, fruits, and seeds. This study indicates that the population uses a considerable number of medicinal plants for gastrointestinal disorders. The plants that are frequently used are culantro and trinitaria, both native plants. There have also been ten mentions for some widely distributed plants, such as ortiga or chini, manzanilla, hierba buena, and paico, the latter frequently used together (39).

In another study carried out in peasant communities in the rural area of the San Carlos parish, Quevedo, Los Ríos Province, it was determined that 43 species of plants had medicinal use, the leaves were the most used structures (76.7%), the form of the principal preparation was the infusion (83.7%), and the most used route of administration was the drink (86.0%). The best-represented category of medicinal use is related to the gastrointestinal system (44.2%). Among the most used medicinal plants are lemon verbena (Cymbopogon citratus), oregano (Origanum vulgare), and hierba buena (Mentha Sativa) (40).

In several countries, medicinal plants' study is developing widely because its purpose is to be used in the health sector. For example, in Brazil, a list of 71 medicinal plants was released by the Ministry of Health to research and develop medicines based on these plants for use in the Unified Health System (41).

1.4.1. *Aloysia citrodora*

Figure 1. *Aloysia citrodora* (42)

1.4.1.1. Taxonomy (43)

Kingdom	Plantae
Division	Angiosperms
Class	Equisetopsida C. Agardh
Order	Lamiales Bromhead
Family	Verbenaceae
Genre	Aloysia
Species	Triphylla
Scientific name	*Aloysia citrodora*

Table 2. Taxonomy of *Aloysia citrodora*

1.4.1.2. Description

Aloysia citrodora known as Cedrón is native to South America and belongs to the family *Verbenaceae* and the genus *Aloysia* (43). It is a perennial plant, widely used in traditional medicine (44).

1.4.1.3. Chemical composition

It is one of the richest plant species in terms of essential oils, whose main component is citral, a mixture of geranial and neral isomers (45). Its fragrance is due to this compound, generally extracted from its leaves. The presence of flavonoids such as salvigenin, eupapholine, cirsiol, eupatorin, hispidulin, apigenin, diosmetin, among others, has been recorded as well. Besides, lemon verbena contains heterosidic iridoids, such as geniposidic acid, derivatives of hydroxycinnamic acid (7%), especially verbascoside (5%) and mucilages, tannins and alkaloids, nonanal and phytosterols (42).

In a study carried out in Argentina, they used several species of *Aloysia* for the extraction of their essential oils. The main components isolated from leaves and flowers were myrcenone (31-37%) and a-thujone (13-17%) (46)(45). On the other hand, a Turkish species, after studying its essential oil from dried leaves and stems, contained limonene (15-19%), geranial (12-19%), and neral (6-8%), together with the sesquiterpenoids arcurcumene (5-6%), ß-caryophyllene (3.5-4%) and its oxide (3-5%), and spatulenol (4-4.5%) (47)(48).

1.4.1.4. Medicinal properties

Lemon verbena is used in traditional medicine thanks to its multiple healing properties. It is mainly used to treat problems related to the digestive system, such as flatulence, diarrhea, and it helps to stimulate digestion. Concerning the nervous system, it is beneficial for sedation, treating seizures, cramps, and involuntary contractions of the skeletal muscles. In addition, it relieves inflammations of the respiratory tract such as those related to bronchitis, asthma, pneumonia, and others. Most of the time, infusions or tea is used to treat each of these pathologies. Leaves, flowers, and stems are recollected for its secondary metabolites extraction.

In some countries such as Argentina, Brazil, and Uruguay, lemon verbena is used as a mild sedative to deal with insomnia (48). The use of this species is widespread as a cardiotonic and stimulant treatment in Paraguay and as an emmenagogue in Brazil. The infusion of the leaves is used in Bolivia when someone suffers from “fear” by using hot water cloths to make the patient feels better.

Throughout Latin America, USA, and Western Europe, *Aloysia citriodora* is widely commercialized and can be found in various presentations, as drinks, tea bags, or desserts (because its leaves are used as a flavoring). In recent years its essence has been used in the perfumery, although there are still several limitations, and adjustments are still being made so that this product becomes more widely available in the coming years (42).

1.4.1.5. Pharmacological effects

Using the method of extraction, maceration, with polar and apolar solvents (methanol and hexane) they obtained the bioactive components of lemon verbena. Subsequently, they were tested against pathogenic organisms such *as E. coli, S. aureus and P. aeruginosa* to demonstrate the presence of antimicrobial activity (44).

In a study conducted in Argentina, essential oils of this species from a farm were used, and it was demonstrated that oils that appear to be derived from oxygenated terpenoids, such as alcoholic and phenolic terpenes, shows an antimicrobial effect. The antimicrobial activity of essential oils was tested against *Gram-positive* and harmful bacteria and yeasts. The results showed that yeasts were the most sensitive microorganisms to the effect of essential oils, followed by *Gram-positive* bacteria, and finally, the *Gram negatives* (44).

Finally, the presence of antioxidant activity was also demonstrated for high concentrations of lyophilisate from the infusion. This effect has been related to the presence of phenolic compounds, in particular, derivatives of verbascoside and luteolin**.**

1.4.2. *Ilex Guayusa*

Figure 2. *Ilex Guayusa Loes* (49)

1.4.2.1. Taxonomy (50)

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Equisetopsida C. Agardh
Order	Aquifoliales Senft
Family	Aquifoliaceae
Genre	*Ilex L.*
Species	*Guayusa*
Author	Loes
Scientific name	*Ilex Guayusa*

Table 3. Taxonomy of *Ilex Guayusa*

1.4.2.2. Description

Ilex Guayusa is one of the most widely used plant species in traditional medicine. Guayusa belongs to the family *Aquifoliaceae* and the genus *Ilex* (50). It is wild-growing part of the forest ecosystems of the Amazon, but management and cultivation of *Ilex guayusa* are carried out traditionally (51).

1.4.2.3. Chemical composition

Several studies have shown that *Ilex Guayusa* leaves have methylxanthines, phenols, carotenoids, tannins, reducing sugars, steroids, terpenes, flavonoids, quinones, among others (52). The presence of phenols in this species is of great importance due to the biological effects that these compounds entail, which can be of great benefit to humans. The presence of 14 phenolic compounds has been registered, including hydroxycinnamic acids (p-coumaric, caffeic, ferulic acids, among others) and flavonoids (quercetin-3-O'Hexose). Hydroxycinnamic acids eliminate free radicals, acting as antioxidants, and this is precisely the property that confers their neuroprotective and anticancer activity (53).

Other compounds are carotenoids (β-carotene, α-carotene, violaxanthin, neoxanthin, and lutein) and methylxanthines (caffeine, theophylline, and theobromine). Methylxanthines come from the purine ring that is formed through the condensation of a pyrimidine with an imidazole. Also, they have a crystalline structure and their molecular formula is $C3H4N2$ (52)(53)(54).

1.4.2.4. Medicinal properties

Guayusa is part of the forest ecosystems of the Amazon and has been used by several indigenous nationalities to carry out ancestral rituals and also for the preparation of medicinal beverages (55). Being an aromatic plant, it is used to make hot beverages such as tea, in which different vitamins, minerals and antioxidants are included (53). In the case of rituals, it is said that natives made infusions with this plant very early in the morning with the aim of transmitting the values, culture, survival techniques, and traditions to their descendants that were transmitted from generation to generation in the past (55). For this reason, it is considered a sacred plant and is widely used in the Amazon region.

Since ancient times, *Ilex Guayusa* has been used as a powerful natural energizer as many of the indigenous people ingested this infusion before hunting, collecting plants, or generally carrying out their activities (56).

Over the years, this plant has been used for new medicinal purposes to treat various pathologies, including venereal diseases, diarrhea, arthritis, colds, a variety of pains, and infertility (49)(55). Likewise, the presence of carotenoids in health improves the immune response towards pathogens such as bacteria, viruses, and fungi (53). It also contributes to the reduction of degenerative diseases such as cancer and cardiovascular diseases.

1.4.2.5. Pharmacological effects

The solid extract of *Ilex guayusa* was tested in strains of *Porphyromonas gingivalis ATCC* (American Type Culture Collection) *33277*, *Fusobacterium nucleatum ATCC 25586*, and *Prevotella intermedia ATCC 25611* to demonstrate the presence of antimicrobial activity. These pathogenic organisms are associated with periodontal disease, that is, inflammation of the gums. In another study, it was observed that methanolic extracts of *Ilex guayusa* leaves have significant activity against *Candida albicans* and the hydroalcoholic extracts against *Microsporum canis*. This study was performed using plant species from *Ilex Guayusa* from the Northeast of Peru through a maceration technique (53).

In addition, the solid extract obtained by the ultrasound-assisted extraction technique (EAU) revealed its antioxidant activity. Elimination tests with 2,2-azinobis (3-ethylbenzothiazoline) -6 sulfonic acid (ABTS) and the antioxidant reducing power of iron (III) (FRAP), demonstrated the antioxidant capacity (57). Each of its antioxidant properties can be related to the presence of phenolic compounds and flavonoids.

1.4.3. *Croton lechleri*

Figure 3. *Croton lechleri* (58)

1.4.3.1. Taxonomy (59)(60)

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Celastrales
Family	Euphorbiaceae
Genre	*Croton*
Species	*Lechleri*
Scientific name	*Croton Lechleri*

Table 4. Taxonomy of *Croton Lechleri*

1.4.3.2. Description

Croton lechleri, also known as sangre de drago is a native species of the Amazon region and has numerous medicinal properties; for this reason, it is widely used in traditional medicine. It belongs to the family *Euphorbiaceae* and the genus *Croton*

(60). In addition, it is known throughout South America by multiple names such as sangre de drago, palo de drago and Balsa macho (61).

1.4.3.3. Chemical composition

Several studies show that *Croton lechleri* has a large number of compounds, mainly its latex, including alkaloids, phenolic compounds, polyphenols, and quinones (62). The alkaloids' presence, especially of Taspina, is of utmost importance because they have pharmacological properties demonstrated in various investigations. Its bark contains diterpenes (type clerodano) such as hardwickiic acid, bincatriol, crolechinol, crolequinic acid, korberine-A, and korberine-B. Moreover, the leaves and roots contain mostly alkaloids. In addition, the essential oil obtained from Amazonian Ecuador included seventy-four compounds such as sesquiterpenes sesquicineole, α-calacorene, 1,10-di-epi-cubenol, β-calacorene, epi-cedrol, p-cymene, limonene, and borneol (63).

In other research, several compounds of several molecular sizes have been isolated from sangre de drago. Such as catechin, epicatechin, gallocatechin, epigallocatechin (monomeric flavan-3ols) and proanthocyanidins (64).

1.4.3.4. Natural properties

Various investigations show that the latex of *Croton lechleri* has antibacterial, anti-inflammatory and healing properties, among others, due to their metabolites such as catechins, proanthocyanidins oligomeric (lignans) and alkaloids (taspine), which help with antimicrobial activity, allow healing, work as antivirals, anti-inflammatories, anti-ulcer medications, and help with diarrhea (64). Furthermore, its chemical components such as flavonoids provide various biological activities, including anti-inflammatory, antibacterial, anti-allergic, antiviral, antithrombotic and as a vasodilator. Proanthocyanidin, also known as "condensed tannins" with antioxidant, antibacterial properties inhibit the adhesion of bacteria. In the same way, it helps to prevent cardiovascular diseases by granting vascular permeability and avoiding free radical damage in the arteries. Additionally, the metabolites have antioxidant, anti-allergic, anticancer, anti-inflammatory, and antimicrobial properties (63). The antimicrobial effect of polyphenols is evidenced by inhibition of the development of microorganisms that affect human health, including *Helicobacte*r *pylori*, *Escherichia coli* and *Staphylococcus aureus* (65).

1.4.3.5. Pharmacological effects

Several studies support the antiviral and antibacterial actions of *Croton lechleri* latex in *in vitro* research due to the presence of polyphenols, polyacetylenes, flavonols, terpenoids, steroids, alkaloids, propolis, chloroquine acid, cooberins A and B and 1,3,5-trimethoxybenzene potent against *Bacillus subtilis* (63). The antibacterial activity was also ratified for phenolic compounds, flavonoids, anthocyanins (58).

It should be noted that sangre de drago presents antimicrobial activity against *Gram-positive* bacteria, such as *Staphylococcu aureus ATCC 6538*. It is considered that the latex of *Croton lechleri* has biological activity, especially antimicrobial, anti-inflammatory, healing, antioxidant and cytotoxic effects. At least 25 of the chemical compounds responsible for the antimicrobial action against *Gram positive* bacteria would be the phenolic compounds. Cai et al.evaluated the cytotoxicity and antibacterial activity in three *in vivo* tests of *Croton lechleri* (62). The results show that latex lacks cytotoxic activity and confirms that phenolic and terpenic compounds are responsible for the antibacterial effect. Finally, the presence of polyphenols (gallic acid) and catechins contribute antioxidant, anti-allergic, anticancer, anti-inflammatory, and antimicrobial properties (58). Likewise, the antimicrobial activity of phenolic compounds is related to their concentration, and the action mechanism consists of destroying the cell wall and membrane by inactivating the enzyme systems. Therefore, phenols have bacteriostatic or bactericidal, fungicidal, and viricidal activity. Being influential in *Gram positive* bacteria (64)(65).

1.4.4. *Clinopodium nubigenum*

Figure 4. Clinopodium nubigenum (66)

1.4.4.1. Taxonomy (67)

Kingdom	Plantae
Class	Equisetopsida C. Agardh
Subclass	Magnoliidae Novák ex Takht
Order	Lamiales Bromhead
Family	Lamiaceae Martinov
Genre	*Clinopodium* L.
Species	*C. nubigenum*
Scientific name	*Clinopodium nubigenum*

Table 5. Taxonomy of *Clinopodium nubigenum*

1.4.4.2. Description

Clinopodium nubigenum, commonly known as Sunfo, sunfillo, surumba is a plant that belongs to the *Lamiaceae* family (67). Taxonomic synosyms are *Micromeria nubigena* (Kunth) Benth., *Satureja nubigena* (Kunth) Briq., *Thymus nubigenus* Kunth (66).

1.4.4.3. Chemical composition

Various studies show that *Clinopodium nubigenum* contains secondary metabolites such as alkaloids, tatinos, saponins, flavonoids, anthraquinones, coumarins, triterpenes and steroids, such as cardiotonic glycosides (68). Its essential oils contain borneol, borneol acetate, butyric acid, carvacrol, citroneol, p-cymene, geraniol, limonene, nerol, valeric acid and acetic acid (69).

1.4.4.4. Medicinal properties

Sunfo is a plant widely used in traditional medicine for relieving stomach pain, muscle pain, and respiratory diseases (70). As it is an aromatic plant, many people make infusions to aid in digestion. It is used to treat chills and also to make aromatic extracts (69).

In several indigenous communities of the Andes region, such as Saraguro and Quechua, this plant has been used to treat colds as well as the flu. Furthermore, people in several other provinces have adopted the same usage. However, in Cañar, they use this plant as a tea infusion to treat urinary incontinence, especially in children and older people. At other localities of the provinces of Tungurahua, Azuay, and Chimborazo, *Clinopodium nubigenum* has been used as a tonic as well as a remedy for menstrual symptoms (71).

As this plant can be found in the field, it has always been used for its pharmacological properties. For example, it serves as an anti-inflammatory, analgesic, antioxidant, antibacterial and helps to provide strength and as a sedative (72).

1.4.4.5. Pharmacological effects

For the antimicrobial resistance test, used *sunfo* essential oil extract against four ATCC certified strains of *Gram-positive* bacteria: *Staphylococcus aureus ATCC®: 25923* ™*, Streptococcus pyogenes ATCC®: 19615* ™*, Streptococcus pneumoniae ATCC®: 49619* ™ and *Streptococcus mutans ATCC®: 25175* ™ (68). In this experiment the presence of antimicrobial activity was shown; however, the essential oil extract was more effective against *S. mutans, S. pyogenes, S. pneumoniae*, and less so with *S. aureus* strains. Furthermore, the presence of antioxidant activity attributed to the presence mainly of carvacrol acetate was evidenced, but it may also be due to other compounds (69)(72).

CHAPTER II

2. METHODOLOGY

The present research work has the aim of making a comparison of the antimicrobial properties of four native plants *(Ilex guayusa, Aloysia citrodora, Clinopodium nubigenum, Croton lechleri*), from studies carried out in Ecuador. The following obtained document was based on national and international protocols with bibliographic research and information analysis and synthesis.

2.1. Bibliographic search and information analysis

Academic search engines were used with the following keywords: antimicrobial properties, *Ilex guayusa*., *Aloysia citrodora, Clinopodium nubigenum, Croton lechleri.*

Indexed journals, databases, publications of international organizations were used as information sources. Among the databases used are the following: National Center for Biotechnology Information (NCBI), Latindex, SciELO, Science Direct, Google Scholar, Scopus, and Springer. In addition, articles and publications from international organizations and institutions were obtained: World Health Organization (WHO), Food and Drug Administration (FDA), Food and Agriculture Organization of the United Nations (FAO), and BioMed Central (BMC). While the public information was collected from the Ministry of Health, Ecuadorian articles referring to antimicrobial activity in the four native plants of Ecuador were also reviewed.

Mendeley is a reference manager that allows management and share bibliographic references, research documents, find new connections, and documents permit the classification of information.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Resistance

The antimicrobial properties of a plant are its ability to kill microorganisms, or in turn, inhibit their growth. They can be grouped in different ways, one of the most important ones is according to the microorganisms they act on; for example, antibiotics are used against bacteria, antivirals are used against viruses, and antifungals against fungi (73)(Table 6).

Table 6. Comparison of the antimicrobial properties of four native plants.

Plants	**Family**	**Active principle**	**Pathogenic organisms**		**Properties**
			Gram- positive bacteria	**Gram- negative bacteria**	
Aloysia citrodora	Verbenaceae	Phenols (thymol)	*S. aureus* *B. cereus* *B. subtilis*	*Helicobacter pylori* *E. coli* *P. aeruginosa* *S. Typhimurium* *K. pneumoniae* *P. vulgaris*	Antimicrobial Antifungal Antioxidant
Ilex guayusa	Aquifoliaceae	Phenols Phenolic acid Quinones	*S. aureus*	*P. gingivalis* *F.nucleatum* *P. intermedia* *E. coli* *Helicobacter pylori*	Antimicrobial Antioxidant
Clinopodium nubigenum	Lamiaceae Martinov	Phenols (carvacrol acetate) Thymol	*S. aureus* *B. subtilis*	*E. coli*	Antimicrobial Anti-inflammatory Antioxidant
Croton Lechleri	Euphorbiaceae	Alkaloids (taspine) Phenols	*S. aureus* *B. subtilis.*	*E. coli* *P. aeruginosa*	Anti-inflammatory Antiviral, Antimicrobial Antiparasitic Antioxidant

Aloysia citrodora

Aliaga Mamani (74) shows that *Aloysia citrodora* exhibits antimicrobial activity because, from its essential oil and disk diffusion method, they determined that there is activity against *E. Coli*. Lemon verbena has among its main components, monoterpenes hydrocarbons, and monoterpenes aldehydes. These act on the bacterial membrane, increasing the permeability of the membrane to small ions. Consequently, this action affects the membrane's structural stability and destabilizes the lipid bilayer packaging, and causes the bacterial cell (75)

At Bazil, the essential oil of *Aloysia citrodora* was obtained by steam distillation. Subsequently, the minimum inhibitory concentration of the oil was determined by the microdilution method. *Aloysia citrodora* showed moderate inhibition against *Staphylococcus aureus, Candida albicans, Enterococcus faecium*, and *Salmonella cholerasuis.* The oil was analyzed by GC and GC-MS techniques in order to determine the significant compounds. The organic phase was dried over sodium sulfate, filtered, and the solvent was evaporated to dryness. The oil was solubilized in ethyl acetate for gas chromatography and mass spectrometry analysis. The main constituents are the monoterpenes linalol, eugenol, and thymol. These compounds are previously known for their antimicrobial activity. However, the antimicrobial action is attributed to thymol due to its phenolic character, causing membrane-altering activities (76).

N. Kiran Kumar (77) in their study shows that *A. citrodora* exhibits antimicrobial activity against the pathogenic organisms *E. coli, B. subtilis, S. aureus, K. pneumoniae*, and *P. vulgaris.* The minimum inhibitory concentration (MIC) was determined for the active acetone extract using the agar diffusion method. The aqueous extract did not show any inhibition against the microorganisms tested. However, the organic extracts showed different levels of antibacterial activity against the organisms. The methanol extract's phenolic content was very high compared to other organic extracts, but the antibacterial activity was found to be higher in the acetone extract. The most significant inhibitory activity was observed with the acetone extract with zones of inhibition ranging from 30 mm with *K. pneumoniae* to 37 mm with *B. subtilis*. These results suggest that the acetone fraction's total phenols can function as healthy antibacterial compounds against these pathogenic organisms.

Seyed Mohammad Bagher Hashemi (78) in their study evaluated the influence of ultrasonic pretreatment (continuous and pulsed), before hydrodistillation (USHD and PUSHD), on the extraction of essential oil from dried *Aloysia citriodora* leaves to determine its antimicrobial activity against Gram-positive bacteria (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli* O157: H7, *P. aeruginosa*, and *S. Typhimurium*). To determine the antibacterial efficacy of essential oils obtained through hydrodistillation, continuous, and pulsed ultrasonic-assisted hydrodistillation (USHD and PUSHD), they measured the diameter of the inhibition zones (mm) against pathogenic bacteria. The results showed that the zones of inhibition observed using the essential oil extracted by USHD and PUSHD were greater than those of the HD samples ($p < 0.05$) due to the higher content of secondary metabolites. The *S. aureus* strains showed high susceptibility to essential oils extracted by HD, USHD, and PUSHD. The most susceptible microorganism was *B. cereus* with 19.64 ± 0.24 mm of the HD sample's inhibitory zone, 27.57 ± 0.19 mm for PUSHD at 30 or 45 min 26.92 ± 0.21 mm for USHD at 30 min sample. However, the most resistant microorganism was *P. aeruginosa* with an inhibitory zone of 10.42 ± 0.14 mm for 14 HD samples, 18.84 ± 0.22 mm for PUSHD at 30 min or 45 min, and 18.32 ± 0.18 mm for USHD at 30 min.

Ilex guayusa

Portela L (79) in his study, obtained antimicrobial activity from extracts of *Ilex guayusa* against *Helicobacter pylori* strains in vitro study at a concentration of 1000 ug / mL. For the biological activity tests, they used extracts of the species *I. guayusa* maceration in 40% ethanol, reflux in 40% ethanol and aqueous infusion. Different investigations have revealed that the antimicrobial potential is related to the presence of different classes of compounds, mainly phenolic, whose leading representatives are gallic, syringic, caffeic, ferulic and ρ-coumaric acid. It is essential to mention that the concentrations of 500 and 1000 ug/ mL have a weak inhibitory activity, so toxicity studies for higher concentrations should be considered.

In other studies, it was found that the ethanolic and hydroalcoholic extracts of the leaves of *I. guayusa* have activity against strains of *Candida* and *Microsporum canis*, which can be attributed to the presence of triterpenes, especially the rotundic acid (80).

In their study, Fredy Gamboa (53) demonstrated antimicrobial activity using ethanol extracts of *Ilex guayusa* against pathogenic organisms associated with Periodontitis such as *P. gingivalis, F. nucleatum*, and *P. intermedia*. The fractions obtained from Ilex guayusa presented diverse activities on the three microorganisms studied. However, results were too satisfactory concerning *P. gingivalis*, with which it showed greater activity. Phytochemical analyzes were performed on total ethanol extracts and revealed several classes of compounds, including phenols and flavonoids. Phenols, phenolic acid, and quinones are the main components in plants with antimicrobial activity. Therefore, the total extracts' phenol concentration was 57.7 mg of gallic acid per gram of *I. guayusa.* Phenols are attributed to the antimicrobial activity present in this plant.

A study where the influence of the age of the leaves on the phenolic and carotenoid compounds and the bioactivity and digestibility (in vitro) of aqueous and hydroalcoholic foliar extracts was evaluated. When analyzing with young leaves due to its higher phenolic content, it was shown that guayusa did not show any antibacterial activity against E. coli ATCC 25922 or S. aureus ATCC 25923 (81).

Clinopodium nubigenum

According to analyzes carried out by Fonseca (72), antibacterial activity was obtained from *C. nubigenum* essential oil for pathogenic bacteria related to respiratory infections. A higher effect was recorded for *S. pyogenes* and lower one for *S. mutans*. One component of its essential oil is acetate carvacrol, a monoterpenoid phenol to which the plant's antibacterial capacity is attributed. Carvacrol inhibits the growth of various strains of bacteria (82).

In another study carried out by Gómez (83) the antimicrobial activity of this plant was corroborated. When carrying out tests at different concentrations of essential oils of *C. nubigenum*, positive results were obtained for the antimicrobial activity against *C.*

Albicans, B. Subtilis, S. Aureus and finally *E. Coli* and *S. Abaetetuba*. However, in these latter two species, the inhibition halos were smaller in diameter.

Estefano Bedini (84) in their study, demonstrated the presence of antimicrobial activity using the essential oil of *C. nubigenum* against *E. coli, S. aureus, B. subtilis, Salmonella* enterica subsp. enterica serovar *Abaetetuba*, and *C. albicans*. Also, for the antimicrobial activity test, they opted for *E. coli, S. aureus*, and *B. subtilis* strains were grown on nutrient agar; *C. albicans* strain was grown on malt agar; *S. enterica* was grown on trypticase soy agar. At the highest dose, which was ten μL of disc-1, the zone of inhibition of the essential oil of *C. nubigenum* ranged from 14.7 ± 0.7 to 45.0 ± 0.0 mm for *S. abaetetuba* and *C. albicans*, respectively. Furthermore, *E. coli* presented a zone of inhibition of 20.9 ± 0.5, 36.0 ± 4.9 for *S. aureus*, and finally 33.0 9 ± 2.0 for *B. subtilis*.

In a study in Brazil, the antimicrobial activity of essential oil was demonstrated, which inhibits Candida albicans. From the aqueous MeOH extract of the plant's aerial parts, two nonvolatile compounds, named schizonepetoside A and schizonepedoside C, have been isolated. They are rare glycosyl terpenoids previously isolated from only one plant but never found before in the genus Clinopodium (71). By mass-coupled gas chromatography (GC MS), carvacrol acetate was identified as the majority compound in a species from Loja, Ecuador (85).

Croton lechleri

According to an *in vitro* study carried out by Elliot et al., *Croton lechleri* has been found to have an inhibitory effect on the growth of *E. coli* at a 1:10 dilution, while at dilution at 1: 100, the inhibitory effect was not manifested (86). Were found similar results when using H. pylori. Three were commercial, using, and the remaining natural, an antimicrobial activity test against the pathogen, was performed. The results show that Sangre de drago inhibits the growth of *Helicobacter pylori* at high concentrations. Also, not evidenced its effect in dilutions of 1/10 or more significant.(87).

In a study carried out at the Universidad Regional Autónoma de Los Andes, the addition of *C. lechleri* extract to several samples of infected tissues inhibited bacterial growth, which indicates that this product helps prevent the bacterial proliferation that is present before and after tooth extraction (88).

Dragon´s blood latex (*Croton lechleri)* shows antimicrobial activity against the *S. aureus* strain. Hydrogels prepared based on chitosan-PVA, cross-linked with gamma radiation, and soaked in hydroalcoholic and aqueous solution show antimicrobial activity against the *S. aureus* strain and not against the *E. coli* and *P. aeruginosa* strains. The best results were obtained with the hydrogels soaked with the hydroalcoholic solution of dragon´s blood related to the better solubility of the latex components in this solvent system. The minimum inhibitory concentration against *S. aureus* is 0.025g / 10mL (89).

In Colombia, research was carried out to evaluate the in vitro antibacterial potential of Croton lechleri against aerobic bacterial isolates from patients with skin ulcers at the Sanatorio de Agua de Dios, Cundinamarca, Colombia. Among the main bacterial agents of aerobic growth that have been associated with skin ulcers are Streptococcus of groups A and G, Staphylococcus aureus, and Pseudomonas aeruginosa. Regarding the results obtained at the level of antimicrobial sensitivity tests, it was evidenced that the ethanolic extract obtained an antibacterial efficacy (absence of growth) of 100% of the evaluated bacteria: S. agalactiae, the two different isolates of S. uberis, S. sanguis, S. aureus (ATCC); S. maltophilia, B. cepacia, P. aeruginosa, and E. coli (ATCC). Also, with the petroleum ether extract, antibacterial efficacy (no growth) was obtained in 55.55% of the study bacteria: S. agalactiae, S. uberis, S. aureus (ATCC) and E. coli (ATCC) (90).

The antimicrobial activity of C. lechleri is due to taspine belonging to the group of alkaloids (91). Furthermore, the antimicrobial activity against Gram-positive bacteria would be mainly due to simple phenolic compounds.

According to studies carried out by Cortéz & Perales (92), *Aloysia triphylla* has antifungal activity against *Rhizopus* spp. From an oily ethanol extract of A. citrodora, they obtained a positive inhibition result against this microorganism at a maximum concentration of 7.2 mg / well.

3.2. Extraction methods and solvents used

The extraction of the compounds can be carried out from fresh, dry, and semi-dry plants. In addition, any portion of the plant can be used, that is, either leaves, stem, root, or fruit. The most common extraction methods are maceration and Soxhlet extraction (93). Modern methods include microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), among others. Separation techniques such as TLC, thin-layer chromatography, column chromatography, HPLC are used for the isolation of the bioactive compounds. Furthermore, there are different solvent systems available for the extraction of the bioactive compound from natural products.

Table 7. Comparison of extraction method and solvent used of four native plants

	Plants						
	Aloysia citrodora		*Ilex guayusa*		*Clinopodium nubigenum*		*Croton lechleri*
Extraction method	Maceration	Steam diatillation	Maceration	Steam diatillation	Maceration	Steam diatillation	Steam diatillation
Solvent used	Ethanol Ethyl acetate	Water	Ethanol	Water	Methanol Ethanol	Water	Ethanol Alcohol
Time required	~ 1 to3 days	~1 to18 hours	~1 to2 days	~3 to 12 hours	~1 to 3 days	~2 to 10 hours	~1 to 24 hours
Dry raw material mass (kg/g)	~2,00 to 25,00 kg		~10,00 to 80,00 g		~3,00 to 6, 00 kg		None

Aloysia citrodora

Some researchers have studied the chemical composition and antibacterial activity of *A. citrodora* essential oil using steam distillation and maceration extraction methods. It has been possible to isolate various phenolic and flavonoid compounds responsible for antimicrobial and antioxidant activity. In Table 7, it is disclosed that one of the solvents used is ethanol because they are safe and non-toxic solvents. Also, to obtain high-quality botanical extracts, drying is at 40 ° to avoid loss of compounds. The amount of raw material required in each process is between 2.00 to 25.00 kg. In the case of maceration, large amounts of raw material are used. Besides, in maceration, the extraction of compounds ranges from 1 to 3 days, which represents a very long time. For steam distillation, the extraction time lasts from 1 to 18 hours. The duration of each of the processes varies according to the protocol established by each of the researchers. The results obtained with *A. citrodora* were successful with both methods. (74).

Ilex guayusa

I. guayusa is one of the wealthiest plant species in phenols. Several researchers demonstrated the phenol content using steam distillation and mashing techniques. One of the studies reported that the leaves are disinfected with sodium hypochlorite and dried at room temperature for an approximate period of 14 days. One of the disadvantages of using air drying is that it can be contaminated by secondary factors such as fungi. For this reason, the results may be altered. Such is the case of one of the studies in which it was demonstrated that due to the volume and disinfection factors, *I. guayusa* did not present activity against previously studied pathogenic organisms such as *E. coli* or *S. aureus*. However, other studies used these pathogens and showed antimicrobial activity. On the other hand, to obtain the bioactive extracts, the researchers used 90% ethanol in a volume of 80 g for approximately 12-72 hours. Likewise, a chromatographic analysis was performed to identify compounds (74). With both extraction methods, the antimicrobial activity of *I. guayusa* against various pathogenic organisms can be determined. (79).

Clinopodium nubigenum

According to several researchers, sunfo (Clinopodium nubigenum) 's active principles are mainly found in its essential oil, and they demonstrated this using steam distillation and maceration techniques. It was possible to extract secondary metabolites such as phenols responsible for antimicrobial and antioxidant activity. Also, for the extraction of the bioactive compounds, they used methanol and ethanol at 90%. Additionally, for the efficient and gentle removal of solvents from the samples by evaporation, they used a rotary evaporator (68)(72). The average duration of each of the extraction processes is a maximum of 3 days and 18 hours for maceration and steam distillation, respectively.

Croton lechleri

Numerous studies support the antimicrobial activity of dragon's blood, and it has been achieved using the latex of the plant. Dragon's blood is one of the most widely used products in Central and South America's tropical and humid areas. In some cases, the researchers used leaves, bark, and latex. The latex samples were refrigerated at 4 ° C for two weeks, during which time their analysis began from their acquisition. In the case of the leaves and bark, the solvent used was 70% alcohol. For the isolation of the bioactive compounds, they used the technique of thin-layer chromatography (94).

3.3. Antioxidant properties

Phenolic compounds have the appropriate chemical structure to exert an antioxidant action, acting as free radical scavengers, such as reactive oxygen species (ROS). ROS-mediated lipid peroxidation is a significant cause of destruction and damage to cell membranes. It has been involved in the pathogenesis of ethanol-induced acute injury of the gastric mucosa and processes that affect the liver, intestine, and pancreas (95).

Aloysia citrodora, its antioxidant activity, can be attributed to the presence of polyphenols, whose chemical structure is especially suitable to act as free radical scavengers (96). The antioxidant activity of lemon verbena essential oil was evaluated relative to ascorbic acid by two different bleaching methods: DPPH and ABTS. There is no further data on the antioxidant capacity in the essential oil of this plant. However, it has been evaluated in aqueous extracts and alcoholics, and their polyphenol content has been determined (97).

C. nubigenum according to an investigation carried out by the antioxidant molecules identified were carvacrol, which is a phenol, and in turn is a molecule already known to have antioxidant activity, ubiquitous in species such as thyme and varieties of oregano (98).

Fernando Noriega (99) assessed the antioxidant activity of *Ilex guayusa* through the DPPH method. In this study, it was possible to show that Ilex guayusa has a greater capacity to eliminate free radicals than *Camellia Sinensis*. Besides, it presented a higher concentration of phenols. In another study, the antioxidant capacity and the polyphenol content were determined by the ABTS and Folin-Ciocalteu methods, respectively. A simple ANOVA analysis of variance was performed with the multiple range comparison test (LSD) with 95% reliability. They were concluding that ethanol is a solvent that favors the extraction of antioxidant compounds in the powder of dry guayusa leaves (100).

Croton lechleri, has as active principles proanthocyanins (antioxidants), tannins, a lingan called dimethyl cedrusin, and the alkaloid Taspine. Taspine has been documented as wound healing, anti-inflammatory, antiviral, with activity against the development of sarcomas (87).

CONCLUSION

In conclusion, the low economic conditions and the limited accessibility of the population to health institutions allow revaluing medicinal plants with palliative, preventive or curative actions on some conditions or symptoms. Among these products are Ilex guayusa, Aloysia citrodora, Clinopodium nubigenum, and Croton lechleri, which have been shown to have a high pharmacological potential.

A bibliographic review was carried out about the characterization of the antimicrobial properties of four native plants of Ecuador (*Ilex guayusa*, *Aloysia citrodora*, *Clinopodium nubigenum, and Croton lechleri*) from different bibliographic sources. A great variety of studies were found that demonstrate the great importance of continuing to carry out new research with medicinal plants.

It is necessary to highlight the importance of choosing the techniques and methodologies to be used to carry out investigations related to the antimicrobial activity of extracts from plants depending on the chosen technique. The results will vary, as well as their sensitivity and reproducibility according to the extract's characteristics. Like polarity, the secondary metabolites obtained in each of the extracts depend on the solvent used to obtain them. These metabolites are responsible for plants' pharmacological properties; this is reflected in the greater efficacy presented by the ethanolic extract in most plants.The differences in sensitivity between Gram-positive and Gram-negative bacteria are centered on the membranes' structure and the bacterial cell wall, both at a morphological and physiological level. In addition to these two structures, Gram-negative bacteria have an outer membrane responsible for a certain degree of resistance against antibacterial agents. The cell walls of Gram-negative bacteria contain peptidoglycan, exterior lipoprotein, and lipopolysaccharides, which emerge up to the bacterial surface. In general, it is permeable without specific selectivity. However, the outer membrane is semi-permeable, restricting the passage of relatively large molecules, such as the secondary metabolites present in ethanolic extracts.

Regarding *Aloysia citrodora*, several researchers have demonstrated the presence of antimicrobial activity against *S. aureus, B. cereus, B. subtilis, Helicobacter pylori, E. coli, P.*

aeruginosa, S. Typhimurium, K. pneumoniae, and *P. Vulgaris*. Lemon verbena has among its main components, monoterpenes hydrocarbons and monoterpenes aldehydes. These act on the bacterial membrane, increasing the permeability of the membrane to small ions. Consequently, this action affects the membrane's structural stability, destabilizes the lipid bilayer packaging, and causes the bacterial cell. Also, it is attributed to other properties such as antifungal and antioxidant.

I. guayusa is one of the wealthiest plant species in phenols. Several investigators have demonstrated antimicrobial activity against *S. aureus, P. gingivalis, F. nucleatum, P. intermedia, E. coli,* and *Helicobacter pylori.* Phenols, phenolic acid, and quinones are the main components in plants with antimicrobial activity. A study where the influence of the leaves' age on the phenolic and carotenoid compounds and the bioactivity and digestibility (in vitro) of aqueous and hydroalcoholic foliar extracts was evaluated. When analyzing young leaves due to its higher phenolic content, it was shown that guayusa did not show any antibacterial activity against *E. coli* ATCC 25922 or *S. aureus* ATCC 25923.

According to several researchers, sunfo's active principles are mainly found in its essential oil. The antimicrobial activity has been demonstrated against *S. aureus, B. subtilis,* and *E. coli*. One component of its essential oil is acetate carvacrol, a monoterpenoid phenol to which the plant's antibacterial capacity is attributed. Carvacrol inhibits the growth of various strains of bacteria. Other properties attributed to it are anti-inflammatory and antioxidant.

Croton lechleri is one of the most popular plants used in the tropical, humid areas of Central and South America. It was possible to demonstrate its antimicrobial activity against pathogenic organisms such as *S. aureus, B. subtilis, E. coli,* and *P. aeruginosa*. Dragon's blood latex has several biological properties: anti-inflammatory, antioxidant, antiviral, antiparasitic, healing, and cytotoxic activity. The antimicrobial activity against Gram-positive bacteria would be mainly due to simple phenolic compounds. Furthermore, the anti-inflammatory and cytotoxic activities are due to the presence of taspine in the latex, although it is not ruled out that other compounds may be involved.

RECOMMENDATIONS

Finally, for future research is recommended to consider using other unconventional extraction techniques to perform antimicrobial activity tests using *Ilex guayusa*, *Aloysia citrodora*, *Clinopodium nubigenum*, and *Croton lechleri* and to demonstrate the effectiveness of each of the methods and verify the viability of each one of them.

Besides, it has been shown that several researchers must focus on studying the active components of each of the plants to reveal, which is the specific component that acts before each biological activity. In this way, the species' study can be facilitated, and new fields such as the pharmacological industry can be opened.

BIBLIOGRAPHY

1. OMS. El primer informe mundial de la OMS sobre la resistencia a los antibióticos pone de manifiesto una grave amenaza para la salud pública en todo el mundo [Internet]. WHO. Ginebra: World Health Organization; 2014 [cited 2019 Sep 3]. Available from: https://www.who.int/mediacentre/news/releases/2014/amr-report/es/

2. Economou V, Gousia P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. Infect Drug Resist. 2015 Jan;8:49–61.

3. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. The global threat of antimicrobial resistance: Science for intervention. New Microbes New Infect. 2015 Apr;

4. Kaya I, Yigit N, Benli M. Antimicrobial activity of various extracts of *Ocimum basilicum* l. and observation of the inhibition effect on bacterial cells by use of scanning electron microscopy. African J Tradit Complement Altern Med [Internet]. 2008 Oct 20 [cited 2020 Jul 30];5(4):363. Available from: /pmc/articles/PMC2816579/?report=abstract

5. Gómez R. Medicinal plants in a small village in the state of tabasco, méxico. 2011 [cited 2020 Jul 29];25. Available from: https://www.researchgate.net/publication/287586417_Medicinal_plants_in_a_small_village_in_the_state_of_tabasco_mexico

6. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science (80-) [Internet]. 1994 [cited 2020 Jul 30];264(5157):375–82. Available from: https://pubmed.ncbi.nlm.nih.gov/8153624/

7. Kalemba D, Kunicka A. Antibacterial and Antifungal Properties of Essential Oils. Curr Med Chem [Internet]. 2005 Mar 23 [cited 2020 Jul 30];10(10):813–29. Available from: https://pubmed.ncbi.nlm.nih.gov/12678685/

8. Soto Ortiz R, Vega Marrero G, Tamajón Navarro AL. Instructivo técnico del cultivo de Cymbopogon citratus (D_C) Stapf (caña santa). Rev Cuba plantas med [Internet]. 2002 [cited 2020 Jul 30]; Available from:

http://bvs.sld.cu/revistas/pla/vol7_2_02/pla07202.htm

9. FAO. El estado de los recursos de tierras y aguas del mundo para la alimentación y la agricultura. Organ las Nac Unidas para la Aliment y la Agric [Internet]. 2012 [cited 2019 Sep 4];338. Available from: http://www.fao.org/3/b-i1688s.pdf

10. 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 [cited 2020 Sep 1];12(1):29. Available from: http://ethnobiomed.biomedcentral.com/articles/10.1186/s13002-016-0100-4

11. Bailon-Moscoso N, Romero-Benavides JC, Tinitana-Imaicela F, Ostrosky-Wegman P. Medicinal plants of Ecuador: A review of plants with anticancer potential and their chemical composition. Vol. 24, Medicinal Chemistry Research. Birkhauser Boston; 2015. p. 2283–96.

12. Kalemba D, Kunicka A. Antibacterial and Antifungal Properties of Essential Oils. Curr Med Chem [Internet]. 2005 Mar 23 [cited 2020 Aug 7];10(10):813–29. Available from: https://pubmed.ncbi.nlm.nih.gov/12678685/

13. Torres-Guevara FA, Ganoza-Yupanqui ML. Etnobotánica y sistemas de extracción para compuestos fenólicos, actividad antioxidante y toxicidad de plantas de páramos y bosques nublados del norte peruano. Rev Peru Med Integr [Internet]. 2017 Oct 2 [cited 2020 Dec 5];2(2):101. Available from: http://www.rpmi.pe/ojs/index.php/RPMI/article/view/51

14. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants. 2015;04(03).

15. QF Glicerio León Méndez, MSc. María del Rosario Osorio Fortich, Sonia Ruby Martínez Useche. Comparación de dos métodos de extracción del aceite esencial de C itrus sinensis L. Rev Cuba Farm [Internet]. 2015 [cited 2020 Dec 5];49(4):1561–2988. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0034-75152015000400014

16. Zhang Q-W, Lin L-G, Ye W-C. Techniques for extraction and isolation of natural

products: a comprehensive review. Chin Med [Internet]. 2018;13:20. Available from: https://doi.org/10.1186/s13020-018-0177-x

17. Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment [Internet]. Vol. 8, Environmental Microbiology. Environ Microbiol; 2006 [cited 2020 Aug 6]. p. 1137–44. Available from: https://pubmed.ncbi.nlm.nih.gov/16817922/

18. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! [Internet]. Vol. 18, Trends in Molecular Medicine. Trends Mol Med; 2012 [cited 2020 Aug 6]. p. 263–72. Available from: https://pubmed.ncbi.nlm.nih.gov/22480775/

19. Von Wintersdorff CJH, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB, et al. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer [Internet]. Vol. 7, Frontiers in Microbiology. Frontiers Media S.A.; 2016 [cited 2020 Aug 6]. p. 173. Available from: /pmc/articles/PMC4759269/?report=abstract

20. Rosewich UL, Kistler HC. Role of horizontal gene transfer in the evolution of fungi [Internet]. Vol. 38, Annual Review of Phytopathology. Annu Rev Phytopathol; 2000 [cited 2020 Aug 7]. p. 325–63. Available from: https://pubmed.ncbi.nlm.nih.gov/11701846/

21. Heuer H, Smalla K. Horizontal gene transfer between bacteria [Internet]. Vol. 6, Environmental Biosafety Research. EDP Sciences; 2007 [cited 2020 Aug 7]. p. 3–13. Available from: www.ebr-journal.org

22. Mosquera WG, Criado LY, Guerra BE. Antimicrobial activity of endophytic fungi from the medicinal plants Mammea americana (Calophyllaceae) and Moringa oleifera (Moringaceae). Biomedica [Internet]. 2020 [cited 2020 Aug 6];40(1):55–71. Available from: https://pubmed.ncbi.nlm.nih.gov/32220164/

23. (PDF) Metabolitos secundarios de las Plantas, una alternativa para el manejo de enfermedades en cultivos de interés económico [Internet]. [cited 2020 Oct 15]. Available from:

https://www.researchgate.net/publication/341295118_Metabolitos_secundarios_de_las_Plantas_una_alternativa_para_el_manejo_de_enfermedades_en_cultivos_de_interes_economico

24. L. Andrew Staehelin. Biochemistry and Molecular Biology of Plants [Internet]. Segunda. Bob B. Buchanan, Wilhelm Gruissem, Russell L. Jones, editors. Vol. Vol. 1. California, Beckerly; 2015 [cited 2020 Nov 26]. 205–265 p. Available from: https://books.google.com.ec/books?hl=es&lr=&id=yRIWCgAAQBAJ&oi=fnd&pg=PA2&dq=biochemistry+and+molecular+biology+of+plants+buchanan+pdf&ots=LPUxAp4XSd&sig=fWFlyy8k1PNzFCBVjVpukyMLjZ4#v=onepage&q=biochemistry and molecular biology of plants buchanan pdf&f=false

25. Gabriel Bueno-Sánchez J, René Martínez-Morales J, Stashenko E, Bueno JG. Artículos Originales Actividad antimicobacteriana de terpenos Antimycobacterial activity of terpenes.

26. Arrázola G, Grané N, Martin ML, Dicenta F. Revista Colombiana de Química. Vol. 42, Química Aplicada y Analítica Rev. Colomb. Quim. 2013.

27. Jaramillo CJ, Jaramillo Espinoza A, D'armas H, Troccoli L, Rojas De Astudillo L. Concentraciones de alcaloides, glucósidos cianogénicos, polifenoles y saponinas en plantas medicinales seleccionadas en Ecuador y su relación con la toxicidad. Vol. 64, Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN. 2016.

28. Vista de La Producción de Metabolitos Secundarios en la Familia Brassicaceae [Internet]. [cited 2020 Oct 15]. Available from: https://revistas.unimilitar.edu.co/index.php/rfcb/article/view/388/167

29. Biosíntesis de los alcaloides indólicos: Una revisión crítica [Internet]. [cited 2020 Oct 15]. Available from: http://www.scielo.org.mx/scielo.php?pid=S0583-76932004000100013&script=sci_arttext

30. Salazar EA, Asesor S, Delgado DR. Efecto bacteriostático y bactericida de extractos de ají panca (Capsicum chinense) y pimiento (Capsicum annuum var. annuum) sobre cultivos de Escherichia coli ATCC 25922 y Staphylococcus aureus ATCC 25923. 2016.

31. Londoño JL, Químico Farmacéuti-Co P. Antioxidantes: importancia biológica y métodos para medir su actividad PARTE III / PART III.

32. Al-Snafi AE. IJPT THERAPEUTIC PROPERTIES OF MEDICINAL PLANTS: A REVIEW OF PLANTS WITH ANTIOXIDANT ACTIVITY (PART 1). / Int J Pharm Ther [Internet]. 2015 [cited 2020 Sep 3];6(3):159–82. Available from: www.ijptjournal.com

33. Katiyar S, Patidar D, Gupta S, Singh P. Some Indian Traditional Medicinal Plants With Antioxidant Activity: A Review [Internet]. Vol. 3297, International Journal of Innovative Research in Science, Engineering and Technology (An ISO. 2007 [cited 2020 Sep 3]. Available from: www.ijirset.com

34. Ana M. Mesa-Vanegas, Carlos A. Gaviria, Felipe Cardona, Jairo A. Sáez-Vega, Silvia Blair Trujillo, Benjamín A. Rojano. Actividad antioxidante y contenido de fenoles totales de algunas especies del género Calophyllum. Rev Cuba Plantas Med [Internet]. 2010 [cited 2020 Dec 5];15(2):1028–38. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1028-47962010000200003

35. B CC, Ramos E QL, Ibáñez L D V. Evaluación de la capacidad antioxidante de siete plantas medicinales peruanas.

36. Service RF. Antibiotics that resist resistance [Internet]. Vol. 270, Science. Science; 1995 [cited 2020 Aug 7]. p. 724–7. Available from: https://pubmed.ncbi.nlm.nih.gov/7481757/

37. Nicolás C, Pava R, Gabriel A, Sanabria Z, Consuelo L, Leal S. Actividad antimicrobiana de cuatro variedades de plantas frente a patógenos de importancia clínica en Colombia [Internet]. 2017 [cited 2020 Oct 9]. Available from: http://www.scielo.org.co/pdf/nova/v15n27/1794-2470-nova-15-27-00119.pdf

38. MSP. Plantas Medicinales de la Sierra. 2008.

39. Ansaloni R, Wilches I, León F, Orellana A, Peñaherrera E, Tobar V. Estudio Preliminar sobre Plantas Medicinales Utilizadas en Algunas Comunidades de las Provincias de Azuay, Cañar y Loja, para Afecciones del Aparato Gastrointestinal

[Internet]. 2010 [cited 2020 Oct 9]. Available from: https://www.researchgate.net/publication/267253360_Estudio_Preliminar_sobre_Plantas_Medicinales_Utilizadas_en_Algunas_Comunidades_de_las_Provincias_de_Azuay_Canar_y_Loja_para_Afecciones_del_Aparato_Gastrointestinal

40. Fabián Zambrano-Intriago L, Patricia Buenaño-Allauca M, Javier Mancera-Rodríguez N, Jiménez-Romero E. Estudio etnobotánico de plantas medicinales utilizadas por los habitantes del área rural de la Parroquia San Carlos, Quevedo, Ecuador Ethnobotanical study of medicinal plants used by rural inhabitants of the parish San Carlos Quevedo in Ecuador [Internet]. 2015 [cited 2020 Oct 9]. Available from: http://www.scielo.org.co/pdf/reus/v17n1/v17n1a09.pdf

41. Feitosa V, Dantas R, Wanderley Y, Wilney W. Actividad antimicrobiana de las plantas medicinales para su uso en el Sistema Único de Salud | Alves | Rev Cubana Estomatol [Internet]. 2019 [cited 2020 Oct 9]. Available from: http://www.revestomatologia.sld.cu/index.php/est/article/view/1159

42. Dellacassa E, Bandoni A. Cedrón, Aloysia citriodora Palau. Rev Fitoter [Internet]. 2003 [cited 2020 Aug 31];19–25. Available from: https://www.fitoterapia.net/php/descargar_documento.php?id=4744&doc_r=sn&num_volumen=8&secc_volumen=5953

43. Dodson CH. Aloysia citrodora Paláu [Internet]. [cited 2020 Sep 1]. Available from: http://legacy.tropicos.org/Image/100000673?projectid=2

44. Rojas Armas J, Palacios Agüero O, Ortiz Sánchez JM, López de la Peña L. Evaluación de la toxicidad del aceite esencial de Aloysia triphylla britton (cedrón) y de la actividad anti-Trypanosoma cruzi del citral, in vivo. An la Fac Med. 2015 Jul 10;76(2):129.

45. Santos-Gomes PC, Fernandes-Ferreira M, Vicente AMS. Composition of the essential oils from flowers and leaves of vervain [aloysia triphylla (L'Herit.) britton] grown in Portugal. J Essent Oil Res [Internet]. 2005 [cited 2020 Aug 31];17(1):73–8. Available from: https://www.tandfonline.com/doi/abs/10.1080/10412905.2005.9698835

46. CDC (Centros para el Control y la Prevención de enfermedades). SRAS | Información básica sobre el SRAS | CDC [Internet]. 2020 [cited 2020 Aug 8]. Available from: https://www.cdc.gov/sars/about/fs-sars-sp.html

47. Bellakhdar J, Idrissi A Il, Canigueral S, Iglesias J, Vila R. Composition of lemon verbena (Aloysia triphylla (L'Herit.) Britton) oil of Moroccan origin. J Essent Oil Res [Internet]. 1994 [cited 2020 Sep 3];6(5):523–6. Available from: https://www.tandfonline.com/doi/abs/10.1080/10412905.1994.9698440

48. Medicinales Aromáticas P. Boletín Latinoamericano y del Caribe de. Plantas Med y Aromáticas [Internet]. 2010 [cited 2020 Sep 3];9:29–37. Available from: http://www.redalyc.org/articulo.oa?id=85612108005

49. Politécnica Salesiana Ecuador Radice U. LA GRANJA. Revista de Ciencias de la Vida. [cited 2020 Sep 3]; Available from: http://www.redalyc.org/articulo.oa?id=476047390002

50. Loesener LET. Taxonomy of Ilex guayusa Loes [Internet]. [cited 2020 Sep 1]. Available from: https://www.tropicos.org/name/2000086

51. Loesener LET. Tropicos | Name - Ilex guayusa [Internet]. Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum 78: 310. 1901. [cited 2020 Sep 1]. Available from: https://www.tropicos.org/name/2000086

52. Alejandro V, Gallegos M, Fabara C. Composición y análisis químico de la especie Ilex guayusa Loes [Internet]. Quito, 2014; 2014 [cited 2020 Sep 3]. Available from: http://repositorio.usfq.edu.ec/handle/23000/3269

53. Gamboa F, Muñoz CC, Numpaque G, Sequeda-Castañeda LG, Gutierrez SJ, Tellez N. Antimicrobial activity of piper marginatum jacq and ilex guayusa loes on microorganisms associated with periodontal disease. Int J Microbiol. 2018;2018.

54. Rocha Santafé MR, Rocío M. Determinación del contenido de cafeína en un cultivo comercial de guayusa (ilex guayusa) [Internet]. Quito: UCE; 2018 [cited 2020 Sep 3]. Available from: http://www.dspace.uce.edu.ec/handle/25000/14259

55. Lewis WH, Kennelly EJ, Bass GN, Wedner HJ, Elvin-Lewis MP, W. DF. Ritualistic

use of the holly Ilex guayusa by Amazonian Jivaro Indians. J Ethnopharmacol [Internet]. 1991 May 1 [cited 2020 Sep 3];33(1–2):25–30. Available from: https://linkinghub.elsevier.com/retrieve/pii/0378874191901568

56. Patino VM. Guayusa, a neglected stimulant from the eastern andean foothills. Econ Bot [Internet]. 1968 Oct [cited 2020 Sep 3];22(4):311–6. Available from: https://link.springer.com/article/10.1007/BF02908125

57. Mosquera Bolaños JD. Estudio preliminar de diversidad genética de Ilex guayusa en la amazonía ecuatoriana mediante marcadores moleculares ISSR [Internet]. Quito, Ecuador: Quito, 2015.; 2015 [cited 2020 Sep 3]. Available from: http://repositorio.usfq.edu.ec/handle/23000/4555

58. Lopes MILE, Saffi J, Echeverrigaray S, Henriques JAP, Salvador M. Mutagenic and antioxidant activities of Croton lechleri sap in biological systems. J Ethnopharmacol [Internet]. 2004 Dec [cited 2020 Aug 31];95(2–3):437–45. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378874104004210

59. Tropicos | Reference - Renner, S. S., H. Balslev & L.B. Holm-Nielsen. 1990. Flowering plants of Amazonian Ecuador—A checklist. AAU Rep. 24: 1–241. [Internet]. [cited 2020 Sep 1]. Available from: http://legacy.tropicos.org/Reference/43828?projectid=2

60. Campos de la Cruz JR. Croton lechleri Müll. Arg. [Internet]. [cited 2020 Sep 1]. Available from: http://legacy.tropicos.org/Image/100000615?projectid=2

61. Borges JR, King SR. Croton lechleri: sustainable utilization of an Amazonian pioneer species. Med Plant Conserv. 2000;6:24–6.

62. Cai Y, Evans FJ, Roberts MF, Phillipson JD, Zenk MH, Gleba YY. Polyphenolic compounds from Croton lechleri. Phytochemistry. 1991 Jan 1;30(6):2033–40.

63. Rossi D, Guerrini A, Maietti S, Bruni R, Paganetto G, Poli F, et al. Chemical fingerprinting and bioactivity of Amazonian Ecuador Croton lechleri Müll. Arg. (Euphorbiaceae) stem bark essential oil: A new functional food ingredient? Food Chem. 2011 Jun 1;126(3):837–48.

64. Jones K. Review of Sangre de Drago (Croton lechleri) - A South American Tree Sap in the Treatment of Diarrhea, Inflammation, Insect Bites, Viral Infections, and Wounds: Traditional Uses to Clinical Research [Internet]. Vol. 9, Journal of Alternative and Complementary Medicine. Mary Ann Liebert Inc.; 2003 [cited 2020 Sep 3]. p. 877–96. Available from: https://www.liebertpub.com/doi/abs/10.1089/107555303771952235

65. Risco E, Ghia F, Vila R, Iglesias J, Álvarez E, Cañigueral S. Immunomodulatory Activity and Chemical Characterisation of Sangre de Drago (Dragon's Blood) from Croton lechleri. Planta Med [Internet]. 2003 Sep [cited 2020 Aug 31];69(9):785–94. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-2003-43208

66. Carrera. UNIVERSIDAD POLITÉCNICA SALESIANA SEDE QUITO.

67. Kuntze C (Karl) E (Eduard) O. Tropicos | Taxonomy- Clinopodium nubigenum (Kunth) Kuntze [Internet]. Revisio Generum Plantarum . 1891 [cited 2020 Sep 3]. p. 515. Available from: http://legacy.tropicos.org/Name/17605046?langid=66

68. Paco FN, Tatiana de LÁM, Edison AO, Pablo G, Andrea F. Clinopodium nubigenum (Kunth) Kuntze essential oil: Chemical composition, antioxidant activity, and antimicrobial test against respiratory pathogens. J Pharmacogn Phyther [Internet]. 2018 Sep 30 [cited 2020 Sep 3];10(9):149–57. Available from: http://www.academicjournals.org/JPP

69. Ruiz S, Malagón O, Zaragoza T, Valarezo E. Composition of the essential oils of artemisia sodiroi hieron., siparuna eggersii hieron., tagetes filifolia lag. and clinopodium nubigenum (kunth) kuntze from loja ecuador. J Essent Oil-Bearing Plants [Internet]. 2010 [cited 2020 Sep 3];13(6):676–91. Available from: https://www.tandfonline.com/doi/abs/10.1080/0972060X.2010.10643879

70. Aldemar A:, Salazar P, Segundo M, Chuga IM. UNIVERSIDAD TECNICA DEL NORTE FACULTAD DE CIENCIAS AGROPECUARIAS Y AMBIENTALES ESCUELA DE INGENIERIA AGROINDUSTRIAL TITULO: Obtención de aceites esenciales de Cedròn (Aloysia Triphylla), Sunfo (Clinopodium nubigenum (Kunth) Kuntze) y Hierba luisa (Cymbopogon Citratus), en un alambique tipo cachimbo por

Cohobaciòn TESIS PREVIA A LA OBTENCION DEL TITULO DE INGENIERO AGROINDUSTRIAL [Internet]. 2011 [cited 2020 Sep 3]. Available from: http://repositorio.utn.edu.ec/handle/123456789/556

71. Gilardoni G, Malagon O, Morocho V, Negri R, Tosi S, Guglielminetti M, et al. Phytochemical researches and antimicrobial activity of Clinopodium nubigenum Kunth (Kuntze) raw extracts. Brazilian J Pharmacogn [Internet]. 2011 [cited 2020 Sep 3];21(5):850–5. Available from: http://dx.doi.org/10.1590/S0102-

72. Fonseca E. Evaluación in vitro de la actividad antimicrobiana del aceite esencial de sunfo (CLINOPODIUM NUBIGENUM (KUNTH) KUNTZE) frente a patógenos de enfermedades respiratorias (STAPHYLOCOCCUS AUREUS ATCC: 25923, STREPTOCOCCUS PYOGENES ATCC: 19615, STREPTOCOCCUS PNEUMONIAE ATCC: 49619 Y STREPTOCOCCUS MUTANS ATCC: 25175. [Internet]. Quito; 2016 [cited 2020 Sep 2]. Available from: https://dspace.ups.edu.ec/bitstream/123456789/13227/1/UPS-QT10425.pdf

73. Paola D, Barrera A. ACTIVIDAD ANTIMICROBIANA DE PLANTAS SOBRE MICROORGANISMOS CARIOGÈNICOS DIANA PAOLA ARICAPA BARRERA PONTIFICIA UNIVERSIDAD JAVERIANA FACULTAD DE CIENCIAS BÁSICAS BACTERIOLOGÍA BOGOTÀ 2009 ACTIVIDAD ANTIMICROBIANA DE PLANTAS SOBRE MICROORGANISMOS CARIOGÈNICOS [Internet]. Pontificia Universidad Javeriana; 2009 [cited 2020 Sep 2]. Available from: http://repository.javeriana.edu.co/handle/10554/8357

74. Aliaga Mamani PA. Evaluación de la actividad antibacteriana in vitro del aceite esencial de hojas de Aloysia triphylla P. “Cedrón” frente a Escherichia coli ATTC 25922 y Staphylococcus aereus 25923 [Internet]. Universidad Nacional Jorge Basadre Grohmann. Universidad Nacional Jorge Basadre Grohmann; 2013 [cited 2020 Sep 2]. Available from: http://repositorio.unjbg.edu.pe/handle/UNJBG/2967

75. Tavares W. Problem gram-positive bacteria: Resistance in staphylococci, enterococci, and pneumococi to antimicrobial drugs [Internet]. Vol. 33, Revista da Sociedade Brasileira de Medicina Tropical. Sociedade Brasileira de Medicina

Tropical; 2000 [cited 2020 Sep 2]. p. 281–301. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0037-86822000000300008&lng=en&nrm=iso&tlng=pt

76. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian J Microbiol. 2004;35(4):275–80.

77. Kumar NK, Kumar KS, Raman B, Reddy I, Ramarao M, Rajagopal S. Antibacterial activity of Lippia citriodora a folklore plant [Internet]. Vol. Vol. 2, JJOURNAL OF PURE AND APPLIED MICROBIOLOGY,. 2008 [cited 2020 Oct 28]. p. 249–52. Available from: https://www.academia.edu/25982907/Antibacterial_activity_of_Lippia_citriodora_a_folklore_plant

78. Hashemi SMB, Mousavi Khaneghah A, Koubaa M, Barba FJ, Abedi E, Niakousari M, et al. Extraction of essential oil from Aloysia citriodora Palau leaves using continuous and pulsed ultrasound: Kinetics, antioxidant activity and antimicrobial properties. Process Biochem [Internet]. 2018 Feb 1 [cited 2020 Nov 5];65:197–204. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1359511317313478

79. Portela L. EVALUACIÓN DE LA ACTIVIDAD ANTIMICROBIANA in vitro DE EXTRACTOS VEGETALES DE Ilex guayusa e Ilex paraguariensis FRENTE a Helicobacter pylori. [Internet]. 1996 [cited 2020 Sep 2]. Available from: https://repository.javeriana.edu.co/bitstream/handle/10554/50076/Documento escrito TG Laura Portela- Repositorio .pdf?sequence=1&isAllowed=y

80. Farmacéutica B. UNIVERSIDAD TÉCNICA DE MACHALA UNIDAD ACADEMICA DE CIENCIAS QUÍMICAS Y DE LA SALUD CARRERA DE BIOQUÍMICA Y FARMACIA TRABAJO DE TITULACIÓN PREVIA LA OBTENCIÓN DEL TÍTULO DE.

81. Villacís-Chiriboga J, García-Ruiz A, Baenas N, Moreno DA, Meléndez-Martínez AJ, Stinco CM, et al. Changes in phytochemical composition, bioactivity and in vitro digestibility of guayusa leaves (Ilex guayusa Loes.) in different ripening stages. J Sci

Food Agric [Internet]. 2018 Mar 30 [cited 2020 Nov 9];98(5):1927–34. Available from: http://doi.wiley.com/10.1002/jsfa.8675

82. Guerra P. EVALUACIÓN DE LA ACTIVIDAD ANTIOXIDANTE BIOAUTOGRÁFICA DE DOS VARIEDADES DE ACEITES ESENCIALES ANDINOS Clinopodium nubigenun (Kunt) Kuntze y Baccharis latifolia. [Quito]: UNIVERSIDAD POLITÉCNICA SALESIANA SEDE QUITO ; 2016.

83. Gómez E. SENSIBILIDAD MICROBIANA Y PODER INSECTICIDA DE LOS ACEITES ESENCIALES DE Clinopodium nubigenum Y Ambrosia arborescens. [Internet]. Ibarra; 2017 [cited 2020 Sep 2]. Available from: http://repositorio.utn.edu.ec/bitstream/123456789/8457/1/03 BIOT 006 TRABAJO DE GRADO.pdf

84. Bedini S, Flamini G, Cosci F, Ascrizzi R, Echeverria MC, Gomez E V., et al. Toxicity and oviposition deterrence of essential oils of Clinopodium nubigenum and Lavandula angustifolia against the myiasis-inducing blowfly Lucilia sericata. Scott MJ, editor. PLoS One [Internet]. 2019 Feb 20 [cited 2020 Nov 9];14(2):e0212576. Available from: https://dx.plos.org/10.1371/journal.pone.0212576

85. Ruiz S, Malagón O, Zaragoza T, Valarezo E. Composition of the essential oils of artemisia sodiroi hieron., siparuna eggersii hieron., tagetes filifolia lag. and clinopodium nubigenum (kunth) kuntze from loja ecuador. J Essent Oil-Bearing Plants [Internet]. 2010 [cited 2020 Nov 11];13(6):676–91. Available from: https://www.tandfonline.com/doi/abs/10.1080/0972060X.2010.10643879

86. Elliott SN, Buret A, McKnight W, Miller MJS, Wallace JL. Bacteria rapidly colonize and modulate healing of gastric ulcers in rats. Am J Physiol - Gastrointest Liver Physiol [Internet]. 1998 [cited 2020 Sep 6];275(3 38-3). Available from: https://pubmed.ncbi.nlm.nih.gov/9724253/

87. Ortiz J, Capcha R, Palomino E, Aguilar J. Actividad antibacteriana de la Sangre de Grado (Croton lechleri) frente al Helicobacter pylori [Internet]. 2003 [cited 2020 Sep 6]. Available from: http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1018-

130X2003000200008

88. Condo A. "ESTUDIO IN VITRO DE LAS PROPIEDADES ANTIBACTERIANAS DEL CROTON LECHLERI (SANGRE DE DRAGO) COMO MEDICAMENTO ALTERNATIVO PREVENTIVO EN LA PROLIFERACIÓN DE BACTERIAS EXISTENTES EN CAVIDAD BUCAL DESPUÉS DE UNA EXTRACCIÓN DENTAL". [Internet]. Ambato ; 2014 [cited 2020 Sep 6]. Available from: http://dspace.uniandes.edu.ec/bitstream/123456789/2857/1/TUAODO014-2014.pdf

89. León K, Santiago J. Propiedades antimicrobianas de películas de quitosano-alcohol polivinílico embebidas en extracto de sangre de grado. Rev la Soc Química del Perú [Internet]. 2007 Sep [cited 2020 Nov 11];V. 73:180–6. Available from: http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1810-634X2007000300005

90. Lucia Corrales Ramírez, Adriana Castillo Castañeda, Andrea Melo Vargas. Evaluación del potencial antibacterial in vitro de Croton lechleri frente a aislamientos bacterianos de pacientes con úlceras cutáneas. Nova [Internet]. 2013 May 16 [cited 2020 Nov 11];Vol. 11:179–85. Available from: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1794-24702013000100006

91. Risco E, Vila R, Henriques A, Cañigueral S. Bases químicas y farmacológicas de la utilización de la sangre de drago. Rev Fitoter [Internet]. 2005 [cited 2020 Nov 29];Vol. 2(5):101–14. Available from: https://www.fitoterapia.net/php/descargar_documento.php?id=4673&doc_r=sn&num_volumen=13&secc_volumen=5955

92. Cortéz A, Perales T. Obtención y evaluación de las propiedades antifúngicas de los extractos vegetales de Equisetum hyemale, Aloysia triphylla y Anagallis arvensis en plagas de interés agrícola (Aspergillus flavus, Rizhopus spp, Mucor ssp, Fusarium ssp) [Internet]. 2014. 2014 [cited 2020 Sep 2]. Available from: https://repositorioinstitucional.buap.mx/handle/20.500.12371/6334?locale-attribute=en

93. de Boer J. Polychlorinated Biphenyls. In: Encyclopedia of Analytical Science: Second Edition. Elsevier Inc.; 2004. p. 214–25.

94. Guevara CB, Montaluisa L, Rodriguez MEM. The antimicrobial activity of alcoholic extracts of leaves and bark from two varieties of ”sangre de drago” compared with the antimicrobial activity present in the latex of the same species [version 1; peer review: 1 approved with reservations, 1 not approved]. F1000Research. 2018;7.

95. Rafael A. RICCO, Marcelo L. WAGNER, Erika PORTMANN, Claudia REIDES, Susana LLESUY, Alberto A. GURNI, et al. Análisis de polifenoles, actividad antioxidante y genotoxicidad en especies argentinas de Lippia y Aloysia (Verbenaceae). Bol Latinoam y del Caribe Plantas Med y Aromáticas [Internet]. 2010 [cited 2020 Dec 4];Vol. 5(9):388–96. Available from: http://www.redalyc.org/articulo.oa?id=85615225010

96. Aguado M, Nuñez M, Bela A, Okulik B, Bregni C. Caracterización fisicoquímica y actividad antioxidante de un extracto etanólico de Aloysia polystachya (Griseb.) Mold. (Verbenaceae) [Internet]. 2013 [cited 2020 Sep 6]. Available from: http://www.scielo.org.mx/scielo.php?pid=S1870-01952013000300006&script=sci_arttext

97. GARCÍA JOSÉ LUIS. EXTRACCIÓN DE ACEITE ESENCIAL DE CEDRON RECOLECTADA EN ALTATONGA Y EVALUACIÓN DE SU ACTIVIDAD ANTIOXIDANTE. [Puebla]: Benemérita Universidad Autónoma de Puebla ; 2018.

98. Guerra L. El presidente Lenín Moreno decreta Estado de Excepción para evitar la propagación del COVID-19 – Secretaría General de Comunicación de la Presidencia [Internet]. 2020 [cited 2020 Aug 5]. Available from: https://www.comunicacion.gob.ec/el-presidente-lenin-moreno-decreta-estado-de-excepcion-para-evitar-la-propagacion-del-covid-19/

99. NORIEGA F. FLAVONOIDES Y ACTIVIDAD ANTIOXIDANTE EN LA ESPECIE DE ILEX GUAYUSA . [QUITO]: UNIVERSIDAD POLITÉCNICA SALESIANA SEDE QUITO; 2017.

100. Tamayo Olalla EC. Estudio de la capacidad antioxidante y contenido de polifenoles

en la extracción etanólica del polvo de hojas de guayusa (Ilex guayusa Loes) deshidratada [Internet]. [Quito]: CIENCIAS DE LA INGENIERÍA E INDUSTRIAS FACULTAD:INGENIERÍA DE ALIMENTOS; 2017 [cited 2020 Dec 4]. Available from: http://repositorio.ute.edu.ec/xmlui/handle/123456789/16677