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Headspace Volatiles of the Leaves and Flowers of *Malvaviscus arboreus* Cav. (Malvaceae)

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Abstract. *Malvaviscus arboreus Cav.*, commonly known as Sleeping Hibiscus, is a plant species that belongs to the family Malvaceae with ornamental, culinary, and ethnomedical importance. This medicinal herb was reported to exhibit noteworthy antioxidant, cytotoxic, hepatoprotective, and anti-infective activities attributed to the presence of a variety of phytochemicals. In this work, the volatile compositions of the leaves and flowers of *M. arboreus* were studied and compared for the first time using the headspace gas chromatography-mass spectrometry (GC–MS) technique. Overall, 39 components were identified, comprising 36 from the leaves and 11 from the flowers, with an evident greater contribution of oxygenated compounds (89.54% in leaves and 89.35% in flowers) to their total volatiles. Phenolic ethers (41.64%) and ketones (21.57%) were the major chemical groups emitted by the flowers, while ketones (27.40%) and carboxylic acids (18.16%) dominated the volatile blends of the leaves. Anethole (32.32%), methyl isobutenyl ketone (19.18%), and methyl chavicol (9.32%) were the most abundant floral volatiles, whereas acetic acid (18.16%) was the major constituent given off by the leaves, followed by 2-cyclohexenone (9.60%) and anethole (7.39%). Additionally, from a biosynthetic point of view, the floral volatiles of *M. arboreus* showed the prevalence of phenylpropanoids/benzenoids (41.64%); however, fatty acid derivatives (54.30%) predominated among those produced by the leaves. The obtained results revealed noteworthy qualitative and quantitative variations in *M. arboreus* leaves and flowers' headspace volatiles that would help complement our phytochemical knowledge on this limitedly studied plant.
Keywords: GC–MS; headspace; malvaceae; *Malvaviscus arboreus*; volatile constituents.

Resumen. *Malvaviscus arboreus* Cav., comúnmente conocida como "Sleeping Hibiscus", es una especie vegetal que pertenece a la familia Malvaceae con importancia ornamental, culinaria y etnomédica. Se ha reportado que esta hierba medicinal exhibe actividades antioxidantes, citotóxicas, hepatoprotectoras y antiinfecciosas notables que se atribuyen a la presencia de una variedad de fitoquímicos. En este trabajo se estudiaron las composiciones volátiles de las hojas y flores de *M. arboreus* y fueron comparadas utilizando la técnica de cromatografía de gases-espectrometría de masas (GC-MS). En total, se identificaron 39 componentes comprendiendo 36 de las hojas y 11 de las flores, con un evidente mayor aporte de compuestos oxigenados (89,54% en hojas y 89,35% en flores). Los éteres fenólicos (41,64%) y cetonas (21,57%) fueron los principales grupos químicos emitidos por las flores, mientras que las cetonas (27,40%) y los ácidos carboxílicos (18,16%) dominaron las mezclas volátiles de las hojas. Anetol (32,32%), metil isobutenil cetona (19,18%) y metil chavicol (9,32%) fueron los volátiles florales más abundantes, mientras que el ácido acético (18,16%) fue el principal componente desprendido por las hojas, seguido de la 2-ciclohexenona. (9,60%) y el anetol (7,39%). Adicionalmente, desde el punto de vista biosintético, los volátiles florales de *M. arboreus*

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mostraron la prevalencia de fenil propanoides/benzenoides (41,64%); sin embargo, los derivados de ácidos grasos (54,30%) predominaron entre los producidos por las hojas. Los resultados obtenidos revelaron variaciones cualitativas y cuantitativas notables en los volátiles de las hojas y flores de *M. arboreus* que ayudarán a complementar nuestro conocimiento fitoquímico en esta planta estudiada hasta ahora de forma limitada.
Palabras clave: GC–MS; espacio de cabeza; malváceas; *Malvaviscus arboreus*; constituyentes volátiles.

Introduction

Malvaviscus is a small genus of perennial herbs or shrubs in the Hibiscus or mallow family, Malvaceae. It includes about 11 species with a natural distribution in the tropics and subtropics [1, 2]. Despite their close resemblance to *Hibiscus* plants, members of the genus *Malvaviscus* are generally distinguished by having ten, rather than five, capitate stigmas overlying a central style, in addition to the production of schizocarpic fruits formed of five separate parts [1, 3]. *Malvaviscus arboreus* Cav. (syn. *Malvaviscus mollis* (Aiton) DC. and *Hibiscus malvaviscus* L.) is a perennial deciduous shrub natively found in Central and South America but was also cultivated and naturalized in some tropical and subtropical regions of Africa, Asia, and Australia [4-6]. It is commonly known as Wax mallow, Turk's cap, and Sleeping Hibiscus; the latter name comes from its tightly wrapped petals that are slightly expanding only at the top [1]. The fast-growing shrubs of Sleeping Hibiscus are up to 1 m in length carrying ovate to broadly cordate leaves and red solitary flowers [7]. *Malvaviscus* plants, including *M. arboreus*, are widely grown as both garden ornamentals and medicinal plants with varied ethnomedical uses, mostly for wounds, fever, hypertension, sore throat, bronchitis, gastritis, and liver problems [1, 8-11]. The flowers, fruits, and leaves of *M. arboreus* are also useful for preparation of jellies, salads, herbal dyes, and herbal teas [1]. Previous phytochemical work on *M. arboreus* leaves and flowers revealed the presence of a range of phenolic metabolites, e.g. flavonoids, anthocyanins, and phenolic acids, along with sterols, terpenoids, and fatty acids [10, 12, 13]. Moreover, different extracts of this plant species were shown to possess antioxidant, hepatoprotective, cytotoxic, and anti-infective properties [14, 15]. Yet no reports are available in the literature on the volatile composition of *Malvaviscus* plants except for that by Vazquez-Cahuich *et al*. [16], where solely two compounds, namely eugenol and 1,4-dichlorobenzene, were detected at very low concentrations among the hydrodistilled leaf volatiles of *M. arboreus* growing in Mexico. Hence, in continuation of our research on *M. arboreus* plants cultivated in Egypt [12, 14, 17–19], the present work was designed to identify and compare the volatile constituents of its leaves and flowers using the headspace GC–MS technique.

Experimental

Plant material

The fresh leaves and flowers of *M. arboreus* were collected at the flowering stage from plants cultivated in the campus of Minia University, Minia, Egypt. The plant was identified by Prof. Mahmoud A. Hassan, Department of Horticulture, Faculty of Agriculture, Minia University. A voucher sample with the number Mn-Ph-Cog-027 was kept in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University.

Headspace GC-MS analysis

Volatiles were extracted using a solid phase microextraction (SPME) fiber coated with polydimethylsiloxane-divinylbenzene (65 µm film thickness; Supelco, Bellefonte, PA, USA). For each sample, the SPME device was inserted into the sealed vial by penetrating the silicone septum, followed by exposing the fiber to the headspace of the plant material (10.0 g) after 30 min. The fiber was exposed to each sample at 40 °C for 30 min. After extraction, the fiber was immediately inserted into the heated GC injector port for 10 min for desorption in the splitless mode. Samples were investigated in duplicate. GC–MS analyses were carried out

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using a Shimadzu GC–MS (QP-2010 Ultra, Kyoto, Japan) equipped with a Rtx-5MS column (5% diphenyl/ 95% dimethyl polysiloxane; 30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent, Palo Alto, CA, USA) and a headspace AOC-5000 auto-injector. Injections were performed in the splitless mode at 210 °C. A flame ionization detector was used at 230 ºC, whereas the temperatures of the interface and ion-source were kept at 280 and 230 ºC, respectively. Helium was used as a carrier gas at a flow rate of 1 mL/min. The programmed temperature technique was started at 40 ºC (for 2 min), then increased at 5 ºC/min to 210 °C with a 5-min hold at that final temperature, with the total run time was 40 min. Electron impact mass spectra were obtained at 70 eV; scanning from 35 to 500 *m/z*. A blank system with no plant material was also run at the same conditions as a control. The percentage composition of the identified volatiles was calculated from the total ion chromatogram (TIC), while they were identified by comparing their retention indices (RI) and mass spectra with some databases, including Wiley library 9, the National Institute Standard and Technology (NIST), and The Pherobase (Database of insect pheromones and semiochemicals) in addition to the literature [20-23].

Results and discussion

Headspace GC–MS analysis of *M. arboreus* leaves and flowers revealed notable qualitative and quantitative variations of their volatile constituents. A total of 39 compounds were detected and identified, including 11 from the flowers and 36 from the leaves, which indicates the higher abundance of volatiles in the latter (Table 1; Supplementary information: Fig. S1–S4). As shown in Table 1, the volatile blends of both plant parts were found to comprise varying levels of isoprenoids, fatty acid derivatives, phenyl propanoids/benzenoids, in addition to a group of other miscellaneous compounds. The chief proportion of floral volatiles was occupied by phenyl propanoids (41.64%) owing to the noticeable content of anethole (32.32%), the major volatile constituent identified from the flowers, along with its related compound, methyl chavicol (estragole; 9.32%). Both compounds were also detected in the leaves, albeit at much lower levels (7.39 and 1.66%, respectively). In contrast, fatty acid-derived compounds were detected as the major contributors to the volatile composition of *M. arboreus* leaves; accounting for 54.30% of their total volatiles, of which acetic acid (18.16%) was the main identified component followed by 2-cyclohexenone (9.60%). Although *M. arboreus* leaves enjoyed a chemically varied group of fatty acid derivatives, its flowers displayed an evidently lower emission level (8.68%) of these volatiles, with acetic acid (5.06%) and *n*-hexanol (3.62%) were merely observed, but also at a lesser extent as compared with the leaves (Table 1).

Table 1. Volatile compounds in the headspace of *Malvaviscus arboreus* flowers and leaves.

		Composition %		
Compound	**RI**	**Flowers**	**Leaves**	**Main fragment ions (*m/z*)[a]**
1) Isoprenoids:				
a) Monoterpenoids:		**7.42**	**5.88**	
Limonene	1033	5.03	2.34	136, 121, 107, 93, 79, <u>68</u>, 53, 41
1-((*E*)-but-1-enyl)cyclohex-1-ene	1039	–	0.70	136, 107, 94, 79, <u>68</u>, 53, 39
Linalool oxide	1072	–	0.74	137, 111, 94, 79, <u>59</u>, 43
Fenchone	1083	2.39	–	152, 109, <u>81</u>, 69, 41
β-Fenchol	1117	–	0.97	154, 139, 111, 93, <u>81</u>, 67, 55, 41
5-Hydroxy-2,7-dimethyl-4-octanone	1142	–	1.13	172, 113, 98, 85, 72, <u>69</u>, 59, 43
b) Diterpenoids:		**5.62**	**0.00**	

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Phytane	1810	5.62	–	183, 155, 141, 127, 113, 99, 85, 71, 57, 43
c) Irregular terpenoids:		**19.18**	**14.51**	
Methyl isobutenyl ketone	838	19.18	4.80	98, 83, 55, 43, 39
3-Methylene-2-pentanone	845	–	2.68	98, 83, 67, 55, 43, 39
6-Methyl-5-heptene-2-one	997	–	3.07	126, 108, 93, 69, 55, 43
Safranal	1196	–	1.13	150, 135, 121, 107, 91, 79, 65, 39
β-Cyclocitral	1219	–	0.74	152, 137, 120, 109, 91, 81, 67, 55, 39
1,5,7-Trimethyl-1,2,3,4-tetrahydronaphthalene	1250	–	0.67	174, 159, 143, 131, 117, 105, 91
Vitispirane	1268	–	0.74	192, 177, 163, 149, 136, 121, 107, 93, 77, 65, 55, 41
Geranyl acetone	1453	–	0.68	194, 151, 136, 125, 107, 93, 69, 43
Total isoprenoids (%)		**32.22**	**20.39**	
2) Fatty acid derivatives:				
Acetic acid	621	5.06	18.16	60, 45, 43
1-Hydroxy-2-propanone (Hydroxy acetone)	662	–	2.59	74, 43
2-Methylbutanal (Isovaleraldehyde)	669	–	5.28	86, 72, 57, 41
Amyl alcohol (1-Pentanol)	762	–	1.89	70, 55, 42
1-Hydroxy-2-butanone	768	–	1.56	88, 72, 57
cis-3-Hexenol	846	–	5.85	100, 82, 67, 55, 41
1-Hexanol	851	3.62	5.51	84, 69, 56, 43
2-Heptanone	889	–	1.19	114, 106, 91, 71, 58, 43
2-Cyclohexenone	914	–	9.60	96, 68, 39
3-Methyl-2-butenyl isovalerate	1181	–	2.67	84, 69, 55, 41
Total fatty acid derivatives (%)		**8.68**	**54.30**	
3) Phenyl propanoids/ bezenoids:				
p-Methylbenzyl alcohol	978	–	0.66	122, 107, 91, 79, 66, 40
p-Allylanisole (Methyl chavicol; Estragol)	1195	9.32	1.66	148, 133, 121, 117, 105, 91, 77, 65, 51, 39
p-Propenylanisole (Anethole)	1285	32.32	7.39	148, 133, 117, 105, 91, 77, 65, 51
p-Vinyl guaiacol (2-Methoxy-4-vinylphenol)	1310	–	0.84	150, 135, 107, 89, 77, 63, 51, 39

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Apiole	1677	–	0.85	222, 207, 191, 177, 161, 149, 133, 121, 106, 91, 77, 65, 53, 39
Total phenyl propanoids/ benzenoids (%)		**41.64**	**11.40**	
4) Miscellaneous compounds:				
2-Ethylfuran	704	–	1.88	96, 81, 67, 53, 39
2,5-Dimethylfuran	712	7.08	–	96, 81, 67, 53, 43
Furfural	836	7.59	0.84	96, 67, 39
Dihydro-2-methyl-3-furanone	850	–	0.10	100, 72, 43
2-(Methoxymethyl)-furan	861	–	0.64	112, 81, 53
2-Ethyl-5,5-dimethyl-1,3-cyclopentadiene	834	–	5.29	122, 107, 91, 79, 65, 51
2-Pentylfuran (2-Amylfuran)	993	2.79	3.07	138, 121, 105, 95, 81, 67, 53, 39
2-Ethenyl-2-methyl-5-(1-methylethenyl) tetrahydrofuran	1208	–	0.63	152, 137, 123, 110, 96, 82, 67, 55, 43
3,3-Dimethyl-1-phenylbutane	1374	–	1.46	162, 147, 106, 91, 71, 57, 41
Total Miscellaneous compounds (%)		**17.46**	**13.91**	
Total oxygenated compounds (%)		**89.35**	**89.54**	
Total non-oxygenated compounds (%)		**10.65**	**10.46**	
Total identified compounds (%)		**100**	**100**	

[a]Underlined *m/z* values indicate base peaks.

Furthermore, the volatile profile of *M. arboreus* exhibited a significant range of terpenoids that amounted to 20.39% in the leaves and 32.22% in the flowers (Table 1). This considerable isoprenoid contents of both the leaves and flowers were largely dominated by irregular terpenoids that accounted for 14.51 and 19.18% of their total volatiles, respectively. Generally, irregular terpenoids are volatile carotenoid-derived compounds that essentially contribute to the odour profile of plants. They are produced through several fragmentation patterns of various carotenoids found in different plant parts, mostly by enzymatic oxidation and photo-oxidation [24]. As depicted in Table 1, a variety of irregular terpenoids was emitted by the leaves of *M. arboreus*, encompassing small acyclic C_6 and C_8 ketones (e.g. methyl isobutenyl ketone and 6-methyl-5-heptene-2-one), C_{10} aldehydic derivatives (e.g. safranal and β-cyclocitral), in addition to some C_{13}-norisoprenoids (e.g. vitispirane and geranyl acetone), among which, methyl isobutenyl ketone (4.80%) was found to predominate. On the contrary, none of these volatile components was detected in the flowers except for methyl isobutenyl ketone, which was given off at a higher percentage (19.18%). In the same context, although *M. arboreus* flowers exhibited a relatively higher content of total monoterpenoids (7.42%) in comparison with its leaves (5.88%), only two monoterpenoids were identified from the flowers, namely limonene (5.03%) and fenchone (2.39%). The monoterpenoid fraction of the leaves, on the other hand, was dominated by limonene (2.34%), whereas β-fenchol (0.97%), rather than fenchone, was observed among their montoerpenoids. It is also worth noting that the volatile mixtures of both plant parts of *M. arboreus* have totally

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lacked sesquiterpenoids, while only one diterpene hydrocarbon, namely phytane, was exclusively emitted from the flowers at a considerable level (5.62%) (Table 1).

Aside from the aforementioned groups of biosynthetically related volatiles, a number of miscellaneous compounds have also contributed to the headspace composition of *M. arboreus* leaves and flowers, constituting 13.91 and 17.46% of their total volatile blends, respectively (Table 1). Among such varied volatiles, a considerable concentration of furanoids was noticed, including furfural that was emitted at a marked level from the flowers (7.59%) followed by 2,5-dimethylfuran (7.08%). Conversely, only negligible amount of furfural (0.84%) was recorded from the leaves, while 2,5-dimethylfuran was totally absent. Some other furan derivatives, e.g. 2-pentylfuran, 2-ethylfuran, and 2-(methoxymethyl)-furan were also characterized at different quantitative ratios, particularly from the leaves. Nevertheless, the assortment of miscellaneous compounds identified from the leaves was predominated by 2-ethyl-5,5-dimethyl-1,3-cyclopentadiene (5.29%) that, on the other hand, was not detected in the flowers (Table 1).

Finally, it is also worth mentioning that the volatile mixtures of the two investigated plant parts of *M. arboreus* displayed comparable amounts of hydrocarbons (10.46% in leaves and 10.65% in flowers) and oxygenated compounds (89.54% in leaves and 89.35% in flowers), with a noticeable greater contribution of the latter to their total volatiles. Among the family of oxygenated volatiles described from *M. arboreus*, a diversity of chemical classes was recorded (Table 2; Fig. 1). In this regard, phenolic ethers (41.64%) have dominated the oxygenated compounds emitted from the flowers followed by ketones (21.57%), with anethole (32.32%) being the major identified component. In contrast, the main proportion of the volatile principles of the leaves was primarily represented by ketones (27.40%), although acetic acid (18.16%) was the chief constituent given off by the leaves. Moreover, while methyl isobutenyl ketone (19.18%) has dominated the ketone content of the flowers, 2-cyclohexenone (9.60%) was the principal ketone detected from the leaves. Another important difference between the volatile compositions of the studied plant parts from *M. arboreus* is the marked prevalence of alcohols in the leaves (14.88%) as compared with the flowers (3.62%) (Table 2; Fig. 1). Of these, *cis*-3-hexenol (5.85%) and *n*-hexanol (3.62%) were the major alcohols identified from the leaves and flowers, respectively. On the other hand, both the leaves and flowers of *M. arboreus* demonstrated relatively comparable levels of aldehydes, accounting for 7.99 and 7.59% of their headspace volatiles, respectively, with furfural (7.59%) being the major aldehyde observed in the flowers, whereas 2-methylbutanal (5.28%) was uniquely characterized from the leaves as the chief component of their aldehyde fraction. Moreover, minor concentrations of esters (2.67%), phenols (0.84%), and oxides (0.74%) were found to contribute to the volatile emission of *M. arboreus*, but only from the leaves (Table 2; Fig. 1).

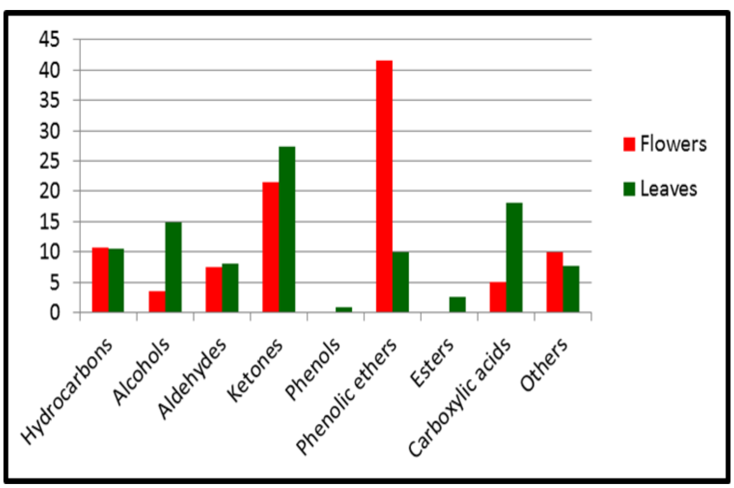

Fig. 1. Comparison of different classes of volatile compounds identified from *Malvaviscus arboreus* flowers and leaves.

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Table 2. Different classes of volatile compounds of *Malvaviscus arboreus* flowers and leaves.

Chemical class	Flowers		Leaves	
	No. of compounds	Total %	No. of compounds	Total %
1) Hydrocarbons	2	10.65	5	10.46
2)Oxygenated compounds:	9	89.35	31	89.54
a) Alcohols	1	3.62	5	14.88
b) Aldehydes	1	7.59	4	7.99
c) Ketones	2	21.57	10	27.40
d) Phenols	–	–	1	0.84
e) Phenolic ethers	2	41.64	3	9.90
f) Esters	–	–	1	2.67
g) Carboxylic acids	1	5.06	1	18.16
h) Others	2	9.87	6	7.70

Conclusion

Comparative headspace GC–MS investigation of *M. arboreus* leaves and flowers has revealed different qualitative and quantitative patterns of their volatile organic compounds, with a marked abundance of volatiles in the leaves. Volatile emissions from both plant parts were dominated by a structurally varied pool of oxygenated compounds, of which ketones and phenolic ethers prevailed in the leaves and flowers, respectively. The volatile profile of the leaves was also characterized by the prevalence of acetic acid, while anethole was the most abundant compound in the flowers. Besides, the volatile composition of *M. arboreus* showed considerable amounts of irregular terpenoids and furanoids, particularly in the flowers, whereas no sesquiterpenoids were detected in both organs. As the first comprehensive report on the volatile metabolites of *M. arboreus*, this work would help expand both the current phytochemical knowledge and future research on plants belonging to the genus *Malvaviscus*.

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