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Ilex guayusa: A systematic review of its Traditional Uses, Chemical Constituents, Biological Activities and Biotrade Opportunities

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Abstract:
Ilex guayusa is an emblematic tree of the Ecuadorian Amazon Region (EAR), widely used in folk medicine, ritual uses and household and industrial beverages. Despite the daily consumption only a few number of studies have been carried out and the species deserves a deepener bioprospecting activity, also in order to define a new Biotrade strategy for the EAR. This review summarizes the ethno pharmacological data and the researches concerning *I. guayusa*. Promising biological activities have been detected, especially as new source of antioxidant agents due to the presence of phenolic compounds. Also a preliminary study as antidiabetic natural product accounts for new researches

Keywords: *Ilex guayusa*, Ecuadorian Amazon Region, folk medicine, antioxidant, antidiabetic, Biotrade

1. Introduction

Ilex guayusa is an emblematic tree of the Amazonian region, widely present in the Amazonian region of Ecuador, Colombia, Peru and Bolivia. As reported by many authors (1 – 8) *I. guayusa* was mentioned in several researches regarding pre-Columbian archaeological collections, old historical manuals and ethnobotanical studies, legitimizing the fame of this specie as the most important plant in the daily life of Kichwa Amazonian communities (5) and local farmer from "colono" and "mestizos" communities. Despite the wide presence of ethnobotanical studies regarding several uses in folk medicine (6), there are only few researches about the phytochemistry and biological activities of *I. guayusa*, and this lack compromises a complete understanding about the concurrence between folk medicine and pharmaceutical applications. Moreover, deepener studies may propose new promising applications as nutraceutical or cosmetic ingredient. We aimed to compile an up to date and comprehensive review of *I. guayusa* that mainly covers the phytochemistry and pharmacology

information, in order to suggest new researches and to offer a complementary paper to the ethnobotanical research.

2. Results and Discussion

2.1 Botanical description, historical information and folk medicine

Ilex guayusa is an evergreen tree belonging to Aquifoliaceae family, native to the Amazon. The plant is dioecious and reaches between 6 to 10 meters tall. The leaves are simple, pinnate, glabrous, oblong, elliptic with serrate margin; they are 7-20 cm long and 2.5-7 cm wide (4). *I. guayusa* is distributed from 200 to 2000 m above sea level along the Andes and contiguous Amazonian piedmont (8). Historical information about *I. guayusa* was mentioned by Schultes (9). Describing an archeological finding from a shaman excavated tomb in Bolivia (Tihuanacoid culture), the author described the presence of dried and pressed leaves, a mortar and pestle. The finding probably describes the use of the species as snuff during ritual activities and it is feasible that the species has been used for at least 1,500 years. Since the XVI century until today, many authors described folk medicine and commercial activities related to *I. guayusa*. Even for Jesuit missionary in Ecuador the species was an important source of income (1,3) and, currently, a few companies from the EAR are selling beverages and infusions obtained from *I. guayusa*.

Regarding the folk medicine information, the Table 1 summarized several traditional uses which include ritual and magical application. According to the "cosmovisión" concept of the Amazonian ethnic groups, *I. guayusa* can be used for multiple porpoises, from human health remedy to the custom of cleansing the stomach daily as a ritual purification. *I. guayusa* tea is considered a "magical drink" and is also given to the hunting dogs, before a hunting expedition, in order to improve their abilities and skills. For indigenous people, the infusion can also provokes a soft hypnotic effect in which "little dreams" can inspire or dissuade in advance a hunting expedition (1).

2.2 Phytochemistry

Leaves contain caffeine, theobromine, phenolic compounds and flavonoids as the main components (10-13). Also guanidine was mentioned as an important component of I. guayusa leaves extracts (14,15). Another research performed by Ruiz and Roque (16), mentioned a phytochemical preliminary assay on ethanolic, methanolic and hydroalcoholic extracts of *I. guayusa*, the study revealed the presence of tannins, alkaloids, flavonoids, glycosides, phenolic compounds and quinones.

A study performed by liquid chromatography with tandem mass spectrometry (17) of a *I. guayusa* leaves extract detected several amino acids (Table 2), which provide an interesting information about nutraceutical profile and taste. From the same author (18), another research on *I. guayusa* leaves extracts, using gas and liquid chromatography and mass spectrometry, revealed the presence of two pentacyclic triterpenoid, oleanolic (1,18 mg/g) and ursolic acid (18,22 mg/g) respectively.

2.3 Biological activity

Stimulant and protective effect of caffeine and theobromine are extremely reported in literature (19-23). As reported by Jara *et al*. (13), dried leaves of *I. guayusa* were extracted with ethanol (EtOH) and ethyl acetate (EtOAc). The total phenolic content was determined spectrophotometrically according to Folin–Ciocalteu's phenol method and calculated as gallic acid equivalent (GAE). The total flavonoids content (TFC) was determined spectrophotometrically, the antioxidant activity was determined using free radical DPPH (2,2-diphenyl-1-picrylhydryzyl) scavenging method and the β-Carotene bleaching. Results are reported in Table 3.

Anyway, the presence of phenolic compounds and flavonoids may indicate a protection against cellular damage induced by free radical oxidative injury or reactive oxygen species. These antioxidant properties are associated with the presence of phenolic compounds and flavonoids.

Although guanidine was reported but wasn't quantified, its presence explains the preliminary hypoglycemic effect of *I. guayusa* leaves extracts in animal model. Guayusa may reduce hyperglycemia without affect the parameter of glucose homeostasis in non-diabetic mice (14).

Oleanolic and ursolic acid are recognized as antiviral and anti-inflammatory bioactive compounds and also were reported the in vitro inhibition activity of these molecules against various cancer cell type (18). Finally, estrogenic activity of ethanolic extracts from leaves of I. guayusa was tested in an *in vivo* model (female albino rats) shoving a remarkable increase of serum estradiol levels and ovaries and uteri weights. This finding is a preliminary but promising data in order to confirm the traditional use of *I. guayusa* against female infertility (24). In another study, it was observed that methanolic and hydroalcoholic extracts from I. guayusa have fungicidal action against *Candida albicans*, the hydroalcoholic extracts was effective also against *Microsporum canis* (16).
Moreover, toxicological study was conducted on ethanolic and water extracts using a Brine-Shrimp assay (25), in both cases the test shown respectively low (LC50 500–1000 µg/ml) and median toxicity (LC50 250–499 µg/ml), in according with safe traditional use, especially for the aqueous extract. Another research performed by Ames test and a chromosome aberration study in human lymphocytes demonstrated a no harmful effects (26).

2.4 Biotrade opportunities

In the EAR, ritual infusion, beverages and tea bags obtained from I. guayusa leaves are widely present in local market, restaurants and houses. Furthermore, there are some experiences about the development of a local and international Fair Trade market, based on social sustainability approaches and eco-friendly criteria. Actually, in order to valorize the Ecuadorian Amazonian biodiversity, the *I. guayusa* derivatives may be a sustainable alternative to design natural products, relevant for local economies, such as: tea (27), phytopharmaceuticals (12). In order to maintain national and international markets, it is necessary to design a bi-commercial business strategy that enhances the balance between conservation politics and entrepreneurs needs.
A recent study performed by Sidali and Garrido Pérez (8), focuses on a food tourism model, based on guayusa case, as a strategy of sustainable development for Kichwas communities in Napo (Ecuador). The qualitative research confirms as food tourism may be a viable strategy and a future trend for EAR. Moreover, the research identifies four principles of Kichwa communities' cosmovision (worldview) which are compatible with Western-based theory on niche tourism, respectively: mutual learning, empowerment, regulated access to intellectual property and community legislation**.**

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Table 1. Traditional, magical and ritual uses of *Ilex guayusa*.

Traditional, magical and ritual uses	Country	Ethnic group	Ref.
Glycemic effect	n.r.	Amaguajes	14, 15
Ritual uses	Brazil (outskirts of Manaus)	n.r.	11
Diabetes, venereal diseases, improving digestion and appetite, strengthening the body and treat pain, increase fertility, daily purging. Promoting conviviality, stimulant, stomach tonic, diuretic, and flu remedy. Increase fertility and libido. Can help to avoid insect and snakebites. Guayusa helps people to dream.	Ecuador, Colombia, Perú	Amazonian Kichwa, Shuar, Achuar, Cofán, Secoya, Awajún Mestizo and white people	6

Daily morning drink, can help to avoid insect and snakebites, improves hunting and fishing ability.	Ecuador	Amazonian Kichwa	5
Ritual uses, scatological purification, ailment, emetic, narcotic, hypnotic, stimulant or tonic, diaphoretic and diuretic, purgative. Increase woman fertility and helps people to dream for knowing in advance.	Ecuador	Several Amazonian communities	1
Health tonic, emetic, venereal diseases, improved the digestion and appetite, can cure dysentery and amenorrhea.	Ecuador, Peru	Amazonian communities	3
Use before and after drinking ayahuasca. Stomach trouble aphrodisiac.	Colombia, Ecuador, Peru	Amazonian communities	4
Emetic and stimulant tea	Ecuador	n.r.	9
Gastritis, relaxant, helping woman fertility.	Ecuador	Saraguros, Shuar	28
Energizing and stomach pain.	Ecuador	Kichwa, mestizo	7
Additives for hallucinogenic rituals and ritual snuff.	Ecuador, Peru	Shuar	29, 30

Table 2. Amino acids in *Ilex guayusa* extract (mg/g).

Gly	Asn	Ser	Asp	Gln	Thr	Ala	Glu	Pro	Lys
0.0100	0.2795	0.0107	0.0533	0.0502	0.0136	0.1069	0.0501	0.0253	0.0092
Val	His	Met	Arg	Tyr	Ile	Leu	Phe	Trp	Total
0.0174	0.0129	0.0052	0.0429	0.0129	0.0132	0.0125	0.0110	0.0794	0.8161

Table 3. Phenolic content, flavonoid content and antioxidant activity in Ilex guayusa extract

Sample	Total Phenolics (GAE mg/g)	Total flavonoids (RE mg/g)	DPPH IC_{50} (μg/mL)	β-carotene IC_{50} (μg/mL)
Guayusa EtOH	54.0±3.8	46.0±2.0	17.5±1.4	55.6±1.6
Guayusa EtOAc	36.0±2.2	20.0±1.8	52.7±4.3	85.7±3.7

3. Materials and Methods

The present systematic review was achieved adopting the following electronic databases: SciFinder, PubMed, Google Scholar, SciElo, Taylor & Francis and Scopus. Data were independently extracted from three reviewers and the final paper selections were completed avoiding duplication of data. The following keywords were selected: *Ilex guayusa*, guayusa. The reviewers selected articles were in English and Spanish language and were excluded data from patents. The above mentioned criteria allowed selecting 20 eligible articles; we also considered some additional key papers for introduction, discussion and result chapters. Anyway, it is deserved to remember that many authors mentioned remarkable letters and historical sources from XVI until XIX century

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4. Conclusions

Despite the widespread presence of beverages and commercial products obtained from *I. guayusa*, mainly ethnobotanical research has been realized in the last decades. All phytochemical researches known until today were developed exclusively on leaves extracts, without a deepener studies on other parts of the plant. A recent review about Ilex genus (31) reported wide information about active constituents and their biological activities, but

present basic information on *I. guayusa* regarding the presence of caffeine. For many others Ilex species were been identified many molecules as triterpenoids, saponins, flavonoids, alkaloids, anthocyanins and other phenolic compounds which can explain the mentioned biological activities. The lack of a deepener phytochemical research about I. guayusa is undeniable and the future trend may be to increase the researches about antidiabetic and estrogenic activity above-mentioned.

Furthermore, others studies about plants which contain caffeine (32,33) reported the effect of leaf age effects on the quantitative contents of caffeine, theobromine, methylxanthines and total phenolic compounds, essentially showing a decrease amount of mentioned active compounds in old leaves. Moreover was observed that caffeine presence seems to be cultivar-specific, tissue-specific, and season-dependent. In order to optimize nutraceutical and cosmetic formulations based on *I. guayusa* extracts, all these findings suggest a deepener research about caffeine presence and phenolic compounds focusing different parts of the plant, different plant ages and harvest seasons. Finally, *I. guayusa* represents a promising bio-active compound source and an alternative income wellspring for local farmers from the Ecuadorian Amazonian Region.

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Conflicts of Interest

State any potential conflicts of interest here or "The authors declare no conflict of interest".

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