SHORT REPORT

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Headspace Analysis of Volatile Compounds Coupled to Chemometrics in Leaves from the Magnoliaceae Family

Mohamed A. Farag [1,2*], Riham Salah El Din [2] and Sherifa Fahmy [2]

[1] *Pharmacognosy Department, College of Pharmacy, Kasr El-Aini St, Cairo University, Egypt P.B.11562.*

[2] *Center for Regulatory and Environmental Analytical Metabolomics, Department of Chemistry, University of Louisville 2320 S. Brook St., Louisville, Egypt KY 40292*

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Abstract: Headspace volatile analysis has been used for volatiles profiling in leaves of 4 *Magnolia* species with a total of 75 compounds were identified. Monterpene hydrocarbons dominated the volatile blend of *M. calophylla* (86%), *M. acuminata* (78%), *M. virginiana* (70%) and *M. grandiflora* (47%) with β-pinene and *β*-ocimene occurring in the largest amounts, whereas sesquiterpenes were the most abundant compounds in *M. grandiflora* (39%). High levels of oxygenated compounds were only found in *M. virginiana* volatile blend (11.4%) with 2-phenylethyl alcohol as major component. Hierarchical cluster analysis performed on volatiles content revealed the close relationship between *M. acuminata* and *M. calophylla*.

Keywords: GC-MS; *Magnolia acuminata*; *Magnolia calophylla*; *Magnolia grandiflora*; *Magnolia virginiana*; Headspace volatiles; Hierarchical cluster analysis.

1. Plant Source

The family Magnoliaceae is an ancient lineage of plants represented by approximately 223 species of trees and shrubs in 7 genera, and *Magnolia* is the largest genus with 128 species [1] distributed in temperate and tropical regions of the world. *Magnolia* plant produces a large number of highly odiferous, cup-shaped flowers mostly used as ornamental plants. In terms of its medicinal use, *Magnolia* exhibit anti-inflammatory, antioxidant, anti-angiogenic and anti-tumor effects [2, 3]. Leaves were collected from cultivated flower-producing trees in the University of Kentucky arboretum and identified by Dr. Robert Pratley (University of Kentucky Herbarium. Voucher specimens of *M. acuminata* L. (UKH-246), *M. calophylla* L. (UKH-259), *M. grandiflora* L. (UKH-854) and *M. virginiana* L. (UKH-934) are deposited at the University of Kentucky Herbarium.

2. Previous Studies

Chemical analyses of floral scent of temperate Asia and North American species of *Magnolia* identified more than 75 chemicals in a variety of classes including terpenoids, benzenoids, fatty acid derivatives, and N-containing compounds [4-6]. Most research on volatile constituents in *Magnolia* species

* Corresponding author:E-mail: mfarag73@yahoo.com; Phone: +202-24178151; Fax:+ 202-26328426

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has focused on its flowers and bark [7, 8] with little information on other organs as leaf. *Magnolia* leaf exhibits a characteristic scent which suggests the presence of volatile constituents. Nevertheless, few studies have focused on examining its volatile constituents. Azuma et al. [9] reported on the change in volatiles profile in several *Magnolia* species leaves in response to wounding. A total of 10 components were identified from damaged *M. grandiflora* leaves including (*Z*)-3-hexenyl acetate, β-myrcene, limonene, β-ocimene, 4,8-dimethyl nonatriene, β-caryophyllene, α-humulene, β-elemene and γ-cadinene. Attempts to characterize *Magnolia* intact leaves volatile blend precluded the detection of any constituent likely to experimental setup or age of leaves used in the study [9]. Only, recently solid phase microextraction (SPME) headspace analysis coupled to GCMS was conducted to determine the chemical composition of *M. grandiflora* flower & leaf volatile constituents, with a total of 48 constituents and having γ-elemene, 2,6-dimethyl-6-bicyclo[3.1.1]hept-2-ene and β-caryophyllene as major leaf constituents [10, 11].

3. Present study

In this study, dynamic headspace volatile analysis combined with GC/MS was utilized for profiling of leaf volatiles in 4 *Magnolia* sp., *M. acuminata* (Cucumber tree), *M. calophylla, M. grandiflora* (Southern magnolia) and *M. virginiana* (Sweetbay magnolia). Extraction and analysis of leaf volatiles as headspace followed the procedure described by Farag [12] an in supplenmnetary S1.

The volatile compounds identified from *Magnolia* leaves are listed in Table 1, Supplementary Figure. S2. Components were categorized into aliphatic, aromatic, monoterpene and sesquiterpene hydrocarbons, alcohols, carbonyls (aldehydes/ketones), and esters for comparative convenience. A total of 75 compounds were detected of which only 7 were previously reported from leaves of *M. grandiflora* [10]. Monoterpene hydrocarbons constituted the most dominant chemical group among *Magnolia* volatiles: *M. calophylla* 86%, *M. acuminata* 78%, *M. virginiana* 70% and *M. grandiflora* 47%. Predominant volatile forms in *M. calophylla* and *M. virginiana* were β-pinene measured 64% and 37%, respectively, whereas (*Z*/*E*)-β-ocimene were the most abundant monoterpenes in *M. acuminata* 67% and *M. grandiflora* 17%. β-ocimene was previously identified as major volatile component in the floral scent of *M. kobus* [6] & *M. grandiflora* [13]. All *Magnolia* species released α-pinene, β-pinene, D-limonene, γ-terpinene and α-terpinolene in agreement with reports in *M. kobus* & *M. grandiflora* flowers [6, 13].

Next to monoterpenoids, sesquiterpenes were the most abundant compounds in the volatile blend of *M. grandiflora* (39%) with germacrene A and β-bisabolene accounting for up to 26 % among sesquiterpenes content. Common sesquiterpenes found in most *Magnolia* species leaves included germacrene A and β-farnesene. β-caryophyllene and γ-elemene, both previously identified as major components in *M. grandiflora* leaves [10] were also detected from our study, though at much lower levels. Discrepancies between present and earlier study, could be due to different type of adsorbent used for volatiles trapping, although nature of stationary phase used by Wang group [10] was not specified to confirm such hypothesis.

A total of 35 oxygenated compounds were found amounting to 11% in *M. virginiana* and to a lesser extent in other species (3-5%). Compounds belonged to carbonyl compounds (aldehydes/ketones), alcohols, esters and ethers/oxides. Among alcohol compounds, low levels were detected in most species (2-3%), except for *M. virginiana* (10%) owing to its high 2-phenylethyl alcohol content. *M. acuminata and M. calophylla* possessed more or less a similar alcohol volatiles profiles differing from that of *M. virginiana* and *M. grandiflora.* Low emission levels in aromatic (0.9-2.8%) and aliphatic hydrocarbons (0.2-0.8%) were observed in all species with p-cymene and naphthalene being the most common.

Table 1. Relative percentage of volatile aroma compounds in the headspace of *M. acuminata, M. calophylla, M. grandiflora* and *M.virginiana* leaves (n=3).

RRI	Compound	Concentration (%)[b]			
		M. acuminata	***M. calophylla***	***M. grandiflora***	***M.virginiana***
906	(2*E*, 4*E*)-hexadienal	0.1	-	-	3.5
926	α-thujene	t.	0.1	-	-
933	α-pinene[a]	t.	0.1	0.8	0.1
948	α-fenchene	t.	-	-	-
961	benzaldehyde [a]	-	-	-	0.3
966	5-ethyl-2(5H)-furanone	0.8	-	-	0.3
973	3,4-dimethyl-2-hexene	0.5	-	-	-
977	β-thujene	-	-	4.0	0.4
979	β-pinene[a]	2.7	64.4	1.8	37.4
1000	α-phellandrene[a]	0.8	7.0	-	0.1
1001	(2*E*, 4*E*)-heptadienal	0.2	1.1	-	0.6
1007	(*Z*)-3-hexen-1-ol-acetate [a]	-	0.2	0.2	-
1008	δ-3-carene	2.0	-	-	-
1018	δ-4-carene	t.	-	-	0.2
1021	*p*-cymene[a]	1.7	3.2	0.6	7.6
1023	pseudocumene	-	0.1	0.2	-
1029	limonene[a]	1.4	7.0	1.4	1.9
1031	eucalyptol [a]	-	-	-	0.2
1033	benzyl alcohol[a]	-	-	0.2	-
1037	(*Z*)-β-ocimene[a]	36.5	0.5	15.2	7.6
1045	benzene acetaldehyde	0.7	0.4	0.7	-
1049	(*E*)-β-ocimene	30.8	1.4	2.1	3.1
1054	phenyl ethyl ketone	-	-	0.1	-
1059	γ-terpinene	0.2	1.4	0.2	1.7
1071	1-octanol	0.3	-	0.1	-
1089	α-terpinolene	1.9	0.6	1.1	6.3
1099	linalool [a]	0.3	0.4	1.2	3.0
1101	3, 4-dimethyl styrene	0.1	-	-	3.6
1102	nonanal [a]	0.2	0.2	1.4	0.3
1107	unknown hydrocarbon	0.1	0.7	4.0	-
1108	2-phenylethyl alcohol	0.9	0.7	-	6.3
1128	unknown monoterpene	-	-	19.5	tr.
1139	2,6-dimethyl-2,4,6-octatriene	0.3	-	0.2	-
1178	terpinen-4-ol	-	0.1	-	0.3
1180	naphthalene[a]	-	0.3	0.7	0.3
1184	*p*-cymen-8-ol	0.4	0.1	-	0.2
1188	α-terpineol[a]	-	0.1	-	0.3
1191	methyl salicylate [a]	0.4	0.6	-	0.2
1198	decanal [a]	-	-	0.3	-
1199	dodecane[a]	-	-	0.3	0.2
1219	β-citronellol[a]	0.4	0.3	-	-
1232	δ-3-caren-10-al	0.2	-	-	-
1249	methyl citronellate	-	0.1	-	-
1282	bornyl acetate	0.1	-	-	-
1291	tridecane[a]	t.	t.	-	-

1295	(*E*)-pinocarvyl acetate	-	-	0.6	-
1337	δ-elemene	0.2	0.1	0.1	-
1352	citronellyl acetate	0.1	-	-	-
1364	neryl acetate	-	0.1	-	-
1380	β-cubebene	-	-	0.1	0.5
1385	β-bourbonene	0.3	-	-	0.1
1400	tetradecane	0.1	-	0.6	-
1411	α-gurjunene	1.1	0.1	-	-
1412	α-cedrene	-	-	0.2	-
1418	β-caryophyllene[a]	-	-	0.8	3.2
1430	γ-elemene	0.3	-	2.6	-
1432	α-bergamotene	-	-	1.1	0.1
1435	α-guaiene	0.1	-	-	-
1450	α-humulene[a]	-	-	-	2.2
1459	(*E*)-β-farnesene[a]	0.3	3.5	1.5	-
1481	γ-gurjunene	-	-	0.6	-
1491	τ-muurolene	-	-	-	1.0
1495	β-selinene	-	-	0.2	-
1497	α-zingiberene[a]	-	0.2	-	-
1506	germacrene A	9.6	1.4	12.9	3.8
1508	(*E*, *E*)-α-farnesene	0.7	0.5	-	-
1511	β-bisabolene[a]	-	-	13.3	-
1513	ledene	-	-	0.8	-
1523	τ -cadinene	-	-	0.8	-
1529	δ-cadinene	0.2	-	0.8	1.4
1549	unknown sesquiterpene	-	-	3.3	-
1560	β-cadinene	0.3	-	-	-
1575	germacrene-D-4-ol	0.1	-	-	-
1588	viridiflorol	-	-	0.1	-
1651	ledene oxide	0.1	-	0.3	-
Total identified%		**73**	**74**	**72**	**74**
Total monoterpene hydrocarbons %		**(78.3)**	**(85.7)**	**(47.1)**	**(66.9)**
Total sesquiterpene hydrocarbons %		**(12.8)**	**(5.8)**	**(39.1)**	**(12.3)**
Total oxygenated compounds %		**(5.3)**	**(2.0)**	**(5.2)**	**(11.4)**

[a]Constituents identified by (i), (ii), (iii) and (iv) co-injection. RRI, retention index on DB-Wax column, MS, identification was based on comparison of mass spectra. All other oil constituents were identified by (i) mass spectral database match, (ii) comparison of mass spectrum with literature data and (iii) RRI.
[b] Relative concentration based on triplicate measurements;**—**, not detected; RRI-retention index; t-traces, <0.05%.
MS data for unknown monoterpene RI 1128, *m/z* (rel. int.): 150(11%), 135(11%), 107(12%), 69(100%).
MS data for unknown sesquiterpene RI 1549, *m/z* (rel. int.): 204(16%), 121(32%), 119(32%), 93(100%), 80(25%).
MS data for unknwon hydrocarbon RI 1107, *m/z* (rel. int.): 130(3%), 103(26%), 85(54%), 57(100%)

Hierarchical cluster analysis (HCA) was performed to define both similarities and differences across *Magnolia* species in a fairly intuitive graphical way. Cluster analysis of the different *Magnolia* species showed two major clear clusters, each of 2 genotypes (Supplementary Figure. S3) referred to as groups 1A and 1B. *M. calophylla* and *M. acuminata* represent one group (1B) as evident from their more or less comparable monoterpene hydrocarbons/alcohol profile and content, differing from that of *M. virginiana and M. glandiflora.* Inspection of group 1A revealed that *M. virginiana* leaf scent is more closely related to *M. grandiflora, in* support of similar clustering results based on their floral scent [5] and or molecular analysis [12]. Principal component analysis (PCA) was further performed to explore the relative variability within the

different species. The PC1/PC2 scores plot (Supplementary Fig. S4) shows that 3 major, distinct clusters are formed corresponding to the 4 different species studied mostly along PC1 and PC2 overall explaining 100% of the variance. Except for *M. grandiflora*. triplicate measurements from the same sample were found to be highly reproducible, as the scores of replicate measurements were more or less superimposed. On the right side of the plot, *M. calophylla* samples are positioned (positive PC1 values), whereas on the far left side, *M. acuminata* samples are located (negative PC1 values) whereas *M. virginiana* and *M. grandiflora* are spread in between. The metabolite loading plot, which exposes the most important components with respect to scattering behavior, reveals that β-pinene, limonene and 2-phenylethanol contributed the most, positively along PC1. The second group had a negative effect on PC1, mostly from β-(*Z/E*)-ocimene enrichment in *M. acuminata* leaf.

In conclusion, these results provide the first comprehensive volatile profile of leaves in family Magnoliaceae and help provide further information for phylogenetic relationship based on volatiles composition across *Magnolia* taxa.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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