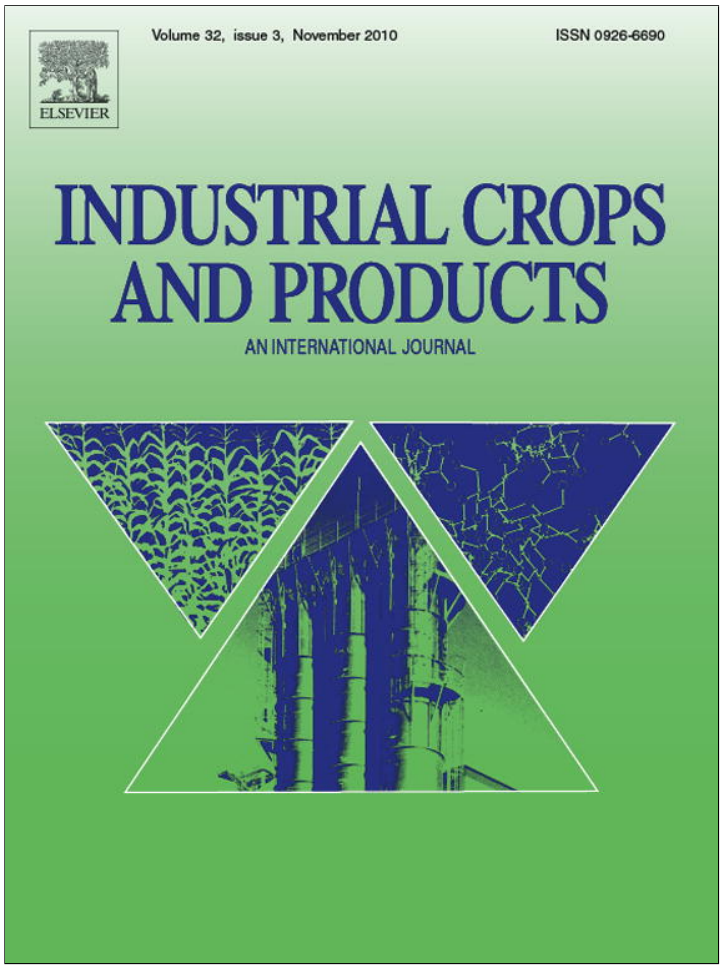

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Short communication

Comparison of extraction methods of *Mimusops elengi* L. flowers

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ABSTRACT

Maulsiri (*Mimusops elengi* L.) belongs to the *Saponaceae* family. It is a large evergreen ornamental tree about 50 ft in height with white flowers and the corolla preserves its fragrance even after drying. The present work includes a comparative analysis of the fragrance obtained from Maulsiri flowers by different methods viz. water soluble volatiles, hexane extract and liquid CO_2 extract. The extracts were analyzed by GC and GC/MS. The chemical compositions of the extracts obtained were rich in bezenoids (61.7%), being phenyl ethyl alcohol (23.6–32.5%) the major compound. The shade dried flowers were extracted by liquid CO_2 and the extract contained high percentage of waxy materials (59.6%). The recovery of water soluble volatiles from the distilled water was carried out by partition with diethyl ether. The diethyl ether extract contained polar compounds (oxygenated terpenoids and benzenoids) and very few percentages of waxy materials. It was observed that the liquid CO_2 extract of fresh flowers, which was free from solvent residue, was organoleptically superior in comparison to the extracts obtained by the conventional processes.

1. Introduction

Maulsiri (*Mimusops elengi* L.) belongs to the family *Saponaceae* (Anon., 2003). It is a large evergreen ornamental tree of about 50 ft in height. It is regarded as a sacred plant among Hindus as a symbol of love and beauty and holds an important place in religious texts (Mitra, 1981). The plants are distributed throughout the tropics of Asia, Africa, Australia and American continents. The family *Saponaceae* consists of about 40 genera spread over 600 species (Misra et al., 1974) and is distributed wild in the Asian forests of South India, Burma and Malaya Peninsula. The flowers of *M. elengi* are white and very fragrant, and the corolla preserves fragrance even after drying (Anon., 2003). The essential oil obtained from flowers has been described for use in perfumes as a stimulant (Anon., 1986). The ethanol extract and headspace volatile composition of the flowers of Malaysian origin were reported by Wong and Teng (1994). The major compounds in ethanol extract and headspace of flowers were identified as p-methylanisole (1.33, 9.94%), methyl benzoate (3.82, 13.40%), methyl salicylate (1.88, 0.48%), 2-phenyl ethyl acetate (2.55, 7.16%), benzyl alcohol (1.75, 4.41%), 2-phenyl ethyl alcohol (38.79, 37.80%), nerolidol (3.19, 1.46%), methyl-E-cinnamate (3.63, 6.83%), 3-hydroxy-4-phenyl-2-butanoate (4.74, 2.85%), E-cinnamyl alcohol (13.72, 0.85%) and 2-phenyl ethyl benzoate (1.13, 0.37%), respectively.

Supercritical CO_2 (SC-CO_2) extraction is one of the advanced processes used for extraction of natural materials because of the non-toxic, non-flammable characteristics of CO_2 and its availability in high purity with low cost (Reverchon and De Marco, 2006). Naik et al. (1989) have studied the advantages of extraction of aroma compounds by liquid CO_2 over the SC-CO_2. Liquid CO_2 is a solvent of interest for fragrance and flavour compounds with medium molecular weight. The main advantages lie in the low extraction temperature and pressure and inert extraction atmosphere. Whereas, the SC-CO_2 extraction requires high pressure (<74 bar) and high temperature (<31 °C), the trapping of volatiles after extraction requires collecting apparatus, which is maintained at low temperature. Therefore, liquid CO_2 extraction has got advantage of recovery of volatile and thermally labile compounds with zero solvent residues.

Limited work on volatile fragrance composition of flowers has been reported in the literature. Therefore, in the present work, the floral extractions are carried out by liquid CO_2 and hexane as well as the recovery of the water soluble components from distilled water through partition with diethyl ether. Finally, the chemical compositions of the different extracts are compared.

2. Material and methods

The fresh flowers were collected from Delhi (28.38°N, 77.12°E) in the early morning from April to May, 2009. All the solvents used in the experiment were reagent grade and distilled in the laboratory before extraction. The standards viz. benzyl alcohol, 2-phenyl ethyl alcohol and E-cinnamyl alcohol were procured from Sigma Aldrich, Bangalore. The liquid CO_2 cylinder was supplied by Laser gas, New Delhi with purity more than 98%. The flowers were shade dried for 3 days and the dried flowers were extracted with liquid CO_2.

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There after, the yields presented in all the experiments are average of three runs and the range of deviation is indicated.

2.1. Recovery of hydrodistilled volatiles

Fresh flowers (1 kg) were distilled with water (4 L) for 3 h in a Clevenger type apparatus. Since, the oily layer did not appear and then the total Clevenger water was extracted with diethyl ether and concentrated. The extracts were kept in refrigerated condition for further analysis.

2.2. Preparation of concrete and absolute

Fresh flowers (500 g) were taken in an aspirator bottle and kept in contact with hexane (2 L) for 6 h at room temperature. After draining out the hexane soluble extract, the raffinate flowers were again contacted in fresh hexane for 6 h followed by a quick wash with hexane (500 mL). The combined extracts on solvent removal in a rotary evaporator under *vacuo* below 40 °C afforded a light yellow waxy residue, the so called concrete. Addition of 40 mL methanol to the residue, warming to 50 °C for 5 min to get a homogeneous mixture followed by refrigeration for 24 h at −15 °C, precipitated most of the waxes. Filtration through a sintered funnel followed by evaporation of methanol below 40 °C afforded a partially dewaxed absolute.

2.3. Extraction of flowers with liquid CO_2

The apparatus consisted of a modified specially designed Soxhlet apparatus suitably positioned in a pressure vessel. Fresh flowers (50 g) were taken in the glass apparatus which filled it upto 3/4 of the volume. The experimental set up and process optimization for floral extraction has already been described in our earlier publications (Rout et al., 2010, in press, 2007). The extraction was continued for 3 h; then CO_2 was released from the extractor slowly through a Teflon tube connected to a glass bottle placed in an ice bath. Analogously, the extraction of dried flowers was also carried out. The extracts were kept in refrigerator for analysis.

2.4. GC and GC/MS analysis

The hydrodistilled volatiles, concrete, absolute and liquid CO_2 extract of flowers were analyzed by GC-FID and GC/MS. The GC analysis was carried out on Varian CP-3800 Gas Chromatograph equipped with an FID and 25 m × 0.25 mm × 0.25 μm WCOT column coated with 5% diphenyl dimethyl siloxane (HP-5). Helium was used as carrier gas at a flow rate of 1.2 mL/min at a column pressure of 22 kPa. Samples (0.2 μL) were injected into the column with a split ratio of 50:1. Component separation was achieved following a linear temperature program of 60–200 °C at 2 °C/min and then held at 200 °C for 50 min, with a total run time of 120 min. The percentage composition was calculated using peak normalization method assuming equal detector response. The samples were then analyzed on a Varian Saturn 2200 GC/MS fitted with the same column and following the same temperature program as given above. The MS parameters were as follows: ionisation voltage (EI) 70 eV, peak width 2 s, mass range 20–400 amu and detector voltage 1.5 V. Peak identification was carried out by comparison of the mass spectra with mass spectra available on database of NIST-1, NIST-2, Adams and Wiley libraries. The compound identification was finally confirmed by comparison of their relative retention indices with literature values (Adams, 1995; Rout et al., 2007).

2.4.1. Determination of volatile matter in floral extracts

The concrete and liquid CO_2 extract are expected to contain some non-volatile material co-extracted from flowers. In order to determine the volatile portion in these extracts, benzyl alcohol, phenyl ethyl alcohol, E-cinnamyl alcohol were quantitatively determined by GC-FID by internal standard (ISTD) method using n-octanol as internal standard. From the amounts of these constituents and their percentage composition as found by GC in each of these extracts, the amount of volatile materials were calculated.

3. Results and discussion

The yields of concrete and absolute were 3.8 ± 0.2 g and 2.8 ± 0.2 g, respectively. They contained oxygenated terpenoids (1.6, 1.0%), benzenoids (45.6, 51.6%), fatty acids/esters (10.8, 10.9%) and mixture of hydrocarbons (18.7, 12.6%), respectively, as presented in Table 1. Twenty-seven compounds were identified by GC/MS analysis. The major components in concrete and absolute are phenol (3.3, 3.4%), benzyl alcohol (3.4, 3.8%), 2-phenyl ethyl alcohol (23.6, 28.1%), anisyl alcohol (2.3, 2.9%), E-cinnamyl alcohol (3.3, 3.5%), ethyl-p-anisate (3.2, 3.5%) in concrete and absolute, respectively. The absolute also contained considerable amounts of fatty acids/esters, which may be act as fixative for volatile fragrance components. The weight of shade dried flowers was reduced to half as compared to the fresh flowers. The yields of the liquid CO_2 extract of fresh and dried flowers were 0.27 ± 0.02 g and 0.45 ± 0.02 g, respectively. The percentage yield of extract in dry flowers was nearly twice in comparison to the fresh flowers. The liquid CO_2 extract of fresh flowers were enriched with chief benzenoids (61.7%) along with few percentages of waxy materials (15.9%), whereas the dry flowers extract contained improved percentages of waxy components (59.6%). The percentage of major compound, viz. 2-phenyl ethyl alcohol is 32.5% and 5.8% in liquid CO_2 extract of fresh and dry flowers, respectively. The dry flowers may have lost the volatile compounds in the process of drying, therefore the recoveries of chief components were very less and the product was inferior in quality. All the extracts contain a part of non-volatile (waxy) materials. Since, the volatility of components is an important factor for perfumery and fragrance application. The total percentage of volatile fraction of each extract is also presented in Table 1. The results show that the volatility of liquid CO_2 extract of fresh flowers (87%) was higher in compared to conventional solvent extraction process (75–82%).

The essential oil could not be observed in hydrodistillation process, which may be attributed to the high solubility of components in water or less volatility of components in steam. Thus, the water soluble organic compounds were recovered (0.2 g) in diethyl ether. The major compounds in ether extract were phenol (4.0%), benzyl alcohol (20.0%), 2-phenyl ethyl alcohol (24.1%), anisyl alcohol (1.5%), carvacrol (2.5%) and E-cinnamyl alcohol (17.0%). It was observed that the compounds detected in diethyl ether extract were mostly oxygenated terpenoids and bezenoids, which were more likely soluble in distilled water in comparison to non-polar compounds (Rout et al., 2001). It was also explained that, the improved percentage of benzenoids in distilled water is due to less volatility and poor recovery of waxy materials in water vapours. The earlier experiments have also revealed that, the solubility of phenyl ethyl alcohol was 0.8 g/100 mL in water (Bohra et al., 1994). Thus, rose concrete (solvent extract) contained more than 60% of phenyl ethyl alcohol; whereas, essential oil (hydrodistilled oil) contained about 1%. Similarly, the solubilities of other major compounds were phenol (~8 g/100 mL), benzyl alcohol (~4 g/100 mL) and cinnamyl alcohol (~0.18 g/100 mL) in water (Hirano et al., 2008; Chem Blink, 2010). It is known that, the oxygenated/polar compounds act as emulsifier between the aqueous and organic phase. Therefore, the separate oily layer was not visible in the Clevenger type hydrodistillation. Finally, less volatility of components along with high solubility in water is the reason for poor recovery of essential oil.

Table 1
Composition of extracts of liquid CO_2, concrete and absolute of *Mimusops elengi* L. flowers.

Compound	Concrete (wt%)	Absolute (wt%)	Liquid CO_2 extract of fresh flowers (wt%)	Liquid CO_2 extract of dry flowers (wt%)	Hydrodistilled volatiles (wt%)	RRI cal.	RRI lit.
Yield	0.76	0.56	0.54	0.9	0.02		
Volatile matter	75 ± 1.3	82 ± 1.0	87 ± 1.1	75 ± 1.2	–		
Heptanal	2.6 ± 0.3	0.2 ± 0.1	0.1	0.4 ± 0.1	0.6 ± 0.1	991	899
Phenol	3.3 ± 0.3	3.4 ± 0.2	3.6 ± 0.4	0.2 ± 0.1	4.0 ± 0.2	1002	–
P-cresol, methyl ether	0.5 ± 0.1	0.1	0.2 ± 0.1	0.1	2.3 ± 0.3	1018	1020
Benzyl alcohol	3.4 ± 0.3	3.8 ± 0.2	6.2 ± 0.4	1.6 ± 0.2	20.0 ± 0.4	1038	1032
Nonanal	1.7 ± 0.3	0.2 ± 0.1	0.9 ± 0.2	0.1	0.6 ± 0.1	1093	1102
Phenyl ethyl alcohol	23.6 ± 0.4	28.1 ± 0.5	32.5 ± 0.6	5.8 ± 0.3	24.1 ± 0.4	1121	1110
P-methoxy cresol	1.1 ± 0.2	0.9 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	1197	1190
P-anisaldehyde	0.5 ± 0.1	0.4 ± 0.1	0.1	t	0.2 ± 0.1	1246	1252
2-Phenyl ethyl acetate	2.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.3	0.2 ± 0.1	2.2 ± 0.2	1258	1256
E-cinnamaldehyde	0.7 ± 0.2	1.3 ± 0.2	1.2 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	1275	1266
Anisyl alcohol	2.3 ± 0.2	2.9 ± 0.4	3.0 ± 0.3	0.8 ± 0.2	1.5 ± 0.2	1282	1279
Carvacrol	1.6 ± 0.2	1.8 ± 0.2	5.9 ± 0.3	2.6 ± 0.3	2.5 ± 0.2	1306	1298
E-cinnamyl alcohol	3.3 ± 0.3	3.5 ± 0.4	3.8 ± 0.5	1.3 ± 0.2	17.0 ± 0.5	1308	1300
Geranyl acetone	0.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	1.6 ± 0.3	1457	1453
Ethyl-P-anisate	3.2 ± 0.3	3.5 ± 0.3	2.6 ± 0.2	0.8 ± 0.2	0.2 ± 0.1	1510	–
E-nerolidol	0.6 ± 0.2	0.6 ± 0.1	0.9 ± 0.2	1.0 ± 0.3	0.5 ± 0.1	1568	1564
Hexadecane	0.1	t	t	t	0.4 ± 0.1	1592	1600
(E,E)-farnesyl acetate	0.2 ± 0.1	0.1	0.4 ± 0.1	1.0 ± 0.2	0.4 ± 0.1	1838	1843
Methyl palmitoleate	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	1.7 ± 0.2	1894	–
Methyl palmitate	0.8 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	t	1917	1927
Ethyl palmitoleate	1.9 ± 0.3	2.0 ± 0.2	0.3 ± 0.1	1.1 ± 0.2	0.3 ± 0.1	1960	–
Palmitic acid[a]	1.2 ± 0.3	0.8 ± 0.2	0.4 ± 0.2	0.7 ± 0.3	0.1	1984	–
Ethyl palmitate	3.2 ± 0.5	3.5 ± 0.6	0.2 ± 0.1	1.0 ± 0.3	0.5 ± 0.1	1995	1993
Heneicosane	5.6 ± 0.4	3.6 ± 0.4	3.8 ± 0.5	16.9 ± 1.0	0.6 ± 0.3	2100	2100
6,13-Octadecadienyl acetate[a]	2.7 ± 0.3	3.4 ± 0.3	5.0 ± 0.5	19.0 ± 1.2	0.1	2112	–
Ethyl stearate[a]	0.5 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	–	2189	–
5-Methyl tricosane	13.0 ± 1.5	9.0 ± 0.8	5.3 ± 0.6	19.5 ± 1.8	0.1	2353	2363
Oxygenated terpenoids	1.6	1.0	2.1	2.7	2.5		
Benzenoids	45.6	51.6	61.7	14.1	74.6		
Long chain fatty acids/esters	10.8	10.9	6.8	23.2	2.7		
Long chain hydrocarbons	18.7	12.6	9.1	36.4	1.1		

RRI cal.: relative retention indices calculated, RRI lit.: relative retention indices literature reported.
[a] Tentative identification, t: trace.

4. Conclusion

This is the first report on extraction of *M. elengi* flowers with hexane and liquid CO_2. Though, hexane extract (concrete) provided improved yield but contained 30% of waxy materials. The absolute obtained from concrete by partitioning in methanol, but it was not free from waxy components and solvent residue. The bezenoids such as phenol (4.0%), p-cresol-methyl ether (2.3%), benzyl alcohol (20.0%), E-cinnamyl alcohol (17.0%) showed improved percentage in diethyl ether extract but the yield of the extract to be poor. The liquid CO_2 extract of fresh flowers was enriched with chief benzenoids such as phenol (3.6%), benzyl alcohol (6.2%), phenyl ethyl alcohol (32.5%), anisyl alcohol (3.0%), carvacrol (5.9%), E-cinnamyl alcohol (3.8%). The extract is free from solvent residues with little percentage of waxy materials and thus product is organoleptically superior. The liquid CO_2 extract of fresh flowers is more demanding for pharmaceutical application and in high grade perfumery. The compositions of liquid CO_2 extracts of fresh and dry flowers were compared. The liquid CO_2 extract of dry flowers contained 60% of waxy materials with poor recovery of benzenoids (14.1%), which suggests that the liquid CO_2 extract of dry flowers was inferior in quality.

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References

Adams, R.P., 1995. Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press, San Diego, pp. 17–28.
2003. The Wealth of India: Raw Materials. Publication and Information Directorate. CSIR, New Delhi, pp. 134–135.
Anony., 1986. The Useful Plants of India. Council of Scientific and Industrial Research, New Delhi, p. 375.
Bohra, P.M., Vaze, A.S., Pangarkar, V.G., Taskar, A., 1994. Adsorptive recovery of water soluble essential oil components. J. Chem. Tech. Biotechnol. 60, 97–102.
Chem Blink, 2010. Cinnamyl alcohol, accessed in month of May at: http://www.chemblink.com/products/104-54-1.htm.
Hirano, A., Arakawa, T., Shiraki, K., 2008. Arginine increases the solubility of coumarin: comparison with salting-in and salting-out additives. J. Biochem. 144, 363–369.
Naik, S.N., Lentz, H., Maheshwari, R.C., 1989. Extraction of perfumes and flavours from plant material with liquid CO_2 under liquid-vapour equilibrium condition. Fluid Phase Equilib. 49, 115–126.
Misra, G., Nigam, S.K., Mitra, C.R., 1974. Studies on *Mimusops* Spp. Planta Medica 26, 155–165.
Mitra, R., 1981. Bakula a reputated drug of Ayurveda, its history, uses in Indian medicine, Indian. J. History Sci. 16 (2), 169–180.
Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. J. Supercrit. Fluid 38, 146–166.
Rout, P.K., Naik, S.N., Rao, Y.R., 2010. Liquid CO_2 extraction of flowers of *Pandanus fascicularis* Lam and fractionation of floral concrete and comparative composition of the extracts. J. Food Biochem. doi:10.1111/j.1745-4514.2010.00398.x (on-line).
Rout, P.K., Naik, S.N., Rao, Y.R., in press. Composition of absolutes of *Jasminum sambac* L. flowers fractionated with liquid CO_2 and methanol and comparison with liquid CO_2 extract. J. Essent. Oil Res.
Rout, P.K., Naik, S.N., Rao, Y.R., Jadeja, G., Maheshwari, R.C., 2007. Extraction of essential oil from *Zanthoxylum rhesta* using subcritical CO_2 and conventional processes. J. Supercrit. Fluid 42, 334–341.
Rout, P.K., Jena, K.S., Rao, Y.R., 2001. Fractional hydrodistillation of citronella (*Cymbopogan winterianus* Jowitt). Fragr. Flavor Assoc. India 3, 57–62.
Wong, K.C., Teng, Y.E., 1994. Volatile components of *Mimusops elengi* L. flowers. J. Essent. Oil Res. 6 (5), 453–458.