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Antioxidant activity, total flavonoids and volatile constituents of *Magonia Pubescens* A.St.-Hil

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Magonia pubescens **A.St.-Hil (Sapindaceae) ('tingui') is a typical medicinal plant of the Cerrado biome. This plant is used as a larvicide and employed in poison fishing. However, little is known of its secondary metabolites. In this study, it is described for the first time as the volatile constituents of *M. pubescens*, collected via headspace. Qualitative phytochemical analyses were performed. In addition, the antioxidant activity of the ethanol extracts of flowers and leaves were evaluated, and the total flavonoids were quantified. The ethanol extracts of flowers (12.67 ± 0.05 rutin equivalent (EQ) g^{-1}) and leaves (11.81 ± 0.05 rutin EQ g^{-1}). The leaf extracts exhibited higher IC_{50} values (18.14 ± 0.02 rutin EQ g^{-1}) than did the flower extracts (31.19 ± 0.05 rutin EQ g^{-1}). Twenty volatile compounds were identified in *M. pubescens* flowers through gas chromatography coupled with mass spectrometry (GC-MS), being identified as benzilic acid (17.9%) and styrene (13.9%) as the major compounds. The antioxidant activity of *M. pubescens* could be related to the presence of flavonoids and tannins, but further studies need to be conducted to fully understand that correlation. The identified volatiles have the potential to be used in the cosmetics industry due to their socio-economic relevance, and the may also contribute to the understanding of the reproductive success of this species.**

Key words: *Magonia pubescens*, 'tingui', headspace, phytochemical screening, gas chromatography coupled with mass spectrometry (GC-MS).

INTRODUCTION

The Cerrado (Brazilian savannah) exhibits high biodiversity and includes a number of species with high bioactive potential (Rocha et al., 2008; Mendonça et al., 2008), such as *Magonia pubescens* A.St.-Hil, which is

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locally known as ‘tingui’ or‘timbó’. This plant is widely distributed in the central region of Brazil in the states of Goiás,Mato Grosso, Mato Grosso do Sul and Minas Gerais (Souza and Lorenzi, 2005). *M. pubescens* is a medium-size tree that reaches between five and nine meters in height and has paripinnate leaves, commonly used as an ornamental plant due to the lacy appearance of its foliage, and indicated for replantation of degraded areas (Lorenzi, 2000).

The fruits and seeds of *M. pubescens* are used to prepare a soap for the treatment of dermatitis and seborrhea and lice infestations and to also be used as insecticide (De Mesquita et al., 2009) and larvicidal (De Mesquita et al., 2009; Fernandes et al., 2005; Fernandes et al., 2007; Figueiredo et al., 2008; Vallotto et al., 2011). The roots infusions can also be used as a tranquilizer (De Mesquita et al., 2009).

Volatile compounds of this plant are associated with survival functions in the ecosystem; there chemical composition changes according to the gentic diversity and habitat of the plants (Siani et al., 2000).

Certain volatile compounds can also have antioxidant properties (Sacchetti et al., 2005). The most active and frequently occurring antioxidants of the plant origin are phenols, which include flavonoids (Pietta, 2000; Koleva et al., 2002; Pourmoradi et al., 2006). Flavonoids are the main flower chromophores, and flavonols (chalcones and aurones) are responsible for the yellow color of *M. pubescens* flowers (Brouillard and Harbone, 1998).

Plant secondary metabolites are important resources for biological applications, and their identification may contribute to the discovery of new biomolecules with potential applications on several areas of science (Sousa et al., 2007). *M. pubescens* has such potential; however, limited studies have been performed on this plant. The goal of the present study was to perform a phytochemical study of the main secondary metabolites to quantify the total flavonoids, evaluate the antioxidant activity of the flowers and leaves, and identify the volatile chemical composition of the flowers of *M. pubescens.*

METHODOLOGY

Collection of plant material

The flowers and leaves of *M. pubescens* were collected in Montes Claros, state of Minas Gerais, Brazil during spring in September of 2013. They were conservated in plant bags at -80°C and 0% relative humidity, in the dark. Voucher number: 106750- Herbarium of Montes Claros-HMC.

Plant extracts preparation

The leaves and flowers of *M. pubescens* were dried in an oven at 40°C (± 2°C) until a constant weight. The leaves were ground using a mill (Willey IKA A11B), and the flowers were ground using a mortar and pestle.

Crude leaf extracts (25%) were obtained through exhaustive maceration of the dried plant material (20 g) in ethanol: water (7:3) for seven days. Subsequently, the extract was filtered and evaporated.

The filtration residue was resuspended in the same solvent, and the extraction was repeated for three consecutive weeks. The resulting extract was stored in dark and cold (± 4°C) conditions until use.

Crude flower extracts (30%) were obtained by drying, grinding and homogenizing flowers (0.1 g ml^{-1}) in ethanol: water (6:4); subsequently, the samples were placed in an ultrasonic bath (UNIQUE) for 20 min. After 24 h of contact with the solvent, the extract was placed in an ultrasonic bath for an additional 20 min. Subsequently, the extract was filtered and evaporated. The resulting extract was stored in dark and cold (±4°C) conditions until use.

Chemical characterization

Qualitative tests to detect the contents of tannins, saponins, flavonoids, alkaloids, and terpenes were performed for the dry leaves and flowers: 10% neutral lead acetate and 2% iron choride reactions for tannins, 2% iron chloride and Shinoda reactions for flavonoids, Mayer, Bouchadart, Bertrand and Dragendorf reagents for alkaloids, Lieberman-Burchard reaction for sterols/triterpenoids, persistent foam test for saponins (Mouco et al., 2003; Barbosa., 2001).

Flavonoids content

The crude ethanol extracts (6:4) of flowers (0.33 g ml^{-1}) and leaves (0.38 g ml^{-1}) were diluted 400 times in the same solvent. Aliquots (0.5 ml) of the resulting solutions were transferred into tubes containing 0.5 ml 2% aluminum chloride (w/v). The mix was homogenized and left to stand for 30 min, and the absorbance was read at 410 nm using a spectrophotometer (Shimadzu). A calibration curve was obtained using a rutin commercial standard (Sigma Aldrich) at 0.01, 0.02, 0.04, 0.06 and 0.08 mg ml^{-1} (Fernandes et al., 2010). Measurements were performed in triplicate, and the results were expressed as rutin equivalent (EQ) g^{-1}. The statistic program used to calculate the standard deviation was Microsoft Office Exel 2007.

Antioxidant activity of leaves and flowers of *M. pubescens*

The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH). 0.04% of DPPH solution reacted with crude ethanol (6:4) plant extracts (0.1 ml) at concentrations of 5, 10, 15, 20, 25 and 30 µg ml^{-1}. The mix was homogenized, and the free-radical scavenging capacity of the extract was measured as the absorbance at 517 nm using a spectrophotometer (Shimadzu) (Ramírez-Mares and De Mejía, 2003). Measurements were performed in triplicate, and the results were used to calculate the IC_{50}, which is the effective concentration at which 50% of the DPPH radicals are scavenged.The statistic program used to calculate the standard deviation was Microsoft Office Excel 2007.

Volatile characterization

The plant material (0.87, 0.96g, 0.67g, and 0.74 g) was stored individually in glass vials (20 ml) and placed in an auto sampler (HS combi-PAL). The flowers were homogenized at 500 rpm and incubated at 75°C for 5 min. The released volatiles were determined via headspace extraction and analyzed by gas chromatography coupled with mass spectrometry (GC-MS) under the conditions described in Table 1 (Aguiar et al., 2014).

GC-MS was performed using a gas chromatograph (Agilent

Table 1. Auto-sampler conditions for volatile extraction via static headspace (HS Combi-PAL).

Auto-sampler system for headspace extraction	Value
Injection volume (µl)	1000
Incubation temperature (°C)	75
Incubation time (m/s)	5 (300)
Syringe temperature (°C)	75
Agitation speed (rpm)	500
Fill speed ($\mu ls^{-1)}$	500
Fill strokes	0
Pullup delay (s)	500
Injection speed ($\mu ls^{-1)}$	500
Pre-injection delay time (ms)	0
GC run time (min)	47
Sample weight (g)	0.87, 0.96, 0.67, 0.74

7890A; Agilent Technologies), coupled with a mass spectrophotometer (MS 5975C) equipped with a fused silica capillary column HP-5 ms (30 m × 0.25 mm × 0.25 µm) using helium as the carrier gas (1 ml min^{-1}). Sample injection (1000 µl) was performed by split less injection using an auto injector (Combi PAL). The rate of temperature increase was 2°C min^{-1} from 35 to 80°C and then 4°C min^{-1} up to150°C, with a total run time of 42 min. The system was operated in the scan mode (monitoring) with electron impact ionization at 70 eV and scan mass range of 40 to 550 (*m/z*) (Aguiar et al., 2014).

The resulting data were analyzed using the software MSD Chemstation along with the National Institute of Standards and Technology Mass Spectral Library (NIST, 2009). The relative abundance (%) of the constituents was calculated from peak areas of the gas chromatogram (CG) and organized according to the order of elution. The percentage of each component was calculated using the normalized means of the chromatogram areas, and the compounds were identified through a comparison with the spectra of compounds deposited in the mass spectral library (NIST 2.0, 2009).

RESULTS AND DISCUSSION

Phytochemical screening, flavonoid quantification and antioxidant activity of *M. pubescens*

A qualitative evaluation of secondary metabolites, tannins, alkaloids, flavonoids, saponins and terpenes from the flowers and leaves of *M. pubescens* is described in Table 2. The leaves and flowers, showed a strong positive result for saponin heterosides and hydrolysable tannins was obtained and the presence of gallic tannins was observed. Isoflavonoids and chalcones were only identified in the leaves, while flavonols, flavones and flavonones were only found in the flowers.

Total tannins were moderately detected in the leaves, and total flavonoids were observed in the flowers. Total alkaloids were weakly detected in the leaves and flowers, and gallic tannins were observed in the flowers. Certain tests did not detect the presence of tannins and alkaloids in the leaves and flowers.

In a previous study of *M. pubescens* leaves, a negative result was observed for steroids/triterpenoids, a strong positive result was observed for saponins, a positive result was observed for tannins, a weak positive result was observed for alkaloids, and a negative result was observed for flavonoids (Silva et al., 2010). To our knowledge, studies on the phytochemistry of *M. pubescens* flowers have not been performed.

In the same study, a negative result was obtained for flavonoids using the Shinoda test (Silva et al., 2010), which is consistent with the results of the present study,wherein a negative result was observed for flavonoids using the Shinoda test and a positive result was observed using the aluminum chloride test (Table 2).

Regarding flavonoid concentration, the results for flowers and leaves was 12.67 ± 0.05 mg rutin EQ. g^{-1} and 11.81 ± 0.05 mg rutin EQ. g^{-1}, respectively. These values are similar to those reported in a study analyzing the aqueous extracts of *Achillea millefolium*, an herb with medicinal potential (Eghdami and Sadeghi, 2010; Masika and Alfalayan, 2003).

The antioxidant potential of the leaves and flowers was evaluated through their capacity to inhibit the oxidation of DPPH free-radicals, and the potential was expressed as the IC_{50} (Huang et al., 2005), which was 18.14 ± 0.02 µg ml^{-1} for the leaves, 31. 19 ± 0.05 µg ml^{-1} for the flowers, and 1.47 µg ml^{-1} for the gallic acid standard.

In addition, *Aristolochia bracteata* has been reported to be an accessible source of natural antioxidants and exhibited IC_{50} values similar to the ones observed for *M. pubescens* (Farias et al., 2013).

Volatile profile in flowers of *M. pubescens*

Twenty volatile compounds were identified in *M. pubescens* flowers (Table 3), and they belonged to five different classes: furanoids (3.9%), esters (9.3%), alcohols (43.8%), aldehydes (24.5%) and hydrocarbons

Table 2. Phytochemicalscreening of the leaves and flowers of *Magoniapubescens.*

Class	Test	Leaf	Flower
Tannins	Ferric chloride	++	+
	Alkaloid aqueous solution	-	-
	Neutral lead acetate	+++	+++
	Copper acetate	++	++
	Tanninspecific	+++	+
Saponins	Persistent foam	+++	+++
Flavonoids	Shinoda	-	+++
	Aluminum chloride	+++	++
Alkaloids	Mayer reagent	-	+
	Bouchadart reagent	+	-
	Bertrand reagent	+	+
	Dragendorf reagent	+	+
Triterpenes	Liebermann-Burchard reaction -	+++	++

(-) Negative, (+) Weak positive, (++) Moderate positive, (+++) Strong positive.

(18.5%).

Benzyl alcohol (8) is a methylated derivative of benzenoid, and it was the most abundant compound (17.9%), thus indicating the presence of O-methyltransferase, an enzyme that catalyzes the transfer of methyl groups to hydroxyl or carboxyl groups within a vast range of receptor molecules. Benzenoids are involved in the biosynthesis of odoriferous substances (Alves et al., 2005) and many of them are important to the cosmetics industry.

Volatile compounds are widely used as a flavoring for foods, confections and spices and as a fragrance in perfumes and cosmetics. They are also used in the production of several skin products because of the complexity of their active compounds, significant aromatic properties and market value (Zellner et al., 2009)

Linalool [10 (1.9%)] and methyl salicylate [13 (7.8%)] are important compounds for the perfume industry (Sell, 2003; Lapczynski et al., 2007). Methyl salicylate is more abundant than linalool, and it is found in several pharmaceutical forms and used in cosmetic and non-cosmetic products, such as cleaning products (Lapczynski et al., 2007). Methyl salicylate is responsible for the refreshing character in the scent of oil, and octanal [7 (1.7%)] is responsible for citrus scents (Zellner et al., 2009; Mahattanatawee et al., 2007).

Linalool oxide [9 (3.9%)] occurs n *M. pubescens* flowers and is a common chemical in floral aromas that is known to be an important mediator of pollination (Knudsen and Tollslen, 1993), particularly with bees for *M. pubescens* (Dewick, 2009).

During evolution, plants developed the ability to attract pollinators as well as defense mechanisms against herbivores and pathogens. These chemical defenses are composed of compounds that are produced and stored for immediate lease following an attack and through the synthesis of new compounds induced in response to attacks (Almeida et al., 2003).

Styrene is the second most abundant compound found in *M. pubescens*, and it appears to possess defensive properties. The high production of styrene compared with other compounds is not clearly explained in literature, and there are almost no studies directly reporting the effects of styrenein plants (Gatehouse, 2002).

Styrene [4 (13.9%)] is a toxic hydrocarbon with a balsamic scent (Stolarska et al., 2010), and it has deleterious effects on the health of organisms in general and is a potential carcinogen. In high concentrations, styrene has an inhibitory effect on seed germination. Winter wheat grown under atmospheric concentrations of styrene between 570 and 2.280 mg m^{-3} was observed to grow more slowly than controls and exhibit a number of anatomical changes (Leffingwell and Alford, 2005).

Methyl salicylate [13 (7.8%)], is a salicylic acid ester that may also be associated with the defense mechanisms of *M. pubescens*. Methyl salicylate occurs widely in plants (Jayasekara et al., 2002), because its production is associated with induced resistance to phytopathogens (Seskar et al., 1998).

A repellent effect of this compound higher than 80% was observed in the larvae of *Boophilus microplus* (Novelino et al., 2007), and terpenoid volatile compounds

Table 3. Volatile constituents detected in flowers of *Magonia pubescens* by gas chromatography coupled to mass spectrometry (GC-MS).

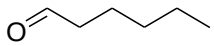
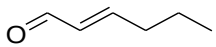
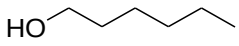
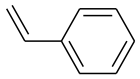
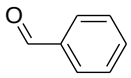
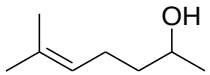
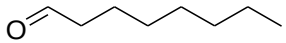
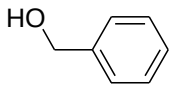
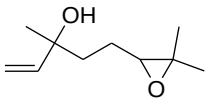
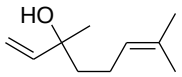
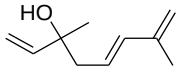
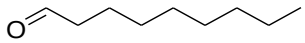
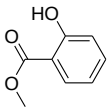
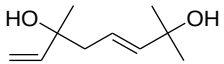
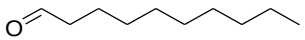
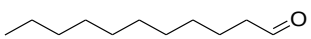
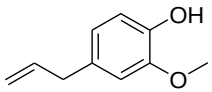
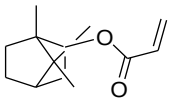
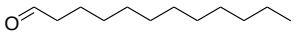
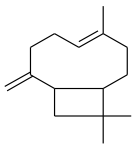
S/N	R.T.[a]	Compounds	Area (%)	M.F.[b]	M.S.[c]	C.I.[d]
1	6.5	Hexanal	10.4	$C_6H_{12}O$		82 (22), 72 (23), 67 (18), 57 (53), 56 (87), 45 (18), 44 (87), 43 (51), 41 (73), 40 (100)
2	8.8	2-Hexenal	3.2	$C_6H_{10}O$		98 (M+35), 83 (83), 80 (18), 70 (24), 69 (83), 57 (51), 55 (88), 43 (23), 42 (68), 39 (71)
3	9.7	1-Hexanol	13.1	$C_6H_{14}O$		84 (5), 69 (36), 56 (100), 55 (53), 54 (4), 45 (4), 43 (48), 42 (33), 41 (36), 39 (12)
4	10.6	Styrene	13.9	C_8H_8		104 (M+100), 103 (49), 102 (9), 78 (40), 77 (22), 75 (4), 52 (6), 51 (17), 50 (8), 40 (7)
5	14.9	Benzaldehyde	2.3	C_7H_6O		106 (M+86), 105 (100), 78 (21), 77 (81), 51 (25), 50 (18), 40 (22), 39 (8)
6	17.2	6-methyl 5-hepten-2-ol	1.8	$C_8H_{16}O$		128 (M+12), 110 (22), 95 (100), 71 (19), 69 (33), 68 (13), 67 (19), 55 (17), 53 (14), 45 (19)
7	18.0	Octanal	1.7	$C_8H_{16}O$		85 (30), 84 (66), 82 (48), 81 (68), 57 (77), 56 (70), 55 (26), 45 (35), 43 (46), 41 (100)
8	20.1	Benzyl alcohol	17.9	C_7H_8O		108 (M+100), 107 (68), 91 (15), 80 (9), 79 (100), 78 (12), 77 (61), 51 (22), 50 (9)
9	23.6	Trans-Linalool oxide	3.9	$C_{10}H_{18}O_2$		155 (15), 111 (38), 94 (65), 93 (32), 81 (23), 79 (15), 68 (31), 67 (26), 59 (100), 55 (41)
10	24.7	Linalool	1.9	$C_{10}H_{18}O$		121 (31), 93 (76), 80 (24), 77 (18), 71 (100), 69 (47), 67 (21), 55 (47), 43 (49), 41 (45)
11	24.9	Hotrienol	2.3	$C_{10}H_{16}O$		82 (81), 81 (18), 79 (12), 71 (100), 67 (31), 55 (12), 53 (11), 44 (8), 43 (35), 41 (11)

Table 3. cont'd

12	25.1	Nonanal	3.1	$C_9H_{18}O$		82 (53), 81 (40), 70 (55), 57 (100), 56 (89), 55 (61), 44 (49), 43 (71), 42 (36), 41 (81)
13	29.8	Methyl salicylate	7.8	$C_8H_8O_3$		153 (4), 152 (M^+ 54), 121 (29), 120 (100), 93 (14), 92 (59), 65 (17), 64 (10), 63 (10)
14	29.9	2,6-dimethyl-3,7-Octadiene-2,6-diol	4.9	$C_{10}H_{18}O_2$		85 (5), 83 (6), 82 (100), 72 (4), 71 (64), 67 (40), 55 (7), 43 (41), 41 (10), 40 (5)
15	30.7	Decanal	1.2	$C_{10}H_{20}O$		71 (67), 70 (74), 68 (54), 57 (97), 56 (58), 55 (88), 44 (41), 43 (83), 41 (100), 40 (18)
16	35.1	Undecanal	1.4	$C_{11}H_{22}O$		126 (30), 95 (51), 82 (75), 81 (55), 71 (50), 68 (45), 57 (100), 55 (84), 43 (65), 41 (66)
17	36.6	Eugenol	1.9	$C_{10}H_{12}O_2$		164 (M^+100), 149 (37), 137 (21), 131 (44), 103 (34), 91 (33), 77 (28), 51 (13) 39 (16)
18	37.4	Isobornyl acetate	1.5	$C_{13}H_{20}O_2$		136 (52), 121 (58), 108 (32), 95 (100), 93 (46), 69 (38), 67 (26), 55 (73), 43 (5), 41 (16)
19	38.8	Dodecanal	1.2	$C_{12}H_{24}O$		140 (24), 97 (43), 83 (46), 82 (88), 69 (82), 67 (63), 57 (100), 56 (46), 55 (91), 41 (86)
20	38.9	Caryophyllene	4.6	$C_{15}H_{24}$		204 (M^+8), 133 (88), 120 (48), 107 (49), 105 (56), 93 (100), 91 (86), 79 (72), 69 (65), 41 (56)

Total compounds (%)	
Furanoid	3.9
Esters	9.3
Alcohols	43.8
Aldehydes	24.5
Hydrocarbons	18.5

[a]Retention time, [b] Molecular formula, [c]Molecular structure, [d]Characteristic ion, [e]Molecular weight according to the NIST 2.0 library.

such as methyl salicylate [13 (7.8%)] were reported to repel aphids and other insects by inhibiting their attraction to host plants (Norin, 2001). Methyl salicylate appears to have a number of functions and is involved in different chemical signaling pathways (Norin, 2001).

A repellent effect was also observed for isobornylacetate [18 (1.5%)], and it has been suggested that the toxicity of essential oils to insects depends on the chemical composition, including the presence of isobornylacetate [18 (1.5%)] (Lee et al., 2001). A repellent effect of linalool [10 (1.9%)] has been described for several insects (Labinas and Crocomo, 2002; Castro et al., 2006; Lima et al., 2009; Wang et al., 2011; Niculau et al., 2013), and this compound has been found to exhibit higher toxicity than other tested compounds in certain studies (Wang et al., 2011; Niculau et al., 2013).

Eugenol [17 (1.9%)] was found in the same percentage as linalool, and it is also a strong repellent that causes behavioral reactions in a number of insect species (Krell and Kramer, 1998). In addition, several volatile compounds, especially eugenol [17 (1.9%)], are associated with plant stress (Silva et al., 2012).

Caryophyllene [20 (4.6%)] is known for its anti-inflammatory and anti-fungal properties, and it is used as local anesthetic and was observed to have cytotoxic effects on a wide range of cell lines (Fernandes et al., 2007; Ashour et al., 2007). Anti-fungal effects were also observed for the aldehydes hexanal [1 (10.4%)] (Almenaret al., 2007; Neri et al., 2006; Baggio et al., 2014) and nonanal [12 (3.1%)], which prevented the germination of *Penicillium digitatum* and *Penicillium italicum* conidia at high concentrations (Droby et al., 2008). At low concentrations, however, it favored conidia development.

To our knowledge, the volatile composition of *M. pubescens* flowers has not been studied. Benzaldehyde [5 (2.3%)], hexanal [1 (10.4%)], and nonanal [12 (3.1%)], which were observed in *M. pubescens* flowers in this study, have been previously observed through head space extraction of the volatiles of fresh, unheated, and desalinated (NaCl) samples and in heated and salted (NaCl) samples of the fruits of *Nephelium lappaceum* (Sapindaceae)(Laohakunjit et al., 2007). Linalool [10 (1.9%)] and 1-hexanol [3 (13.1%)] were also found in cultivars of *Dimocarpus longan* (Sapindaceae) (Zhang et al., 2009). The remaining detected compounds (2, 6, 11, 14, 15, 16, 19) are involved in the synthetic pathways of these volatiles, few studies are focused on these type of compounds.

Conclusion

The leaves and flowers of *M. pubescens* have high bioactive potential that could be related with the presence of some secondary metabolites detected in the present study. Saponins, flavonoids and tannins were the main classes of compounds detected, having the last two classes effective action against free radicals. The volatile compounds identified in the present study are promising molecules for the cosmetic and pharmaceutical industries and may be involved in interactions that promote the reproductive success of *M. pubescens*.

Conflict of Interests

The authors have not declared any conflict of interests.

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