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TÍTULO: Phytochemical study and biological activities of Ecuadorian plants traditionally used in the treatment of skin diseases

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

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Dedicatoria

A mis sobrinas Rafa y Dominique

A mis sobrinos Juan David, Samuel y Daniel

Que sepan que todo lo que se propongan,

con esfuerzo y dedicación, lo podrán lograr.

Que no permitan que les digan que no pueden,

las capacidades y dones de cada uno son maravillosos.

Que se mantengan curiosos y aprendan cada día cosas nuevas.

Que descubran su pasión y le pongan el corazón a cada cosa que hacen.

Que sepan que siempre los apoyaré en cada etapa de su vida.

Camila Anabell Salvador Tinoco

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Camila Anabell Salvador Tinoco

Resumen

En Ecuador, un país megadiverso, cada año se descubren nuevas especies de plantas. Esto hace imposible estudiar muchas de ellas, en particular lo relacionado a sus metabolitos secundarios que pueden ser de interés médico terapéutico, como una alternativa a las drogas sintéticas. Esta tesis estudia la fitoquímica y la bioactividad de cuatro especies de plantas nativas del Ecuador en base a extractos metanólicos: *Juglans neotropica, Duranta triacantha, Mikania* sp. y *Brugmansia aurea*. Primero, se realizó un screening fitoquímico cualitativo para detectar fenoles, alcaloides y terpenos, así como sus derivados. Segundo, para la especie con mayor cantidad de resultados positivos en las pruebas cualitativas, se cuantificó los contenidos de flavonoides y fenoles. Además, se realizaron pruebas de bioactividad antibacteriana y antifúngica para las cuatro especies.

De las cuatro especies, *J. neotropica* fue la que tuvo mayor rendimiento de extracción y presentó mayor presencia de metabolitos secundarios, a excepción de glicósidos cardiacos y cumarinas. Respecto a la cuantificación de flavonoides y fenoles de esta especie, se midieron 244.8 mg QE/g extracto y 225.8 mg GAE/g extracto, respectivamente. Además, resultó resultó ser moderadamente activa en contra de cepas bacterianas de *Enterococcus faecalis, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* y *Pseudomonas aeroginosa*, como también en contra de la cepa fúngica *Candida albicans*. Por otra parte, *D. triacantha* dio positivo para la mayoría de metabolitos secundarios probados, aunque no se detectaron ni fenoles ni alcaloides. Esta especie también mostró actividad antibacterial y antifúngica, pero solo en contra de *Listeria monocytogenes* y *Aspergillus niger*, respectivamente. Los extractos metanólicos de *Mikania* sp. y *B. aurea* presentaron metabolitos secundarios en común, como fenoles, taninos y saponinas, pero solo *Mikania* sp. tuvo bioactividad antibacteriana en contra de *Staphylococcus aureus* y *Listeria monocytogenes* y bioactividad antifúngica contra *Aspergillus niger*.

En conclusión, todas las especies estudiadas podrían tener potenciales aplicaciones médicas. *J. neotropica*, por tener muchos metabolitos secundarios y gran actividad antibacteriana y antifúngica, podría ser utilizada para tratar enfermedades de piel. *D. triacantha* posee una interesante variedad de metabolitos secundarios, por lo que se pueden hacer más estudios al respecto, mientras que *Mikania* sp. y *B. aurea*, que presentaron pocos metabolitos secundarios, podrían ser objeto de desarrollo de hidrogeles por el mucílago que aparentemente presentan.

Palabras clave: fitoquímica, metabolitos secundarios, bioactividad, *Juglans neotropica*, *Duranta triacantha*, *Mikania* sp., *Brugmansia aurea*.

Abstract

In Ecuador, a megadiverse country, new plant species are discovered every year. This makes it impossible to study many of them, particularly in relation to their secondary metabolites that may be of medical therapeutic interest, as an alternative to synthetic drugs. This thesis studies the phytochemistry and bioactivity of four native plant species from Ecuador based on methanolic extracts: *Juglans neotropica*, *Duranta triacantha*, *Mikania* sp. and *Brugmansia aurea*. First, a qualitative phytochemical screening was performed to detect phenols, alkaloids and terpenes, as well as their derivatives. Second, for the species with the highest number of positive results in the qualitative tests, the flavonoid and phenol contents were quantified. In addition, antibacterial and antifungal bioactivity tests were performed for the four species.

Among the four species, *J. neotropica* had the highest extraction yield and the highest presence of secondary metabolites, with the exception of cardiac glycosides and coumarins. Regarding the quantification of flavonoids and phenols of this species, 244.8 mg QE/g extract and 225.8 mg GAE/g extract, respectively, were measured. In addition, it was found to present moderate bioactive against bacterial strains of *Enterococcus faecalis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*, as well as against the fungal strain Candida albicans. On the other hand, *D. triacantha* was positive for most secondary metabolites tested, although neither phenols nor alkaloids were detected. This species also showed antibacterial and antifungal activity, but only against *Listeria monocytogenes* and *Aspergillus niger*, respectively. The methanolic extracts of *Mikania* sp. and *B. aurea* had secondary metabolites in common, such as phenols, tannins and saponins, but only *Mikania* sp. had antibacterial bioactivity against *Staphylococcus aureus* and *Listeria monocytogenes* and antifungal bioactivity against *Aspergillus niger*.

In conclusion, all the species studied could have potential medical applications. *J. neotropica*, having many secondary metabolites and high antibacterial and antifungal activity, could be used to treat skin diseases. *D. triacantha* possesses an interesting variety of secondary metabolites, so more studies can be done on this area, while *Mikania* sp. and *B. aurea*, which presented only a few secondary metabolites, could be the object of hydrogel development because of the mucilage they apparently exhibit.

Keywords: phytochemistry, secondary metabolites, bioactivity, *Juglans neotropica*, *Duranta triacantha*, *Mikania* sp. and *Brugmansia aurea*.

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CHAPTER I: INTRODUCTION

1.1. Background

The skin is the largest organ in the body and has several vital functions, such as keeping body fluids inside, protecting against bacteria and viruses and, regulating body temperature[1]. Being in contact with external and internal environments, it is more susceptible to disease than any other organ; in fact, skin diseases are the fourth largest non-fatal socio-economic burden in the world[2], but they are not given the importance they need. Skin problems include a wide range of illnesses such as cancer, bacterial infections, viral infections, fungal infections, allergic reactions and parasites; some can be inherited while for others the reasons are still unknown[3,4].

The most commonly used treatments for skin conditions are synthetic drugs. For example, the acne treatment is based mainly on antibiotics such as benzoyl peroxide, tazarotene, adapalene, and isotretinoin, among others[5]. All synthetic drugs are known to have the potential to cause unwanted or adverse side effects[6]. To illustrate with an example, methotrexate, a standard medicine in the treatment of psoriasis, can cause life-threatening side effects; this medicine decreases the number of blood cells, which can lead to liver, stomach or lung damage. In addition, methotrexate can cause milder side effects, such as dizziness, drowsiness, headache, decreased appetite, and hair loss, among others[7]. These latter conditions are prevalent in most medications used for skin diseases or other ailments. The discovery of new treatments based on natural products is relevant, considering the great variety of plants that may have anti-inflammatory and antibacterial properties to treat skin diseases.

Secondary metabolites can be found in nature, often playing an antibiotic role in the organisms responsible for their production. Among the main functions of these molecules is the fact that they act as a weapon against fungi, amoebae, insects and bacteria, which is an important factor in the development of new drugs based on natural products[8]. They are classified into three general groups according to where their metabolic pathway comes from. These are phenolic compounds derived from acetate and shikimic acid, alkaloids derived from amino acids, and terpenic compounds from the derivation of mevalonic acid[9].

Phenolic compounds which embrace simple phenols and phenolic acids such as cinnamic and benzoic acid derivatives, are characterized by the benzene ring in their structure with at least one hydroxyl group. These are subclassified into simple phenolic compounds, lignans, chromones, stilbenes, quinones, coumarins, flavonoids, tannins and benzofurans[9–11]. In the

plant kingdom these molecules are involved in plant pigmentation, act as coating agents against UV light, and behave as antioxidants, among others. The UV/VIS spectrophotometric method is widely used in laboratory techniques for colorimetric reactions of this type, by measuring the total content of phenolic hydroxyl groups in plant extracts. The method of action of these quantifications is by means of a blue complex formed by the redox reaction that occurs between the extract and the redox reagent. The concentration of phenols and the alkaline solution are dependent variables in determining the maximum absorbance of the chromophores[10].

Alkaloids are nitrogenous compounds that are subclassified according to the derivation depending on the nature of their chemical synthesis (for example, tyrosine and phenylalanine, nicotinic acid, tryptophan and ornithine, and lysine[9]).

Terpenic compounds result from the polymerization that occurs between their dimethylallylpyrophosphate isomer and their isoprene units. These are subclassified into iridoids, monoterpenes, diterpenes, triterpenes, tetraterpenes, and sesquiterpenes[9].

The main components in natural products are flavonoids and phenolic acids, which are currently of great interest due to their great capacity to eliminate free radicals[12]. Because each plant species has a very characteristic chemical composition with uniform phenol groups present in all individuals, it is expected that similar chemical structures provoke similar chemical interactions[10].

This project aims to evaluate extracts of four native Ecuadorian plant species through phytochemical studies and bioactivity assessments. These species are traditionally used to treat skin infections in some Ecuadorian cultures. This work may be useful for future applications of these extracts in some medical forms that might help obtain less invasive treatments for skin diseases.

1.2. Problem Statement

Ecuador is one of the countries with the greatest biodiversity globally; in fact, every year new species are discovered throughout the country[13]. This scenery, and the lack of resources for research in the country, means that there are many plants without scientific studies that validate the traditional medical knowledge accumulated over thousands of years[13]. Fortunately, at present, Ecuador has many scientists who have awakened the interest to assess the chemical and biological properties of native plants in order to explore medical applications with potential benefits to the society.

Plants are a potential source for creating pharmaceuticals based on natural products because of their biological properties[13]. Hopefully, this research will help to define potential treatments of certain skin diseases that affect millions of people worldwide. In this thesis, phytochemical evaluations of four native plant species from Ecuador are carried out, including qualitative and quantitative determinations of certain secondary metabolites, and the evaluation of antibacterial and antifungal biological activity of the different extracts.

1.3. Objectives

General Objective

Evaluate the phytochemical composition and bioactivity of four focal native plant species known to be traditionally used to treat skin diseases.

Specific Objectives

- Conduct a methanolic extraction of each selected species.
- Assess the composition of secondary metabolites of the extracts via qualitative phytochemistry experiments.
- Quantify the content of flavonoids and phenols from the plant species with the strongest presence of secondary metabolites.
- Analyze the biological activity of the extracts against bacterial and fungal strains.

CHAPTER II: MATERIALS AND METHODS

2.1. Identification of Potential Taxa for Skin Diseases Treatment

An exhaustive investigation was carried out using the "Enciclopedia de Plantas Útiles del Ecuador", wrote by De la Torre et al. (2008)[14]. It presents traditional cures or treatments for certain diseases and ailments accumulated throughout the years by humans (ancestral knowledge). This book reports 82 types of skin diseases cure by many different plant species. Table 1 summarizes the number of species per family thereby reported.

Table 1: Family and number of species traditionally used for skin diseases treatments.

Family	# spp.	Family	# spp.	Family	# spp.	Family	# spp.
Asteraceae	67	Brassicaceae	7	Betulaceae	3	Combretaceae	1
Fabaceae	53	Plantaginaceae	7	Lauraceae	3	Coriariaceae	1
Rubiaceae	49	Polygalaceae	7	Phytolaccaceae	3	Ebenaceae	1
Gesneriaceae	43	Polygonaceae	7	Rutaceae	3	Iridaceae	1
Solanaceae	39	Clusiaceae	6	Tropaeolaceae	3	Marcgraviaceae	1
Euphorbiaceae	39	Cactaceae	6	Valerianaceae	3	Myricaceae	1
Araceae	34	Clusiaceae	6	Achariaceae	2	Ochnaceae	1
Piperaceae	34	Loranthaceae	6	Agavaceae	2	Olacaceae	1
Amaranthaceae	23	Amaryllidaceae	5	Alismataceae	2	Oleaceae	1
Malvaceae	23	Anacardiaceae	5	Alstroemeriaceae	2	Quinaceae	1
Melastomataceae	20	Ericaceae	5	Asphodelaceae	2	Batidaceae	1
Urticaceae	19	Cleomaceae	5	Calceolariaceae	2	Bixaceae	1
Apocynaceae	17	Onagraceae	5	Cannabaceae	2	Bombacaceae	1
Myristicaceae	17	Begoniaceae	5	Capparaceae	2	Dipsacaceae	1
Lamiaceae	15	Lecythidaceae	5	Crassulaceae	2	Haemodoraceae	1
Commelinaceae	14	Zingiberaceae	5	Cyperaceae	2	Heliconiaceae	1
Acanthaceae	13	Meliaceae	4	Erythroxylaceae	2	Hippocrateaceae	1
Poaceae	13	Myrtaceae	4	Menispermaceae	2	Hydrangeaceae	1
Rosaceae	13	Sapotaceae	4	Musaceae	2	Hydrophylaceae	1
Moraceae	12	Campanulaceae	4	Schlegeliaceae	2	Juglandaceae	1
Cucurbitaceae	11	Costaceae	4	Berberidaceae	2	Linaceae	1
Arecaceae	10	Cyclanthaceae	4	Dichapetalaceae	2	Linderniaceae	1
Boraginaceae	10	Myrsinaceae	4	Dioscoreaceae	2	Loasaceae	1
Apiaceae	8	Oxalidaceae	4	Lythraceae	2	Loganiaceae	1
Orchidaceae	8	Salicaceae	4	Phyllanthaceae	2	Nyctaginaceae	1
Marantaceae	8	Sterculiaceae	4	Picramniaceae	2	Picrodendraceae	1
Verbenaceae	8	Bromeliaceae	4	Theophrastaceae	2	Plumbaginaceae	1
Passifloraceae	8	Burseraceae	4	Alliaceae	1	Pontederiaceae	1
Violaceae	8	Papaveraceae	4	Araliaceae	1	Portulacaceae	1
Sapindaceae	7	Annonaceae	3	Aristolochiaceae	1	Primulaceae	1
Gentianaceae	7	Caricaceae	3	Cannaceae	1	Proteaceae	1
Geraniaceae	7	Convolvulaceae	3	Caryocaraceae	1	Ranunculaceae	1
Scrophulariaceae	7	Monimiaceae	3	Caryophyllaceae	1	Rhamnaceae	1
Bignoniaceae	7	Smilacaceae	3	Chloranthaceae	1	Tiliaceae	1

2.1.1. Species and Individuals Selected for Phytochemistry and Bioactivity Studies

Different but complementary criteria were used to select the four species focused in this study. Fist, the species had to present many records of traditional treatments or cures for skin diseases, according to De la Torre et al. (2008)[14]. Second, the number of scientific

phytochemistry studies reporting these species had to be low or nonexistent. Third, the natural distribution of the species selected had to occur in the province of Imbabura. Applying these criteria, four species were finally selected: *Juglans neotropica, Duranta triacantha, Mikania* sp. and *Brugmansia aurea*.

Juglans neotropica Diels is a native and cultivated species of Ecuador commonly called "Nogal" or "Tocte" in Spanish. Traditionally, it has been used for food, beekeeping, as raw material, and social and medicinal purposes. The latter is of interest in the present research because this species has been used ancestrally to treat skin diseases such as gout, pimples, herpes, sores, rashes, and syphilis, and also as a healing agent[14]. The Juglandaceae family to which this species belongs comprises about 10 genera and 50 species. Trees belonging to this family are deciduous, monoecious, and resinous. The terminal buds are larger than the lateral buds. The aromatic leaves are alternate with serrated margins. This family has pistillate unisexual flowers, with fruits as nuts. The seeds do not contain endosperm, but have fleshy and oily cotyledons. This family is found in greater proportion in Central America, along the Andes, and in the temperate zone of Asia. The trees are of great economic importance as they are mostly fruit and timber producers[15–17]. For this study, a representative individual of this species was randomly selected and its shoots (stems and leaves) were collected at Yachay Tech University campus, at coordinates -78.1795890 (Longitude), 0.4105411 (Latitude), on October 14$^{th.}$, 2021.

Duranta triacantha Juss. is a small shrub or tree with three long thorns with sympodial branching. This species is native to Ecuador and is commonly called "Espino Chivo" in Spanish or "Mote Kasha" in Spanish-Kichwa. Traditionally, it has been used for food, raw material, and environmental and medicinal purposes. The latter is vital in this research because this species has been used ancestrally to treat skin diseases such as dermatomycosis, spots and skin burns[14]. The Verbenaceae family to which this species belongs comprises around 91 genera and 2000 species. In this family, we can find trees and climbing bushes whose leaves can be opposite without stipules and trifoliate. The inflorescences can be racemose, cymose, and terminal. One of the characteristics of their flowers is that they are bisexual or polygamous and not very often can be actinomorphic. Fertile stamens can be found inserted in the corolla. The fruits are small indehiscent capsules. Some genera are of economic importance because they are suppliers of gum, oil, tannins, and wood; while others have medicinal use[18–20]. For this study, a representative individual of this species was randomly selected and its shoots (leaves, stems and nascent flowers) were collected at Chachimbiro area, with coordinates -78.2189642 (Longitude), 0.4601753(Latitude), on November 16th, 2021.

Regarding *Mikania*, the goal was to find *Mikania micrantha* Kunth, commonly named "Matico Silvestre" in Spanish, which has been traditionally used in the medicinal field because of its healing capacity for skin wounds, healing of sores, and infection control[14]. However, this species was not located in the field and thus another unidentified native species of *Mikania* was studied (hereby refered as *Mikania* sp.). The Asteraceae family to which this species belongs is of great importance due to its biological and morphological diversity, as it groups more than 30,000 species. Its morphology includes herbs ranging from 1 cm in height to trees that can reach 30 m in height. Its flowers can present diverse forms, colors, and arrangements. The leaves can be alternate, spiral and rarely verticillate, lacking stipules. On the other hand, its flowers can be hermaphrodite, unisexual, or sterile, and its fruits are cypselae. The importance of this family lies in the fact that some species may contain latex and essential oils. Moreover, some phytochemical assays have revealed that this family contains oligosaccharides, polyacetylenes, aromatic oils, terpenoids, and lactones[21,22]. For this study, representative individuals of this species were randomly selected and their shoots (leaves and young unsuberized stems) were collected at "El Gallinazo" creek at the Yachay Botanical Garden (YBG), with coordinates -78.1821173 (Longitude), 0.4224937 (Latitude), on December 03rd, 2021.

Brugmansia aurea Lagerh. is native and cultivated in Ecuador, and is commonly called "Floripondio Amarillo". Traditionally, it is used for environmental and ritual purposes, beekeeping, and as a source for natural medicine. It has been reported to cure rashes and control inflammations[14].The Solanaceae family to which this species belongs comprises about 96 genera and 2300 species. Most species in this family can be shrubs or perennial herbs, with stems showing heteroblastic growth, and often spines. Leaves are alternate and without stipules. The flowers are mostly hermaphrodite and pollination is entomophilous. The fruits can be berries or capsules, although drupes are also found. Some species can have alkaloids which makes them pharmaceutically interesting because they can contain scopolamine, atropine, nicotine, and hyoscyamine. This family has a wide geographical distribution, with many species found in Central and South America. In addition to the medicinal interest, many species also have value for nutrition[23–25]. For this study, a representative individual of this species was randomly selected and its shoots (leaves, stems and flowers) were collected at a house garden in Urcuquí town, with coordinates -78.1957379 (Longitude), 0.4179304 (Latitude), on December 03rd, 2021.

2.2. Laboratory Procedures

2.2.1. Equipment

Zuzi Spectrophotometer Model 4211/50, Ultrasons H-D Selecta Model E4211050, Hot & Stirrer MS300HS, Rotavapor Butchi R-210 w/ Heating Bath Butchi B4-91, Vacuum Pomp Butchi V-300, Vitrina de Gases FL6000, Estufa POL-EKO SLW115 STD, Balanza Gramera COBOS TX3202L, Balanza COBOS HR-150A, Metter Toledo GmbH MS105DU.

2.2.2. Samples Collection and Preparation

Juglans neotropica Diels (Juglandaceae)

The fresh sample of *J. neotropica* weighed 1396.96 g and it was dried for three days at the plant dryer located at YBG. The sample dry weight was 470.4 g, from which 50.13 g were weighed to be used during the laboratory procedures.

Duranta triacantha Juss. (Verbenaceae)

The sample of *D. triacantha* was dried for one day at the plant dryer at YBG and three days at 40°C in an oven of the Chemistry Laboratory at Yachay Tech University. The sample dry weight was 93,73 g, from which 50,01 g were weighed to be used during the laboratory procedures.

Mikania sp. (Asteraceae)

The fresh sample of *Mikania* sp. weighed 208,20 g and it was dried for four days at 45°C in an oven of the Chemistry Laboratory at Yachay Tech University. The sample dry weight was 45,18 g, from which 44,97 g were weighed for use during the laboratory procedures.

Brugmansia aurea Lagerh. (Solanaceae)

The fresh sample of *B. aurea* weighed 406.21 g and it was dried for four days at 45°C in an oven of the Chemistry Laboratory at Yachay Tech University. The sample dry weight was 50,27 g, from which 50,03 g were weighed for use during the laboratory procedures.

2.2.3. Extraction

For each species, each plant sample of ca. 50 g was first placed in an Erlenmeyer flask, to which ca. 500 ml of methanol were added. The mixture was macerated on a magnetic stirrer for 24 hours. After that time period, the first extraction was collected by gravity filtering and stored in a glass bottle. The residual sample of the plant was put back in the Erlenmeyer flask

with more methanol added, always keeping the solvent level above the sample. After 24 hours of maceration, the gravity filtering procedure was repeated using the same glass bottle. This procedure was repeated one more time. Repetitive maceration and subsequent filtering for three days assured that most metabolites from the sample were successfully extracted. The filtered mixture (extract in methanol) was placed in a 1000 ml round bottom flask in order to evaporate the solvent (methanol) using a rotary evaporator configured at 38°C with a rotation speed of 5 rpm. The sample was allowed to rotate for a few minutes until most of the solvent evaporated. The remaining mixture was transferred to a 100 ml round bottom flask previously weighed in order to evaporate the residual solvent for approximately 4 hours, using the rotary evaporator. The final dry extract was transferred to a glass vial, labeled and stored in a refrigerator for later use.

2.2.4. Qualitative Phytochemical Screening

The following phytochemical tests were carried out based on protocols described by Shaikh et al. (2020)[26], Friedman (2004)[27], Simoni et al. (2002)[28], and Benedict (1909)[29].

Detection of Flavonoids

Shinoda's Test (Mg-hydrochloride reduction): In 5 ml of each methanolic extract, a few fragments of magnesium ribbon were added and 0,5 ml of concentrated hydrochloric acid. If the solutions turn into a pink to crimson color, the presence of Flavonal Glycosides will be confirmed.

Concentrated H_2SO_4 Test: In 1 ml of each methanolic extract were added 0,5 ml of concentrated sulfuric acid. If the solutions turn into an orange color, the presence of Flavonoids will be confirmed.

Alkaline reagent Test 1: In 1ml of each methanolic extract were added 1ml of 10% ammonium hydroxide solution. If the solutions turn yellow with fluorescence, the presence of Flavonoids will be confirmed.

Ammonia Test: In 2ml of each methanolic extract were added 5ml of diluted ammonia solution and 3 drops of concentrated sulfuric acid. If the solutions turn into a yellow color, the presence of Flavonoids will be confirmed.

Shibata's reaction Test: In 2ml of each methanolic extract were added metal magnesium and 6 drops of concentrated hydrochloric acid. If the solutions turn into a red color, the presence of Flavonols will be confirmed. On the other hand, if solutions turn into an orange color, it will

be indicative of the presence of Flavones.

Alkaline reagent Test 2: In 1ml of each methanolic extract were added 2ml of 10% NaOH solution and 5 drops of diluted hydrochloric acid. If the solutions turn into an intense yellow color at the first step and turn colorless at the second step, the presence of Flavonoids will be confirmed.

Detection of Simple Phenolic Compounds

Iodine Test: In 1ml of each methanolic extract were added 3 drops of diluted iodine solution. If the solutions turn into a transient red color, the presence of Phenols will be confirmed.

Ferric Chloride Test: In 1ml of each aqueous solutions of methanolic extracts were added 4 drops of 5% ferric chloride solution. If the solutions turn into a bluish black or dark green color, the presence of Phenols will be confirmed.

Cartenoids Test: Each dry extract was added 10ml of chloroform, shaken, and carefully concentrated sulfuric acid was placed. If the solutions turn into a blue color at the interference, the presence of Cartenoids will be confirmed.

Detection of Alkaloids

Mayer's Test: In 1ml of each methanolic extract were added 2 drops of Mayer's reagent. If the solutions turn into a creamy yellow or white precipitate, the presence of Alkaloids will be confirmed.

Wagner's Test: In 1ml of each methanolic extract were added 2 drops of Wagner's reagent carefully placed along the sides of a test tube. If the solutions turn into a precipitate of reddish or brown color, the presence of Alkaloids will be confirmed.

Dragendroff's Test: In 1 ml of each methanolic extract were added 1ml of Dragendroff's reagent. If the solutions turn into a reddish-brown precipitate, the presence of Alkaloids will be confirmed.

Iodine Test: In 3ml of each methanolic extract were added 3 drops of iodine solution, then heated. If the solutions turn into a blue color at the first step and turn colorless at the second step, the presence of Alkaloids will be confirmed.

Detection of Carbohydrates

Molish's Test: In 2ml of each methanolic extract were added 2 drops of alcoholic 1-

naphtol, then carefully along the sides of test tubes were added 1ml of sulfuric acid. If the solutions show a violet ring, the presence of Carbohydrates will be confirmed.

Resorcinol Test: In 2ml of each aqueous solution of methanolic extracts, were added a few crystals of resorcinol and 2ml of hydrochloric acid, then heated. If the solutions turn into a rose color, the presence of ketones will be confirmed.

Detection of Glycosides

Borntrager's Test: In 2ml of each methanolic extract were added 3ml of chloroform, stirred until two layers were obtained and then 1ml of 10% ammonia solution were added. If the extract layer turns into a pink solution, the presence of Glycosides will be confirmed.

Modified Borntrager's Test: In 1ml of each methanolic extract were added 1ml of 5% ferric chloride solution and 1ml of hydrochloric acid, then the solutions were boiled into a water bath for 5 minutes and allowed to cool. 2ml of benzene were added, shaken, and allowed to stand. Then this benzene layer was removed. Finally, 1ml of ammonia solution were added. If the solutions turn into a blood red or rose-pink color, the presence of Glycosides will be confirmed.

Aqueous NaOH Test: In each dry extract were added 1ml of water and 5 drops of aqueous NaOH solution. If the solutions turn into a yellow color, the presence of Glycosides will be confirmed.

Concentrate H_2SO_4 Test: In 5ml of each methanolic extract were added 2ml of glacial acetic acid and 2 drops of 5% ferric chloride solution, then 3 drops of concentrated sulfuric acid were gently added. If the solutions show a brown ring, the presence of Glycosides will be confirmed.

Detection of Cardiac Glycosides

Keller-Killiani Test: In 1ml of each methanolic extract were added 1.5ml of glacial acetic acid and 1 drop of 5% ferric chloride solution. Along the side test tubes were placed 3 drops of concentrated sulfuric acid. If the acetic acid layer of solutions turns into a blue color, the presence of Cardiac Glycosides will be confirmed.

Detection of Proteins and Amino Acids

Ninhydrin Test: In a beaker were placed 2g of ninhydrin in 100ml of acetone in order to prepare the ninhydrin solution. In 2ml of each methanolic extract were added 2 drops of ninhydrin solution. If the solutions turn into a purple color, the presence of Amino Acids will

be confirmed.

Xanthoproteic Test: In 1ml of each methanolic extract were added 4 drops of concentrated nitric acid. If the solutions turn into a yellow color, the presence of Proteins and Amino Acids will be confirmed.

Detection of Tannins

Braymer's Test: In 1ml of each methanolic extract were added 3ml of distilled water and 3 drops of 10% ferric chloride solution. If the solutions turn into a green or blue color, the presence of Tannins will be confirmed.

10% NaOH Test: In 1ml of each methanolic extract were added 4ml of 10% sodium hydroxide solution and shaken well. If the solutions form an emulsion, the presence of Hydrolysable Tannins will be confirmed.

Detection of Saponins

$NaHCO_3$ Test: In 1ml of each methanolic extract were added 0,5ml of sodium bicarbonate solution and 0,5ml of distilled water and shaken vigorously. If the solutions form a stable honeycomb like froth, the presence of Saponins will be confirmed.

Foam Test 1: In each dry extract were added 2ml of distilled water and shaken vigorously. If the solutions form a persistent foam for 10 minutes, the presence of Saponins will be confirmed.

Foam Test 2: In each dry extract were added 5ml of distilled water, shaken vigorously and heated to boiling. If the solutions create an appearance of creamy miss of small bubbles, the presence of Saponins will be confirmed.

Detection of Phytosterols

Salkowki's Test: In 1ml of each methanolic extract were added 0,5ml of concentrated sulfuric acid, shaken well and allowed to stand. If the solutions turn into a red color at lower layer, the presence of Phytosterols will be confirmed.

Detection of Coumarins

NaOH Test: In 1ml of each methanolic extract were added 0,5ml of 10% sodium hydroxide solution and 0,5ml of chloroform. If the solutions turn into a yellow color, the presence of Coumarins will be confirmed.

2.2.5. Quantitative Phytochemical Screening

Quantitative phytochemical screening was only conducted for methanolic extracts of *Juglans neotropica*, based on protocols described in Sepahpour et a. (2018)[12].

Quantification of Total Flavonoid Content (TFC)

Three reagent solutions were prepared with 50ml of distilled water each one: 5% NaNO2 solution (2.5g), 10% AlCl3 solution (5.10g), and 4% NaOH solution (2g). Solutions were stored in capped amber bottles and kept at room temperature.

To prepare the Quercetin stock solution, weigh 10mg of it and dissolve it in 5ml of 96% EtOH, obtaining a concentration of 2mg/ml. With this, the points of the standard curve were prepared at different concentrations in mg/ml: 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10. These solutions were stored at -20°C protected from light.

Since the *J. neotropica* extract is not soluble in water, it is dissolved in methanol at a concentration of 1mg/ml and then diluted to obtain a concentration of 0.4mg/ml, which is ideal because it fits perfectly into the standard curve.

The total flavonoid content protocol is followed with the 3 previously mentioned points ready. To each previously labeled test tube, add 1ml of distilled water and 250µl of the extract or quercetin, respectively (to the test tube corresponding to the blank, 250µl are distilled water). 75µl of NaNO2 at 5% are added and it is shaken and then left to incubate for 5 minutes in the dark. Then 150 µl of 10% AlCL3 are added, shaken and left to incubate for 6 minutes in the dark. After that time, 500µl of 4% NaOH is added and it is stirred, then 3025µl of distilled water is added and it is stirred again to incubate for 15 minutes in the dark. In the end, the absorbance of each sample at 415nm is read in the spectrophotometer.

The calibration curve was developed by triplication, obtaining excellent reproducibility. Different extract concentrations were tested until finding the appropriate concentration within the curve. The determination of the absorbance of the samples was carried out nine times to obtain the minimum error.

Quantification of Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used for phenol counting. To prepare the calibration curve, Gallic Acid stock solutions are prepared from a concentration of 10 mg/ml. With this, the points of the standard curve were prepared at different concentrations in mg/ml: 0.02, 0.04, 0.06, 0.08, 0.10 and 0.12. These solutions were stored at -20°C protected from light.

The Folin-Ciocalteu reagent is diluted in a 1:10 (v/v) ratio with distilled water. The extract of *J. neotropica* is prepared at 0.4mg/ml concentration in methanol. A 7.5% (w/v) sodium carbonate solution was prepared for the experiment.

Having the previously mentioned points ready, continue with the total phenol content protocol. To each previously labeled test tube, add 300µl of the extract or gallic acid (for calibration curve), respectively (to the test tube corresponding to the blank, the 300µl are distilled water). 1500µl of the Folin-Ciocalteu solution are added and it is stirred and then left to incubate for 5 minutes in a dark place at room temperature. Then 1200 µl of the sodium carbonate solution are added, shaken and left to incubate for 30 minutes in the dark. After that time, the absorbance of each sample at 765nm is read in the spectrophotometer.

The calibration curve was developed by triplication, obtaining excellent reproducibility. Different extract concentrations were tested until finding the appropriate concentration within the curve. The determination of the absorbance of the samples was carried out nine times to obtain the minimum error.

2.2.6. Biological Activity

Assessment of Antibacterial and Antifungal Bioactivity

The antibacterial activity of the extracts against Gram-positive bacteria was evaluated following protocols by Valarezo et al. (2022)[30]: *Enterococcus faecalis* ATCC ® 19433, *Enterococcus faecium* ATCC ® 27270, *Staphylococcus aureus* ATCC ® 25923 y *Listeria monocytogenes* ATCC ® 19115 as well as in Gram-negative bacteria: *Escherichia coli* (O157:H7) ATCC ® 43888, *Pseudomonas aeruginosa* ATCC ® 10145, *Salmonella enterica* subs *enterica* serovar *Typhimurium* WDCM 00031, derived ATCC ® 14028. The antifungal activity was evaluated against the following yeast and sporulated fungi: *Candida albicans* ATTC ® 10231 and *Aspergillus niger* ATCC ® 6275. These analyses were conducted with the collaboration of Universidad Técnica Particular de Loja.

For this, the microdilution broth method was used. The incubation of the bacterial strains was carried out in a Müeller-Hinton (MH) broth. For the positive control Ampicilin (1 g/ml), Ciprofloxacin (1 mg/ml) and Amphotericin B (250 µg/ml) were used; while for negative control, Dimethylsulfoxide (DMSO 5%) were used. Strains were placed in fluid thioglycolate medium supplemented with 5% horse serum for reactivation. A portion of a cryogenic stock was resuspended in thioglycolate and incubated in a microaerophilic atmosphere at 37°C for 48h. 40mg of each extract was separately dissolved in 1ml of DMSO. The medium used for the

assay was in a Müeller-Hinton (MH) broth at pH 7.3 with 5% lysed horse blood, where double serial dilutions of decreasing concentrations of each extract from 2000 to 500 MCI in µg/ml were placed. The inoculum was prepared from the thioglycate culture by adjusting it to 0.5 McFarland. In an atmosphere of 5% CO_2 of an Oxoid CampyGen (microaerophilic), the microplate was incubated at 37°C for 48h. With the same range of concentrations of the extracts, a blank was prepared to carry out measurements at 405nm to rule out TTZ reduction due to contamination.

CHAPTER III: RESULTS AND DISCUSSION

First, the yield of the extractions will be calculated. Then, a phytochemical screening of all extracts will be performed, followed by a quantitative analysis (total flavonoid and phenol content) of the most promising plant. Finally, an analysis of the antibacterial and antifungal activity of all extracts will be carried out.

3.1. Extraction

Table 2 shows the yields obtained for each plant by the methanol maceration method. It can be seen that *J. neotropica* gave the highest yield of the four extractions with 20.39% followed by *D. triacantha* with 17.95%. While *Mikania* sp. was the lowest yielding with 10.62%, together with *B. aurea* with 14.02%. The polarity of the solvent causes the extraction yield of the plants to vary, however in this study the same solvent (methanol) was used for the four plants, so the difference in extraction yields between the species probably reflects differences in bioactive compounds or their concentrations[31]. In other words, the variation in the extraction yields of the four plants studied in this work is related to quantitative and/or qualitative differences in the components[32].

Table 2: Yields obtained from methanolic plant extracts

Sample	Plant (g)	Extract (g)	Yield (%)
Juglans neotropica	50.13	10.22	20.39
Duranta triacantha	50.01	8.98	17.95
Mikania sp.	45.18	4.80	10.62
Brugmansia aurea	50.27	7.05	14.02

Unlike the first two, a dry powder extract was not obtained from the latter two, but rather a sticky mass which is why it can be said that these two species could contain mucilage, which by definition is a substance or soluble fiber of viscous texture from plants that are coagulable to alcohol[33]. Several applications have been reported for mucilage as a natural emulsifier, thickener, or an alternative for synthetic additives and polymers since they present hydrogen bonds between different functional and polar groups. One application that could be given to *Mikania* sp. and *Brugmansia aurea* extracts is the development of mucilage-based hydrogels for healing skin wounds. They are biocompatible, non-toxic, and biodegradable. This substance is a polysaccharide polymeric complex composed mainly of carbohydrates and glycoproteins. Tannins, alkaloids, and steroids are also reported[34].

Table 3: Qualitative phytochemical screening in extracts of *Juglans neotropica*, *Duranta triacantha*, *Mikania* sp. and *Brugmansia aurea*.

SECONDARY METABOLITES	TEST	EXTRACT	RESULT
FLAVONOIDS	Shinoda's	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Conc. H_2SO_4	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Alkaline reagent	*J. neotropica*	-
		D. triacantha	+
		Mikania sp.	-
		B. aurea	-
	Ammonia	*J. neotropica*	-
		D. triacantha	+
		Mikania sp.	-
		B. aurea	-
	Shibata's reaction	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Alkaline reagent	*J. neotropica*	-
		D. triacantha	+
		Mikania sp.	-
		B. aurea	+
ALKALOIDS	Mayer's	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Wagner's	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Dragendroff's	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Iodine	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
SAPONINS	$NaHCO_3$	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	+
	Foam	*J. neotropica*	+
		D. triacantha	+
		Mikania sp.	+
		B. aurea	+
	Foam	*J. neotropica*	+
		D. triacantha	+
		Mikania sp.	+
		B. aurea	-
TANNINS	Braymer	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	10% NaOH	*J. neotropica*	+
		D. triacantha	+
		Mikania sp.	+
		B. aurea	+

SECONDARY METABOLITES	TEST	EXTRACT	RESULT
SIMPLE PHENOLIC COMPOUNDS	Iodine	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Ferric Chloride	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Carotenoids	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	+
		B. aurea	+
CARBOHYDRATES	Molish's	*J. neotropica*	+
		D. triacantha	+
		Mikania sp.	+
		B. aurea	-
	Resorcinol	*J. neotropica*	+
		D. triacantha	+
		Mikania sp.	+
		B. aurea	-
PROTEINS AND AMINOACIDS	Nihydrin	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Xanthoproteic	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
GLYCOSIDES	Borntrager's	*J. neotropica*	+
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Modified Borntrager's	*J. neotropica*	-
		D. triacantha	-
		Mikania sp.	-
		B. aurea	-
	Aqueous NaOH	*J. neotropica*	-
		D. triacantha	+
		Mikania sp.	-
		B. aurea	-
	Conc. H_2SO_4	*J. neotropica*	+
		D. triacantha	+
		Mikania sp.	+
		B. aurea	-
CARDIAC GLYCOSIDES	Keller-Killani	*J. neotropica*	-
		D. triacantha	+
		Mikania sp.	-
		B. aurea	-
COUMARINS	NaOH	*J. neotropica*	-
		D. triacantha	+
		Mikania sp.	-
		B. aurea	-
PHYTOSTEROLS	Salkowski	*J. neotropica*	+
		D. triacantha	+
		Mikania sp.	-
		B. aurea	-

3.2. Qualitative Phytochemical Screening

Table 3 shows the results of each test for each secondary metabolite and methanolic extract. Table 4 summarizes the secondary metabolites that tested positive to one or more of the colorimetric tests applied to each extract; as well as the negative results of these tests.

Table 4: Summary of secondary metabolites (based on Table 3) detected in extracts of *Juglans neotropica*, *Duranta triacantha*, *Mikania* sp. and *Brugmansia aurea*.

		EXTRACT			
		J. neotropica	***D. triacantha***	***Mikania* sp.**	***B. aurea***
SECONDARY METABOLITES	FLAVONOIDS	+	+	-	+
	PHENOLS	+	-	+	+
	ALKALOIDS	+	-	-	-
	CARBOHYDRATES	+	+	+	-
	PROTEINS AND AMINOACIDS	+	-	-	-
	GLYCOSIDES	+	+	+	-
	CARDIAC GLYCOSIDES	-	+	-	-
	TANNINS	+	+	+	+
	SAPONINS	+	+	+	+
	COUMARINS	-	+	-	-
	PHYTOSTEROLS	+	+	-	-

The analysis of the results will be carried out for the extracts of each species. Starting with *J. neotropica*, where a positive result can be observed for most of the metabolites tested except for cardiac glycosides and coumarins. It is also worth noting that, compared to the other species studied in this work, *J. neotropica* has the highest number of secondary metabolites. Other species of the genus Juglans have been recorded: *J. Regia, J. Cathayensis, J. Mandshurica, J. Sigillata, J. Nigra, and J. Sinensis*; according to the literature, they present flavones, terpenes, and glycosides. In addition, quinones and polyphenols are abundant in any part of the plant. Finally, hydrolyzable tannins have also been reported in Juglans species[35]. With all this background, the qualitative phytochemical analysis of *J. neotropica* can be confirmed, adding the presence of alkaloids, saponins, and phytosterols in this species.

The extract of *D. triacantha* was positive for flavonoids, carbohydrates, glycosides, cardiac glycosides, tannins, saponins, coumarins, and phytosterols. A study by Luna (2015)[36]

confirms the presence of tannins and flavonoids in the genus *Duranta*. According to the review by Puri (2018)[37], the genus also contains saponins, alkaloids, and glycosides. From the same genus, but in different species such as *D. Repens* and *D. Erecta*, in addition to the secondary metabolites already mentioned, the presence of steroids, terpenoids, triterpenes, and phenols is reported[37,38]. This allows us to compare the main secondary metabolites of the genus and those of the species in question. In the colorimetric study, no alkaloids were detected in the extract, so it could be said that there is no presence of alkaloids in the leaves, stems, or nascent flowers of the plant.

Mikania sp. extract presents phenols, carbohydrates, glycosides, tannins, and saponins. The main chemical groups of the genus *Mikania* according to Rufatto et al. (2012)[39] are coumarins, sesquiterpenes lactones, sesquiterpenes, diterpenes, flavonoids, and terpenoids. Medeiros et al. (2018)[40] reported *Mikania* glomerata rich in coumarins. According to several sources, the main metabolite of this genus is coumarins and their derivatives; however, we can note that it does not possess this compound in the colorimetric analysis of the collected species.

The methanolic extract of *B. aurea* showed the lowest number of secondary metabolites in the colorimetric analysis: flavonoids, phenols, tannins, and saponins are the main chemical groups of this species. Some studies support that *B. aurea* possesses alkaloids such as atropine, hyoscyamine, and scopolamine[41,42]. Literature on the genus Brugmansia suggests the presence of alkaloids, terpenes, and flavonoids as the main components of the genus[43]. However, colorimetric tests were negative for these types of secondary metabolites. These results may be because the alkaloids of this species are not found in the aerial part of the plant, but the bark or fruit.

3.3. Quantitative Phytochemical Screening in *Juglans neotropica* Methanolic Extract

3.3.1. Quantification of Total Flavonoid Content (TFC)

Figure 1 shows the calibration curve obtained with quercetin giving a Pearson coefficient of R^2=0.9987, which implies a very accurate preparation of the solutions with the pipetting technique. The points of the absorbance of the *J. neotropica* extract at 0.4 mg/ml, which are in orange color, were also plotted to show that they fall within the calibration curve. Values are expressed as mg quercetin equivalent (QE) per gram of dry sample.

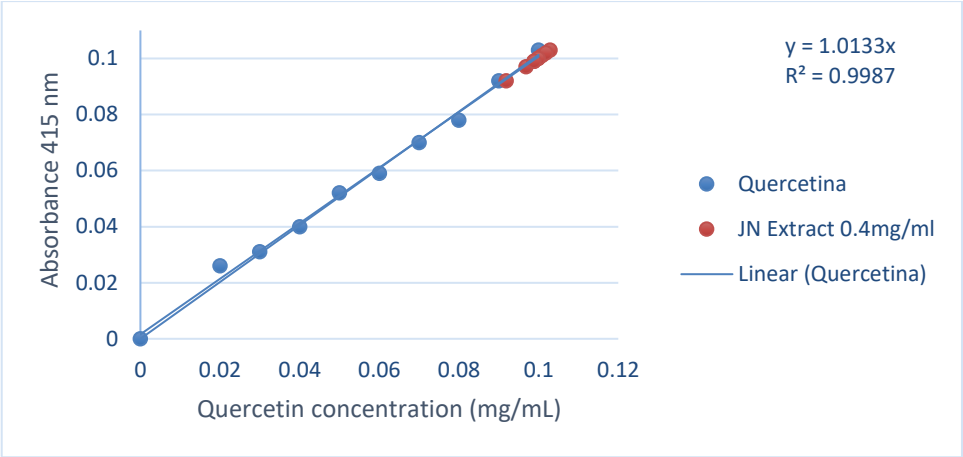

Figure 1. Calibration Curve to calculate TFC values

The equation obtained through linear regression is as follows:

$$\boldsymbol{y = 1.0133x} \text{ (Eq.1)}$$

Where:

y = Absorbance average value of *J. neotropica* extract.

x = Quantification of flavonoids in the sample at 0.4 mg/ml.

With the nine absorbance values of the sample, a total average of 0.0992 nm was obtained. Performing the calculation of Eq.1, an x of 0.0978 mg of QE/0.4 ml extract is obtained, therefore the corresponding conversion was done and it is obtained that the total flavonoid content in the methanolic extract of *J. neotropica* is **244.8 ± 0.003 mg of QE/g extract.**

The total flavonoid content in the Folin-Ciocalteau assay confirms the presence of flavone compounds in the extract of *J. neotropica* as we could observe in the qualitative phytochemical screening performed previously. The dielectric constant of methanol is 32.78 at 25°C, indicating good solvent polarity, which stimulates the polar components of the extract to dissolve quickly in alcohol[12].

Qu et al. (2016)[44] quantified flavonoids in dried nuts of *J. regia* obtaining 53.66µg/ml values. Evaluations of leaves and stems of the mentioned species were performed by Muzaffer et al. (2018)[45] in methanolic extracts reporting a TFC of 144.62 ± 2.40 mg/g. The same research work reports previous studies on the same species with TFC of 48.90 ± 0.7 mg QE/g of extract[45]. Another study shows that the TFC of aerial parts of *J. regia* resulted in a value of 149.00 ± 2.55 mg QEs/g[46].

3.3.2. Quantification of Total Phenolic Content (TPC)

Figure 2 is the calibration curve obtained with gallic acid giving a Pearson's coefficient of $R^2 = 0.9998$, which supposes a very exact and excellent preparation of the solutions with the pipetting technique. The points of the absorbance of the *J. neotropica* extract at 0.4 mg/ml that are in orange color were also plotted to demonstrate that they fall within the calibration curve. The values are expressed as mg gallic acid equivalent (GAE) per gram of dry sample.

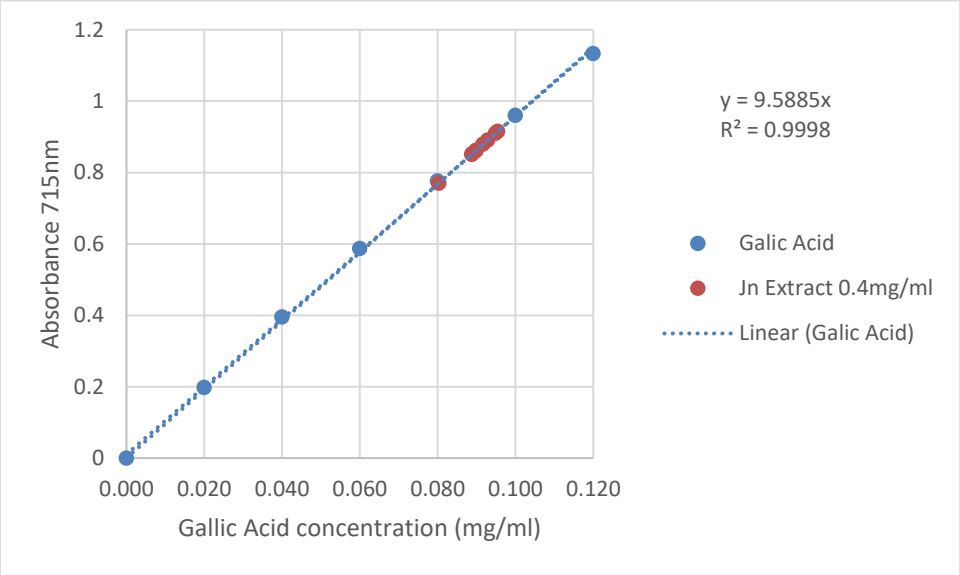

Figure 2. Calibration Curve to calculate TPC values

The equation obtained through linear regression is as follows:

$$\boldsymbol{y = 9.5885x} \text{ (Eq.2)}$$

Where:

y = Absorbance value of *J. neotropica* extract.

x = Quantification of phenols compounds in the sample at 0.4 mg/ml.

With the nine absorbance values of the sample, a total average of 0.866 nm was obtained. Performing the calculation of Eq.2, an x of 0.0903 mg of GAE/0.4 ml extract is obtained, therefore the corresponding conversion was done and it is obtained that the total content of phenolic compounds in the methanolic extract of *J. neotropica* is **225.8 ± 0.04 mg GAE/g extract**.

The total phenol content in the Folin-Ciocalteau assay confirms the presence of phenolic compounds in the extract of *J. neotropica*; as we could observe in the qualitative phytochemical screening performed previously.

Qu et al. (2016) [44] quantified phenols in dried nuts of *J. regia* obtaining values of 589 µg/ml. However, evaluations to leaves and stems of the mentioned species were performed by Muzaffer et al. (2016)[45] in methanolic extracts reporting a TPC of 129.76 ± 3.11 mg/g. In the same research work they report previous studies on the same species with TPC of 116.39 ± 5.63 mg GAE/g[45]. Another study shows that the TPC of aerial parts of *J. regia* resulted in a value of 267.30 ± 2.19 mg GAEs/g[46].

3.4. Biological Activity

3.4.1. Antibacterial Bioactivity

The evaluation results of antibacterial activity against the strains previously mentioned in section 2.2.6. are reflected in Table 5. It can be seen that the extract that showed the greatest activity against most of the bacteria was that of *J. neotropica*, giving positive antibacterial results for *Enterococcus faecalis, Staphylococcus aureus, Listeria monocytogenes, Echerichia coli* and *Pseudomonas aeroginosa*. On the other hand, no antibacterial activity was found in the *B. aurea* extract for any of the strains tested at the mentioned concentrations. *Mikania* sp. was only positive for *Staphylococcus aureus* and *Listeria monocytogenes*. Finally, the extract of *D. triacantha* has a moderate antibacterial activity against the gram-positive rod bacteria *Listeria monocytogenes*. It should be noted that none of the extracts tested showed antibacterial activity against *Enterococcus faecium* and *Salmonella enterica* bacteria strains at concentrations tested.

Table 5: Antibacterial capacity of methanol extracts against pathogenic reference strains measured as minimum inhibitory concentration (MIC) and expressed in µg/ml.

		Gram-positive cocci			Gram-positive rod	Gram-negative rods		
		E. faecalis ATCC ® 19433	*E. faecium* ATCC ® 27270	*S. aureus* ATCC ® 25923	*L. monocytogenes* ATCC ® 19115	*E. coli* ATCC ® 43888	*P. aeruginosa* ATCC ® 10145	*S. enterica* ATCC ® 14028
SAMPLE	*Juglans neotropica*	1000	-	1000	1000	2000	1000	-
	Duranta triacantha	-	-	-	2000	-	-	-
	Mikania sp.	-	-	2000	1000	-	-	-
	Brugmansia aurea	-	-	-	-	-	-	-
CONTROL (+)	Ampicillin (1 g/mL)	0.7812	<0.3906	<0.3906	n/a	n/a	n/a	n/a
	Ciprofloxacin (1 mg/mL)	n/a	n/a	n/a	1.5625	1.5625	<0.3906	<0.3906
	Amphotericin B (250 µg/mL)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
CONTROL (-)	Dimethylsulfoxide (5%)	+	+	+	+	+	+	+

According to Bi et al. (2016)[35], the species of the genus Juglans mentioned previously in section 4.2. present antioxidant, antitumor, and especially antibacterial activity due to the presence of Juglone, a quinone derivative found in walnut leaves[46]. Due to the antibiotic resistance that many organisms have developed, the use of natural antimicrobial compounds is of great interest, and several researchers have demonstrated the antimicrobial activity of phenolic extracts. The antibacterial activity of the male flower of *J. regia* showed significant antibacterial activity against strains of *Staphylococcus aureus* and *Escherichia coli*[45]. It is important to know that *Staphylococcus aureus* strains cause the majority of skin infections[47], so finding natural products that are positive for antibacterial assays is a great advance for the future development of new therapeutic options that are much less invasive for the human body.

3.4.2. Antifungal Bioactivity

The evaluation results of antifungal activity against the strains mentioned in section 2.3.5. are reflected in Table 6. The *B. aurea* extract showed no fungal activity against the tested two strains at the mentioned concentrations. The sample of *J. neotropica* showed moderate antibacterial bioactivity against *Candida albicans* fungi, while the methanolic extracts of *D. triacantha* and *Mikania* sp. were positive for *Aspergillus niger* fungi.

Table 6: Antifungal capacity of methanol extracts against pathogenic reference strains measured as minimum inhibitory concentration (MIC) and expressed in µg/ml

		Yeast and sporulated fungi	
		Candida albicans ATTC ® 10231	*Aspergillus niger* ATCC ® 6275
EXTRACT	*Juglans neotropica*	2000	-
	Duranta triacantha	-	1000
	Mikania sp.	-	2000
	Brugmansia aurea	-	-
CONTROL (+)	Ampicillin (1 g/mL)	n/a	n/a
	Ciprofloxacin (1 mg/mL)	n/a	n/a
	Amphotericin B (250 µg/mL)	<0.098	<0.098
CONTROL (-)	Dimethylsulfoxide (5%)	+	+

The aerial part of the genus Juglans shows favorable results in curing ringworm[35], a skin disease caused by the zoophilic dermatophyte *Trichophyton verrucosum*[48] and is highly contagious in humans[49]. Circular gray lesions characterize this fungal infection[48], especially on the scalp, ears, neck, and shoulders[49]. The antibacterial activity of the male flower of *J. regia* showed significant antifungal activity against *Candida albicans* strains[45].

Although the methanolic extracts of *B. aurea* studied in this work were negative for antifungal activity in the mentioned strains at the concentrations tested, an antifungal study of this same species by Singh et al. (2017)[41] confirmed a high degree of bioactivity for endophytic fungi due to the presence of the ketoacyl synthase domain and concluded that this species has great potential in the production of therapeutic compounds. The species *B. candida* Pers. has been recorded as an antifungal as one of the therapeutic applications[50].

CHAPTER IV: CONCLUSIONS AND RECOMMENDATIONS

Among the four plants studied, *Juglans neotropica* was the one that gave the best results, starting with the highest yield (20.39%) when extracted. This same species tested positive for many of the secondary metabolites analyzed, among the main ones: flavonoids, phenols, alkaloids and carbohydrates and with the exception of cardiac glycosides and coumarins. The results of flavonoid and phenol counts in *J. neotropica* were very favorable with 244.8 ± 0.003 mg QE/g extract and 225.8 ± 0.04 mg GAE/g extract respectively. This species presents moderate bioactivity for antibacterial procedures against strains of *Enterococcus faecalis* (1000 MIC µg/ml), *Staphylococcus aureus* (1000 MIC µg/ml), *Listeria monocytogenes* (1000 MIC µg/ml), *Escherichia coli* (2000 MIC µg/ml) and *Pseudomonas aeruginosa* (1000 MIC µg/ml) and antifungal processes against the fungus *Candida albicans* (2000 MIC µg/ml), which makes this extract an excellent candidate for possible treatments for certain skin diseases caused by the aforementioned strains.

Duranta triacantha extract was positive for flavonoids, carbohydrates, glycosides, cardiac glycosides, tannins, saponins, coumarins, and phytosterols; although neither phenols nor alkaloids were detected. It also showed moderate antibacterial bioactivity against *Listeria monocytogenes* strains (2000 MIC µg/ml) and antifungal bioactivity against *Aspergillus niger* fungi (1000 MIC µg/ml).

The extracts of *Mikania* sp. and *Brugmansia aurea* had secondary metabolites in common such as phenols, tannins and saponins, but only *Mikania* sp. had moderate antibacterial bioactivity against *Staphylococcus aureus* (2000 MIC µg/ml) and *Listeria monocytogenes* (1000 MIC µg/ml) and antifungal bioactivity against *Aspergillus niger* (2000 MIC µg/ml).

Mikania sp. and *B. aurea*, which presented only a few secondary metabolites, could be the object of hydrogel development because of the mucilage they apparently exhibit, this will contribute to a better comprehension of these species.

For future work it is suggested to continue with the counting of flavonoids of *D. triacantha* and *B. aurea*; and also, the counting of phenols of *Mikania* sp. and *B. aurea* to complete the phytochemical profiles of the missing plants.

It is suggested to carry out studies of antioxidant and anti-inflammatory activity of the methanol extracts of the four plants studied here. For the most active plant, it is suggested to perform a bioguided fractionation for the isolation of specific secondary metabolites.

BIBLIOGRAPHY

(1) Honari, G.; Andersen, R.; Maibach, H. Sensitive Skin Syndrome - Google Libros https://books.google.es/books?hl=es&lr=&id=9jsPEAAAQBAJ&oi=fnd&pg=PA16&dq=skin+function&ots=Qyv4Ut59n2&sig=dXEG4ZLT3I4yEcQ9mGvUzmJCabQ#v=onepage&q=skin function&f=false (accessed Apr 8, 2022).

(2) Seth, D.; Cheldize, K.; Brown, D.; Freeman, E. E. Global Burden of Skin Disease: Inequities and Innovations. *Curr. Dermatology Reports 2017 63* **2017**, *6* (3), 204–210. https://doi.org/10.1007/S13671-017-0192-7.

(3) Tang, L.; Zhou, F. Inflammasomes in Common Immune-Related Skin Diseases. *Front. Immunol.* **2020**, *0*, 882. https://doi.org/10.3389/FIMMU.2020.00882.

(4) Wei, L. S.; Gan, Q.; Ji, T. Skin Disease Recognition Method Based on Image Color and Texture Features. *Comput. Math. Methods Med.* **2018**, *2018*. https://doi.org/10.1155/2018/8145713.

(5) Halder, R. M.; Nootheti, P. K. Ethnic Skin Disorders Overview. *J. Am. Acad. Dermatol.* **2003**, *48* (6 SUPPL.). https://doi.org/10.1067/MJD.2003.274.

(6) Rolfe, H. M. A Review of Nicotinamide: Treatment of Skin Diseases and Potential Side Effects. *J. Cosmet. Dermatol.* **2014**, *13* (4), 324–328. https://doi.org/10.1111/JOCD.12119.

(7) Kroft, E. B. M.; Creemers, M. C. W.; Van Den Hoogen, F. H. J.; Boezeman, J. B. M.; De Jong, E. M. G. J. Effectiveness, Side-Effects and Period of Remission after Treatment with Methotrexate in Localized Scleroderma and Related Sclerotic Skin Diseases: An Inception Cohort Study. *Br. J. Dermatol.* **2009**, *160* (5), 1075–1082. https://doi.org/10.1111/J.1365-2133.2008.09017.X.

(8) Demain, A. L.; Fang, A. The Natural Functions of Secondary Metabolites. *Hist. Mod. Biotechnol. I* **2000**, *69*, 1–39.

(9) Soraya Tipantuña Guamán, C.; Soraya Tipantuña Guamán, C. C. Contenido de Flavonoides Totales y Actividad Antioxidante En Inflorescencias, Hojas y Tallos de Plantas Endémicas: Verbena Litoralis Kunth y Duranta Triacantha Juss, 2019.

(10) Blainski, A.; Lopes, G. C.; Carlos, J.; De Mello, P. Molecules Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from Limonium Brasiliense L. *Molecules* **1980**, *18*, 6852–6865. https://doi.org/10.3390/molecules18066852.

(11) Attard, E. A Rapid Microtitre Plate Folin-Ciocalteu Method for the Assessment of

Polyphenols. *Cent. Eur. J. Biol.* **2013**, *8* (1), 48–53. https://doi.org/10.2478/S11535-012-0107-3/machinereadablecitation/ris.

(12) Sepahpour, S.; Selamat, J.; Manap, M. Y. A.; Khatib, A.; Razis, A. F. A. Comparative Analysis of Chemical Composition, Antioxidant Activity and Quantitative Characterization of Some Phenolic Compounds in Selected Herbs and Spices in Different Solvent Extraction Systems. *Molecules* **2018**, *23* (2), 1–17. https://doi.org/10.3390/molecules23020402.

(13) Armijos, C.; Ramírez, J.; Salinas, M.; Vidari, G.; Suárez, A. I. Pharmacology and Phytochemistry of Ecuadorian Medicinal Plants: An Update and Perspectives. *Pharmaceuticals* **2021**, *14* (11), 1–41. https://doi.org/10.3390/ph14111145.

(14) De la Torre, L.; Navarrete, H.; Muriel, P.; Macía, M.; Balslev, H. *Enciclopedia de Las Plantas Útiles Del Ecuador*; 2008.

(15) Muñoz, F.; Navarro, C. 13 Juglandaceae_18 Hyssopus.Qxd.

(16) Narave Flores, H. Flora de Veracruz. **1983**.

(17) Stone, D. Juglandaceae in Flora of North America @ efloras.org http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=10460 (accessed Apr 7, 2022).

(18) Wagstaff, S. J.; Olmstead, R. G. Phylogeny of Labiatae and Verbenaceae Inferred from RbcL Sequences. *Syst. Bot.* **1997**, *22* (1), 165–179. https://doi.org/10.2307/2419684.

(19) Atkins, S. Verbenaceae. *Flower. Plants · Dicotyledons* **2004**, 449–468. https://doi.org/10.1007/978-3-642-18617-2_25.

(20) Chen, S.; Gilbert, M. G. Verbenaceae in Flora of China @ efloras.org http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=10941 (accessed Apr 7, 2022).

(21) Bohm, B. A.; Stuessy, T. F. Flavonoids of the Sunflower Family (Asteraceae) - Bruce A. Bohm, Tod F. Stuessy - Google Libros https://books.google.com.ec/books?hl=es&lr=&id=-d8yb_8F320C&oi=fnd&pg=PR13&dq=asteraceae+family&ots=caxZkvL7Rb&sig=iR_TTe_qyLEifLdc6eaOmP5H344&redir_esc=y#v=onepage&q=asteraceae family&f=false (accessed Apr 7, 2022).

(22) Singh, S. B.; Devi, W. R.; A, M.; Devi, W. I.; Swapana, N.; Singh, C. B. Ethnobotany, Phytochemistry and Pharmacology of Ageratum Conyzoides Linn (Asteraceae). *J. Med. Plants Res.* **2013**, *7* (8), 371–385. https://doi.org/10.5897/JMPR12.897.

(23) Barboza, G. E.; Hunziker, A. T.; Bernardello, G.; Cocucci, A. A.; Moscone, A. E.;

Carrizo García, C.; Fuentes, V.; Dillon, M. O.; Bittrich, V.; Cosa, M. T.; Subils, R.; Romanutti, A.; Arroyo, S.; Anton, A. Solanaceae. *Flower. Plants. Eudicots* **2016**, 295–357. https://doi.org/10.1007/978-3-319-28534-4_29.

(24) Olmstead, R. G.; Bohs, L. A Summary of Molecular Systematic Research in Solanaceae: 1982-2006. *Acta Hortic.* **2007**, *745*, 255–268. https://doi.org/10.17660/ACTAHORTIC.2007.745.11.

(25) Wu, F.; Tanksley, S. D. Chromosomal Evolution in the Plant Family Solanaceae. *BMC Genomics* **2010**, *11* (1), 1–11. https://doi.org/10.1186/1471-2164-11-182/FIGURES/5.

(26) Shaikh, J. R.; Patil, M. Qualitative Tests for Preliminary Phytochemical Screening: An Overview. *Int. J. Chem. Stud.* **2020**, *8* (2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834.

(27) Friedman, M. Applications of the Ninhydrin Reaction for Analysis of Amino Acids, Peptides, and Proteins to Agricultural and Biomedical Sciences. *J. Agric. Food Chem.* **2004**, *52* (3), 385–406. https://doi.org/10.1021/JF030490P.

(28) Simoni, R. D.; Hill, R. L.; Vaughan, M. Benedict's Solution, a Reagent for Measuring Reducing Sugars: The Clinical Chemistry of Stanley R. Benedict. *J. Biol. Chem.* **2002**, *277* (16), e5–e6. https://doi.org/10.1016/S0021-9258(19)61050-1.

(29) Benedict, S. R. A Reagent for the Detection of Reducing Sugars. *J. Biol. Chem.* **1909**, *5* (5), 485–487. https://doi.org/10.1016/S0021-9258(18)91645-5.

(30) Valarezo, E.; Ludeña, J.; Echeverria-Coronel, E.; Cartuche, L.; Calva, J.; Morocho, V.; Meneses, M. A. Enantiomeric Composition, Antioxidant Capacity and Anticholinesterase Activity of Essential Oil from Leaves of Chirimoya (Annona Cherimola Mill.) Eduardo. *Plants* **2022**, *11* (367). https://doi.org/https://doi.org/10.3390/plants11030367.

(31) Ho, K. L.; Tan, C. G.; Yong, P. H.; Wang, C. W.; Lim, S. H.; Kuppusamy, U. R.; Ngo, C. T.; Massawe, F.; Ng, Z. X. Extraction of Phytochemicals with Health Benefit from Peperomia Pellucida (L.) Kunth through Liquid-Liquid Partitioning. *J. Appl. Res. Med. Aromat. Plants* **2022**, 100392. https://doi.org/10.1016/J.JARMAP.2022.100392.

(32) Pino, J. A.; Garcia, J.; Martinez, M. A. Comparative Chemical Composition of the Volatiles of Coleus Aromaticus Produced by Steam Distillation, Solvent Extraction and Supercritical Carbon Dioxide Extraction. *http://dx.doi.org/10.1080/10412905.1996.9700643* **2011**, *8* (4), 373–375. https://doi.org/10.1080/10412905.1996.9700643.

(33) Villa-Uvidia, D. N.; Osorio-Rivera, M. Á.; Villacis-Venegas, N. Y. Extracción,

Propiedades y Beneficios de Los Mucílagos. *Dominio las Ciencias* **2020**, *6* (2), 503–524.

(34) Tosif, M. M.; Najda, A.; Bains, A.; Kaushik, R.; Dhull, S. B.; Chawla, P.; Walasek-Janusz, M. A Comprehensive Review on Plant-Derived Mucilage: Characterization, Functional Properties, Applications, and Its Utilization for Nanocarrier Fabrication. *Polym. 2021* **2021**, *13* (7), 1066. https://doi.org/10.3390/POLYM13071066.

(35) Bi, D.; Zhao, Y.; Jiang, R.; Wang, Y.; Tian, Y.; Chen, X.; Bai, S.; She, G. Phytochemistry, Bioactivity and Potential Impact on Health of Juglans: The Original Plant of Walnut. *Nat. Prod. Commun.* **2016**, *11* (6), 869–880. https://doi.org/10.1177/1934578x1601100643.

(36) Luna, A. Estudio Fitoquìmico de Espino Chivo (Duranta Triacantha), 2015.

(37) Puri, A. Duranta Repens Linn. (Verbenaceae): A Comprehensive Review Of Pharmacognostic, Ethnomedicinal, Pharmacological, And Phytochemical Aspects. *Artic. Asian J. Pharm. Clin. Res.* **2018**, *11* (11), 91–96. https://doi.org/10.22159/ajpcr.2018.v11i11.28509.

(38) Subsongsang, R.; Jiraungkoorskul, W. An Updated Review on Phytochemical Properties of "Golden Dewdrop" Duranta Erecta. *Pharmacogn. Rev.* **2016**, *10* (20), 115. https://doi.org/10.4103/0973-7847.194042.

(39) Rufatto, L. C.; Gower, A.; Schwambach, J.; Moura, S. Genus Mikania: Chemical Composition and Phytotherapeutical Activity. *Rev. Bras. Farmacogn. Brazilian J. Pharmacogn.* **2012**, *22* (6), 1384–1403. https://doi.org/10.1590/S0102.

(40) Medeiros-Neves, B.; Teixeira, H. F.; von Poser, G. L. The Genus Pterocaulon (Asteraceae) – A Review on Traditional Medicinal Uses, Chemical Constituents and Biological Properties. *J. Ethnopharmacol.* **2018**, *224*, 451–464. https://doi.org/10.1016/j.jep.2018.06.012.

(41) Singh, G.; Katoch, A.; Razak, M.; Kitchlu, S.; Goswami, A.; Katoch, M. Bioactive and Biocontrol Potential of Endophytic Fungi Associated with Brugmansia Aurea Lagerh. *FEMS Microbiol. Lett.* **2017**, *364* (21), 194. https://doi.org/10.1093/FEMSLE/FNX194.

(42) Bedoya, J. G.; Bernal, M. E.; Castaño, É. Descripción de Relaciones Ecológicas de Brugmansia Aurea Con Plantas, Insectos y Hongos En Manizales y Villamaría. *Bol. Cient. Mus. Hist. Nat. Univ. Caldas* **2009**, *13* (2).

(43) Algradi, A. M.; Liu, Y.; Yang, B. Y.; Kuang, H. X. Review on the Genus Brugmansia: Traditional Usage, Phytochemistry, Pharmacology, and Toxicity. *J. Ethnopharmacol.* **2021**, *279*, 113910. https://doi.org/10.1016/J.JEP.2021.113910.

(44) Qu, Q.; Yang, X.; Fu, M.; Chen, Q.; Zhang, X.; He, Z.; Qiao, X. Effects of Three

Conventional Drying Methods on the Lipid Oxidation, Fatty Acids Composition, and Antioxidant Activities of Walnut (Juglans Regia L.). *Dry. Technol.* **2016**, *34* (7), 822–829. https://doi.org/10.1080/07373937.2015.1081931.

(45) Muzaffer, U.; Paul, V. I. Phytochemical Analysis, in Vitro Antioxidant and Antimicrobial Activities of Male Flower of Juglans Regia L. *Int. J. Food Prop.* **2018**, *21* (1), 345–356. https://doi.org/10.1080/10942912.2017.1409762.

(46) Jahanban-Esfahlan, A.; Ostadrahimi, A.; Tabibiazar, M.; Amarowicz, R.; Pellati, F.; Mercolini, L.; Sardella, R. A Comparative Review on the Extraction, Antioxidant Content and Antioxidant Potential of Different Parts of Walnut (Juglans Regia L.) Fruit and Tree. *Mol. 2019* **2019**, *24* (11), 2133. https://doi.org/10.3390/MOLECULES24112133.

(47) Daum, R. S.; Miller, L. G.; Immergluck, L.; Fritz, S.; Creech, C. B.; Young, D.; Kumar, N.; Downing, M.; Pettibone, S.; Hoagland, R.; Eells, S. J.; Boyle, M. G.; Parker, T. C.; Chambers, H. F. A Placebo-Controlled Trial of Antibiotics for Smaller Skin Abscesses. *N. Engl. J. Med.* **2017**, *376* (26), 2545–2555. https://doi.org/10.1056/NEJMOA1607033/SUPPL_FILE/NEJMOA1607033_DISCLOSURES.PDF.

(48) Tartor, Y. H.; El-Neshwy, W. M.; Merwad, A. M. A.; Abo El-Maati, M. F.; Mohamed, R. E.; Dahshan, H. M.; Mahmoud, H. I. Ringworm in Calves: Risk Factors, Improved Molecular Diagnosis, and Therapeutic Efficacy of an Aloe Vera Gel Extract. *BMC Vet. Res.* **2020**, *16* (1), 1–15. https://doi.org/10.1186/S12917-020-02616-9/FIGURES/4.

(49) Hussein, M. F. Ringworm (Dermatomycosis). *Infect. Dis. Dromedary Camels* **2021**, 207–210. https://doi.org/10.1007/978-3-030-79389-0_34.

(50) Tene, V.; Malagón, O.; Finzi, P. V.; Vidari, G.; Armijos, C.; Zaragoza, T. An Ethnobotanical Survey of Medicinal Plants Used in Loja and Zamora-Chinchipe, Ecuador. *J. Ethnopharmacol.* **2007**, *111* (1), 63–81. https://doi.org/10.1016/j.jep.2006.10.032.