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Flavonoids in Malpighiaceae: A Comparative Study of Four Species from the Northern Minas Gerais Cerrado

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Abstract

This study investigated the diversity of flavonoids in four Malpighiaceae species occurring in the northern Minas Gerais cerrado. The species share the same environment as sympatric congeners, two belonging to the genus Banisteriopsis (B. gardneriana and B. cf. anisandra), and two to the genus Diplopterys (D. lutea and D. pubipetala). The study was conducted using hydroethanolic extracts and thin-layer chromatography (TLC). The elution system employed was specific for flavonoids, and detection was carried out using 5% aluminum chloride. The results indicated similar retention factors (Rf) among the species, as well as unique markers for each sample, demonstrating both diversity and the presence of specific metabolite markers. These findings support a possible chemotaxonomic relationship among the species, suggesting the conservation of biosynthetic pathways within the family.

Keywords: *metabolite markers, flavonoids, diversity, species, genus*

INTRODUCTION

Malpighiaceae is a botanical family currently comprising 77 genera and approximately 1,350 species distributed across tropical and subtropical regions of the world [1]. In Brazil, 46 genera are found, with a significant concentration of species diversity in the cerrado biome. Notable genera occurring in this biome include *Byrsonima, Banisteriopsis, Diplopterys, Heteropterys, Malpighia,* and *Stigmaphyllon* [2,3]. Certain species have been the focus of phytochemical investigations, such as *Diplopterys pubipetala*, which has been reported to contain flavonoids with antifungal activity, including high efficacy against yeasts of the genus *Candida* [4]. Studies conducted on *Banisteriopsis* species from the cerrado have revealed a chemical profile rich in flavonoids [5], suggesting that species and genera within this family may serve as sources of such metabolites and exhibit correlated chemical profiles.

Beyond taxonomic classification, the occurrence of sympatric species—particularly congeners sharing the same habitat or ecosystem—raises questions regarding their metabolic richness. This is especially relevant in the context of recent taxonomic reclassifications, such as the division of the genus *Banisteriopsis* into three genera: *Diplopterys, Bronwenia,* and *Banisteriopsis* [6], with reported distributions across different geographic regions [7,8]. With regard to metabolite composition, both *Banisteriopsis* and *Diplopterys* have been studied for their phenolic content, including glycosylated flavonoids and aglycones [4,9,10]. In relation to other studies in species of the same family, metabolomic and chemical profiling assays have already been performed [11,12].

Therefore, the aim of this study is to evaluate the phytochemical characteristics of four Malpighiaceae species occurring in a cerrado fragment in northern Minas Gerais, Brazil, which share taxonomic traits, such as yellow flowers.

MATERIALS AND METHODS

Plant Material

Leaves of *Banisteriopsis gardneriana* (Bg), *Banisteriopsis cf. anisandra* (Ba), *Diplopterys lutea* (Dl), and *Diplopterys pubipetala* (Dp) were collected in the district of Nova Esperança, municipality of Montes Claros, Minas Gerais, Brazil, in February 2025 (coordinates: 16W 33' 41", 43S 55' 30"). Voucher specimens of each species were deposited at the Herbário Norte Mineiro – UFMG under the following voucher numbers: 6223 (Bg), 6225 (Ba), 6222 (Dl), and 6221 (Dp). The samples were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number A7ED1E1.

Extract Preparation

The leaves were washed with running water and dried in a forced-air oven (insert brand/model) at 40 °C ± 2 °C for five days. The dried material was ground using an IKA A11 analytical mill (IKA, Germany), and the resulting powder was stored in paper bags inside a freezer (Consul, Brazil) at a temperature between 0 and 4 °C.

The hydroethanolic extract (70:30, v/v) was prepared using the exhaustive maceration method, at a ratio of 1 g of plant material to 10 mL of solvent, for seven days in the dark with occasional agitation. The material was filtered, and the solid plant residue was re-extracted with the same volume of solvent. After a second filtration, the combined extracts were concentrated under reduced pressure in a rotary evaporator (SP Labor, Brazil)

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at 40 °C ± 2 °C. The extraction yields for Bg, Ba, Dl, and Dp were 14.7% (1,470 mg), 16% (1,600 mg), 19.7% (1,970 mg), and 7.1% (710 mg), respectively.

Thin-Layer Chromatography (TLC)

TLC was performed on aluminum plates precoated with silica gel 60 (0.2 mm thickness) containing a fluorescent indicator F_{254} (Macherey-Nagel, Düren, Germany). The mobile phase specific for flavonoids consisted of ethyl acetate: glacial acetic acid: formic acid: and water (100:11:11:26, v/v/v/v). Detection was carried out using UV light at wavelengths of 254 nm and 365–395 nm, and chemically by spraying with 5% aluminum chloride ($AlCl_3$) solution. Rutin and quercetin were used as flavonoid standards. The alignment of solvent front distances for each sample was performed using the dimension tool in CorelDRAW (2021).

RESULTS AND DISCUSSION

The results obtained under UV light at 254 nm revealed compounds with corresponding retention factors (Rf) shared among some species. *B. gardneriana* (Bg), *D. lutea* (Dl), and *B. cf. anisandra* (Ba) exhibited shared Rf values of 0.70; Bg, *D. pubipetala* (Dp), and Ba showed Rf values of 0.81; and Bg and Ba shared an Rf of 0.89. Under UV light at 365–395 nm, Bg, Dl, Dp, and Ba shared Rf values of 0.60 and 0.64. After spraying with $AlCl_3$ and subsequent observation under UV 365–395 nm, Rf values of 0.75 and 0.64 were observed across all species. These Rf values may be closely associated with flavonoids common to the species, or to the family or genus level; given their taxonomic proximity, *Diplopterys* and *Banisteriopsis* may contain similar compounds. The retention factors are detailed in Table 1.

Table 1. Retention factor (Rf) values determined by TLC for the four species.

B. gardneriana	*B. cf. anisandra*	*D. lutea*	*D. pubipetala*
UV-254nm			
0.70	0.39	0.50	0.43
0.81	0.70	0.60	0.65
0.89	0.81	0.70	0.75
	0.89		0.81
365nm			
0,09	0,09	0,09	0.60
	0.60	0,41	0.64
	0.64	0,60	
		0.64	
365nm + 5% aluminum chloride			
0.09	0.09	0.09	0.48
0.41	0.41	0.17	0.64
0.58	0.58	0.31	0.68
0.64	0.64	0.41	0.75
0.75	0.75	0.51	0.82
0.86	0.86	0.64	
0.93	0.93	0.75	
		0.82	
Rutin: 0,41. Quercetin: 0,92~0,94			

Rf values corresponding to rutin were observed in three samples: *B. gardneriana* (Bg), *B. cf. anisandra* (Ba), and *D. lutea* (Dl), while quercetin was detected in Bg and Ba. A study on TLC of flavonoids [13], using the same mobile phase as in the present work, indicates typical flavonoid Rf regions as follows: 0.05–0.3 (flavonoid oligosides), 0.25 (flavonol triosides), 0.40 (rutin, quercetin-, kaempferol-, isorhamnetin-3-O-(2''–6''-di-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside), 0.45 (narcissin, isorhamnetin-rutinoside, iso-orientin, isovitexin, vitexin), 0.5 (6-hydroxykynurenic acid, kaempferol-, quercetin-3-O-(6''-trans-p-coumaroyl-4''-glucosyl)-rhamnoside), 0.6 (quercitrin), 0.75 (isoquercitrin, astragalin, dihydrokaempferol-7-O-glucoside), and front (flavonol aglycones, biflavonoids). These data corroborate the results obtained here, with well-defined zones corresponding to possible flavonoid groups.

Regarding *Diplopterys pubipetala*, previous investigations reported the presence of orientin and vitexin with an Rf of 0.49 [4]. Geographic aspects may directly influence the ecological and ecophysiological interaction networks of the studied flora.

The use of aluminum chloride favors the formation of fluorescent complexes in flavonoids, allowing visualization under UVA light, as occurred in the present study [14,15]. which indicates not only the presence but also the diversity of flavonoids in the samples analyzed. Other Rf values were observed sporadically, showing zones of higher and lower intensity, which may relate to the chemical identity of each analyzed species. Thus, the findings indicate not only chemotaxonomic proximity but also the individual chromatographic profile of each species and the richness of flavonoids present in the samples. This may suggest a chemical identity or even physiological and phenotypic plasticity of the species in response to their environmental conditions.

CONCLUSION

The results obtained in this study highlight the presence of flavonoids in the four species analyzed, with common retention factors as well as specific markers for each sample. The recurrence of similar retention factors, including those corresponding to rutin and quercetin, supports a possible chemotaxonomic relationship among the species, suggesting the conservation of biosynthetic pathways within the family. These findings demonstrate the chemical diversity produced by the species. Future work will include spectroscopic and biological analyses to further characterize the metabolite profiles and potential bioactivities of the extracts.

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