ILEX GUAYUSA LOES (AQUIFOLIAECEAE): AMAZON AND ANDEAN NATIVE PLANT

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Abstract

Ilex guayusa Loes (Aquifoliaceae) is native to the Andean Amazon (Colombia, Ecuador, and Perú) commonly known as guayusa. From ancestral times up to today Guayusa has been employed by indigenous and urban communities as a herbal infusion, for the treatment of diabetes, infertility, or venereal diseases. As an antiinflammatory, diuretic or energizing-agent. In addition, it can be used as a regulator of the menstrual cycle and during the lactation period. Other benefits include for weight loss, and as a mouth wash, among others. This study encompasses Ilex guayusa taxonomy, etnobotany, geographical distribution and habitat (elevation), ecology, phytochemistry, biological activity, and toxicity. Few investigations have been devoted to its phytochemical and pharmacological properties, thus other studies could suggest new medicinal effects for future alternative medicinal development.

Key words: Ilex guayusa, taxonomy, biogeography, ecology, ethnobotany, phytochemistry, biological activity, medicinal plant, toxicity.
Introduction
The Andean Amazon is located in the countries of Colombia, Ecuador, Peru, and Bolivia, encompassing approximately three fourths of Peru and Bolivia territory, one half of Ecuador, and one third of Colombia. The Andean Amazon is characterized by its richness in biodiversity for food, medicine, cosmetic, and raw material for industry production (1). *ilex guayusa* is among the many plant species in this region, also known as guayusa, guañosa, huayusa, aguayusa, and wuayusa (2). For centuries aborigens in this region have employed *ilex guayusa* as a diuretic, hypoglycemic agent, stimulant and in ritual ceremonies, among others (3). *ilex guayusa* production has increased in the Andean Amazon zone in the past years aiming to export, as well as introduce it to other countries due to its medicinal and stimulant properties (4-6). Other medicinal attributes include aiding in scar formation and as a diaphoretic. It can be used to treat asthma or as an expectorant. Its antiinflammatory properties are known, thus it can be used against rheumatism. It can also be employed as a mouth wash, against gastritis, as an emetic, digestive, and diuretic. It is known to reduce head and body aches, for muscular pain, and to treat flu symptoms. Among its various uses are as an emmenagogue, during the lactation period, to treat venereal diseases, for dissynergy, and weight loss. Furthermore, it can be consumed as a herbal tea. It is a blood fortifying agent, blood pressure regulator with hypolegymic and antioxidant properties. Other beneficial effects include fatigue suppressant, provides physical and mental agility, stimulant, hallucinogen, toner, energizing, restorative, and aphrodisiac. Last, it aids in the sense of awareness throughout the whole body due to its content of a mix of theophylline, theobromine, and caffeine (7-18).

Methods
The methods utilized to search, gather and analyze information include the following Data Banks: Plantlist, Scopus, PubMed, IsiWeb, Sprink link, Francis & Taylor, SIB Bioinformatics Resource Portal, and Sinab. Books and articles referring *ilex guayusa* ethnobotanical aspects or any subject related to taxonomy or phytochemistry were included. In addition, books and articles describing biological activity, medicinal properties and toxicity among others were also employed. The collection of information was carried out between January 2012 and June of 2016.

Taxonomy
*ilex guayusa* Loes, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 78:310.1901. The plant belongs to the kingdom Plantae, Phylum: Magnoliophyta, Class: Magnoliopsida, Order: Celastrales, Family: Aquifoliaceae, Genus: *ilex*, Species: *ilex guayusa* Loes. The tree can grow between four to 15 m height, with a ramified trunk up to 1 m diameter. It has dentate oblong/elliptic olive green coriaceous leaves, glabrous or subglabrous at the blade as well as the back of the leaf. The leaves are arranged in a simple and alternate manner. It has an acuminate apex with an acute base. The leaves can grow between 15 - 21 cm long and 5 - 8 cm wide, with a short 1 cm petiole. Flowers have a persistent calyx and the petals forming the corolla are obtuse. The number of stamens are the same as for the petals, with oblong anthers. The ovary sessile, subglose usually 4-6 celled (locules). The fruit is a globose green berry almost 1 cm wide (3, 19-21). The plant is classified under voucher No. HPUJ 011734 at the Pontificia Universidad Javeriana herbarium. In addition, the National Colombian Herbarium and Bogota Botanical Garden Herbarium have classified this species under vouchers COL 523700 and JBB 10344, respectively.

Geographical distribution
*ilex guayusa* is a plant native to the Neotropics, with natural distribution in Colombia, Ecuador, Peru, and Bolivia (22, 23). According to specimens deposited in the Colombian National Herbarium and the Herbarium at the Universidad de El Valle, this plant is found in the Departments of Nariño and Putumayo, from Mocoa to Sibundoy (20, 24), in the area between the Departments of Putumayo and Caquetá in Colombia (25). In Ecuador this plant is found in the provinces of Sucumbios, Napo, Pastaza, Morona Santiago, and Zamora Chinchipe. In addition, it has been registered in the provinces of Pichincha and Tungurahua (26).

In Colombia *ilex guayusa* was reported in the Department of Amazon in front of the south tip of the Guadual Island, where it is commonly known as “detzacogque” for the Miraña community. Furthermore, in the Department of Caquetá the Tucana indigenous community has named it “Yurugü”. Moreover, it has been found in front of the Mariname Island in poorly drained woods. Likewise, it is found in the environmental path of Mogambo (Figure 1), in the Municipality of Viotá, Department of Cundinamarca. Last, it is also established in the National Research Center for tropical aromatic plant species agroindustrialization (Centro Nacional de
Investigaciones para la Agroindustrialización de Especies Vegetales Aromáticas Medicinales Tropicales: Cenivam) in Bucaramanga, Santander Colombia (27, 28).

**Altitudinal distribution**

According to deposited samples in the COL herbarium, *Ilex guayusa* grows in Colombia at 2,000 masl. This species is distributed in altitudes between 200 and 2,000 masl (25). It has been collected from Ecuador at 500 masl and Perú at 220 masl (22). Furthermore, in Ecuador this species distribution ranges from sea level up to 1,500 masl (26). Gupta reported this plant can grow between 200 and 350 masl (29).

**Ecology**

*Ilex guayusa* is found in the Colombian lower Neotropical jungle and in Sub-Andean forests (30). This perennial tree is native to the Amazon region, where it grows in the wild. However, it is also present plantations in subtropical Andean regions (26). This species grows in humid tropical forests in the Colombian, Ecuadorian, and Peruvian Amazon forming part of secondary forests (31). This plant was reported in phytosociological association with *Tabebuia insignis–Mauritietum flexuosae*, defined as a vegetation unit encompassing small to medium forests, with a short basal area, high shrub density in the thicket, and a high palm tree percentage (32). *Ilex guayusa* is a tree reported in the literature with monoecious flowers and prone to polygamy; with shrub like physiognomy during the juvenile stage. In addition, it is semi-domesticated in plantations. Its asexual reproduction strategy consists of basal shoots, sprouts, and suckers. Phenological cycles do not report fertile matter activity. Anthropic distribution is limited to the Peruvian-Ecuadorian and Colombian Amazon corridor, thus its main biophysical requirements are the soil and water resource in its three forms: rain-, soil-, and vapor water. Soils where *Ilex guayusa* grows have a sandy-loam characteristic with acid pH between 4.34 and 5.01. It has low cationic capacity, high aluminum and heavy metal content, following the pattern of acid soils with a tendency to become poor depending on the vegetation sustained not including trees (32). Taking into account its light requirements it can be considered a forest species. It is designated as a durable heliophyte, since its natural regeneration can be maintained at low light levels. In fact, semi-dark sites are the most recommended for its proliferation. Originally guayusa seedlings need little light to meet their functions. Moreover, in an environment free of light exposure it tends to ramify and grow shoots, since the terminal bud has been affected by light and grows branches. Forming stems cast a shadow on basal shoots that are generated on dead or little vigorous stems, generating a “soil bed” made of leaves and trunks, which eventually decompose and serve as nutrients for the seedlings. No reproductive phenophases have been reported.

*Ilex guayusa* is not found within the conservation category in the vascular plant catalog, such as the red book, proposed by the International Union for Conservation of Nature and Natural Resources, i.e. it is not vulnerable, it is not endangered, or in critical danger, thus it is not a species at risk (26, 33-35). For many years some botanists speculated *Ilex guayusa* reproduced in an asexual manner, since it had lost its flowering and fruit production capacity through years of selection and vegetative propagation by man. This theory was based in the lack of specimens with reproductive organs, thus its certain taxonomic classification (21). At present it reproduces asexually, despite the presence of seeds. Stems without leaves are planted for propagation (4).

**Ethnobotany**

In 1683 the Jesuit Juan Lorenzo Lucero reported the Shuar natives (known as Jibaros by the Spanish conquistadores) used *Ilex guayusa* in their medicinal-magical acts in the following manner: “They placed together these demonic herbs (Datura, *Banisteriopsis caapi*, Psychotria viridis, *Justicia pectoralis*, *Brugmansia*, *Nicotiana rustica*, and other hallucinogenic plants) in addition to guayusa and tobacco, also invented by the devil. They cooked them in a way the little juice produced became the quintessence, with the belief those who drank were rewarded with the fruit of a curse by the devil for the misfortune of many ...”. Lucero described the Shuar as well disposed people, with good physical appearance, accustomed to take several times a day a decoction referred to as “guayusa”; to stay awake for several nights without losing consciousness, when an invasion by their enemies was feared. In the indigenous view the guayasa ritual has a purification significance and was used as a “bode drink”. It was consumed in high concentrations to dream, foresee the future and guess whether the hunting or fishing would be successful (3, 7, 19, 21, 22, 25, 36-39).

In 1756 Fray Juan de Santa Gertrudis Serra stated: “The most beautiful leafy tree of all I have ever seen, thick trunk, with peaceful and delightful green leaves. The leaves have a very tasty flavor, similar to tea, but finer and appetizing. When the beverage
preparing with cooked leaves is drank it produces sweating and eliminates phlegm, represses blood ardor and eliminates heaviness, aids in digestion with a satiety feeling, gives robustness and removes all moodiness. When it is drank with honey obtained from apate bees, women become pregnant” (20, 24). Registries from 1756 indicate high Putumayo Indians (Amahuayas and Paraguayaguas), in addition to White people have employed *Ilex guayusa* leaves as a stimulant infusion. It would be consumed in the mornings to alleviate hunger, it was argued they did not feel hungry from early in the morning until noon (20). In 1943 the Botanical Institute of the Central University in Ecuador issued a bulletin informing *Ilex guayusa* leaves were used by people from the oriental region as an infusion for breakfast with the belief this plant would “lift them up”. Moreover, it had a fertilizing power and could be related to getting married (20, 24, 25). In 1857 Richard Spruce observed guayusa use among Shuar (Jibaro) natives as an emetic to daily cleanse the stomach, as a purgative, as a narcotic and hypnotic. Likewise, to exonerate the body before the daily tasks, with eschatological purification beliefs, as a ceremonial daily mouth wash (14, 18, 22, 25, 38, 40-42). Pleasant taste leaf infusion in the form of tea it was used to treat all chills, venereal diseases, and for women to become pregnant when they were sterile many years back.

In the mid-XIX century guayusa was used for poisoned people. In addition, burned leaves then mixed with barley and honey was employed to treat amenorrhea; cooked leaves to treat diarrhea and stomach pain. Around the third quarter of the XIX century botanists found the presence of caffeine in leaves (38). Prehispanic Bolivian culture possibly employed leaves as an enema (18, 43, 44). Kallawaya’s from the Province of Bautista Saavedra in Bolivia are known to be expert herbalists. With over a millennium in traditional medicine practice they are characterized for curing physical and spiritual illnesses. One particular distinctive of this culture is to perform brain surgery. Furthermore, they employ over 1,000 plants, among them *Ilex guayusa* a holly-like plant as an anesthetic. This use has been described as early as 700 A.C. (45, 46). Kallawaya are recognized by Andean people (Peru, Bolivia, and Argentina) as “The Lords of the Medicine Bag” (4, 47). Natives of some localities of the Department of Nariño (Colombia) use *Ilex guayusa* as a medicinal plant, in particular to regulate menstrual cycles. The bark and wood are used as a medicinal stimulant.

Leaf infusion against chills, as a narcotic, and stimulant beverage. With dried leaves and branches a beverage is prepared similar to mate from Paraguay (*Ilex paraguayensis*) (29, 48). Whole fresh plant cooked and drank with lemon and orange serves as a diuretic, against anemia and sorec. *Ilex guayusa* cooked leaf intake with fresh *Pilea microphylla* L. (prefadilla) and fresh *Lycaste gigantea* Lindl. (simayuca) fruit is used for masculine fertility. Mixed with the juice of two *Citrus aurantium* L (bitter orange) serves as a vitamin supplement, together with burned bitter orange skin is used as incense in ceremonies. These last two preparations are also employed against scurvy, stomach ache, high blood pressure, as a deodorant, and for “mal de aire” (syndrome of culture filiation-bad air).

*Ilex guayusa* decoctions with whole *Pilea microphylla* L. (prefadilla), with *Eucalyptus globulus* Labill (aromatic eucalyptus) and *Lycaste gigantea* Lindl. (simayuca), and sugar can be taken on a daily basis as a diuretic, against venereal diseases, for the lungs, and for fertility purposes (49). Oral administration of dried *Ilex guayusa* leaves are used to treat blood intoxication and diabetes (13, 50). Due to its high caffeine content (2%) it is considered and energizing plant (10, 14). Moreover, *Ilex guayusa* is employed against drug addiction, hangover, and to eliminate the bad taste of ayahuasca consumption (51). In Ecuador this species is frequently used as a refreshing and tonic beverage, with similar effects to Asian tea or to Argentina-Paraguayan mate. It can be purchased in most grocery stores as dried leaves. It is claimed to have fertility properties. In addition it is used as a stimulant, tonic, stomachic, digestive and emetic (3, 52, 53). Aids digestion and it is stated that cleanses the stomach and the intestines, since it has emetic characteristics. Likewise, it has expectorant properties, since its intake produces a warm burst throughout the body, allowing for phlegm expulsion from the lungs resulting from colds (10, 29, 40). In 2003 a descriptive, analytical-comparative research was carried-out in the cities of Quito (Ecuador), Puyo (Ecuador), and Bogota (Colombia) finding the following uses against: sterility, diabetes, asthma, as a diuretic, during pregnancy and lactation period, as a mouth wash, against tiredness, muscular pain, weight loss, as a narcotic/shaman, aphrodisiac, purgative/emetic, and refresher. Data gathered by traditional knowledge demonstrate a main use (12.8%) as an emetic and stimulant. Application techniques include baths, lavage, ointment, poultice, intake or inhalation among others, every eight to 24 h (9). *Ilex guayusa* is the most used and cultured plant by the Kichwa Indians in the Canton Loreto
region in Ecuador. It is the most important plant in daily life, since its consumptions every morning brings about multiple effects such as luck for fishing and hunting, in addition to providing protections against snake bites (54, 55). In Peru its leaves are employed as a dietary supplement, for prostate and kidney protection, favoring kidney stone expulsion (56). From the ethno-veterinary medicine point of view *Ilex guayusa* is used by Shuar and Quichua Indians in Ecuador as a psychoactive plant to improve performance and capacities in hunting dogs, increasing their sense of smell. This use could be implemented by police or guard dogs to detect explosives, illegal drugs, human remains, and other activities of value (57).

**Phytochemistry**

Some studies with this plant reveal its caffeine, triterpene, and chlorogenic content (14, 18, 58-60). Family compound identification has been performed through preliminary phytochemical analysis identifying tannins and flavonoids in leaf aqueous and ethanol extracts, respectively (61). Polyphenol quantification evidenced 0.49 and 0.18 mg tannic acid per gram of sample for the aqueous and ethanol extracts, respectively. Total phenol content present in leaf methanol extract was 116.8 g of gallic acid per g of sample (62). Methanol total extract bio-assay guided fractionation by antioxidant and antihyperglycemic activity identified Uvaol, by GC-MS (63). Racidi and collaborators reported in leaf ether extract the presence of alkaloids, steroids, terpenes and lactonic or coumarin compounds. Moreover, in the aqueous extracts saponins, phenols, tannins, reducing sugars and alkaloids; and in the ethanol extract phenols, alkaloids, reducing sugars, steroids, terpenes, flavonoids and quinones. These authors described *Ilex guayusa* phytochemical knowledge is still very limited and other studies could suggest new medicinal uses for this plant (19).

Other compounds present are methylxanthine, theobromine, theophylline, guanidine, steroids, essential oils, isobutyric acid, nicotinic acid, ascorbic acid, riboflavin, choline, pyridoxine, triterpenes, chlorogenic acid and sugars among others (10, 12, 64). Likewise, polyphenol content 40.1 mg/g, L-theanine (1.3 mg/g), theobromine (0.4 mg/g), and caffeine (32.8 mg/g) have been reported (65, 66).

In 2013 researchers from the Escuela Superior Politécnica del Litoral, in Ecuador performed from *Ilex guayusa* leaves a physicochemical, bromatological, sensorial and microbiological study. Phytochemical analysis revealed alkaloids, flavonoids, reducing sugars phenols, triterpenes, quinones, fats and oils. Bromatological study indicated a protein content between 0.6 and 1.3%, total fat content between 1.6 and 4.0%, total ash content between 5.5 and 6.9%, hydrochloric acid insoluble ash between 0.7 and 0.8%, water soluble substances between 0.9 and 2.9%, carbohydrates (including monosaccharides to structural polysaccharides) between 78.4 and 83.6%.

**Biological activities**

Studies in mice with Diabetes mellitus type I, induced by streptomycin treatment (STZ) demonstrated oral...
administration of *Ilex guayusa* infusion slowed down hyperglycemia development, reduced glycosylated hemoglobin, polydipsia, and weight loss (70, 71). Sarango in 2008 reported leaf methanol extract had an inhibitory effect against a-glucosidase with an IC\textsubscript{50} of 411 µg/mL (72). Other *in vitro* studies evaluating *Ilex guayusa* leaf extracts in hexane, ethyl acetate and ethanol demonstrated hypoglycemic activity with inhibition of a- and b-glucosidases, enzymes associated with diabetes mellitus type I development. For 500 µg/mL hexane, ethyl acetate, and ethanol extracts a-glucosidase was inhibited at 98.4, 79.1, and 58.2%, respectively. Likewise, for b-glucosidase inhibition was 35.0, 52.5, and 84.2% for each extract at 1,000 µg/mL. Results suggest this plant could be considered a possible neutraceutical in the diet of diabetic patients (73).

Colombian medicinal plant Vademecum describes *Ilex guayusa* ethanol leaf extract presents central nervous system and sympathetic nervous system stimulation, possibly due to caffeine high content. Infusion consumption stimulates the cardiac system, augments alertness, and increases the capacity to perform physical tasks (74). Hot tea drunk at a concentration of 10 g/L three times per day is used as a treatment for diabetes (75). In Trujillo, Northern Peru it is traditionally used by the medicine man as an anti-inflammatory and antimicrobial plant. Antibacterial activity results demonstrate leaf aqueous and ethanol extracts have a biological activity against *Staphylococcus aureus* presenting 14 mm inhibition halos (50, 76). Methanol, ethanol and hydroalcoholic extracts at 25 mg/mL presented antifungal activity with 16 mm halos for ethanol extract and 24 mm for the methanol extract against *Candida albicans*. In addition, a 32 mm halo was observed for the hydroalcoholic extract against *Microsporum canis* (11). Inter-institutional work carried-out by Calderon and collaborators with 311 species, including *Ilex guayusa* evaluated antiparasitic effect against Malaria, Chagas disease and Leishmaniasis, finding leaf ethanol extract presented an IC\textsubscript{50} of 47, > 50, and > 50 mg/mL against *Trypanozoma cruzi*, *Plasmodium falciparum*, and Leishmania mexicana, respectively (77).

Estrogen effect of *Ilex guayusa* leaf hydroalcoholic extract was evaluated on ovaries, uterus, and serum estradiol by oral administration given to albino rats (*Rattus novergicus*). Used doses of 9, 18, and 36 mg/kg per day presented such effect on immature rats. These results suggest *Ilex guayusa* potential use for infertility in women (78, 79). DPPH and TEAC assays revealed its probable antioxidant use with an IC\textsubscript{50} of 11.8 and 14.9 [µg/mL], respectively for leaf methanol extracts (62). Furthermore oxygen radical absorbance capacity (ORAC) in aqueous and lipophylic media reported values of 658.9 and 0.3 µmol TE (Trolox equivalent) per gram of sample for ORAC\textsubscript{hydro} and ORAC\textsubscript{lipos} respectively (65).

Researchers of the Department of Pharmacy at Universidad Nacional de Colombia evaluated *in vitro* and *in vivo* antioxidant capacity in leaf aqueous and ethanol extracts. Xanthine/xanthine oxidase anion radical uptake measured as inhibition of nitroblue tetrazolium (NBT) reduction was 64 and 57% for aqueous and ethanol extracts, respectively. Furthermore, peroxy radical uptake was measured in Aroxyl radical absorption capacity ABAP/lysozyme system finding inhibition values of 15 and 18% for aqueous and ethanol extracts, respectively.

Hydroxyl radical uptake generated in the H\textsubscript{2}O\textsubscript{2}/Fe\textsuperscript{3+}/EDTA/ascorbate system demonstrated for the aqueous extract a 42% uptake and 18% uptake for the ethanol extract. Liver microsomal lipid peroxidation using the non-enzymatic Fe\textsuperscript{3+}/EDTA/ascorbate/H\textsubscript{2}O\textsubscript{2} method determined inhibition percentage values of 93% for the aqueous extract and 96% for the ethanol extract (61).

*Ilex guayusa* based cosmetic gel elaboration by Ecuadorian researchers established a skin protective agent in addition to having a lipolytic effect. *Ilex guayusa in vivo* anti-cellulite effect was evaluated in women between the ages of 30 and 50 years old. Their findings evidenced a reduction in body measurements and in the appearance of orange-textured skin known as cellulite, proportional to the time of treatment. This effect is likely due to caffeine plant content (66).

**Toxicity**

*Ilex guayusa* could affect the nervous system if consumed with food in great quantities (29, 52). In the Department of Pharmacy at the Universidad Nacional de Colombia an *in vivo* hepatotoxicity model was evaluated using Wistar rats. Affected animals were induced by CCl\textsubscript{4} administration, and as a positive control Sylimarin was used (61). Histopathological study did not reveal any considerable toxicity signs (80). Colombian medicinal plant Vademecum indicated *Ilex guayusa* infusion or decoction beverage consumption did not present signs of acute toxicity (74). Multidimensional tests using de 1,000, 500, 250, and 125 mg/kg ethanol extract did no cause lethality in animals. In addition,
repeated infusion doses were safe (74, 80). Due to its high caffeine content it is not recommended for pregnant women. Excess consumption can produce vomit and alterations in the CNS (74).

In a collaborative work between the USA and Perú, researchers evaluated alcohol and water extract toxicity of 341 plants, including *Ilex guayusa* using the brine shrimp lethality test. Results demonstrated a LC$_{50}$ > 10,000 µg/mL for the aqueous extract and 300 µg/mL for the ethanol extract (81). Achuar Indians from the Ecuadorian Amazon pointed out *Ilex guayusa* mix with other plants can be toxic. For example, its decoction with *Psidium guajava* produces a poisonous beverage (12). Kapp and collaborators evaluated standardized liquid concentrate of guayusa using *in vitro* genotoxicity tests, with Bacterial reverse mutation test (Ames test). Furthermore, a study of chromosome aberrations in human lymphocytes was performed. Ames test established a negative result. Likewise, no structural or numeric aberrations were observed for the chromosome study. Acute toxicity by oral administration with a 5,000 mg/kg dose in female rats established a salivation response, hypoactivity, abnormal breathing, stooped posture, decreased and soft feces. All animals recuperated at the third day of administration and continued healthy until day 14 of the study. Necropsy did not evidence any severe abnormalities. Results suggested oral median lethal dose in female rats was > 5,000 mg/kg, 90 day subchronic toxicity test by oral administration at 1,200, 2,500, and 5,000 mg/kg in female and male rats revealed no toxicity associated with standardized liquid concentrate of guayusa. In females a high neutrophil and basophil count was found, depending on administered *Ilex guayusa* dose. Additionally, a distinct adaptive hypertrophy in salivary glands was observed, with a greater impact on females, depending on dose. Moreover, blood chemistry was altered with the following values increasing in blood serum: aspartate aminotransferase, serum alanine aminotransferase, and cholesterol. A body weight reduction and food efficiency, decreased triglycerides values, and diminished fat pad weight were observed. However, no noxious effects were observed. Therefore, this study indicated no harmful effect in liquid concentrate of guayusa on this model system (64).

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Figure 1. Flowers, leaves and steams of *Ilex guayusa* (27).