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Chemical Composition of *Diplopterys pubipetala* (Malpighiaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CAA, MONC and KTS contributed running the laboratory work analysis of the data and drafting the paper. Authors FSAF and CFFA contributed in analysis of the LC/MS characterization. Authors DAO and AFMJ contributed to the collection and preparation of plant material. Author EVM supervised the laboratory work and contributed to drafting the paper. Author VAR designed the study, supervised the laboratory work and contributed to several reading of the manuscript. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

In view of the therapeutic potential and popular use of the Malpighiaceae family, with emphasis on the importance of species of the *Diplopterys (Banisteriopsis)* genus, the objective of this study was to identify the metabolites classes of leaves and stems of *Diplopterys pubipetala*. The extracts were analyzed and detected 10 compounds distributed among alkaloids (2), flavonoids (3), terpenes (3), saponin (1) and lactone (1). Among the substances found, there are compounds already reported in the Malpighiaceae family. The therapeutic potentials cited in the literature for the identified

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substances were: antifungal, antiviral, antimicrobial, antioxidant, anti-inflammatory and antitumor actions. This work is a pioneer in the study of the chemical constituents present in *D. pubipetala* and opens new lines of research for this species.

Keywords: Alkaloids; flavonoids; terpenes; Banisteriopsis.

1. INTRODUCTION

The Malpighiaceae family is composed of tropical flowering plants, with approximately 1300 species and 75 genus [1]. The *Diplopterys* genus is found in Latin America, in some subtropical regions, but most species are restricted to tropical regions [2].

The *Banisteriopsis pubipetala* A. Juss. came to be considered synonymous with *Diplopterys pubipetala* (A. Juss.) W.R. Anderson & C. Davis [3].

The species *D. pubipetala* has distribution throughout Brazil, extending up to Colombia, Peru, Bolivia and Paraguay and blooms in September and bears fruit from November [3]. In view of the therapeutic potential, the importance of species of the genus *Diplopterys (Banisteriopsis)*, the absence of studies on *D. pubipetala* and the inexistence of the ethnobotanical use of this species, the objective of this study was to identify the classes of metabolites of leaves and stems of *D. pubipetala*.

The main constituents of the Malpighiaceae plants are the alkaloids, flavonoids and terpenes [4]. In this context, the objective of this work was to study the chemical composition of *D. pubipetala* stem and leaf extracts and thus propose the main classes of metabolites.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Obtaining the Extract

Leaves and stems of young and healthy plants of *D. pubipetala* were collected, identified with the specimen deposited at the Herbário Montes Claros Minas Gerais, of the State University of Montes Claros, Brazil under voucher 4033.

2.2 Obtaining the Crude Extracts and Partitions Crude Extracts

Leaves and stems were separately macerated in ethanol/water (7:3 v/v). Flavonoid partitions: the crude extracts (1 g) were solubilized in methanol/water (10 mL; 9:1) each separately. Extractions were performed (4 x 75 mL) with the solvents: dichloromethane and ethyl acetate. Alkaloid partitions: the crude extracts (1 g) were resuspended in methanol/water (30 mL, 70% v/v) and taken to an ultrasonic bath for 30 minutes. The samples were centrifuged (5000 rpm) for 20 minutes and the supernatant filtered through filter paper (Nalgon - 3µm), with pH adjustment 10.0 using ammonium hydroxide. Extractions (3x 30 mL) were made with dichloromethane. The solvents of the extracts and partitions were evaporated (40ºC) and later stored under refrigeration (4ºC).

2.3 Column Chromatography

Columns (60 cm x 3 cm) were prepared for alkaloids: (a) 50 mg leaf extract and (b) 4.7 mg stem extract and for flavonoids: (c) 32 mg leaf extract and (d) 3.8 mg of stem extract. The packaging was carried out with (40 mL) silica gel 60 (0.063 - 0.200MM / 70 - 230 MESH - VETEC) and hexane. The fractions were eluted with the solvents (120 mL): hexane, dichloromethane, ethyl acetate and butanol, respectively. Fractions of 5 mL in 5 mL were collected in a test tube, the fractions obtained were compared by thin layer chromatography (CCD) and grouped. The solvent was evaporated in a circulating air oven at 40ºC for 24 hours and after drying, the samples were stored at 4ºC (Table 1).

2.4 LC-MS

The mass spectrometry analysis was performed on UHPLC (Hewlett Packard, Agilent Technologies 1290 series) coupled to the Q-ToF iFunnel 6550 mass spectrometer using electrospray ionization source (ESI) without the column. The voltages and temperatures of the mass spectrometer were: VCap 3000 V; shredder voltage at 100 V; OCT 1RF Vpp at 750 V; gas temperature at 250°C; Gas temperature sheath at 350°C; Drying gas at 10 L min^{-1}. The mass spectra were acquired in profile and negative ion mode and the acquisition range was 100-2000 m/z. The data were processed using the Agilent Mass Hunter Qualitative Analysis B0.7 software. The provisional identification of the compound was made using the METLIN library based on the exact mass (3 ppm). Searches were carried out on websites and articles in order to identify each compound. The analyzes were carried out at the Mass Spectrometry Laboratory - Thomson of the Universidade de Estadual de Campinas.

Table 1. Information about part of the plant, solvent and extraction yield

Sample	PP	Solvent	MI	Y (mg e %)	Nº GP
1				1.9 (3.8%)	15 a 30
2			Alkaloid	4.3 (8.6%)	1 a 14
3	Leaf	Dichloromethane		1.9 (3.8%)	31 a 33
4				1.0 (3.1%)	11 a 13
5			Flavonoid	3.5 (11.2 %)	1 a 10
6	Stalk			3.2 (84.2%)	1 a 7
7				2.3 (4.6%)	1 a 9
8				2.2 (4.4%)	9 a 11
9	Leaf	Ethyl acetate	Alkaloid	0.4 (0.8%)	12
10				2.8 (5.6 %)	13 a 33
11				2.4 (7.5 %)	1 a 6
12			Flavonoid	6.3 (19.7%)	7 a 13
13				0.7 (1.4%)	1 a 8
14	Leaf			3.1 (6.2%)	28 a 33
15		Butanol	Alkaloid	3.7 (7.4%)	19 a 27
16	Stalk			0.8 (17.0%)	1 a 7
17			Flavonoid	3.3 (10.3%)	12 a 15
18	Leaf			2.8 (8.8%)	1 a 11
19			Alkaloid	5.6 (11.2%)	*All*
20	Leaf	Hexane		2.8 (8.8%)	1 a 6
21			Flavonoid	2.2 (6.9%)	13 a 16

PP: part of the plant; MI: metabolite of interest; Y: Yield; N° GP: Grouped Partition

3. RESULTS AND DISCUSSION

Ten compounds belonging to the class five distinct classes of secondary metabolites were identified in the leaf and stem partitions *D. pubipetala*: alkaloids, flavonoids, terpenoids, saponin and lactone (Table 2).

The two alkaloids detected in the leaf extracts have the biological importance described for other species. N-*cis*-Feruloyltyramine ($C_{18}H_{19}NO_4$) was identified in the leaf partition with ethyl acetate and is reported in the species *Piper umbellatum* (Piperaceae), *Solanum sordidum* (Solanaceae), *Acorus gramineus* (Araceae), *Celtis africana* (Cannabaceae), *Piper flaviflorum* (Piperaceae) and *Tetrapterys mucronata* (Malpighiaceae) [5,6,7]. This molecule has anticancer, antifungal, antioxidant and anti-inflammatory activities [5]. Simulansamide ($C_{22}H_{23}NO_6$), also an alkaloid, was identified in the leaf partition in ethyl acetate and identified in *Zanthoxylum simulans* (Rutaceae), this molecule being able to inhibit platelet aggregation [8].

Table 2. Compounds present in leaves and stems of *D. pubipetala* identified in extracts and partitions by mass spectrometry (Q-ToF)

CQ	*m/z*	Compound	MF	Score	D (DB, ppm)
	312.1247	N-*cis*-Feruloyltyramine [a]	($C_{18}H_{19}NO_4$)	87.76	-4.33
Alkaloid	396,1467	Simulansamide[a]	($C_{22}H_{23}NO_6$)	83.20	-4.56
	587.1332	Cucumerin A [a]	($C_{29}H_{28}O_{11}$)	97.16	-1.57
Flavonoid	521.0933	Syringetin 3-glucuronide [a]	($C_{23}H_{22}O_{14}$)	99.53	0.65
	407.1866	Macarangaflavanone A [a]	($C_{25}H_{28}O_5$)	99.99	0.09
	617.3835	3-*β*-O-(*cis*-p-coumaroyl) corosolic acid [a]	($C_{39}H_{54}O_6$)	95.09	1.98
Terpenoid	451.3221	25-anidro-alisol F[a]	($C_{30}H_{44}O_3$)	99.27	-0.86
	293.1769	Phytuberina[a,b]	($C_{17}H_{26}O_4$)	91.85	-3.62
Saponin	621.4371	Ginsenoside Rh2[a]	($C_{36}H_{62}O_8$)	99.96	0.18
Lactone	221.1553	S-cucujolide V [a,b]	($C_{14}H_{22}O_2$)	99.16	-1.34

QC: chemical class. m/z: Mass/charge ratio. MF: proposed molecular formula. a: the detected in leaf. b: detected in stem. D: Diff (DB, ppm) error of the analysis, the difference in mass given in ppm of the molecular formula that was assigned in relation to the mass that was measured

Cucumerin A ($C_{29}H_{28}O_{11}$) was one of three molecules identified in the leaf partition (ethyl acetate), being described in the literature in *Cucumis sativus* L. (Cucurbitaceae) [9]. The syringetin 3-glucuronide ($C_{23}H_{22}O_{14}$) was found in the partition leaf (ethyl acetate) and described in the *Spinacia oleracea* species (Amaranthaceae) showing antioxidant, anti-allergic, anti-inflammatory, antithrombotic, anticarcinogenic and antiviral actions [10]. Already macarangaflavanone A ($C_{25}H_{28}O_5$) was identified in the leaf partition (butanol) and with showed antimicrobial activity, described in *Macaranga triloba* ((Euphorbiaceae) and *Flemingia strobilifera* (Leguminosae) [11,12].

The terpenes were 3-*β*-O-(*cis*-p-coumaroyl) corosolic acid ($C_{39}H_{54}O_6$) identified in the leaf partition (butanol), being reported in *Ludwigia octovalvis* (Onagraceae) with cytotoxicity activity in human tumor cells [13]. The 25-anhydro-alisol F ($C_{30}H_{44}O_3$) was detected in the leaf fraction (dichloromethane). This terpene was isolated from *Alisma orientalis* (Alismataceae), being a substance derived from alisol F, which is a terpene with anti-inflammatory action [14,15]. Phytuberina ($C_{17}H_{26}O_4$) was detected in leaf partitions (all solvents) and in the stem partition (dichloromethane), being found in Solanaceae specifically in *Solanum tuberosum* L. and *Nicotiana tabacum* [16,17]. These terpenes have antimicrobial actions and antifungal and defensive function, synthesis and accumulation are induced after the attack of a pathogen [17]. Saponin Ginsenoside Rh2 ($C_{36}H_{62}O_8$) was identified in the leaf partition (butanol), being characteristic of the species *Panax ginseng* (Araliaceae) [18]. This molecule may be able to inhibit the action of glutamate mediated by NMDA receptors [19] and has anti-obesity properties, reducing risk factors for metabolic diseases, such as diabetes [19,20]. And lastly, lactone S-cucujolide V ($C_{14}H_{22}O_2$) was found in all studied partitions, except for the butanol leaf partition.

4. CONCLUSION

This study reports the presence of alkaloids, flavonoids and terpenes in extracts of leaves and stems of *D. pubipetala* (Malpighiaceae), and the proposed molecular structures are already described in the literature with the biological potential applied in the treatment of several human diseases.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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