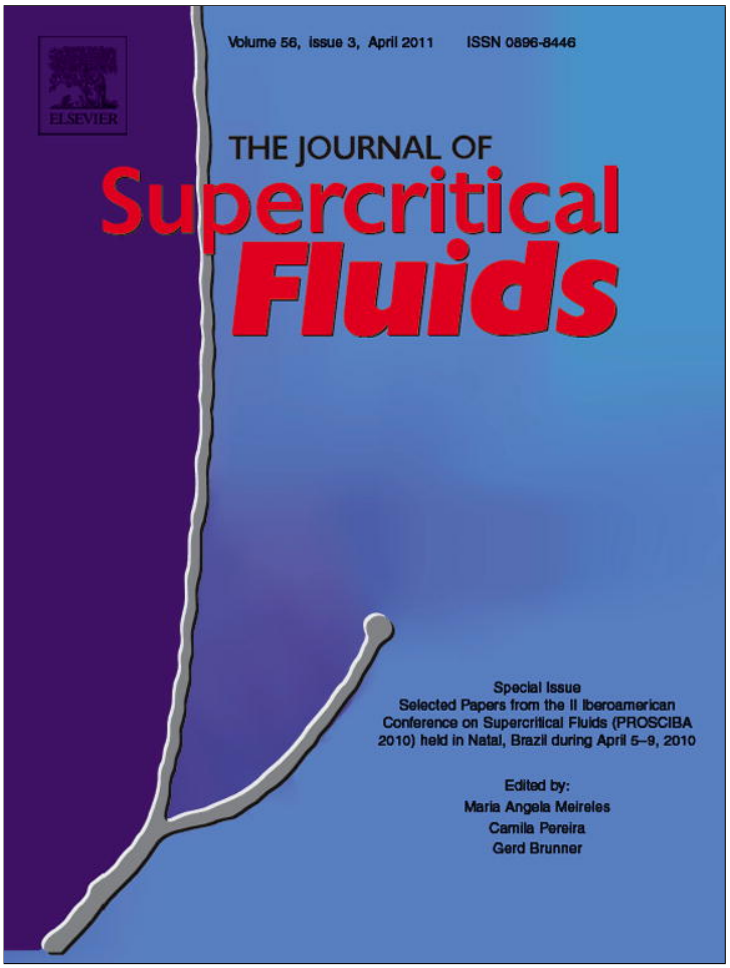

J. of Supercritical Fluids 56 (2011) 249–252

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Liquid CO_2 extraction of flowers and fractionation of floral concrete of *Michelia champaca* Linn

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ARTICLE INFO

Article history:
Received 30 May 2010
Received in revised form 12 January 2011
Accepted 12 January 2011

Keywords:
Michelia champaca L.
Concrete
Absolute
Liquid CO_2 fractions of concrete
Liquid CO_2 extract
Benzenoids

ABSTRACT

Extraction of the fresh flowers of *Michelia champaca* L. with liquid CO_2 provided a floral extract in 1.0 ± 0.04 wt% yields. The extract so obtained contains far less waxes and is organoleptically very superior. Similarly extraction with pentane gave the so-called 'Concrete' in 1.58 ± 0.06 wt%. While the concrete contains co-extracted floral waxes that make it unsuitable for blending with other perfumes, direct extraction with CO_2 is an expensive process mainly due to low bulk density of flowers and their availability during short flowering season. On the other hand, fractionation of the concrete with liquid CO_2 to separate the waxy components has provided solvent and almost wax free fractions. The duration of extractive fractionation has been optimized for selective extraction with liquid CO_2 at 62 bar. These liquid CO_2 fractions of concrete and liquid CO_2 extract of flowers were analyzed by GC and GC/MS and their composition compared with that of concrete and partially de-waxed absolute obtained in the conventional way. The major fragrance compounds enriched in the direct liquid CO_2 extract were methyl benzoate (11.5 ± 0.8%), phenyl ethyl alcohol (5.0 ± 0.6%), phenyl acetonitrile (10.4 ± 1.1%), indole (1.2 ± 0.3%), methyl anthranilate (1.3 ± 0.5%), E-β-ionone (1.5 ± 0.4%), and Z-methyl jasmonoate (1.0 ± 0.3%). The liquid CO_2 fractionation of concrete is a practical process and the first fraction is comparable with direct liquid CO_2 flower extract in terms of composition of the major compounds.

1. Introduction

Michelia champaca Linn (family *Magnoliaceae*) is a tall, evergreen tree growing usually up to 30 m in height [1]. It is native to temperate Himalayan region; however, it is found distributed throughout the subtropical and tropical countries such as India, South China, Indonesia, The Philippines and some pacific islands [2]. The tree blooms in monsoon (June–September) and in spring (January–April) seasons. During the flowering period, the tree is covered with thousands of golden yellow flowers with powerful and diffusive fragrance. Most of the flowers are used for ornamental purposes and for worship in temples. However, small quantities are processed for its essential oil, concrete and absolute. The general terms frequently used in fragrance chemistry [3] are 'concrete and absolute'. 'Concrete' is concentrated form of floral fragrance isolated in a hydrocarbon type solvent such as hexane or pentane. In such a process, some unwanted waxy components and fatty esters are also co-extracted. The concrete is of lower quality due to the presence of the waxy components which cause problems such as clouding during storage in fragrance formulations due to their limited solubility. Therefore, the concrete has to be converted into a wax free and alcohol soluble volatile concentrate, known as 'absolute'. In the process of absolute preparation, the concrete is mixed with ethyl alcohol, warmed to 40–60 °C and strongly agitated to get a homogeneous solution. Once the solution is achieved, it is refrigerated below −5 °C, so that the waxes are precipitated and can be removed by filtration. Meyler-Warnod reported that, the waxes are not soluble in alcohol below −1 °C [4]. Finally, the alcoholic extract is concentrated to obtain the absolute. A literature review on *M. champaca* L. indicates that it has not been much studied. Kaiser [5] and Zhu et al. [6] reported the results of analysis of laboratory prepared concrete and one commercial sample of absolute of *M. champaca* L. produced in India. The major compounds were linalool (0.2–11.0%), methyl benzoate (1.0–5.0%), benzyl acetate (0.1–4.0%), cis-linalool oxide (pyranoid) (0.2–7.0%), phenyl acetonitrile (0.1–4.3%), 2-phenyl ethyl alcohol (2.0–34.0%), dihydro-β-ionone (0.3–10.0%), α-ionone (0.1–6.8%), β-ionone (0.2–3.4%), dihydro-β-ionol (0.3–3.8%), methyl anthranilate (1.4–9.0%), indole (2.5–12.0%) and methyl linoleate (1.0–18.0%). Rout et al. reported the composition of concrete, absolute, essential oil and headspace volatiles of *M. chamapaca* L. of Indian origin [7].

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doi:10.1016/j.supflu.2011.01.005

Table 1
Percentage composition of chief chemical classes of compounds in liquid CO_2 fractions of floral concrete (extract) of *Michelia champaca* Linn.

Chemical classes	1st fraction (%)	2nd fraction (%)	3rd fraction (%)	4th fraction (%)	5th fraction (%)	Wax (%)
Yield (%)	0.32 ± 0.03	0.27 ± 0.02	0.24 ± 0.02	0.17 ± 0.02	0.08 ± 0.01	0.5 ± 0.04
Monoterpenoids	2.0 ± 0.3	1.1 ± 0.3	0.8 ± 0.3	0.2 ± 0.2	–	–
Sesquiterpenoids	5.5 ± 0.6	4.0 ± 0.5	2.5 ± 0.6	1.8 ± 0.3	0.5 ± 0.3	0.2 ± 0.1
Benzenoids	35.0 ± 2.3	30.0 ± 2.1	20.6 ± 1.7	12.6 ± 1.3	4.8 ± 1.3	3.6 ± 0.3
Z-methyl jasmonoate/epijasmonoate	1.8 ± 0.3	1.3 ± 0.2	0.8 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	–
9,12-Octadecadienol[a]	4.8 ± 0.8	6.2 ± 0.8	7.1 ± 0.7	7.5 ± 0.8	8.1 ± 1.2	8.5 ± 1.8
Fatty acids/methyl esters	33.7 ± 5.6	40.7 ± 6.6	45.4 ± 7.6	51.7 ± 8.6	58.9 ± 8.8	62.7 ± 8.9
Long chain hydrocarbons	0.2 ± 0.1	0.4 ± 0.2	2.8 ± 0.3	6.0 ± 0.4	7.8 ± 0.4	8.0 ± 0.5

[a] Tentatively identified based on mass fragmentation data and matching with spectral libraries.
% composition reported is the average of three readings.

The solvent extracts (concrete and absolute) usually contain solvent residues and non-volatile waxy components. On the other hand, hydro distillation provides an essential oil in very low yields containing several artifacts produced in the distillation process. Supercritical CO_2 (SC-CO_2) extraction is one of the 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 and due to its operation at near ambient temperatures. Reverchon and DeMarco [8] have reviewed the numerous works carried out on the application of SC-CO_2 in food processing, pharmaceuticals and nutraceuticals. In the case of floral materials direct extraction with SC-CO_2 poses several problems. Due to the very low bulk densities of flowers, their availability during a short flowering season and occurrence of the fragrance in low concentration, very large capacity plants are required. On the other hand, fractionation of floral concrete with liquid CO_2 is an attractive alternative process.

Naik et al. have studied the advantages of liquid CO_2 over the SC-CO_2 extraction of aroma compounds [9]. Liquid CO_2 is a solvent of interest for fragrance and flavor compounds with medium molecular weight. The main advantages lie in the low extraction temperature and pressure and inert extraction atmosphere, which are an advantage for recovery of volatile and thermally labile components free from solvent residues.

There is neither any reported work on SC-CO_2 or liquid CO_2 extraction of *M. champaca* flowers nor fractionation of floral concrete. Thus, the objective of the present work is to extract flowers of *M. champaca* with liquid CO_2, and also fractionate the concrete (as obtained in a previous work by pentane extraction) using liquid CO_2 and compare the two processes. The yields and composition of the products obtained were compared vis–vis with those reported previously for concrete and absolute reported earlier.

2. Experimental

All solvents used were of laboratory grade and were redistilled before use. Yields reported in all experiments are average of three runs and the range of error is indicated. Standard compounds such as methyl benzoate and phenyl ethyl alcohol are purchased from Sigma–Aldrich, Bengaluru, India.

2.1. Preparation of concrete and absolute

Fresh flowers (500 g) were collected from the campus of the Institute of Minerals and Materials Technology, Bhubaneswar (21°15′E, 85°15′N), Orissa, India. The flowers were taken in an aspirator bottle and contacted with distilled pentane (3 L) for 6 h. After draining the extract, the raffinate flowers were again contacted with fresh pentane (2.5 L) for 6 h followed by a quick wash with pentane (1 L). Removal of pentane from the combined extracts in a rotary evaporator under *vacuo* below 35 °C afforded a deep yellow waxy residue, the so called concrete (7.9 ± 0.2 g). Methanol (40 mL) was added to the concrete, warmed to 50 °C for 5 min to get a homogeneous mixture, which was then refrigerated for 24 h at −15 °C, when most of the waxes got precipitated. Filtration of the precipitate through a sintered funnel followed by evaporation of methanol below 40 °C afforded a light yellow partially dewaxed absolute.

2.2. Liquid CO_2 fractionation and extraction

2.2.1. Liquid CO_2 fractionation of concrete

The apparatus consisted of a modified specially designed glass Soxhlet apparatus suitably positioned in a pressure vessel of volume 935 mL. The details of the experimental set up and the process adopted had appeared in our earlier publications [9–11]. It consisted of an upper compartment for placing the plant materials to be extracted and provided with a siphon for periodical removal of the extract into the bottom receptor glass vessel. The concrete (3 g) was mixed with cleaned 3 mm glass beads and placed in the upper compartment to fill three-fourth (3/4) of the volume. The extraction was carried out at room temperature maintained at 25 ± 1 °C. Fractionation was carried out by successive extraction with liquid CO_2 of pressure 62 ± 1 bar. The lower part of the apparatus was kept in the water bath at 35 °C to evaporate CO_2. Cold water (5 °C) from a thermostatic bath was circulated through the cooling finger of the apparatus. Liquid CO_2 placed in the lower compartment evaporates at the bottom of the apparatus due to the heat supplied from the warm water of the bath, gets condensed at the cold finger and the liquid CO_2 gets collected in the upper compartment of the Soxhlet apparatus over the material to be extracted. When liquid CO_2 gets filled up to the siphon level, the liquid CO_2 soluble floral extract siphons out to the lower receptacle kept in the lower compartment. The cycle of evaporation, condensation and extraction was continued for the desired extraction period; then CO_2 was released from the extractor slowly through a Teflon tube connected to a glass bottle placed in an ice bath. In order to optimize the extraction time for fractionation, five successive extractions were carried out with 45 min intervals. Experiments were carried out in triplicate and yields reported are average of these results. The recoveries of the fractions are 0.6 ± 05, 0.52 ± 04, 0.45 ± 04, 0.32 ± 04 and 0.15 ± 03 g, respectively. Finally, the glass beads were washed with pentane and on evaporation of pentane from the extract under *vacuo*, a waxy residue of 0.96 ± 09 g was obtained. The extracts were collected and stored in a refrigerator for analysis.

From the analytical data presented in Table 1, it can be seen that most of the desired compounds are extracted in the first four extractions, leaving mostly the waxes. It was therefore decided to carry out another set of experiments for fractionation of concrete with liquid CO_2 in two stages only with 90 min extraction time. The chemical compositions of these two fractions are compared.

2.2.2. Liquid CO_2 extraction of flowers

For direct extraction of flowers with liquid CO_2, 50 g of flowers were taken and quickly charged into the extractor. Extraction was

Table 2
Percentage composition of liquid CO_2 extract of flowers and liquid CO_2 fractions of concrete of *Michelia champaca* Linn.

Compound identified	Liquid CO_2 extract of flower (%)	Concrete[a] (%)	Liquid CO_2 fraction (first stage) (%)	Liquid CO_2 fraction (second stage) (%)	Absolute[a] (%)	RRI lit
Volatile matter	85 ± 1.0	78 ± 1.2	83 ± 1.1	81 ± 1.2	80 ± 0.9	
Yield	1.04 ± 0.04	1.58 ± 0.06	0.56 ± 0.02	0.44 ± 0.02	1.32 ± 0.02	
3-Methyl-4-heptanone	1.5 ± 0.2	2.2 ± 0.4	2.5 ± 0.4	1.5 ± 0.3	3.4 ± 0.3	929
Benzaldehyde	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1	<0.1	961
6-Methyl-5-hepten-2-one	0.1	t	t	–	–	985
Decane	0.1	0.3 ± 0.1	t	t	0.2 ± 0.1	1000
1,8-Cineole	1.3 ± 0.3	0.4 ± 0.1	1.0 ± 0.2	0.8 ± 0.3	1.4 ± 0.3	1035
(E)-β-Ocimene	0.2 ± 0.1	0.1	0.2 ± 0.1	0.1	0.2 ± 0.1	1050
p-Cresol	1.8 ± 0.4	1.0 ± 0.3	1.8 ± 0.4	1.2 ± 0.3	1.7 ± 0.4	1075
Methyl benzoate	11.5 ± 0.8	4.1 ± 0.4	10.5 ± 1.0	8.5 ± 0.7	8.6 ± 0.6	1091
Phenyl ethyl alcohol	5.0 ± 0.6	4.3 ± 0.4	4.8 ± 0.5	3.0 ± 0.4	4.2 ± 0.4	1110
Phenyl acetonitrile	10.4 ± 1.1	4.4 ± 0.3	9.5 ± 1.0	7.5 ± 0.8	8.7 ± 0.7	–
Ethyl benzoate	0.7 ± 0.3	0.6 ± 0.2	0.5 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	1170
Phenyl ethyl formate	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.1	1174
Dodecane	0.1	0.2 ± 0.1	–	t	–	1200
Indole	1.2 ± 0.3	0.8 ± 0.2	1.3 ± 0.4	0.7 ± 0.3	0.8 ± 0.3	1288
Methyl anthranilate	1.3 ± 0.5	0.8 ± 0.3	1.2 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	1337
Eugenol	0.1	t	t	t	t	1356
(E)-α-Ionone	0.7 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	1426
Dihydro-β-ionone	1.0 ± 0.3	0.6 ± 0.2	0.9 ± 0.3	0.5 ± 0.2	0.7 ± 0.2	–
Dihydro-β-ionol	0.6 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	–
(E)-β-Ionone	1.5 ± 0.4	0.8 ± 0.3	1.3 ± 0.4	0.8 ± 0.3	1.2 ± 0.3	1485
Pentadecane	t	0.3 ± 0.1	–	t	t	1500
(E,E)-α-farnesene	1.4 ± 0.4	0.7 ± 0.2	1.2 ± 0.4	0.6 ± 0.2	0.7 ± 0.2	1508
Hexadecane	–	0.1	–	t	t	1600
(Z)-methyl jasmonoate	1.0 ± 0.3	0.8 ± 0.3	1.0 ± 0.3	0.7 ± 0.2	0.8 ± 0.3	1647
β-Bisabolol	0.1	0.1	0.1	0.1	–	1671
(Z)-methyl epi-jasmonoate	0.6 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	1676
Benzyl benzoate	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	1762
Octadecane	–	0.1	t	0.1	0.2 ± 0.1	1800
Phenyl ethyl benzoate	0.8 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	1853
Nonadecane	–	0.8 ± 0.3	–	0.1	<0.1	1900
Methyl palmitate	3.7 ± 0.4	5.0 ± 0.6	5.0 ± 0.8	5.8 ± 1.1	5.6 ± 0.5	1927
Eicosane	–	0.1	t	0.2 ± 0.1	0.3 ± 0.1	2000
Palmitic acid	3.2 ± 0.7	4.2 ± 0.6	5.0 ± 1.1	5.3 ± 1.3	5.3 ± 0.6	–
Methyl linoleate	18.0 ± 1.1	24.4 ± 1.5	21.0 ± 1.5	24.8 ± 2.0	25.3 ± 1.2	2092
Methyl linolenate	6.9 ± 1.0	8.0 ± 0.9	7.9 ± 1.0	8.4 ± 1.6	9.0 ± 0.8	2100
Methyl stearate	–	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	2128
9,12-Octadecadienol[b]	5.8 ± 0.8	6.9 ± 2.0	6.5 ± 1.1	6.8 ± 1.4	6.6 ± 1.5	–
Docosane	0.3 ± 0.1	1.7 ± 0.8	0.2 ± 0.1	1.1 ± 0.4	1.1 ± 0.3	2200
Tetracosane	–	8.9 ± 0.5	–	0.4 ± 0.3	0.3 ± 0.1	2400
hexacosane	–	1.8 ± 0.5	–	0.5 ± 0.3	0.5 ± 0.2	2600
Mixed hydrocarbon	–	10.0 ± 2.0	–	0.5 ± 0.2	1.0 ± 0.3	–

t: trace (<0.1), RRI lit: relative retention indices literature reported.
[a] Rout et al. [7].
[b] Tentatively identified based on mass fragmentation data and matching with spectral libraries.
% composition reported is the average of three readings with RSD values.

carried out for 3 h in the same experimental set up as discussed above. The extract was stored in a refrigerator for analysis.

2.3. GC and GC/MS analysis

The liquid CO_2 flower extract and liquid CO_2 fractions of concrete were analyzed by GC and GC/MS. GC analysis was carried out on a Shimadzu GC-17A Gas Chromatograph equipped with an FID and 25 m × 0.25 mm × 0.25 μm WCOT column coated with 5% diphenyl dimethyl siloxane supplied by J & W (DB-5). Helium was used as carrier gas at a flow rate of 1.2 mL/min at a column pressure of 42 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 60 min, with a total run time of 130 min. The percentage composition was calculated using peak normalization method assuming equal detector response. The samples were then analyzed on a Shimadzu QP 5000 GC/MS fitted with the same column and following the same temperature program as above. The MS parameters are as follows: ionisation voltage (EI) 70 eV, peak width 2 s, mass range 40–400 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 the literature values [11,12].

2.3.1. Determination of volatile matter in floral extracts

The pentane extract (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, phenyl ethyl alcohol and methyl benzoate 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 was calculated.

3. Results and discussion

The yield of the concrete was 1.58 ± 0.06 wt%. In the process of precipitating the waxes by addition of methanol and chilling,

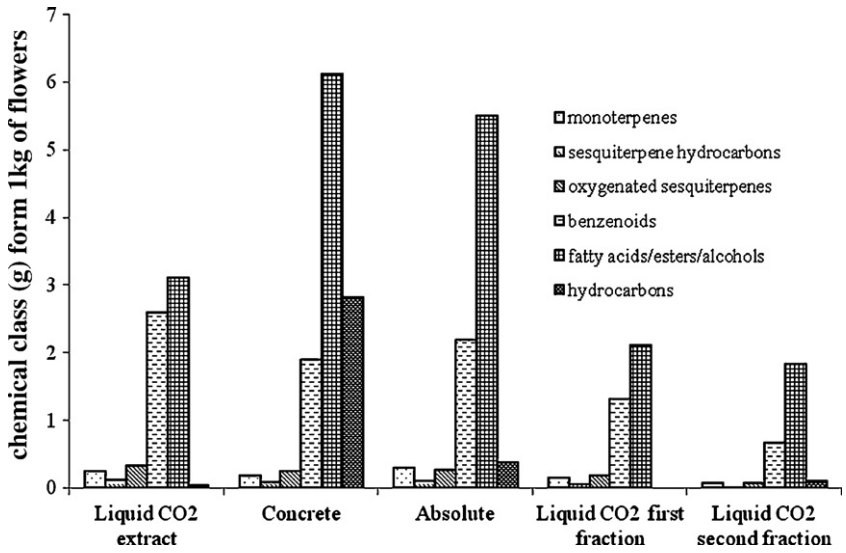

Fig. 1. Comparative chemical classes in different extracts of *Michelia champaca* flowers.

most of the hydrocarbons were separated, but the absolute so obtained (1.32 ± 0.02 wt%), contained still fair amounts of fatty acid/esters/alcohols (52.2%) and solvent residues. In order to obtain, organoleptically superior extract, free from solvent residues and that contains lesser amounts of waxes, the liquid CO_2 fractionation of the concrete was taken up.

Table 1 presents the composition of fractions obtained from the extraction of concrete using liquid CO_2, 45 min in each stage of extraction. It may be observed that the extraction of the desirable class of compounds such as terpenoids, benzenoids and methyl jasmonoates was completed in 180 min. Further extraction with liquid CO_2 (fifth fraction) has provided extract enriched in 9,12-octadecadienol (8.1%), fatty acids/esters/alcohol (58.9%) and long chain hydrocarbons (7.8%) with far lesser quantities of benzenoids (4.8%). Therefore it was decided to carry out two stages of extraction only of 90 min intervals.

The yields of liquid CO_2 extract in the first and second stage were 1.06 ± 0.02 and 0.84 ± 0.02 g, respectively from 3 g of concrete. They contained monoterpenes (1.2 ± 0.2, 0.9 ± 0.2%), sesquiterpene hydrocarbons (1.2 ± 0.4, 0.6 ± 0.2%), oxygenated sesquiterpenes (3.5 ± 0.4, 1.8 ± 0.3%), benzenoids (31.2 ± 1.3, 23.4 ± 1.1%), fatty acids/esters/alcohol (45.9 ± 2.0, 51.5 ± 3.2%) and hydrocarbons (0.2 ± 0.1, 2.9 ± 1.0%) in first and second stage, respectively. The yield of direct liquid CO_2 extract of flowers was 1.0 wt%. The liquid CO_2 extract of flowers contained monoterpenes (1.5 ± 0.4%), sesquiterpene hydrocarbons (1.4 ± 0.4%), oxygenated sesquiterpenes (4.1 ± 1.0%), benzenoids (33.7 ± 3.8%), fatty acids/esters/alcohol (37.6 ± 4.0%) and hydrocarbons (0.5 ± 0.1%).

The amounts of volatile material in the extracts as calculated from the quantity of methyl benzoate and phenyl ethyl alcohol are also presented in Table 2. The volatile fraction in liquid CO_2 extract of flowers is 85%, which means that about 15% of non-volatile material also gets extracted. Fig. 1 represents the quantities of different chemical classes in the volatile portion of the extracts. It is clear from the figure that, the high molecular weight hydrocarbons are extracted relatively in much less amounts in liquid CO_2.

In Table 2 is presented the compositions of concrete, absolute, liquid CO_2 extract of flowers and liquid CO_2 fractions from *M. champaca* L. concrete. In total, 40 compounds have been identified in GC/MS analysis.

4. Conclusions

Liquid CO_2 fractionation of floral concrete is a practical and superior process in comparison to the methanol chilled process. The liquid CO_2 fractions are free from solvent residues and might be suitable for use in aromatherapy or high grade perfumery. Whereas, the direct liquid CO_2 extraction of flowers provides the best extract; but it requires large capacity of the plant involving very high costs making the process uneconomical. Preparation of concrete by conventional method and its fractionation is a practically feasible process, which is recommended for commercial scale of production. This is the first report on extraction of *M. champaca* flowers and fractionation of floral concrete with liquid CO_2.

Acknowledgement

The authors are thankful to Prof. Ram Rajasekharan, Director, CIMAP, Lucknow for encouragement.

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