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Phytochemical screening and bioactivity analysis of extracts from *Helicteres guazumifolia* Kunth (Sterculiaceae)

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Abstract
Crude extracts from *Helicteres guazumifolia* aerial parts were obtained with petroleum ether and methanol, and their biological activities were evaluated. Preliminary phytochemical screening of extracts indicated the possible presence of alkaloids, saponins, sesquiterpenlactones, sterols, and terpenoids in several extracts tested. Almost all of them were lethal against brine shrimp *Artemia salina*, especially polar extracts of the fruit (LC_{50} = 17.82 $\mu g \cdot mL^{-1}$), which suggest the presence of possible potent pharmacological agents in this *Helicteres* specie. Additionally, these extracts showed a sight antimicrobial activity, inhibiting the growth of several bacteria (*Micrococcus lutens*, *Enterobacter cloacae*, *Acinetobacter calcoaceticus*, *Bacillus subtilis*) and fungus (*Aspergillus oryzae*, *Curvularia lunata*, *Mucor* sp.). The results obtained indicate that *H. guazumifolia* Kunth collected in Venezuela is a promising source of bioactive secondary metabolites with pharmacological activity.

Keywords: *Helicteres*, secondary metabolites, cytotoxicity, antimicrobial activity

1. Introduction
Majority of ethnobanical studies has been focused on Sterculiaceae family, which has shown being a source of secondary metabolites with biological properties [1]. A previous study done on *Helicteres baruensis* Jacq collected in the eastern part of Venezuela showed the presence of sterols and alkaloids, and antibacterial activity of the petroleum ether extract against *Salmonella enteritidis*, and of the chloroform extract against *S. enteritidis* and *Bacillus cereus* bacterial strains [2].
The specie *Helicteres guazumifolia* Kunth ("tornillo", "tornillito", "guácimo tornillo") belongs to Sterculiaceae family, and is widely distributed in several regions of Venezuela [3, 4, 5, 6]. Although this specie is used in folk medicine by several communities of Sucre, state, there have been no reports on the biological or phytochemical investigation of this species until now to the best of our knowledge, excepting the constituents of essential oil from its leaves [7]. The purpose of this study was to investigate the phytochemical composition, as well as the antimicrobial and lethal activity of extracts of *Helicteres guazumifolia* Kunth.

2. Materials and Methods
2.1 Sampling
Sample of *H. guazumifolia* was collected in the way between Cumaná city and San Juan de Macarapana sector, Sucre state, Venezuela (Coordinates: 10°38'44''N, 63°02'20''W/Altitude m SNM: 43). Taxonomic identification was realized in the herbarium "Isidro Ramón Bermúdez Romero" (IRBR) of Biology Department of *Universidad de Oriente*, Sucre Campus, Venezuela.

2.2 Extracts
Each botanical part (leaves, fruits, stems, and flowers) was separated from others, dried and powdered, which were extracted with petroleum ether (Class 1A, Fisher Chemical, Fair Lawn, New Jersey, USA). In each case, solvent was evaporated under vacuum in a rotary evaporator Heidolph (~11 mbar, 40 °C), obtaining the lipid crude extracts (PEE). The residues were re-extracted with pure methanol (Fluka Chemie, Seelze, Germany), and after separated the respectively solvent, anhydrous sodium sulfate (Fisher Chemical, Fair Lawn, New Jersey, USA) was added to dry (~5 g/100 mL of solvent). Then, each methanolic filtrate was concentrated under the same conditions to obtain polar crude extracts (ME).

2.3 Phytochemical screening
The phytochemicals screening of plants were performed as per well-established protocols [8, 9].

Reagents used were: Dragendorff (bismuth nitrate in nitric acid and aqueous potassium iodine) for alkaloids, Liebermann-Burchard (acetic anhydride and chloroform with concentrated sulfuric acid) for sterols and triterpenoids, Baljet (picric acid in ethanol and aqueous sodium hydroxide) for sesquiterpenlactones; aqueous ferric chloride for polyphenols, gelatin for tannins, and Shinoda (magnesium and concentrated hydrochloric acid) for flavonoids.

2.4 Brine shrimp (*Artemia salina*) lethality assay

This bioassay was used to detect possible pharmacological properties of extracts through the toxicity or lethality estimation [10]. Solution of 10,000 $\mu g \cdot mL^{-1}$ of each essential oil was prepared using a mix of dimethyl sulfide and sterile sea water (1:1 v/v). Then, dilutions of 1,000.00; 100.00; 1.00, 0.10, and 0.01 $\mu g \cdot mL^{-1}$ were prepared. The same solvent system and dilutions were used as negative controls. Ten nauplii of *A. salina* brine shrimp were put into vials containing those solutions. Bioassay was carried out by triplicate. Mortality quantification after 24 h and 48 h was used to calculate median lethal concentration (LC_{50}) according to statistical program [11].

2.5 Antimicrobial assay

Potential antibacterial [12] and antifungal [13] properties of extracts were evaluated as follow. Sterile discs of filter paper (Whatman Nº 3) of 10 mm of diameter were impregnated with 25µl of extracts (40 $mg \cdot mL^{-1}$) and put on Petri plates with Müller-Hinton for antibacterial activity or PDA agar for antifungal activity determinations, which were previously inoculated with microbial suspensions (10^8 $cells \cdot mL^{-1}$, McFarland 0.5). Plates with bacteria were pre-incubated at 5°C for 12 h and then incubated at 37°C for 24 h; while those with fungus were incubated at room temperature for 48 h. Antimicrobial effect was evidenced by diameter of inhibition halo (mm) around the discs.

3. Results & Discussion (Times New Roman, 12, Bold)

As shown in table 1, methanol extracts (ME) were obtained with major yields (1.58-9.36 %) respect to petroleum ether extracts (PEE) (0.11-3.04 %). In both group, leaves provided major mass (3.04 % PEE-L, 9.36 % ME-L), followed by fruits (2.60% PEE-Fr, 4.17 % ME-Fr). These results suggest that leaves of *H. guazumifolia* have constituents more soluble in solvents used (or more easily extractable with them), respect to the other aerial organs. Besides, polar constituents are in major proportion in this *Helicteres* specie.

Table 1: Yield of extraction of areal parts of *H. guazumifolia* Kunth

Crude extract	Extraction yield* (%)
PEE-L	3.04
PEE-S	0.11
PEE-Fr	2.60
PEE-Fl	0.51
ME-L	9.36
ME-S	1.58
ME-Fr	4.17
ME-Fl	3.97

PEE: petroleum ether extract, ME: methanol extract, L: leaves, S: stems, Fr: fruits, Fl: flowers, *: calculated on basis to dried weight of the respectively botanical part.

Preliminary phytochemical screening revealed the possible presence of alkaloid in all of extracts, while saponins were detected only in methanol extracts (table 2). Polyphenols, tannins and flavonoids were not detected in any extract (table 2), which could indicate that the biosynthesis of this kind of compounds do not occur in this specie or their concentration were very low to be detected by methods for functional groups. Sterols were detected in petroleum ether extract of leaves (PEE-L) and methanol extract of stems (ME-S), terpenoids were detected in PEE-L and methanol extract of fruits (ME-Fr), sesquiterpenlactones and cardiatonic glycosides only were detected in PEE-L and ME-S, respectively (table 2). Detection of several kinds of metabolites has been reported in other *Helicteres* species [2, 14, 15].

Table 2: Secondary metabolites detected in crude extracts of *H. guazumifolia* Kunth

Family of metabolite	Crude extracts							
	PEE-L	PEE-S	PEE-Fr	PEE-Fl	ME-L	ME-S	ME-Fr	ME-Fl
Alkaloids	+	+	+	+	+	+	+	+
Sterols	+	–	–	–	–	+	–	–
Terpenoids	+	–	–	–	–	–	+	–
Sesquiterpenlactones	+	–	–	–	–	–	–	–
Flavonoids	–	–	–	–	–	–	–	–
Polyphenols	–	–	–	–	–	–	–	–
Tannins	–	–	–	–	–	–	–	–
Cardiotonic glycosides	–	–	–	–	–	+	–	–
Saponins	–	–	–	–	+	+	+	+

PEE: petroleum ether extract, ME: methanol extract, L: leaves, S: stems, Fr: fruits, Fl: flowers,. +: detected, –: not detected.

Almost all extracts of *H. guazumifolia* Kunth evidenced lethal activity against *Artemia salina* ($LC_{50} < 1{,}000.0$ $\mu g \cdot mL^{-1}$) until 48 h of exposition, except petroleum ether extract of flowers (PEE-Fl), which was not active at any concentrations used in this bioassay (table 3). This bioassay has correlation with cytotoxic activities [10, 16], which suggest the presence of potential cytotoxic agent in *H. guazumifolia*, such as other extracts of genus *Helicteres* [17, 18].

At the first 24 h, methanol extracts of stem and fruit (ME-S, ME-Fr) were not bioactive against *A. salina*, and methanol extract of leaves (ME-L) showed the highest lethal activity (which had the lowest LC_{50}), followed by petroleum ether extract of fruit (PEE-Fr). However, up to 48 h, ME-Fr was the most bioactive or toxic extract (LC_{50} of 17.82), followed by methanol extract of flowers (ME-Fl, LC_{50} of 18.57). Majority of extracts, excepting PEE-Fl, increased their lethality against *A. salina* from 24 to 48 h (16.81-97.34%). According to CYTED categories [19, 20], ME-Fr and ME-Fl extracts can be considered extremely toxic, and ME-L and PEE-Fr extracts considered highly toxic.

Some of these extracts were more active against *A. salina* than reported for methanol extracts of *H. vegae* [21]; in that study this kind of biological activity was associated to the presence of phenolic compounds. Two pregnane derivatives

and a quinolone alkaloid have been reported from *H. angustifolia*, which showed anti-proliferative activity [22]. In this sense, lethal effect of extracts against *A. salina* could be due to the alkaloids present in *H. guazumifolia* (table 2), because of its well-known toxic effect [23].

Table 3: Lethal activity of crude extracts of H. guazumifolia Kunth against Artemia salina

Crude extract	LC_{50} (µg·mL^{-1})		% IA
	24 h	48 h	
PEE-L	972.42	725.86	25.36
PEE-S	902.82	230.35	74.49
PEE-Fr	143.93	99.55	30.83
PEE-Fl	> 1,000.0	> 1,000.0	–
ME-L	77.92	64.82	16.81
ME-S	> 1,000.0	155.31	–
ME-Fr	> 1,000.0	17.82	–
ME-Fl	698.71	18.57	97.34

PEE: petroleum ether extract, ME: methanol extract, L: leaves, S: stems, Fr: fruits, Fl: flowers, LC_{50}: median lethal concentration, IA: increasing of activity, –: not determined.

Table 4 shows zones of inhibition caused by extracts on growth of microorganisms employed, which were among 11 to 15 mm of diameter. Lipid extracts (PEE-L, PEE-S, PEE-Fr and PEE-Fl) affected several of microorganisms (53.85-61.54 %), and were more bioactive against tested microorganisms than polar extracts (ME-L, ME-S, ME-Fr and ME-Fl), which affected less microorganisms (23.08-46.15%). *Pseudomonas aeruginosa* bacteria and *Candida albicans* fungus were resistant to all extracts at concentration used, while only the pathogen fungi *Curvularia lunata* and *Mucor* sp. showed sensibility to all extracts. Between 25-75% of the extracts showed antimicrobial activity against the remaining microorganisms tested (table 4).

Methanol extracts exhibited more antifungal activity against fungi than petroleum ether extracts, which is similar to antifungal properties reported for extracts of *H. isora* stem [24]. Other studies about antimicrobial properties of *H. isora* indicated that leaves, stems and fruits contain potent antimicrobial agents [25, 26]. Thus, antimicrobial activity exhibited by *H. guazumifolia* could indicate potential of this *Helicteres* specie as source of pharmacological secondary metabolites. Based on the all results obtained, could be say that the extracts have a great pharmacological potential.

Table 4: Antimicrobial activity of crude extracts of *H. guazumifolia* Kunth

Microorganism	Diameters of inhibition halos* (mm)								%EA
	PEE-L	PEE-S	PEE-Fr	PEE-Fl	ME-L	ME-S	ME-Fr	ME-Fl	
Bacteria									
Bacillus subtilis (ICTA07)	12	11	12	12	–	–	–	–	50.00
Staphylococcus aureus (IBE-DOC19)	–	–	11	11	–	–	–	–	25.00
Escherichia coli (ATCC25922)	–	11	11	–	–	–	–	–	25.00
Pseudomonas aeruginosa (ATCC27853)	–	–	–	–	–	–	–	–	0.00
Acinetobacter calcoaceticus (ATCC 23055)	11	12	12	12	–	–	11	–	62.50
Enterobacter cloacae (DAI268JI)	11	12	12	15	11	–	–	–	62.50
Micrococcus lutens (Bionálisis 33810)	–	–	11	11	–	–	–	–	25.00
Fungi									
Aspergillus niger (phyto-pathogen)	–	–	–	–	11	–	11	–	25.00
Aspergillus oryzae (phyto-pathogen)	12	13	–	–	13	14	12	13	75.00
Penicillium expansum (phyto-pathogen)	11	11	–	–	–	–	11	11	50.00
Curvularia lunata (pathogen)	11	11	11	11	11	11	11	11	100.0
Mucor sp. (pathogen)	11	11	11	11	11	11	11	11	100.0
Candida albicans (pathogen)	–	–	–	–	–	–	–	–	0.00
%MS	53.85	61.54	61.54	53.85	38.46	23.08	46.15	30.77	

PEE: petroleum ether extract, ME: methanol extract, L: leaves, S: stems, Fr: fruits, Fl: flowers. MS: Microorganisms sensible to specific extract. EA: Extracts active against specific microorganism. *: included diameter of discs (10 mm). –: inactive.

4. Conclusions

Petroleum ether and methanol extracts of *Helicteres guazumifolia* aerial parts contain bioactive secondary metabolites, which could be important pharmacological agents. Alkaloids and saponins, which were principal constituents detected in extracts of *H. guazumifolia*, could be responsible of bioactivity observed. Leaves and fruits are the main botanical organs to be a promising source of these bioactive constituents, especially those with polar nature.

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