J. Indian Chem. Soc.,
Vol. 94, June 2017, pp. 649-652

Composition and antibacterial activities of volatile oil of *Millingtonia hortensis* flowers from Sultanate of Oman

Jamal Nasser Al Sabahi[a], Nallusamy Sivakumar[b] and Ethirajan Sukumar[*c]

[a]Central Instrumentation Laboratory, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, P.C. 123, Muscat, Sultanate of Oman

E-mail : jamal@squ.edu.om

[b]Department of Biology, College of Science, Sultan Qaboos University, P.O. Box 36, P.C. 123, Muscat, Sultanate of Oman

E-mail : apnsiva@squ.edu.om

[c]Department of Research, Saveetha University, Thandalam, Chennai-602 105, India

E-mail : drsuku3@gmail.com

Manuscript received online 02 March 2016, accepted 12 February 2017

Abstract : *Millingtonia hortensis* commonly known as Tree Jasmine or Indian Cork Tree, is indigenous to Burma and the Malay Archipelago and cultivated as an ornamental plant in some countries. The flowers, root and bark are used medicinally. In this study, the volatile oil (VO) obtained by the hydro-distillation of the flowers of the plant grown in Sultanate of Oman, has been analyzed by GC-MS. A total of 32 compounds have been identified that formed major part (97.7%) of the volatile oil. The main components of VO were L-linalool (29.16%), α-farnesene (11.80%), nerolidol (11.34%), pentacosane (9.69%), 1-octen-3-ol (7.62%), (e)-isoeugenol (4.87%), α-terpineol (3.82%), linalool oxide (3.68%), heptacosane (3.03%) and tricosane (2.63%). The volatile oil has been tested for its antimicrobial activity against eleven bacteria wherein it exhibited significant inhibition against seven, moderate against two and no response against two strains. The minimum inhibitory concentration (MIC) has also been determined for the volatile oil.

Keywords : *Millingtonia hortensis* flowers, volatile oil, composition, antibacterial activity.

Introduction

M. hortensis Linn.f. (Family : Bignoniaceae) is a tall and hardy tree that grows up to 24 m in height with a straight trunk and an elongated pyramidal crown with deep green foliage. It is cultivated as an ornamental plant throughout India and some other countries chiefly for its stately form, handsome foliage and profusion of attractive fragrant flowers that appear from September to December. It grows very well in moist climate but does fairly good in dry conditions also. The bark yields an inferior type of cork[1]. The root, bark and flowers are used in the treatment of asthma, sinusitis and as tonic in Indonesia and Thailand. Earlier studies have revealed antimicrobial activity of leaf extract and inhibition of growth and proliferation of RKO colon cancer cell line by the aqueous extract of the root bark of the plant[2]. The presence of cyclohexylethanoids, glucosides and related compounds are reported from the flowers[3] besides hortensin and a flavone derivative[4]. The bark contains a bitter substance and tannin; it is used as an antipyretic in Indonesia[5]. Flower extract of the plant has been reported to have significant antioxidant and hepato-protective activities[6].

In the present investigation, the volatile oil obtained by the hydro-distillation of flowers has been studied for its chemical composition, antibacterial activity and minimum inhibitory concentration against selected bac-

teria. Earlier study on the essential oil of the flowers from Thailand showed inhibitory effect on six out of ten bacterial strains tested[7].

Results and discussion

GC-MS analysis of VO showed the presence of 38 volatile compounds of which 32 were identified, while 6 others present in very small amounts (2.22%), remained unidentified. The GC-MS profile revealed the presence of a range of compounds comprising of terpenoids, aromatic, long chain hydrocarbons, alcohols, aldehydes and esters. The retention time, components identified and percentage composition of the volatile oil are presented in Table 1. L-Linalool (29.16%) was the main component of the oil followed by (E,E) α-farnesene (11.80%), nerolidol (11.34%), pentacosane (9.69%), 1-octen-3-ol (7.62%), (e)-isoeugenol (4.87%), α-terpinol (3.82%), linalool oxide (3.68%), heptacosane (3.03%) and tricosane (2.63%). The antibacterial potential of VO was studied by disc diffusion method against 11 strains. The inhibition was significant with seven bacteria, moderate with two and no response with the remaining two (Table 2).

Volatile oils derived from plants have wide spectrum of biological activities and since Middle Ages they are extensively used in bactericidal, virucidal, fungicidal, antiparasital, insecticidal, medicinal and aromatic applications especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural, food and perfumery industries[11]. The flowers of *Millingtonia hortensis* Linn.f. (Family : Bignoniaceae) possess a pleasant aroma and contain an appreciable amount of volatile oil. Of the compounds that were present in good amounts, L-linalool constituted a major percentage. The appreciable presence of this compound in flowers might be the cause for its lavender-like fragrance[12]. Plant derived volatile oils due to their antimicrobial components, have potential as natural agents for controlling microbial pathogens. The volatile oil's antimicrobial property might be due to a number of terpenoid and phenolic compounds that accumulate in bacterial membranes because of their lipophilic character causing depletion of energy[13]. Generally, volatile oils are slightly more effective against Gram-positive bacteria than the Gram-negative strains as they are less susceptible to the action of antibacterials[14].

Table 1. Components of volatile oil from *Millingtonia hortensis* flowers

Peak No.	Compound name	Ret. time (min)	Area (%)
1	3-Hexenol (leaf alcohol)	2.59	0.03
2	1-Octanol	2.67	0.07
3	1-Octen-3-ol	3.85	7.62
4	3-Octanol	4.06	0.57
5	Linalool oxide	5.40	3.68
6	L-Linalool	5.53	29.16
7	Trans-linalool oxide	6.63	0.59
8	Alpha-terpineol	6.89	3.82
9	Nerol	7.38	0.13
10	Geraniol	7.72	1.15
11	4-Vinyl guiacol	8.60	0.24
12	Eugenol	9.17	1.03
13	(E)-Isoeuginol	10.35	4.87
14	(Z,E)-alpha-farnesene	10.83	0.17
15	Isohomogenol	10.90	0.12
16	(E,E)-alpha-farnesene	10.99	11.80
17	Nerolidol	11.67	11.34
18	(Z,Z)-alpha-farensene	12.05	0.23
19	Trans-alpha-farnesene	12.92	0.10
20	Heptadecane	13.12	0.12
21	(Z,Z)-farnesal	13.67	0.20
22	Farnesyl methyl ester	14.10	0.32
23	Octadecane	14.18	0.16
24	Tetradecanal	14.34	0.13
25	Cis-farnesal	14.66	0.49
26	Nonadecane	15.18	0.12
27	Tricosane	18.80	2.63
28	Tetracosane	19.63	1.60
29	(Trans)-2-nonadecane	20.24	1.64
30	Pentacosane	20.44	9.69
31	Hexacosane	21.24	0.92
32	Heptacosane	22.05	3.03

Experimental

Plant material and extraction of volatile oil :

The flowers of *M. hortensis* have been collected in Al Khuwair area of Muscat in the month of December and identified by the taxonomist in the Department of Biology, Sultan Qaboos University. About 70 g of freshly collected flowers were soaked in water for 12

Table 2. Antibacterial activity of *Millingtonia hortensis* flower volatile oil

Bacteria	Zone of inhibition (mm)		
	VO (10 µL)	Standard*	MIC (µL/ml)
Gram-positive			
B. subtilis	*ni*	24.0±0.47[a]	*nd*
M. leteus	18.3±0.21	20.0±0.47[b]	10.0
S. aureus	10.0±0.94	13.0±0.94[c]	10.0
S. aureus (ATCC 25921)	12.0±0.47	12.0±0.94[c]	10.0
Gram-negative			
P. aeruginosa	10.7±0.59	11.1±0.36[a]	60.0
K. pneumoniae	*ni*	25.0±1.41[a]	*nd*
E. aerogens	23.0±1.41	22.0±0.47[b]	70.0
E. coli	7.0±0.47	9.0±0.0[b]	40.0
P. vulgaris	8.73±0.58	10.0±0.47[a]	60.0
P. aeruginosa (ATCC 27853)	11.0±0.47	14.0±0.94[a]	40.0
E. coli (ATCC 25922)	9.0±0.94	14.4±0.24[b]	40.0

Diameter of disc : 6 mm ; *ni* – no inhibition ; *nd* – not determined.

*Standards used : [a]Tetracycline – 30 µg; [b]Cephalothin – 30 µg; [c]Ceftazidine – 30 µg.

h prior to hydro-distillation using Clevenger apparatus. The contents were distilled for 3 h and the distillate was extracted with *n*-hexane (3×100 ml). The organic layer was dried over anhydrous sodium sulphate and distilled-off to get the pleasant smelling volatile oil designated as VO (Yield : 0.07% w/w).

Analysis of volatile oil :

GC-MS analysis was performed on a Perkin-Elmer Clarus 600 GC system, fitted with a HP-5MS capillary column (30 m×0.25 mm i.d.×0.25 µm film thickness; maximum temperature, 350 °C), coupled to a Perkin-Elmer Clarus 600C Mass Selective Detector. Ultra-high purity helium (99.9999%) was used as carrier gas at a constant flow of 1.0 ml/min. The injection, transfer line and ion source temperatures were 290, 200 and 200 °C respectively. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range 50–500 amu. The split injection mode of 40 : 1 was employed for the analysis. The sample volume of VO injected with a Perkin-Elmer Clarus 600 injector was 1 µL with a split ratio of 40 : 1. The oven temperature program was 80 °C (2 min)–10 °C/min–290 °C (30 min).

Volatile components were first identified by comparing the spectra obtained with mass spectrum libraries (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003). Corroboration of the identification was then done by matching the mass spectra of compounds with those available in the literature and the retention times of the compounds reported on the equivalent column[8]. Relative percentages of components were calculated from the TIC of the automated integrator.

Determination of antibacterial activity :

The volatile oil has been tested against eleven bacterial strains viz. the local clinical isolates *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter aerogens*, *Eicherichia coli* and *Proteus vulgaris* besides the typed organisms *E. coli* ATCC 25922, *P. aeruginosa* ATCC 37853 and *S. aureus* ATCC 25923. Stock cultures of the test organisms were maintained at 4 °C. Active cultures were prepared by inoculating a loopful of stock cultures to the nutrient broth tubes and incubated at 37 °C for 24 h. After incubation, the turbidity of the cultures were adjusted to 0.5 McFarland. 100 µL of these cultures (5×10^5 CFU/ml) were used as inoculums.

The disc diffusion method of Salie *et al*[9] was used

to evaluate the antimicrobial activity of volatile oil. Mueller Hinton Agar (MHA, Oxoid) was used to screen the antimicrobial activity. The MHA plates were prepared by pouring 15–20 ml of molten media into sterile petriplates. 100 µL of inoculum was swabbed uniformly over the MHA plates using sterile cotton swabs and allowed to dry for 5 min. 6 mm sterile discs (Whatman No. 1) were saturated with 10 µL of volatile oil and allowed to dry. Then these discs were placed on the surface of MHA plates inoculated with different microbes and the compound was allowed to diffuse for 5 min and then incubated at 37 °C for 24 h. At the end of the incubation period, inhibition zones were recorded including the diameter of the discs and the experiment has been carried out in triplicate. Control discs contained 10 µL of pure DMSO. The same procedure was followed for standard antibiotics (Cephalothin, Ceftazidine and Tetracycline – all 30 µg).

Determination of minimum inhibitory concentration :

In order to determine the minimum inhibitory concentration (MIC), VO was added at different concentrations (5–100 µL) to the tubes containing 1 ml nutrient broth. 50 µL of 10^6 cells/ml of 6 h nutrient broth cultures of respective organisms were inoculated into these tubes and incubated at 37 °C for 24 h. The lowest concentration of VO in broth medium that inhibited the growth of the test organism was considered as MIC which was determined by measuring the optical density at 620 nm against the control[10]. The experiment was repeated thrice and the mean values were recorded.

Conclusion

The results showed that VO has been effective against both Gram-positive and Gram-negative bacteria. Further linalool has been reported to possess sedative action in animals[15]. Probably this might be the reason for the mild sedative effect of *M. hortensis* flowers when inhaled.

References

1. Anonymous, "The Wealth of India, Raw Materials", Council of Scientific and Industrial Research, New Delhi, 1962, Vol. VI, p. 380.
2. S. Tansuwanwong, H. Yamamoto, K. Imai and U. Vinitketkumnuen, 'The Third Takeo Wada Cancer Research Symposium', Japan, 2002.
3. T. Hase, Y. Kawamoto, K. Ohtani, R. Kasai, K. Yamasaki and C. Picheansoonthon, *Phytochemistry*, 1995, **39**, 235.
4. N. Bunyapraphatsan, G. Blasko and G. A. Cordell, *Phytochemistry*, 1989, **28**, 1555.
5. R. N. Chopra, I. C. Chopra and B. S. Varma, "Supplement to Glossary of Indian Medicinal Plants", NISCAIR-CSIR, New Delhi, 2005.
6. C. Sittiwet, *J. Pharmacol. Toxicol.,* 2009, **4**, 41.
7. S. Babitha, D. Banji and O. J. F. Banji, *J. Pharm. Bioallied Sci.*, 2012, **4**, 307.
8. R. P. Adams, "Identification of Essential Oil Components by Gas Chromatography–Mass Spectrometry", Allured Publishing Corporation, Illinois, 2007.
9. F. Salie, P. F. K. Eagles and H. M. J. Leng, *J. Ethnopharmacol.,* 1996, **52**, 27.
10. G. G. F. Nascimento, J. Locatelli, P. C. Freitas and G. L. Silva, *Brazilian J. Microbiol.,* 2000, **31**, 247.
11. F. Bakkali, S. Averbeck, D. Averbeck and M. Idaomar, *Food Chem. Toxicol.*, 2008, **46**, 446.
12. A. Lapczynski, S. P. Bhatia, C. S. Letizia and A. M. Api, *Food Chem Toxicol.*, *Supp. No. 1*, 2008, S195.
13. M. Vaara, *Microbial Rev.*, 1992, **56**, 395.
14. D. E. Conner in "Antimicrobials in Foods", eds. P. M. Davidson and A. L. Branen, Marcel and Dekker, New York, 1993, p. 441.
15. E. Elisabetsky, G. P. Coelho de Souza, M. A. C. Santos, I. R. Siqueira and T. A. Amador, *Fitoterapia*, 1995, **66**, 407.