Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

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Mohamed A. Farag [a b] & Dalia A. Al-Mahdy [b]
[a] Chemistry Department , Center for Regulatory and Environmental Analytical Metabolomics, University of Louisville , 529 S. Jackson St., Louisville , KY 40202 , USA
[b] Pharmacognosy Department , College of Pharmacy, Kasr El-Aini St, Cairo University , P.B. 11562 Cairo , Egypt
Published online: 12 Jun 2012.

To cite this article: Mohamed A. Farag & Dalia A. Al-Mahdy (2013): Comparative study of the chemical composition and biological activities of Magnolia grandiflora and Magnolia virginiana flower essential oils, Natural Product Research: Formerly Natural Product Letters, 27:12, 1091-1097

To link to this article: http://dx.doi.org/10.1080/14786419.2012.696256

Natural Product Research, 2013
Vol. 27, No. 12, 1091–1097, http://dx.doi.org/10.1080/14786419.2012.696256

SHORT COMMUNICATION

Comparative study of the chemical composition and biological activities of *Magnolia grandiflora* and *Magnolia virginiana* flower essential oils

Mohamed A. Farag[ab]* and Dalia A. Al-Mahdy[b]

[a]*Chemistry Department, Center for Regulatory and Environmental Analytical Metabolomics, University of Louisville, 529 S. Jackson St., Louisville, KY 40202, USA;* [b]*Pharmacognosy Department, College of Pharmacy, Kasr El-Aini St, Cairo University, P.B. 11562 Cairo, Egypt*

(*Received 26 January 2012; final version received 10 May 2012*)

The biological activities and the determined major volatile components in the *Magnolia grandiflora* and *M. virginiana* flowers extracts were compared. Volatile components were detected in the essential oil by dynamic headspace sampling (HS). 2-Phenylethanol (40% and 61%) was found as the main constituent in the essential oil and HS samples of *M. virginiana,* respectively. In the *M. grandiflora* oil sample, (*E*,*E*)-farnesol (18%) and 2-phenylethanol (10%) were found as main constituents, whereas germacrene D (17%) and β-bisabolene (17%) were the main components of the HS sample. The essential oil in *M. virginiana* displayed a moderate antioxidant activity relative to vitamin E, whereas both essential oils were active against human lung carcinoma and breast carcinoma cell lines, even at concentrations higher than 200 $\mu g\,mL^{-1}$.

Keywords: *Magnolia grandiflora* L.; *Magnolia virginiana* L.; headspace volatiles; essential oil; antioxidant; anticancer; GC-MS

1. Introduction

Magnolia is a large genus of about 210 flowering plant species in the family Magnoliaceae, indigenous to tropical, subtropical Americas and Southeast Asia (Jones, 2005). Many members of this genus, such as *Magnolia grandiflora* (Southern Magnolia) and *M. virginiana* (sweet bay Magnolia), are found as ornamental trees in large parts of North America, chiefly valued for their highly odiferous, large cup-shaped flowers. In regard to pharmacology or biological activities, *M. officinalis* has long been used as a remedy for flatulent dyspepsia, cough and asthma (Pu, Pannell, & Ji, 1990). Essential oil from the bark of *Magnolia obovata* displayed a strong antifungal activity, mostly attributed to its polyphenol content. Studies regarding the volatile components in *M. liliflora* (Fujita, 1989), *M. kobus* (Azuma, Toyota, & Asakawa, 2001) and *M. salicifolia* flowers (Li, Tanaka, Kurasawa, Ikeda, & Nohara, 2007) are available in the literature. Chemical analysis of the floral scent of temperate North American *Magnolia* species revealed distinct chemical profiles: *M. kobus* releases linalool, *M. salicifolia* emits 1,2-dimethoxybenzene, whereas *M. virginiana* emits 2-phenylethanol (Azuma et al., 1997). This variation in floral volatile composition could assist in the differentiation of *Magnolia* subspecies with similar flower morphology (Azuma et al., 2001).

*Corresponding author. Email: mfarag73@yahoo.com

A survey of the literature indicates that *M. grandiflora* and *M. virginiana* have not been much studied. Azuma et al. (1997) reported the results of the headspace analysis of flowers produced in both Japan and USA. The major components were myrcene, geraniol, methyldodecanoate and (*E*)-ocimene in *M. grandiflora* headspace, whereas 2-phenylethanol dominated the *M. virginiana* headspace. In this study, the essential oil and headspace volatiles from both *M. grandiflora* and *M. virginiana* flowers were analysed and compared by GC-MS to provide a more comprehensive profile of its floral scent. This article presents the first detailed comparative study between hydrodistilled oils and headspace volatiles derived from *M. grandiflora* and *M. virginiana* flowers. Moreover, essential oils were also evaluated for their antioxidant and anticancer activities reported for other *Magnolia* species.

2. Results and discussion

2.1. *Chemical composition*

The complete list of identified volatiles in *M. grandiflora* and *M. virginiana* is presented in Table 1. GC-MS analyses resulted in the characterisation of 65 and 45 compounds in the essential oil and HS samples of *M. grandiflora,* which accounted for 99% and 95% of the total composition, compared with 23 components previously reported from flowers of *M. grandiflora* headspace (Azuma et al., 1997). Increase in the score of identification in this study is due to the processing of GC/MS files by the AMDIS software (www.AMDIS.net) that assists in adjacent peak deconvolution, background subtraction and increased detection limit. Various chemical compounds, such as terpenoids, benzenoids and fatty acid derivatives, were identified. In the oil sample of *M. grandiflora*, (*E*,*E*)-farnesol (18%) was found as a main constituent, whereas germacrene D (17%) and β-bisabolene (17%) were detected as major components in the HS sample. In the essential oil and HS samples of *M. virginiana*, 49 and 31 compounds were identified representing 98% and 99% of the total volatile composition, respectively, of which 9 were previously reported in *M. virginiana* headspace (Azuma et al., 1997). Aromatic compounds represented the major constituent in the essential oil of *M. virginiana* (46%) and HS sample (65%), with 2-phenylethanol as the main component in both the samples (40–60%), in agreement with previous reports (Azuma et al., 1997). Technically, with regard to different isolation methods of the volatile compounds, hydrodistilled oils showed a higher number of compounds compared to HS-trapped volatiles for both species, as seen in Table 1. Both techniques also showed notable differences in volatile components. While oxygenated monoterpenes were the main component of the essential oil of *M. grandiflora* (28%), sesquiterpene hydrocarbons predominated in the HS sample (45%). In *M. virginiana,* esters such as methyl myristate (11%) and methyl palmitate (5%) were detected at high levels in the oil sample, being completely absent or at low concentration in the HS sample. In both species, the HS extract was found to be more enriched in terpene hydrocarbons, whereas oxygenated terpenes were present at higher levels in the distilled oil. This inconsistency may be due to differences in volatility and chemical conversions that might have taken place during hydrodistillation, compared to the mild HS technique (Fernando & Grun, 2001).

The chemical composition of both oils showed qualitative and quantitative differences. The most notable difference was the low percentage of oxygenated terpenes in *M. virginiana* (11%), compared to *M. grandiflora* (52%). Another point of interest is the presence of large amount of aromatic components in both oils, 46% in *M. virginiana* and 20% in *M. grandiflora*. Among aromatic compounds, benzene acetaldehyde was found only in *M. grandiflora* samples, whereas phenyl ethyl acetate was found exclusively

Table 1. Volatile composition (%) of *M. grandiflora* and *M. virginiana* flowers.

		M. grandiflora		*M. virginiana*	
RRI	Compound	Essential oil	HS	Essential oil	HS
925	α-Thujene	0.1	–		–
932	α-Pinene[a]	0.4	–	0.2	–
949	Camphene	0.1	–	–	–
958	Benzaldehyde[a]	–	0.1	–	0.2
974	β-Pinene[a]	1.4	0.1	0.3	–
990	β-Myrcene[a]	0.1	0.3	1.1	17.7
1022	p-Cymene[a]	–	–	0.1	1.7
1029	Limonene[a]	–	0.1	–	1.0
1031	1,8-Cineole	1.4	–	0.5	–
1034	Ethyl hexanol	–	–	–	0.2
1029	Benzyl alcohol[a]	0.1	0.5	–	0.2
1035	(*Z*)-β-ocimene[a]	0.9	5.8	–	–
1044	Benzene acetaldehyde	8.5	17.0	0.1	1.6
1049	(*E*)-β-ocimene[a]	–	3.6	–	1.2
1069	1-Octanol	–	0.1	–	–
1084	(*E*)-linalool oxide	0.1	–	–	–
1091	α-Terpinolene[a]	–	–	1.6	2.4
1101	3,4-Dimethyl styrene	–	–	0.1	–
1199	Linalool[a]	0.6	0.2	0.4	1.5
1112	Nonanal[a]	–	0.8	–	0.2
1113	2-Phenylethanol	10.8	2.2	39.9	61.3
1141	(*E*)-Pinocarveol	0.2	–	–	–
1143	Camphor	0.9	–	–	–
1163	(*E*)-2-nonenal	–	0.1	–	–
1165	Pinocarvone	0.2	–	–	–
1168	Borneol	2.9	–	–	–
1171	Epoxy linalool	0.1	–	0.1	–
1175	3-Pinanone	0.1	–	–	–
1178	Terpinen-4-ol	0.9	–	0.3	–
1179	Methyl phenyl acetate	–	0.3	–	–
1180	Naphthalene[a]	–	0.4	–	–
1181	Myrtanal	0.2	–	–	–
1183	p-Cymen-8-ol	0.2	–	0.3	0.1
1188	α-Terpineol	2.3	–	0.4	–
1190	Methyl salicylate[a]	–	–	–	0.6
1192	Myrtenol	0.8	0.1	–	–
1199	Decanal	–	0.3	–	–
1201	Verbenone	2.2	0.3	1.5	0.2
1227	β-Citronellol	0.2	–	0.3	–
1253	(*Z*)-Geraniol	4.0	10.5	–	0.1
1257	2-Phenylethyl acetate	–	–	4.1	0.6
1262	(*E*)-Myrtanol	0.6	–	–	–
1280	α-Citral	–	0.7	0.1	–
1289	Bornyl acetate	3.3	–	0.9	–
1310	Pinocarvyl acetate	1.3	–	–	–
1337	δ-Elemene	0.1	0.2	0.1	–
1342	2-Hydroxycineole acetate	3.7	–	–	–
1357	Eugenol[a]	0.3	–	–	–
1372	α-Ylangene	0.1	–	0.2	–
1376	α-Copaene	0.1	–	0.3	0.1
1381	Geranyl acetate	0.8	0.3	–	–
1390	β-Elemene	1.7	0.9	1.2	–

(*continued*)

Table 1. Continued.

RRI	Compound	*M. grandiflora*		*M. virginiana*	
		Essential oil	HS	Essential oil	HS
1394	(*Z*)-Jasmone[a]	1.1	0.5	–	–
1413	cis-α-Bergamotene	–	–	1.3	0.2
1418	β-Caryophyllene[a]	0.7	–	3.0	0.4
1432	trans-α-Bergamotene	0.2	0.6	0.4	0.2
1435	α-Guaiene	0.2	0.2	–	–
1437	Aromadendrene	0.1	–	–	–
1452	α-Humulene[a]	–	–	4.6	0.5
1459	(*E*)-β-Farnesene[a]	0.3	3.1	–	–
1469	γ-Gurjunene	0.2	0.2	–	0.2
1478	β-Chamigrene	1.5	–	–	0.5
1480	Germacrene D	1.9	16.6	1.5	1.4
1488	β-Selinene	2.0	0.1	0.9	–
1495	α-Selinene	2.7	1.5	1.4	–
1496	τ-Elemene	0.9	2.1	0.4	2.7
1498	β-Himachalene	–	–	–	0.5
1503	α-Muurolene	2.0	–	1.5	–
1505	β-Bisabolene[a]	1.5	16.7	–	–
1508	τ-cadinene	0.6	–	1.5	0.9
1518	δ-Cadinene	3.8	0.5	3.2	0.9
1529	τ-Gurjunene	–	–	0.3	–
1544	Elemol	0.4	–	–	–
1563	(*E*)-Nerolidol[a]	1.0	0.1	1.0	–
1575	Germacrene-D-4-ol	–	0.7	–	–
1584	Globulol	1.1	0.1	0.3	–
1596	Viridiflorol	0.4	–	–	–
1611	δ-Cadinol	–	–	0.7	–
1644	α-Cadinol	1.5	–	0.7	–
1659	Patchouli alcohol	–	–	0.7	–
1682	11-Methyl tetradecenoate	0.8	0.8	0.3	–
1720	(2*E*, 6*Z*)-Farnesol[a]	18.0	2.3	2.3	–
1723	Methyl myristate	4.3	2.7	11.5	–
1922	Methyl palmitate	0.7	–	4.4	–
Identified compounds					
Monoterpene hydrocarbons		3.0	10.6	3.5	24.0
Oxygenated monoterpenes		27.8	12.7	5.3	1.8
Sesquiterpene hydrocarbons		19.5	45.1	22.3	8.8
Oxygenated sesquiterpenes		23.8	3.7	5.9	–
Aromatic compounds		19.8	20.4	45.9	64.7
Hydrocarbons		5.9	7.9	16.9	0.5

Notes: All oil constituents were identified by (i) mass spectral database match, (ii) comparison of mass spectrum with literature data, (iii) RRI, relative retention index to a hydrocarbon series C_6–C_{20}. Relative concentration as % based on duplicate measurements. -, not detected.
[a]Constituents identified by (i), (ii), (iii) and (iv) co-injection to a pure compound matching in RRI and mass data.

in *M. virginiana*. A great contribution to the characteristic rose-like scent of these flowers can be attributed to aromatic compounds such as 2-phenylethanol (Cunningham, Van Buskirk, Hodges, Weimerskirch, & Nevitt, 2006) and other mono- and sesquiterpenes detected in large amounts.

2.2. *Biological activities*

Essential oils were assessed for its capacity to scavenge the DPPH free radical. Oil from *M. virginiana* reduced the concentration of DPPH by $34.3 \pm 4.6\%$ with an efficacy much higher than that of *M. grandiflora* ($17.3 \pm 7.0\%$). Vitamin E, included as positive control in the assay, showed the greatest ability to scavenge the DPPH free radical ($68.5 \pm 1.5\%$) at the same test concentration of $5\,mg\,mL^{-1}$. The notable antioxidant activity in *M. virginiana* is likely associated with the abundance of aromatic compounds such as 2-phenyl ethanol and its ester (44%) in the essential oil. 2-Phenyl ethanol, analogues as 'tyrosol' found in the olive oil, exhibits a strong antioxidant activity comparable to that of vitamin E (Waterman & Lockwood, 2007).

With an increasing interest in the marked anticancer effects for *Magnolia* plant extracts (Lee et al., 2009), essential oils from both plants were assessed for their cytotoxic activity against human lung carcinoma cell line A549 and human breast carcinoma cell line MDA-MB231. Both oils showed no significant anticancer activity (IC_{50} values of 220–$486\,\mu g\,mL^{-1}$) against both cell lines. According to the criteria of the American National Cancer Institute, it requires an IC_{50} lower than $30\,\mu g\,mL^{-1}$ to consider the sample promising (Suffiness & Pezzuto, 1990). Sulforaphane, an anticancer volatile found in crucifers (positive control), exhibited an IC_{50} value of $14.2\,\mu g\,mL^{-1}$ for A549 and $9.7\,\mu g\,mL^{-1}$ for MDA-MB231, respectively.

3. Experimental

3.1. *Plant material and chemicals*

Flowers were collected on June 2008 from cultivated trees in the University of Kentucky Arboretum and authenticated by Dr Robert Pratley. Voucher specimens for *M. grandiflora* (UKH-2076) and *M. virginiana* (UKH-2241) have been deposited at the University of Kentucky Herbarium. All standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.2. *Essential oil extraction and analysis*

Extraction and analysis of the essential oil from the flowers of *M. grandiflora* and *M. virginiana* followed the procedure described by Farag (2009). The oil yields (v/w) on moisture-free basis were determined as 0.004% and 0.001%, respectively.

3.3. *Headspace isolation*

Headspace volatiles were collected and analysed according to Farag (2008). Briefly, 10 g of fresh flowers were placed in a side-arm conical flask (125 mL). Charcoal-purified air was passed over the plant from the top and volatiles were collected by pulling vacuum through Tenax (Sigma, St. Louis, MO) traps located at the flask side arm. Volatiles collected on the Tenax adsorbent traps for 8 h were eluted with 500 µL hexane and analysed by GC-MS. A system blank containing no plant material was run as a control.

3.4. *MTT cytotoxicity assay*

Human lung cancer A549 and breast cancer MDA-MB231 cell lines exponentially grown in the RPMI 1640 medium (MP Biomedicals Inc., Irvine, CA) were plated in 96-well microplates at a density of 3×10^3 cells per well in 100 µL of culture medium and were allowed to adhere for 16 h before the treatment. Increasing concentrations of essential oil at 10, 50, 100 and $500\,\mu g\,mL^{-1}$ in ethanol were then added (100 µL per well). The cells were incubated for 48 h in the presence and absence of the essential oil. Cytotoxicity was

assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) according to the vendor's protocol (Promega, Madison, WI). Cytotoxicity is expressed as IC_{50} value with each measurement performed in triplicate.

3.5. *Free radical-scavenging activity*

The antioxidant activity was assayed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the procedure described by Farag (2009). Essential oil samples were tested at concentrations of 5, 50 and 100 mg mL^{-1}. Measurements were carried out in triplicate.

3.6. *Statistical analysis*

All results were expressed as means ± SD, and the significant difference between the two groups was determined using an unpaired Student's *t*-test. The differences were considered significant when $p < 0.05$.

4. Conclusion

In conclusion, dynamic headspace parallel to essential oil analyses provided the first comprehensive volatiles profile in *M. grandiflora* and *M. virginiana* flowers, which can be further used for investigating other factors such as growth stage, geographical origin and storage on volatile composition. The presence of 2-phenylethanol at such high levels (64%) in *M. virginiana* and the exclusive release of its ester suggest that benzenoid compounds could serve as chemotaxonomic markers for the above species. The volatile oil showed significant antioxidant activity in scavenging the DPPH free radical, but no significant cytotoxic effect against lung and breast cancer cell lines. Nevertheless, screening using some oil constituents (as pure compounds) against a broad range of cancer cell lines could reveal lead compounds for the future design of potent anticancer drugs.

Acknowledgements

Dr Mohamed A. Farag thanks the Alexander von Humboldt foundation, Germany, for financial support.

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