Comparison of subcritical CO_2 extraction with conventional methods of extraction to isolate the flower fragrance from *Michelia champaca* Linn

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Abstract:

The extraction of champa flowers by conventional methods and compared with the subcritical CO_2 extraction. The headspace volatile composition of living flowers and after plucking was determined by solid phase micro extraction on PDMS fiber for comparison. Solvent extraction of fresh flowers of *Michelia champaca* with pentane yields 1.5 ± 0.05 % concrete. The concrete was extracted with subcritical CO_2 and methanol to separate the absolute and the yields are 70% and 80% of the concrete respectively. Hydro-distillation of the fresh flowers in a Clevenger type unit furnished 0.03% of the essential oil. The chemical composition of the concrete, absolute and essential oil were determined by GC and GC/MS. The chemical composition of absolute obtained through CO_2 extraction method is superior in comparison to absolute produced by methanol process. interestingly, the concrete and absolute contain very less amount of sesquiterpene hydrocarbons. The chief components like indole, methyl anthranilate, methyl benzoate, phenyl ethyl alcohol, phenyl acetonitrile are present in higher percentage in the CO_2 extract. The essential oil contains several components responsible for the top note, but quite different from those present in the pentane extract. The essential oil contains more sesquiterpene hydrocarbon components among these β-elemene (19.8%) is a major component. The higher diffusivity of champa fragrance from the fresh flower may be due to the presence of higher amounts of indole and methyl benzoate, which is presented in the headspace analysis of fresh flower.

Keywords: *Michelia champaca* L., Subcritical CO_2, Liquid CO_2, methyl benzoate, indole, methyl anthanilate, β-elemene, (E,E)-α-farnesene, HS-SPME, concrete, absolute, GC/MS

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Introduction:

Michelia champaca is one among many species of Michelia[1]. *Michelia champaca* Linn[1], family Magnoliaceae is a tall, evergreen tree growing usually upto 30m in height. It is native to the temperate Himalayan region; however, it is found distributed through out the subtropical and tropical countries such as India, South China, Indonesia, the Philippines and some Pacific islands [2]. In India, it is found in Eastern Himalayas and Eastern and Western Ghats. The tree blooms once during monsoon and again in spring. When fully in bloom, the tree is covered with thousands of golden yellow flowers with powerful and diffusive fragrance. Although widely distributed, *M.champaca* is found concentrated in some pockets of Eastern Ghats bordering Orissa and Andhra Pradesh. Production of essential oil and *attars* from the flowers is carried out here to a limited extent. Michelia alba DC, a closely related species has the same distribution. The flowers of both the species are highly revered because of their attractive and persistent odor. Major portion of the flowers is used for ornamental purpose and for worshiping in temples.

A survey of the literature indicates that *M. champaca* L. has not been much studied. Kaiser[2-4] reported results of a very detailed analysis of 3 samples of laboratory prepared concrete and one commercial sample of absolute of *M.Champaca* L. produced in India. The major components were linalool (0.2-11.0%), methyl benzoate (1-5%), benzyl acetate (0.1-4.0%), cis-linalool oxide-pyranoid (0.2-7.0%), phenyl acetonitrile (0.1-4.3%), 2-phenethyl 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%), methyl palmitate (t-3.0%), ionone oximes (t-3.0%) and methyl linoleate (1.0-18.0%). A total of more than 250 compounds including trace components have been identified. Zhu et al.[5] studied headspace analysis of mature flower of *M. champaca* and identified 19 compounds. The major compounds were linalool (5.04%), cis and trans linalool oxides (furanoid form) 2-phenethyl alcohol (3.15%), heptanal (17.07%), indole (7.20%), methyl anthanilate (5.15%) and methyl linoleate (14.18%).

There are few literatures available on supercritical CO_2 extraction of Jasmine and Rose concrete6[6,7] to separate absolute but there is no reported work on separation of absolute from champa concrete. In the present paper freshly collected *M.champaca* flowers were used to prepared concrete and essential oil by different extraction processes and compared he chemical composition of the extracts by GC & GC/MS. The fragrance emitted by living flowers on the tree and after plucking also has been trapped on a PDMS fibre using the HS-SPME technique[8,9] and analysed by GC & GC/MS.

Experimental:

All the solvents used were of reagent grade and have been redistilled before use. The yields reported are average of two experiments. Fresh flowers of *M. champaka* L. flowers were collected from two 30-year old trees growing in the Regional Research Laboratory campus, Bhubaneswar (20.15E. 85.52N). 1 kg of flowers collected early morning was subjected to distillation with water in a Clevenger type apparatus for 3 hours and the upper oily layer separated. Yield 0.3g. Cold extraction of flowers (500g) with pentane (2.5 lit, two successive extractions) followed by careful evaporation of the solvent afforded a waxy residue (8.0g). Addition of 50 ml of cold methanol to 5 gm of the residue, warming to 40^0C, followed by refrigeration for 72 hours, precipitated most of the waxes. Filtration through a sintered funnel followed by evaporation of methanol below 50^0C afforded the absolute (4.0g).

2 gm of same champa concrete was taken in a glass soxhlet apparatus in the high-pressure extraction vessel for subcritical CO_2 extraction. The detailed experimental set up (fig-1) and process is appeared in our earlier paper[10]. The concrete was mixed with glass bead (3 mm diameter) and half filled the glass extraction vessel. The extraction was carried out in an air condition room the temperature is maintained at 20^0-22^0C and 60-65 bar pressure. Chilled water of 5^0C from a thermostatic bath was circulated through the cooling finger of the apparatus. The liquid CO_2 extraction was carried out for 2 hours. Then CO_2 was released from the extractor

slowly through tephlon tube connected through a glass bottle placed in ice bath. Yield of absolute is 1.42 gm.

HS-SPME was carried out as follows: A 100 ml conical flask provided with a B40 and a B14 joint was used for collecting the head space fragrance emitted by the flowers. The B14 joint was sealed with a screw cap provided with a silicone rubber septum for introducing the SPME manual holder. A branch of the tree carrying two flowers was carefully introduced into the flask through the wider mouth and the mouth covered with aluminium foil. The SPME sample holder used for outdoor sampling was introduced through the septum within 2 cm distance from the flowers and the PDMS fibre exposed for 30 minutes for achieving equilibrium. After withdrawing the fiber into the holder, it was brought to the laboratory for analysis.

GC analysis was carried out on a Shimadzu GC 17A Gas chromatograph equipped with a flame ionization detector and a 30 X 0.25 mm WCOT column coated with 0.25µ 5% diphenyl dimethyl silicone supplied by J &W (DB-5). Helium was used as the carrier gas at a flow rate of 1.2 mL per min at a column pressure of 42 Kpa. 0.2µl of each sample injected in the split ratio of 50:1. When the SPME fibre was introduced into the injection port for desorption, split less mode of injection was used. Component separation was achieved following a linear temperature program of 60^0-200^0C (2^0C/min), 200^0 (60 min). Percentage composition was calculated using peak normalization method. The oils were analysed using a Shimadzu QP5000 GC MS fitted with the same column and temperature programmed as above. MS parameters: ionization voltage (EI) 70ev, peak width 20sec, mass range 40-400amu and detector voltage 1.5volts. Peak identification was carried out by comparison of the mass spectra with mass spectra available on NIST-1, NIST-2, Wiley and Adams libraries. The compound identification was finally confirmed by comparison of their relative retention indices[11-13] with literature values.

Results and Discussion:

Table 1 presents the compositions of the concrete, absolute and essential oil while in Table 2 presents the compositions of the fragrance in headspace of the flowers while still attached to the tree and immediately after plucking. The values presented are average of two readings recorded from 2 samples obtained in experiments carried out under identical conditions. Considerable variation occurs in the composition of the concrete, absolute and the essential oil of the flowers of *M.Champaca* L. Esters such as methyl benzoate, ethyl benzoate, phenyl ethyl formate, phenyl ethyl benzoate, methyl anthranilate, Z-methyl jasmonate and Z-methyl epi jasmonate and phenyl acetonitrile are detected in much higher amounts in the concrete and absolute whereas they have either totally disappeared or decreased in concentration in the essential oil. Obviously, this has occurred due to hydrolysis during the essential oil recovery. Further, water soluble components such as P-cresol, indole, phenyl ethyl alcohol, dihydro β-ionol, have disappeared from the essential oil and are lost in the distillation water. The absolute obtained through CO_2 extraction method is superior organoleptically in comparison to methanol chilled process. The headspace vapor of the flowers as absorbed on the non polar PDMS fiber gives an altogether different analysis of flowers. Methyl benzoate, phenyl ethyl alcohol, phenyl acetonitrile, indole, methyl anthranilate, sesquiterpene hydrocarbons constitute the body of the headspace. The high concentration of sesquiterpenes may be due to the nonpolar nature of the adsorbing fiber with higher selectivity of non polar compounds. Further the composition of headspace of *M.champaca* flowers reported by us is different from the composition reported by Zhu et al., which may be due to different geographical locations. While (E,E)-α-farnesene and methyl benzoate are the major constituent in the headspace, β-elemene and methyl palmitate contribute to the body of the essential oil. The higher diffusivity of champa fragrance from the live flower may be due to the presence of higher amounts of indole and methyl benzoate.

Conclusions: These results show that the concrete and absolute contain much smaller proportion of sesquiterpenes and the essential oil contains several components quite different from compounds isolated from the pentane extract. Especially, the monoterpene hydrocarbons detected in the essential oil are not found in the extract. These compounds are obtained probably as artifacts during distillation. Further, several thermally labile compounds undergo decomposition during distillation. The absolute obtained by subcritical CO_2 extract process is superior then the absolute obtained by methanol process and close to the fragrance of fresh flowers.

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Table1: Chemical composition of the concrete, absolute and essential oil of fresh flowers of *M. Champaca* L.

GC RT	Compound identified	Concrete	Absolute (methanol)	Absolute (CO_2 extract)	Essential oil	RRI cal.	RRI lit
6.7	3-methyl-4-heptanone	2.2	3.4	2.5	-	932	929
6.8	α-thujene	-	-	-	0.1	934	931
7.0	α-pinene	-	-	-	0.5	938	939
7.4	camphene	-	-	-	0.2	953	953
7.8	benzaldehyde	0.2	<0.1	0.2	-	970	961
8.0	sabinene	-	-	-	0.5	979	976
8.1	β-pinene	-	-	-	1.2	982	980
8.4	6-methyl-5-hepten-2-one	<0.1	-	<0.1	-	992	985
8.5	myrcene	-	-	-	0.2	993	991
9.5	decane	0.3	0.2	<0.1	-	1001	1000
10.4	α-terpinene	-	-	-	0.1	1019	1018
11.0	limonene	-	-	-	0.2	1030	1031
11.3	1,8-cineole	0.4	1.4	1.0	5.1	1035	1035
11.7	Z-β-ocimene	-	-	-	0.2	1040	1040
12.3	E-β-ocimene	0.1	0.2	0.2	0.3	1051	1050
13.1	γ-terpinene	-	-	-	0.2	1061	1062
13.8	p-cresol	1.0	1.7	1.8	-	1069	1075
14.5	terpinolene	-	-	-	0.1	1088	1088
15.4	methyl benzoate	4.1	8.6	10.0	3.3	1097	1091
15.8	linalool	-	-	-	1.5	1105	1098
17.2	phenyl ethyl alcohol	4.3	4.2	4.8	-	1120	1110
17.6	Z-P-menth-2-en-1-ol	-	-	-	<0.1	1128	1121
18.2	E-P-menth-2-en-1-ol	-	-	-	<0.1	1147	1140
19.2	phenyl acetonitrile	4.4	8.7	9.5	-	1150	-
19.3	camphene hydrate	-	-	-	<0.1	1151	1148
19.9	borneol	-	-	-	0.4	1168	1165
20.2	ethyl benzoate	0.6	0.4	0.5	-	1177	1170
20.7	phenyl ethyl formate	0.4	0.1	0.4	-	1178	1174
20.8	terpinen-4-ol	-	-	-	0.9	1179	1177
21.0	α-terpineol	-	-	-	0.3	1194	1189
21.2	dodecane	0.2	-	-	-	1197	1200
28.7	isobornyl acetate	-	-	-	0.1	1285	1285
32.3	indole	0.8	0.8	1.3	0.2	1303	1288
33.6	methyl anthranilate	0.8	0.9	1.2	<0.1	1341	1337
33.7	δ-elemene	-	-	-	0.4	1342	1339
33.9	α-longipinene	-	-	-	0.1	1347	1351
34.3	eugenol	<0.1	<0.1	<0.1	-	1362	1356
34.6	α-ylangene	-	-	-	<0.1	1367	1372
35.0	α-copaene	-	-	-	0.1	1375	1376
35.8	β-elemene	-	-	-	19.8	1392	1391
36.0	tetradecane	<0.1	-	-	-	1395	1400
36.6	E-caryophyllene	-	-	-	2.4	1417	1418
37.0	E-α-ionone	0.3	0.4	0.4	-	1430	1426
37.3	γ-elemene	-	-	-	0.9	1433	1433
37.5	α-E-bergamotene	-	-	-	0.4	1435	1436

37.6	dihydro-β-ionone	0.6	0.7	0.9	-	1440	-
37.7	α-Z-ambrinol	-	-	-	0.7	1441	1436
37.9	aromadendrene	-	-	-	0.2	1444	1439
38.2	α-humulene	-	-	-	1.1	1448	1454
38.6	E-β-farnesene	-	-	-	0.5	1454	1458
38.7	dihydro-β-ionol	0.3	0.3	0.4	-	1455	-
38.8	β-santalene	-	-	-	0.1	1458	1462
39.1	9-epi-E-caryophyllene	-	-	-	0.8	1467	1467
40.4	germecrene-D	-	-	-	4.2	1479	1480
40.8	E-β-ionone	0.8	1.2	1.2	3.2	1488	1485
41.3	pentadecane	0.3	<0.1	-	-	1494	1500
42.0	(E,E)-α-farnesene	0.7	1.0	0.9	4.5	1507	1508
42.1	β-bisabolene	-	-	-	0.2	1508	1509
43.0	δ-cadinene	-	-	-	0.4	1523	1524
45.1	germecrene-B	-	-	-	3.2	1552	1556
45.8	E-nerolidol	-	-	-	0.8	1568	1564
46.3	spathulenol	-	-	-	1.7	1576	1576
46.4	caryophyllene oxide	-	-	-	0.6	1579	1581
46.5	globulol	-	-	-	0.2	1582	1583
46.8	guaiol	-	-	-	0.4	1594	1594
47.1	hexadecane	0.1	<0.1	-	-	1594	1600
49.4	β-acorenol	-	-	-	1.4	1639	1634
49.5	epi-α-cadinol	-	-	-	0.8	1641	1640
49.8	α-muurolol	-	-	-	0.1	1647	1645
51.2	Z-methyl jasmonoate	0.8	0.8	1.0	-	1652	1647
51.3	α-cadinol	-	-	-	0.4	1653	1653
51.4	selin-11-en-4-α-ol	-	-	-	2.2	1655	1652
52.5	Z-nerolidol acetate	-	-	-	0.9	1668	1675
52.6	β-bisabolol	0.1	-	0.1	0.5	1670	1671
53.3	Z-methyl epi-jasmonoate	0.3	0.4	0.5	-	1682	1676
59.2	benzyl benzoate	0.2	0.2	0.3	-	1771	1762
59.5	α-E-bergamotyl acetate	-	-	-	0.5	1779	1778
61.6	octadecane	0.1	0.2	<0.1	-	1796	1800
63.0	phenyl ethyl benzoate	0.5	0.6	0.7	0.2	1854	1853
64.9	nonadecane	0.8	<0.1	-	-	1894	1900
66.6	methyl palmitate	5.0	5.5	5.3	1.5	1930	1927
68.9	eicosane	0.1	0.3	<0.1	0.5	1995	2000
70.8	palmitic acid	4.2	5.1	5.3	-	2021	-
75.8	methyl linoleate	24.4	25.3	21.0	3.2	2092	2092
76.5	methyl linolenate	8.0	9.0	7.9	3.5	2099	2100
78.4	methyl stearate	0.2	0.3	0.2	3.4	2137	2128
82.7	9,12-octadecadienol	6.9	6.6	6.8	-	2192	-
84.3	docosane	1.7	1.1	0.2	-	2207	2200
96.2	tetracosane	8.9	0.3	-	-	2395	2400
111.5	hexacosane	1.8	-	-	-	2600	2600
126.1	mixed hydrocarbon	10.0	-	-	-	3000	-

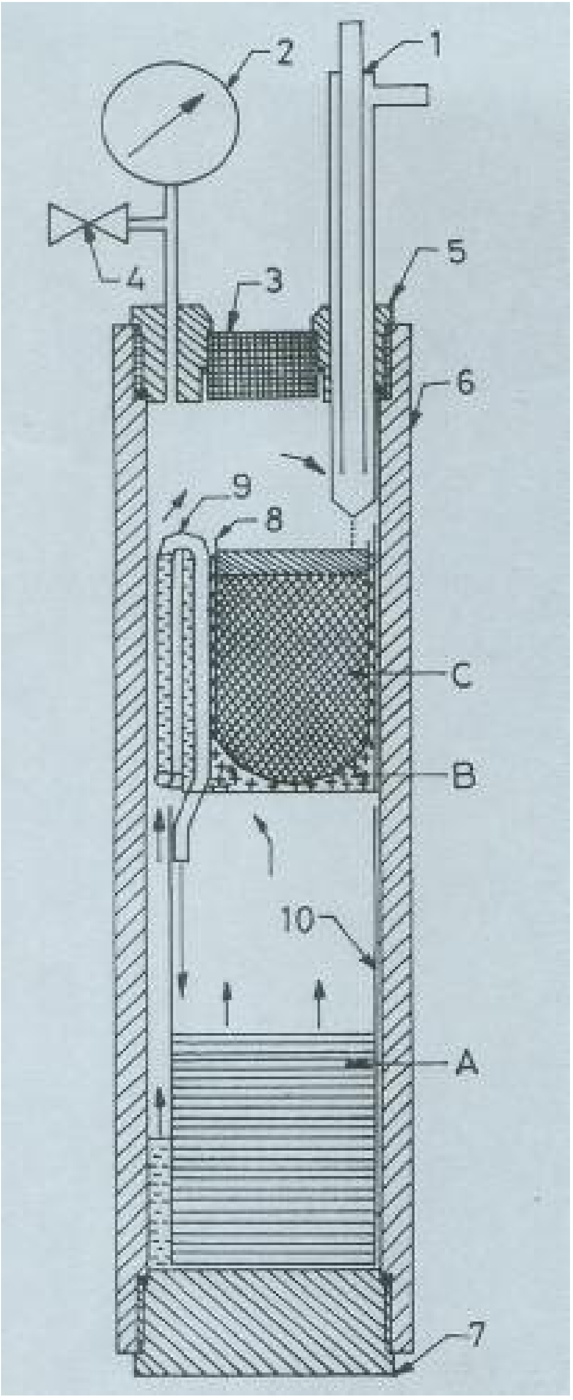
Absolute (methanol) – separate absolute by methanol chilled process
Absolute (CO_2 extract) – separate absolute by subcritical CO_2 process

Table 2: Composition of Headspace of Live flowers of *Michelia champaca* growing at Bhubaneswar.

GC RT	Compound	A	B	RRI cal	RRI lit
12.3	E-β-ocimene	1.0	0.5	1050	1050
15.5	methyl benzoate	23.4	21.2	1097	1091
17.2	phenyl ethyl alcohol	1.3	0.8	1120	1110
19.2	phenyl acetonitrile	4.6	4.0	1149	-
32.3	indole	3.5	3.3	1303	1288
33.6	methyl anthranilate	1.0	0.7	1341	1337
33.7	δ-elemene	1.0	0.7	1342	1339
35.0	α-copaene	1.5	0.9	1375	1376
35.7	β-copaene	0.8	0.6	1388	1390
35.9	β-elemene	5.7	3.3	1391	1391
36.6	E-caryophyllene	4.2	2.8	1415	1418
37.3	β-gurjunene	0.8	-	1427	1432
37.5	γ-elemene	0.6	0.7	1434	1433
37.6	α-E-bergamotene	1.3	1.1	1436	1436
37.9	epi-α-muurolene	1.9	1.7	1442	1441
38.4	E-β-farnesene	1.2	0.9	1450	1458
39.0	9-epi-E-caryophyllene	0.8	0.6	1463	1467
40.4	germacrene-D	7.3	4.8	1478	1480
40.8	E-β-ionone	0.6	0.8	1488	1485
41.1	zingiberene	2.1	1.6	1497	1495
42.2	(E,E)-α-farnesene	32.7	44.6	1513	1508
43.1	δ-cadinene	0.7	0.7	1525	1524
66.7	methyl palmitate	-	0.2	1931	1927

A: Fully bloomed flowers attached to the branch and equilibrated with SPME for 30 minutes in the flask.

B: Plucked flower equilibrated with SPME for 30 minutes in the flask.



1. Cooling finger
2. Manometer
3. Window
4. Valve

5,7. Closures

6. Cylinder
8. Extraction thimble
9. Siphon
10. Receiver

A. Extraction product
B. Extraction with liquid CO_2
C. Extraction vessel

Fig 1: Subcritical CO_2 Extraction apparatus