Antimicrobial, antioxidant, antitumor activities of *Guatteria elliptica* R. E. Fries (Annonaceae) alkaloids and their safety

Agnieszka K. Rajca-Ferreira[a*], Telma M. Kaneko[a], Adilson Sartoratto[b], Damila R. de Morais[c], Marcos N. Eberlin[c], Patricia S. Lopes[d], Ivana B. Suffredini[e], Paulo R. H. Moreno[a,f]

Abstract— *Guatteria elliptica* R. E. Fries (Annonaceae), a native Brazilian plant from the Atlantic Rainforest, belongs to a genus known for its pharmacological properties and a wide diversity of alkaloids with biological activities. However, it has not been assayed for its biological activities and alkaloids. The study aimed to identify its alkaloids and test their antimicrobial, antioxidant, antitumor activities and safety. *G. elliptica* leaves and branches were extracted to obtain the crude extracts and total alkaloids. The total alkaloids were analyzed by GC-MS. The branch alkaloids were chromatographically fractionated yielding two samples that were then analyzed by GC-MS and LC-MS/MS. The extracts and purified fractions were evaluated for their antimicrobial and antioxidant activities. In vitro antitumor activity was determined on cultured breast and prostate tumor cells (MCF-7 and PC-3, respectively). Their safety was evaluated on a normal fibroblast cell line (BALB/c 3T3, ATCC CCL 163). As *G. elliptica* alkaloids, it was possible to identify nine aporphines (nornuciferine, stepharine, corytuberine, asimilobine, dehydronantenine, glaunidine, liriodenine, oliverine *N*-oxide and telkovine) and two protoberberines (discretamine and caseadine). Caseadine, glaunidine, oliverine *N*-oxide and telkovine were not previously reported for other Guatteria spp. The total alkaloids showed moderate antimicrobial and antioxidant activity and a high antitumor potential (IC_{50}=9.32-22.06 µg/mL). The antitumor activity of isolated samples was stronger (IC_{50}=1.37-13.66 µg/mL) than for total alkaloids. All tested samples showed low cytotoxicity against normal fibroblast cell lines. This work showed that *G. elliptica* possesses alkaloids with biological activities and is an interesting target for further phytochemical studies.

Index Terms— Annonaceae, antimicrobial activity, antioxidant activity, antitumor activity, cytotoxic activity, *Guatteria elliptica*, medicinal plants

1 Introduction

Annonaceae, a pantropical plant family, is found throughout the world, especially in tropical lowland evergreen forests. It is a known source of raw materials for cosmetics and perfumery, edible fruits and medicinal plants. Concerning the medicinal uses, many species are traditionally used in India, Malaysia, Philippines, Indo-China and Latin America as febrifuge, anthelmintic, anti-emetic, anti-inflammatory, in urinary and uterine discharges, piles and lumbago, among others [1], [2].

This family contains about 120 genera and more than 2,000 species of trees and shrubs. The largest and most complex genus is Guatteria, which comprises ca. 260 species dispersed from Southern Mexico to Southern Brazil [3]. Guatteria is known for pharmacological properties and a wide diversity of alkaloids. Until now, approximately 130 alkaloids were isolated and identified from this genus. Most of these alkaloids presented an aporphine scaffold, but other types were also reported [4]. Several of these alkaloids presented important biological properties such as antimicrobial, antitumor, antiparasitic, antimalarial and antioxidant activities.

Guatteria elliptica R. E. Fries (Annonaceae) is an endemic Brazilian species which has not yet been chemically investigated. There are only some preliminary studies reported concerning the crude extracts biological activities [5], [6]. Thus, the aim of this work was to analyze the alkaloid composition from *G. elliptica* leaves and branches, as well as its biological activities (antimicrobial, antioxidant, antitumor) and safety.

2 Material and Methods

2.1 Plant material

Guatteria elliptica R. E. Fries branches and leaves were collected from nine individuals in July 2012 in an Atlantic Forest environmental conservation area, located in Paranapiacaba, Santo André, SP, Brazil (23° 46′ 41′′ S and 46° 18′ 16′′ W). All collected plants were taxonomically confirmed by Dr. Inês Cordeiro, a voucher specimen (LIMA 1790) was deposited in the herbarium of the Botanical Institute of Sao Paulo. Fresh plant material was cleaned of impurities and then dried at room temperature for about 3 weeks. Later, the dried leaves and branches were separately ground, using the Knife Mill, Croton type (Tecnal, TE 625).

2.2 Crude extracts

Crude extracts were obtained from the dried ground leaves (1704.2g) and branches (4263.5g) separately using ethanol

- [a] *School of Pharmaceutical Sciences, University of São Paulo, CEP 05389-970, São Paulo, SP, Brazil*
- [b] *Chemical, Biological and Agricultural Pluridisciplinary Research Center, Campinas State University, CEP 13081-970, Campinas, SP, Brazil*
- [c] *Thomson Mass Spectroscopy Laboratory, Institute of Chemistry, University of Campinas, CEP 13083-970, Campinas, SP, Brazil*
- [d] *Department of Exact Sciences and Earth, Federal University of Sao Paulo, CEP 09913-030, Diadema, SP, Brazil*
- [e] *Laboratory of Extraction, University Paulista, CEP 01310-100, Sao Paulo, SP, Brazil*
- [f] *Institute of Chemistry, University of Sao Paulo, PO Box 26077, 05513-970, Sao Paulo, SP, Brazil*
- **Corresponding author: e-mail: arajcaferreira@gmail.com, Tel: (+55) 11960700515, address: Institute of Chemistry, University of Sao Paulo, PO Box 26077, 05513-970, Sao Paulo, SP, Brazil*

(92%) as solvent in the Accelerated Solvent Extractor 300, DIONEX (1500psi, 60oC, 15min, 30g/cell, 15min/cell). Later, the ethanol extracts were concentrated under vacuum and dried in a desiccator.

2.3 Total alkaloid extraction

Crude extracts were re-suspended separately in a hydrochloric acid solution (10% v/v) and filtered, resulting in the acidic aqueous solutions and the remaining crude alkaloids-free extracts. The acidic aqueous solutions were then washed with n hexane to remove lipophilic substances. These solutions were brought to pH 9 with ammonium hydroxide (25% v/v) and then extracted with chloroform, until the negative reaction with the Dragendorff's reagent. The chloroform fractions were concentrated and dried, giving the total leaf and branch alkaloids.

2.4 Alkaloids isolation

The branch alkaloids were fractionated by the Solid Phase Extraction (SPE), using the reverse phase cartridge C18 (Perkin Elmer, N9306479). The conditioning and elution conditions were established by the supplier. For the elution, we used a mixture methanol: ammonium hydroxide 2.5% aq. in the following proportions: (% v/v) 5:95, 10:90, 20:80, 30:70, 40:60, 50:50, 70:30 and 100% MeOH, yielding fractions F1-F8 that were further purified by preparative TLC. The eluent systems were: chloroform-methanol (9:1 for F1-F4, 95:5 for F5, 98:2 for F6-F8). The final purification was performed using chloroform-ethyl acetate (7:3). Samples A and B were isolated from the branch fractions F6 and F7.

2.5 GC-MS analysis

The total leaf and branch alkaloids and Sample A were analyzed using a gas chromatograph (Agilent Technologies model 6890N) coupled to a mass spectrometer (Agilent Technologies model 5975), equipped with an HP5-MS capillary column (30 m x 0.25 mm x 0.24 μm). The samples dissolved in methanol were analyzed (1 μL) in the following conditions: injector temperature 290ºC, initial temperature 100ºC (1 min), final temperature 290ºC (10 min), heating rate 15 ºC/min, gas flow (He) 1 mL/min, flow division 1:40. The compound identification was performed by comparing with the Wiley 275 library and literature.

2.6 LC-MS/MS analysis

Sample B was analyzed using Accurate-Mass Quadrupole Time-of-Flight (Q-Tof) LC/MS (G6550A), Agilent Technology, equipped with the Zorbax Extend C18 column (2.1 x 50 mm x 1.8 μm; Agilent Technologies). The mobile phase was composed from A-water and B-methanol and the mobile phase flow was 0.2 mL/min. The gradient was programmed to as follow: 0-7 min 60% A, 7-10 min 95% A, 10-14 min 20% A e 14-16 min 95% A. The samples dissolved in methanol were analyzed (1 μL) in the following conditions: gas temperature 250ºC, gas flow 11 mL/min, nebulizer 35 psig, sheath gas temp 325 ºC, sheath gas flow 10 mL/min, scan rate 1.0 scan/s, mass range 50-1500 m/z. The compound identification was performed by comparing fragmentation patterns with literature.

2.7 Antimicrobial activity

Antimicrobial activity against *Aspergillus brasiliensis* (ATCC 16404), *Candida albicans* (ATCC 10231), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 8739) was evaluated using the broth microdilution method [7]. Samples were diluted in MeOH-DMSO (1:1) to the concentration 1 mg/mL in well. The change in growth rate was assessed by the change in turbidity of the microbial suspension and it was measured by the microplate reader LM DGL (DGL Biotechnology, 630nm). The growth inhibition reading for *A. brasiliensis* was done visually after 3 and 5 days. In all assays, antibiotics were used as positive controls (Nystatin for fungi; Ciprofloxacin and Erythromycin for bacteria). Samples that were not able to inhibit the microorganism growth were considered inactive and their study was discontinued. Active samples were serially diluted and every concentration was tested in triplicates and the experiment was repeated 3 times. The results were statistically processed by Origin® 9.1 software. The dose-response inhibition curves were obtained and MICs were calculated. Samples that no growth was detected were subcultured, in triplicates, into agar plates to determine Minimum Bactericidal Concentration (MBC).

2.8 Antioxidant activity

The antioxidant activity evaluation was performed by the DPPH scavenging method in 96-well plates [8]. For the preliminary test, samples were diluted in methanol to the final concentration 100 μg/mL in well. The free radical scavenging activity was determined using the Polaris® (Celer) plate reader (517 nm). Quercetin was used as a positive control. Every well contained 50 μL of sample dissolved in methanol and 150 μL of 200 μmol/L DPPH dissolved in the same solvent. Appropriate control and sample blanks were also tested. After adding the DPPH solution to each well, the plates were incubated at room temperature in darkness for 20 min. Samples that did not show inhibition were not further assessed. Active samples were serially diluted and every concentration was tested in triplicates. The results were statistically analyzed by Origin® 9.1 software. The obtained dose response inhibition curves were used to calculate EC_{50}.

2.9 Antitumor activity

The antitumor activity against human breast carcinoma (MCF-7) and prostate carcinoma (PC-3) cell lines was evaluated by the Sulforhodamine B assay [9]. The cells were cultivated in RPMI 1640 (Bio Whittaker) medium with 10% fetal bovine serum and 1% L glutamine (Sigma) for 24 hours. Samples were dissolved in DMSO-H_20 (1:1) and tested six-fold. Doxorubicin was used as a positive control. The IC_{50} were obtained by nonlinear regression using the GraphPad Prism 5.0 software.

2.10 Cytotoxic activity

The cytotoxicity against the normal cell line derived from mouse fibroblasts (BALB/c 3T3, ATCC CCL-163) was tested using the MTS method [10]. Cells were grown in the Dubelcco's Modified Eagle Medium supplemented by antibiotics and antimycotics (penicillin 100 IU/mL, streptomycin 100 mg/mL

and amphotericin 0.025 mg/mL), 4 mM glutamine and 10% (v/v) fetal bovine serum. Samples were compared to appropriate controls (solvent, culture medium and culture medium+cells). The absorbance reading (490 nm) was performed using the BioTek (Synergy HT) [10]. Obtained results were evaluated by the Statistical Software Phototaxy®, Version 2.0. The cell viability dose-response curves were determined and the IC_{10} and IC_{50} values were calculated. The IC_{50} values were used to estimate the median lethal dose (LD_{50}) [10], [11]. Based on LD_{50} thus obtained, the acute oral toxicity of the samples was estimated using the United Nations Globally Harmonized System (GHS) [12].

3 Results and Discussion

From the ground material, crude extracts were obtained with the yield of 12.6% for leaves and 4.3% for branches. The total alkaloid yields were 0.4% for leaves and 0.1% for branches, calculated in relation to the dried plant material. The chemical analysis of the total alkaloids from the leaves and branches were conducted by GC-MS (Fig. 1 A e B, respectively). The alkaloids characterization was done with the comparison of the fragmentation pattern with the Wiley library and literature; all the structures found are represented and numbered (1-11) in Fig. 2.

The leaf alkaloids chromatogram showed the presence of two major compounds: RT=14.14 min (38.50%) and RT=14.75 min (20.72%). The compound with RT=14.14 min could be identified as nornuciferine (2, aporphine), while the second one did not show a recognizable fragmentation pattern. Thus, it should be isolated and analyzed by NMR in a future study. Leaf alkaloids also contained stepharine (3, aporphine) and discretamine (5, protoberberine). In branches, the major alkaloids were nornuciferine (2, RT=14.1 min, 16.09%), as in the leaves, and asimilobine (1, RT=13.9 min, 13.53%). Additionally, in branches were also found the aporphines corytuberine (4) and stepharine (3) plus the protoberberines discretamine (5) and caseadine (6). Three alkaloids identified in leaves (2, nornuciferine; 3, stepharine and 5, discretamine) were also present in branches, showing similarities in alkaloid composition. In total, the GC-MS analysis from *G. elliptica* total alkaloids allowed the identification of four aporphines: nornuciferine (2, leaves and branches), stepharine (3, leaves and branches), corytuberine (4, branches) and asimilobine (1, branches) and two protoberberines: discretamine (5, leaves and branches) and caseadine (6, branches). The results of GC-MS analysis, with RT, molecular formula, experimental m/z, main MS fragments and species in which the alkaloids were previously reported, are given in Table 1 [3], [4], [13], [14], [15], [16], [17]. The leaf and branch alkaloids showed similar composition, but the branches presented more alkaloids. For this reason, only this plant part was considered for the isolation procedure that afforded two samples (Sample A and B). Sample A was composed by the two major alkaloids nornuciferine (2) and asimilobine (1) (Figure 4) as indicated by GC-MS, with the respective areas of 72 and 21%. The other alkaloid fraction, Sample B, was necessary to be analyzed by LC-MS/MS due to its low volatility. The structure identification was based on a similar fragmentation pattern found in literature for aporphines and protoberberines [18], [19]. In this sample, the LC-MS/MS analysis showed the presence of six alkaloids, five aporphines (8, dehydronantenine; 9, glaunidine; 11, liriodenine; 7, oliverine *N*-oxide and 10, telkovine) and one protoberberine (2, caseadine). From these alkaloids, caseadine (2), glaunidine (9), oliverine *N*-oxide (7) and telkovine (10) were not previously reported for Guatteria. The results of the LC-MS/MS analysis, with the respects RT, molecular formula, experimental m/z, main MS/MS fragments and species in which the alkaloids were previously reported, are shown in Table 2 [14], [16], [17], [18], [19], [20], [21], [22], [23]. The two alkaloid types found in *G. elliptica,* aporphines and protoberberines, are characteristic for this genus [4].

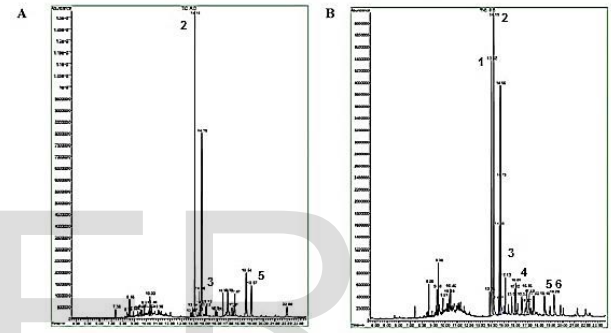

Fig. 1. GC-MS chromatograms of the *Guatteria elliptica* total alkaloids: A – leaf total alkaloids, B - branch total alkaloids: 1-asimilobine, 2-nornuciferine, 3-stepharine, 4-corytuberine, 5-discretamine, 6-caseadine

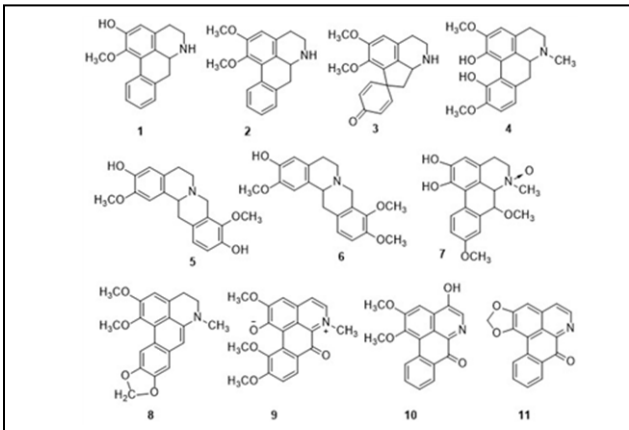

Fig. 2. Structure of alkaloids identified in *Guatteria elliptica* by GC-MS and LC-MS/MS: 1-asimilobine, 2-nornuciferine, 3-stepharine, 4-corytuberine, 5-discretamine, 6-caseadine, 7-oliverine *N*-oxide, 8-dehydronantenine, 9-glaunidine, 10-telkovine, 11-liriodenine

TABLE 1
ALKALOIDS IDENTIFIED IN *GUATTERIA ELLIPTICA* LEAVES AND BRANCHES USING GC-MS ANALYSIS

nº	RT (min)	Molecular formula	m/z $M^{+\cdot}$	Main MS fragments	Proposed alkaloid	Previously reported
1	13.9	$C_{17}H_{17}NO_2$	267	266, 252, 250, 236, 224, 193	asimilobine	*G. scandens*
2	14.1	$C_{18}H_{19}NO_2$	281	280, 266, 252, 250, 237, 221, 178, 165	nornuciferine	*G. blepharophylla, G. hispida,*
3	15.1	$C_{18}H_{19}NO_3$	297	296, 281, 269, 268	stepharine	Xylpia parviflora (Annonaceae)
4	16.8	$C_{19}H_{21}NO_4$	327	326, 312, 310, 296, 284, 281, 269, 253, 207	corytuberine	*G. goudotiana*
5	18.9	$C_{19}H_{21}NO_4$	327	296, 178, 151, 150, 135	discretamine	*G. discolour, G. scandens*
6	19.2	$C_{20}H_{23}NO_4$	341	340, 178, 176, 164	caseadine	*Dasymaschalon sootepense* (Annonaceae)

RT=retention time, m/z=mass to charge ratio

TABLE 2
ALKALOIDS IDENTIFIED IN SAMPLE B, ISOLATED FROM GUATTERIA ELLIPTICA BRANCHES, USING LC-MS/MS

nº	RT (min)	Molecular formula	m/z $(M+H)^+$	Main MS/MS fragments	Proposed alkaloid	Previously reported
6	5.0	$C_{20}H_{23}NO_4$	342.17	342, 327, 192, 178, 165	caseadine	*Dasymaschalon sootepense* (Annonaceae)
7	5.4	$C_{20}H_{21}NO_5$	356.19	340, 192	oliverine *N*-oxide	*Greenwayodendron suaveolens* (Annonaceae)
8	6.3	$C_{20}H_{19}NO_4$	338.14	323, 294	dehydronantenine	*G. goudotiana*
9	6.8	$C_{20}H_{17}NO_5$	353.15	336, 308	glaunidine	*Glaucium fimbrilligerum* (Papaveraceae)
10	7.6	$C_{18}H_{13}NO_4$	308.15	307, 275, 195	telkovine	*Telitoxicum krukovii*
11	8.2	$C_{17}H_9NO_3$	276.06	248	liriodenine	*G. friesiana, G. blepharophylla*

RT=retention time, m/z=mass to charge ratio

In the antimicrobial assay, samples were serially diluted, had their MICs and CBMs determined and those with MIC > 1 mg/mL were not considered active (Table 3). It can be noticed that the crude extract did not show activity to any tested microorganism. However, the total alkaloids obtained from these extracts were more active (Table 3) and the strongest activity was found against *S. aureus* with MICs/MBCs=0.21±0.01/0.28 and 0.12±0.01/0.26 mg/mL (leaves and branches, respectively). Our results showed that *G. elliptica* alkaloids were more active than *G. friesiana* stem alkaloids against *C. albicans* (>1.0 mg/mL), *E. coli* (0.750 mg/mL) and *S. aureus* (0.750 mg/mL) [24]. Only the activity against *P. aeruginosa* (MIC=0.375 mg/mL) was stronger than in our study [24]. Despite the activity, the *G. elliptica* total alkaloids cannot be consider promising for new antibiotic development as their MIC exceeded 100 µg/mL [7].

TABLE 3
ANTIMICROBIAL ACTIVITY OF *GUATTERIA ELLIPTICA* EXTRACTS AND ALKALOIDS AGAINST *CANDIDA ALBICANS* (ATCC 10231), *ASPERGILLUS BRASILIENSIS* (ATCC 16404), *STAPHYLOCOCCUS AUREUS* (ATCC 6538), *PSEUDOMONAS AERUGINOSA* (ATCC 9027) AND *ESCHERICHIA COLI* (ATCC 8739)

	C. albicans		*A. brasiliensis*		*S. aureus*		*E. coli*		*P. aeruginosa*	
	MIC±IC_{95}	MBC	MIC±IC_{95}	MBC	MIC±IC_{95}	MBC	MIC±IC_{95}	MBC	MIC±IC_{95}	MBC
CE-L	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00
CE-B	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00	>1.00
TA-L	0.58±0.06	0.66	>1.00	>1.00	0.21±0.01	0.28	0.31±0.01	>1.00	0.85±0.35	>1.00
TA-B	0.84±0.14	1.00	>1.00	>1.00	0.12±0.01	0.26	0.44±0.02	0.62	>1.00	>1.00
Sample A	na	na	na	na	na	na	na	na	na	na
Sample B	na	na	na	na	na	na	na	na	na	na

MIC=Minimum Inhibitory concentration (mg/mL), MBC=Minimum Bactericidal Concentration (mg/mL), CI_{95}=95% confidence interval, CE=crude extract, TA=total alkaloids, L=leaves, B=branches, na=not applicable

In the antioxidant assay, the leaf and branch crude extracts presented EC_{50}=48.92±4.53 and 42.00±6.29 µg/mL, respectively (Table 4). The activity of leaves and branches total alkaloids were like the crude extracts (EC_{50}=42.48±2.54 and 52.11±6.51 µg/mL, respectively). These could indicate that the crude extracts activity was not only due to alkaloids and there might be other secondary metabolites with this activity. When compared with the positive control quercetin (EC_{50}=3.61±0.26 µg/mL), the *G. elliptica* antioxidant activity was much lower. Similarly, previous research showed that the crude extract of the leaves and bark of *G. megalophylla* and *G. modesta* were not active [25]. Only pure alkaloids isolated from the *G. hispida* trunks (9-methoxyisomoschatoline, *O*-methylmoschatoline, lysicamine, liriodenine, nornuciferine, coreximine and isocoreximine) possessed antioxidant activity more potent than quercetin [3].

Antitumor activity was frequently found in *Guatteria* spp., thus the *G. elliptica* extracts, total alkaloids and isolated fractions (Sample A and B) were investigated for this activity. Sample A and B were investigated for antitumor and cytotoxic activities only because of their small amount isolated. Only samples with IC_{50}<30 µg/mL were considered active because this concentration range in in vitro assay is promising for the anticancer drugs development [26]. From the crude extracts, only the one from leaves was active against MCF-7 (IC_{50}=27.00±0.27µg/mL) (Table 4). In a previous study, extracts from four Annona species (*A. cherimola*, *A. aquamosa*, *A. muricata* e *A. glabra*) presented higher activity (IC_{50}=3.43-18.20 µg/mL) than *G. elliptica*, against the same cell line [27]. Total leaf and branch alkaloids presented stronger activity than crude extracts (Table 4). The leaf alkaloids were very active against MCF-7 (IC_{50}=9.32±0.36µg/mL), while against PC-3 both, leaf and branch alkaloids, presented similar activity. Sample B had antitumor activity increased almost 10-fold when compared to the total alkaloids against MCF-7 (IC_{50}=2.28±0.18 µg/mL) and PC-3 (IC_{50}=1.37±0.36 µg/mL). However, Sample A was ca. twice more active than the total alkaloids against MCF-7 (13.66±0.34 µg/mL) while for PC-3 there was no much increase in activity. These results indicate

TABLE 4
ANTIOXIDANT, ANTITUMOR AND CYTOTOXIC ACTIVITY OF THE *GUATTERIA ELLIPTICA* EXTRACTS, TOTAL ALKALOIDS AND ISOLATED SAMPLES

	Antioxidant	Antitumor		Cytotoxic		LD_{50} estimated	Acute oral toxicity category
Sample	EC_{50}±CI_{95}	IC_{50}±SE MCF-7	IC_{50}±SE PC-3	IC_{50}±SD BALB/c 3T3	IC_{10}±SD BALB/c 3T3		
CE-L	48.92±4.53	27.00±0.27	>30	63.53±9.4	49.45±1.71	495.12	4
CE-B	42.00±6.29	>30	>30	>453.36	293.09±49.11	>1028.48	4 or 5*
TA-L	42.48 ±2.54	9.32±0.36	22.06±0.14	44.61±2.52	8.48±1.80	434.10	4
TA-B	52.11±6.51	21.66±0.15	18.08±0.24	>68.80	27.03±0.36	>510.01	4 or 5*
Sample A	na	13.66±0.34	12.77±0.25	>43.39	3.79±0.92	>429.64	4 or 5*
Sample B	na	2.28±0.18	1.37±0.36	>4.92	2.78±0.12	>191.17	4 or 5*
Q	3.61±0.26	na	na	na	na	na	na
D	na	0.07 ±0.15	0.30 ±0.08	na	na	na	na

*EC_{50}=concentration required to obtained 50% free radical scavenging activity (µg/mL), IC_{50}=concentration required to obtained 50% inhibition of cell growth (µg/mL) , IC_{10}=concentration required to obtained 10% inhibition of cell growth (µg/mL), LD_{50}= median lethal dose (mg/kg) , SE=standard error, SD=standard deviation, CI_{95}=95% confidence interval, MCF-7= human breast cancer cell line, PC-3= human prostate cancer cell line, BALB/c3T3=mouse normal fibroblast cell line, CE=crude extract, TA= total alkaloids, L= leaves, B= branches, Q=quercetin, D=Doxorubicin, *-category estimated approximately based on inaccurate LD_{50} value, na= not applicable*

that the synergism of some alkaloids in different proportions could be further investigated in anticancer drug development studies, not only an isolated pure compound.

Our study demonstrated that some *G. elliptica* samples showed IC_{50}<30 µg/mL against MCF-7 and/or PC-3 and could be promising for the new anticancer drugs development. However, they could also be toxic to normal cells, causing unwanted effects [28]. Therefore, an *in vitro* cytotoxicity assay against normal cells was performed to assess the safety of the samples. The cytotoxic activity against normal cells allowed to estimate the LD_{50} values, representing the acute oral toxicity. All the *G. elliptica* samples could be placed in categories 4 or 5 of the United Nations Globally Harmonized System for toxicity hazards, which characterize these extracts and fractions as safe after oral acute exposure [12]. To further assess the sample safety, additional *in vitro* tests should be performed, including systemic and organ specific toxicity, such as neuro-, nephro- and hepatotoxicity, as well as intestinal absorption, distribution, metabolism, among others [29].

4 CONCLUSIONS

G. elliptica alkaloids possess interesting biological properties, especially antitumor activity present in Sample B. This sample could be a promising candidate for the new anticancer drug development due to its high antitumor activity and safety towards normal cells. In future work, the antitumor activity should be accessed against a broader tumor cell line panel. Moreover, the sample safety ought to be further evaluated by additional *in vitro* tests.

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