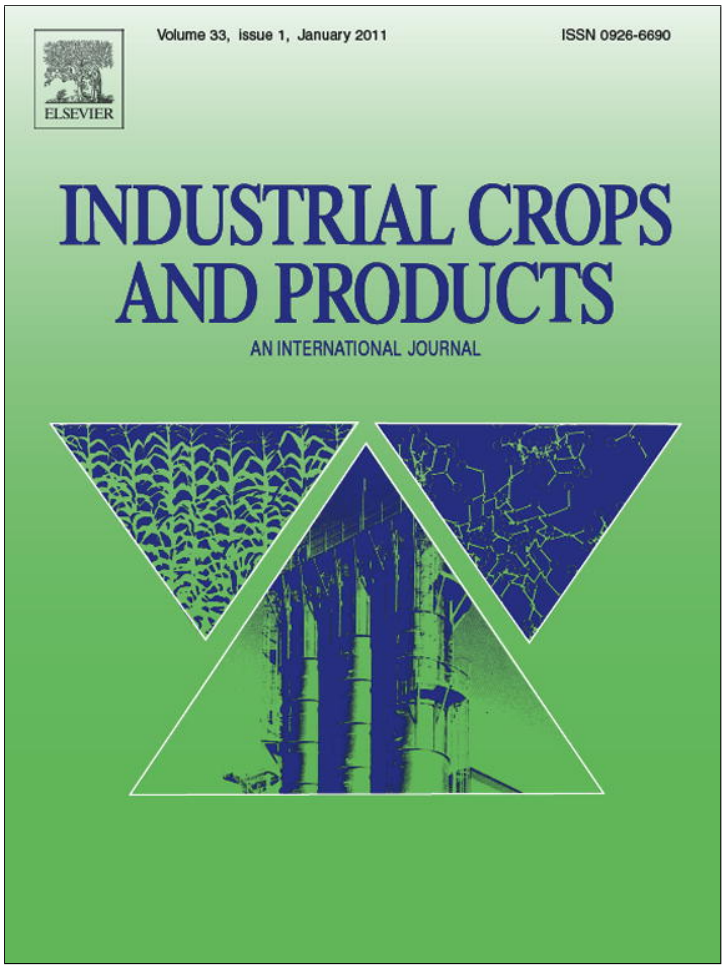

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Composition of essential oil, concrete, absolute and SPME analysis of *Tagetes patula* capitula

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ABSTRACT

The chemical compositions of essential oil, concrete, absolute from the capitula of *Tagetes patula* (family *Asteraceae*) were analysed by GC-FID and GC/MS. The major compounds identified were (*Z*)-β-ocimene, (*E*)-β-ocimene, terpinolene, (*Z*)-ocimenone, (*E*)-ocimenone and δ-elemene. In addition, the volatiles of live and plucked capitula (flowers) were analysed by SPME technique using PDMS/DVB/CAR fiber. The SPME-GC-FID analyses of live capitula showed that the volatiles were rich in monoterpenoids in comparison to the plucked capitula. On the other hand, the plucked capitula recorded with significant increase in sesquiterpenoids in comparison to the living capitula.

1. Introduction

Tagetes patula L. (marigold) belongs to the *Tagetes* genus, family *Asteraceae*. *Tagetes* species are native to Central America, but it is found throughout the world as an important ornamental plant (Soule, 1996). In India, it is cultivated as a floriculture crop and capitula are sold loose or used in garlands for social and religious purposes (Vasudevan, Kashyap, et al. 1997). In the year 2000, commercial cultivation of marigold in India had raised to 13,000 ha with annual production of 200,000 tonnes of capitula, the highest of any flowers grown in India (Raghava, 2000). In India, the estimated land for cultivation of marigold is more than 25,000 ha. Igolen (1936) had studied chemistry of *T. patula* oil in first time; the yield of essential oil was 0.3% by steam distillation and tagetone was reported as the major compound. Garg et al. (1999) have isolated the essential oil (0.09%) from capitula and identified 22 compounds. Piccaglia et al. (1997) have reported the chemical and antimicrobial activity of essential oil of *Tagetes erecta* and *T. patula*. Vasudevan, Tandon, et al. (1997) have isolated the *T. patula* capitula volatiles by using liquid and supercritical CO_2. The chemical compositions of CO_2 extracts were compared with the essential oil. Krishna et al. (2002) have reported the essential oil of *T. patula*, leaves and shoots collected from Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP) research farm, Lucknow, India. The major compounds identified in capitula essential oil were limonene (2.1%), (*Z*)-β-ocimene (19.9%), (*E*)-tagetone (1.4%), (*Z*)-tagetone (1.8%), isoborneol (3.5%), (*Z*)-tagetenone (12.4%), (*E*)-tagetenone (10.4%), pipertitenone oxide (5.8%), β-caryophyllene (15.1%) and (*E*,*E*)-α-farnesene (2.5%). Vidya Sagar et al. (2005) have reported the *T. patula* essential oil of capitula, leaf and whole herb collected from Farukunagar, Haryana, India. The major compounds in the capitula essential oil were limonene (6.2%), dihydrotagetone (6.2%), (*E*)-tagetone (2.5%), P-cymen-8-ol (11.0%), piperitone (10.6%), piperitenone (8.1%) and (*E*)-sesquisabinene hydrate (12.5%).

Solid phase micro extraction (SPME) is a novel approach in sorbent extraction (Pawliszyn, 1997). It totally excludes use of organic solvents and is very simple to operate. The technique integrates extraction, concentration and sampling into a single step. In SPME, a fused silica fiber is coated with a stationary phase and the fiber is exposed to an aqueous or gaseous sample till equilibrium is achieved between the analyte in the sample and on the fiber. The analyte is then thermally desorbed from the fiber in the injection chamber of a gas chromatograph (GC) and analysed by flame ionisation detector (FID). Rout et al. (2006, 2007, 2012) have reported the volatiles of live and plucked flowers in SPME techniques using non-polar PDMS fiber. In that study, we had observed that the terpene hydrocarbons were enriched in floral headspace analysis. In recent literature revealed that, the fiber coated with non-polar, semipolar and polar adsorbing phase was more suitable for analysis of organic volatiles (Bicchi et al., 2007; Prosen et al., 2010).

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There is no reported work on concrete, absolute and SPME analysis of *T. patula* capitula. In this communication, the comparative chemical compositions of essential oil, concrete, absolute and also compositions of volatiles of live and plucked capitula by using PDMS/DVB/CAR fiber are reported.

2. Experimental

2.1. Plant material

The fresh *T. patula* capitula were collected from CSIR-CIMAP research farm, Lucknow (26°55′N, 80°59′E) in the early morning on the month of February 2010. The capitula were immediately extracted as per the procedure given below. Each extraction was carried out three times and yields reported along with the standard deviation.

2.2. Isolation of essential oil

The essential oil was extracted from 250 g of fresh capitula in a Clevenger-type of apparatus for 3 h. The yield of the oil was 0.14 ± 0.02 g. The oil was dried over anhydrous Na_2SO_4 and kept in refrigerated conditions prior to GC-FID and GC/MS analysis.

2.3. Concrete and absolute

Fresh capitula (250 g) were taken in an aspirator bottle and contacted with distilled pentane (1 l) for 6 h. After draining the extract, the raffinate capitula were again contacted with fresh pentane for another 6 h followed by a quick washing in pentane (100 ml). The combined extracts on solvent removal in a rotary evaporator under *vacuo* below 30 °C afforded a deep yellow waxy residue, the so called concrete (1.38 ± 0.05 g). Addition of 20 ml of methanol to the residue, warming to 50 °C for 5 min to get a homogeneous mixture followed by refrigeration for 12 h at −15 °C, precipitated most of the waxes. Filtration through a sintered funnel followed by evaporation of methanol below 40 °C afforded a light yellow partially dewaxed absolute (0.8 ± 0.03 g) with a pleasant odour reminiscent of fresh capitula.

2.4. GC-FID and GC/MS analysis

All the extracts were analysed on a Varian CP-3800 GC fitted a DB-5 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) equipped with a flame ionisation detector (FID). The oven column temperature was ranged from 60 to 240 °C, programmed at 3 °C/min, with a final hold time of 10 min, using H_2 as carrier gas at 1 ml/min constant flow with split ratio of 1:30. The neat sample (0.03 μl) was injected to the capillary column through the injection port of the GC. The injector and detector (FID) temperatures were 300 °C and 310 °C, respectively. The relative percentage of individual compound was determined on the basis of peak area normalisation method without using the correction factors.

GC/MS analysis was performed in a PerkinElmer AutoSystem XL GC interfaced with a Turbomass Quadrupole mass spectrometer fitted with same DB-5 fused silica capillary column. The oven temperature program was the same as that described in GC-FID. The injector, transfer line and source temperatures were 300 °C. The neat sample (0.03 μl) was injected with split ratio 1:30. The carrier gas was He at 10 psi constant pressure; ionisation energy 70 eV; mass scan range 40–450 amu. The compounds were identified on the basis of retention time, Kovats Index and calculation of relative retention index using a homologous series of *n*-alkanes (C_8–C_{24} hydrocarbons, Polyscience Corp. Niles, IL). Finally, the compounds were identified by mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data, 7th edition) and by comparing with the mass spectral literature data (Adams, 1995; Davies, 1990).

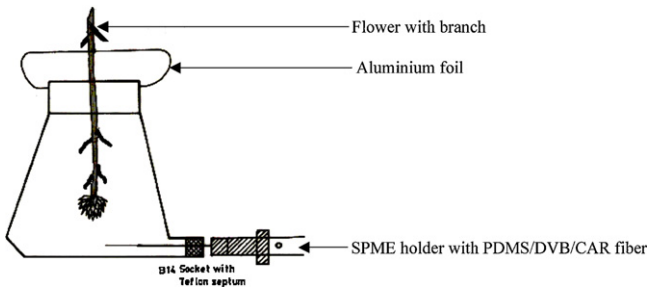

Fig. 1. SPME set up for floral volatiles pre-concentration over PDMS/DVB/CAR fiber.

2.5. SPME-GC-FID analysis

A 100 ml conical flask provided with a B40 and a B14 joint was used for equilibrating the fragrance emitted by the capitula in the headspace with a PDMS/DVB/CAR fiber as presented in Fig. 1. The B14 joint was sealed with a screw cap provided with a silicone rubber septum for introducing the SPME manual holder. The thickness of stationary phase (PDMS/DVB/CAR) coating is 100 μm and the length is 10.6 mm. Before use, the fibers were conditioned at 250 °C for 2 h. A branch of the tree carrying two flowers (living capitula) was carefully introduced into the flask through the wider mouth and the mouth was closed with aluminum foil and then parafilm. The headspace was allowed to attain equilibrium with the volatile compounds emitted by the capitula during the next 30 min. Then SPME manual sample holder used for outdoor sampling was introduced through the septum within 2 cm distance from the capitula and the PDMS/DVB/CAR fiber was exposed for 15–75 min for achieving the equilibrium. The fiber was withdrawn into the needle, and then the needle was removed from the septum and inserted directly into the injection port of the GC. The analytes on the fiber coating were desorbed by heating the fiber in the injection port at 300 °C for 4–5 min. The analytes were transferred directly into the capillary column (DB-5) for analysis. Between the extractions, the fiber was conditioned for 10 min at 300 °C to prevent the carryover of residues. The fragrance emitted by freshly plucked capitula (without branch) was analogously carried out. The percentage composition was determined by GC-FID and compounds were identified by GC/MS.

3. Results and discussion

The capitula on hydrodistillation gave essential oil (0.056%). There were 51 compounds identified; comprise 93% of the total identified compounds (Table 1). The major compounds identified in the essential oil were 1,8-cineole (4.4%), (*Z*)-β-ocimene (11.8%), terpinolene (6.9%), (*Z*)-ocimenone (6.4%), (*E*)-ocimenone (3.0%), δ-elemene (16.9%), piperitenone (3.3%), β-caryophyllene (18.6%) and (*E*)-β-ionone (2.5%). The chemical composition of the essential oil is presented in Table 1. The result is closed with the composition reported by Krishna et al. (2002), besides some variation of minor compounds occurred, which might be due to the seasonal effect. In the present work, the essential oil was extracted from capitula in winter season (February). On the other hand, Krishna et al. (2002) had reported the chemical composition of capitula essential oil of summer season (August). It was observed that, the essential oil yield was better in winter season (0.056%) in comparison to the summer season (0.04%)

The yields of concrete and absolute were 0.56% and 0.32%, respectively. There were 56 compounds identified in the concrete

Table 1
Composition of essential oil, concrete and absolute of *T. patula* capitula.

Compound	Essential oil (%)	Concrete (%)	Absolute (%)	RRI cal	RRI lit
Yield	0.056 ± 0.003	0.55 ± 0.02	0.32 ± 0.01		
α-Pinene	0.2 ± 0.1	0.1	0.7 ± 0.1	931	939
β-Pinene	0.3 ± 0.1	t	0.1	971	980
Sabinene	0.1	–	t	976	976
Myrcene	0.3 ± 0.1	t	0.1	986	991
α-Phellandrene	0.2 ± 0.1	0.1	0.1	1004	1005
α-Terpinene	0.1	–	0.3 ± 0.1	1010	1018
Limonene	0.2 ± 0.1	–	0.2 ± 0.1	1022	1031
1,8-cineole	4.4 ± 0.3	0.2 ± 0.1	0.5 ± 0.2	1027	1033
(*Z*)-β-ocimene	11.8 ± 0.7	1.7 ± 0.1	2.2 ± 0.3	1033	1040
(*E*)-β-ocimene	1.8 ± 0.2	0.3 ± 0.1	0.6 ± 0.2	1043	1050
γ-Terpinene	0.4 ± 0.2	t	0.1	1048	1062
Dihydrotagetone	0.1	0.2 ± 0.1	0.5 ± 0.2	1056	1054
(*Z*)-sabinene hydrate	0.1	0.1	0.3 ± 0.1	1074	1068
Terpinolene	6.9 ± 0.4	0.7 ± 0.1	1.2 ± 0.2	1088	1088
Methyl benzoate	0.2 ± 0.1	0.1	0.3 ± 0.1	1091	1091
Linalool	0.2 ± 0.1	0.1	0.1	1098	1098
P-mentha-1,3,8-triene	0.3 ± 0.1	0.1	0.1	1112	1111
(*Z*)-P-menth-2-en-1-ol	0.1	0.2 ± 0.1	0.3 ± 0.1	1123	1121
(*Z*)-pinene hydrate	2.0 ± 0.3	0.9 ± 0.1	1.5 ± 0.2	1126	1121
Allo-ocimene	2.0 ± 0.3	0.9 ± 0.1	1.3 ± 0.3	1129	1129
(*Z*)-sabinol	2.3 ± 0.3	1.4 ± 0.1	1.6 ± 0.2	1139	1140
(*E*,*E*)-allo-ocimene	0.7 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	1142	1142
Camphor	0.1	–	–	1145	1143
(*E*)-tagetone	0.1	0.1	0.3 ± 0.1	1147	1146
(*Z*)-tagetone	0.6 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	1151	1153
Borneol	0.1	0.1	0.2 ± 0.1	1168	1165
Terpinen-4-ol	0.6 ± 0.2	0.9 ± 0.1	0.3 ± 0.1	1177	1177
P-cymen-8-ol	0.8 ± 0.2	0.2 ± 0.1	0.8 ± 0.1	1185	1183
(*Z*)-ocimenone	6.4 ± 0.4	3.7 ± 0.3	9.5 ± 0.5	1231	1231
(*E*)-ocimenone	3.0 ± 0.2	2.0 ± 0.2	5.8 ± 0.4	1240	1239
Carvone	0.2 ± 0.1	2.0 ± 0.2	2.5 ± 0.3	1246	1242
Piperitone	0.6 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	1257	1252
δ-Elemene	16.9 ± 0.7	7.2 ± 0.4	14.5 ± 0.8	1340	1339
Piperitenone	3.3 ± 0.3	3.8 ± 0.3	6.6 ± 0.4	1346	–
β-Caryophyllene	18.6 ± 0.8	12.6 ± 0.5	25.8 ± 0.5	1418	1418
β-Humulene	0.2 ± 0.1	0.1	0.4 ± 0.1	1434	1440
Geranyl acetone	0.2 ± 0.1	0.1	0.2 ± 0.1	1455	1453
(*E*)-β-farnesene	0.6 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	1459	1458
Germacrene-D	0.1	0.2 ± 0.1	0.4 ± 0.1	1480	1480
Caryophyllene oxide	–	0.1	0.3 ± 0.1	1582	1581
(*E*)-β-ionone	2.5 ± 0.3	t	t	1486	1485
Bicyclogermacrene	1.3 ± 0.3	0.2 ± 0.1	0.4 ± 0.1	1501	1494
(*E*,*E*)-α-farnesene	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	1505	1508
(*Z*)-nerolidol	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	1526	1534
(*E*)-nerolidol	0.1	0.2 ± 0.1	0.4 ± 0.1	1560	1564
(*E*)-sesquisabinene hydrate	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	1586	1580
Caryophyllene oxide	0.2 ± 0.1	0.1	0.4 ± 0.1	1590	1581
(*E*)-sesquilavandulol	0.1	0.9 ± 0.1	1.2 ± 0.2	1647	1632
(*E*,*E*)-farnesyl acetate	0.3 ± 0.1	1.1 ± 0.2	1.5 ± 0.2	1844	1843
Isophytol	0.6 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	1927	1944
Icosane	0.1	1.0 ± 0.2	0.5 ± 0.2	2002	2000
Heneicosane	–	1.3 ± 0.3	0.7 ± 0.2	2092	2100
Methyl linoleate	–	6.1 ± 0.7	3.0 ± 0.6	2096	2092
Ethyl stearate	–	1.8 ± 0.5	1.0 ± 0.3	2192	–
Docosane	–	0.1	–	2208	2200
Tricosane	–	1.7 ± 0.6	0.2 ± 0.1	2310	2300
High molecular mass hydrocarbon (C > 25)[a]	–	20.3 ± 2.0	–	–	–
High molecular mass unidentified components (RT = 74.5–75.5)	–	15.1 ± 1.6	–	–	–
Monoterpene hydrocarbons	25.3	4.1	7.1		
Oxygenated monoterpenes	25.2	17.2	32.9		
Sesquiterpene hydrocarbons	38.0	21.1	42.7		
Oxygenated sesquiterpenes	3.6	3.0	4.8		
Oxygenated diterpenoid	0.6	0.2	0.5		
Fatty components (hydrocarbons/fatty esters, etc.)	0.1	47.4	6.0		
Total	93.0	93.1	94.3		

t, trace (<0.1%); RRI cal, relative retention indices calculated; RRI lit, relative retention indices literature reported; RT, retention time.
[a] The appropriate hydrocarbon compound is not identified.

and absolute. The major compounds were (*Z*)-ocimenone (3.7%, 9.5%), (*E*)-ocimenone (2.0%, 5.8%), δ-elemene (7.2%, 14.5%), piperitenone (3.8, 6.6%) and β-caryophyllene (12.6, 25.8%) in concrete and absolute, respectively. The concrete comprised of waxy compounds (47.4%). The last peak, which appeared at RT 75 min was not identified in mass spectra. Addition of methanol in concrete and then after chilling the methanol solution was used to remove most of the long chain hydrocarbons. Thus, the absolute was

Table 2
SPME analysis of plucked and live *T. Patula* capitula.

Compound	15 min		30 min		45 min		60 min		75 min
	Plucked (%)	Live (%)	Plucked (%)	Live (%)	Plucked (%)	Live (%)	Plucked (%)	Live (%)	Plucked (%)
α-Pinene	1.0 ± 0.2	2.2 ± 0.3	1.0 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
β-Pinene	1.4 ± 0.2	3.8 ± 0.4	1.3 ± 0.1	2.7 ± 0.3	1.1 ± 0.2	2.8 ± 0.3	1.0 ± 0.2	2.3 ± 0.3	0.9 ± 0.2
Sabinene	0.5 ± 0.1	1.6 ± 0.3	0.3 ± 0.1	0.9 ± 0.2	0.4 ± 0.1	1.2 ± 0.3	0.4 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
Myrcene	1.7 ± 0.3	2.4 ± 0.3	1.2 ± 0.3	1.8 ± 0.2	1.1 ± 0.2	2.1 ± 0.3	0.8 ± 0.2	1.5 ± 0.2	0.9 ± 0.1
α-Phellandrene	0.5 ± 0.1	0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	–
α-Terpinene	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
Limonene	–	0.5 ± 0.1	–	0.6 ± 0.1	t	0.8 ± 0.1	0.1	0.7 ± 0.2	0.2 ± 0.1
1,8-Cineole	4.5 ± 0.3	1.0 ± 0.2	4.6 ± 0.4	0.8 ± 0.2	5.0 ± 0.3	0.8 ± 0.2	5.7 ± 0.2	1.1 ± 0.3	6.0 ± 0.3
(*Z*)-β-ocimene	36.8 ± 0.5	50.5 ± 1.5	32.8 ± 0.5	48.8 ± 1.2	27.7 ± 1.2	50.0 ± 1.0	21.8 ± 0.6	46.7 ± 0.9	21.4 ± 0.7
(*E*)-β-ocimene	4.2 ± 0.3	10.3 ± 0.3	3.6 ± 0.3	9.9 ± 0.3	3.0 ± 0.3	10.8 ± 0.4	2.1 ± 0.3	8.5 ± 0.4	2.5 ± 0.4
γ-Terpinene	3.8 ± 0.3	0.7 ± 0.2	4.2 ± 0.3	0.4 ± 0.2	4.1 ± 0.3	0.3 ± 0.1	4.5 ± 0.3	1.2 ± 0.3	4.6 ± 0.5
Dihydrotagetone	0.1	0.3 ± 0.1	t	0.2 ± 0.1	0.1	0.5 ± 0.1	–	0.6 ± 0.1	–
(*Z*)-sabinene hydrate	–	0.3 ± 0.1	–	0.2 ± 0.1	–	0.4 ± 0.1	0.1	0.3 ± 0.1	0.2 ± 0.1
Terpinolene	5.7 ± 0.3	–	12.2 ± 1.0	0.2 ± 0.1	12.2 ± 1.2	0.7 ± 0.1	16.7 ± 0.6	0.7 ± 0.1	16.5 ± 0.4
Methyl benzoate	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	t	0.5 ± 0.1	0.1	0.4 ± 0.1	0.1
Linalool	0.2 ± 0.1	–	0.1	–	0.1	–	0.1	–	0.1
P-mentha-1,3,8-triene	–	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
Allo-ocimene	3.8 ± 0.3	5.8 ± 0.4	3.9 ± 0.4	6.8 ± 0.5	3.0 ± 0.3	7.4 ± 0.5	3.4 ± 0.3	9.9 ± 0.5	3.8 ± 0.3
(*Z*)-sabinol	0.4 ± 0.1	1.0 ± 0.2	0.3 ± 0.1	1.5 ± 0.3	0.2 ± 0.1	0.8 ± 0.2	0.3 ± 0.1	0.7 ± 0.2	0.4 ± 0.1
(*E*,*E*)-allo-ocimene	1.0 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	t	0.7 ± 0.2	0.2 ± 0.1	0.8 ± 0.2
(*E*)-tagetone	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
(*Z*)-tagetone	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Terpinen-4-ol	–	0.4 ± 0.1	–	0.5 ± 0.1	–	0.4 ± 0.1	–	0.3 ± 0.1	–
P-cymen-8-ol	0.1	0.8 ± 0.2	0.1	1.0 ± 0.2	t	0.6 ± 0.1	–	0.5 ± 0.1	–
(*Z*)-ocimenone	0.8 ± 0.2	0.9 ± 0.2	0.6 ± 0.2	1.2 ± 0.2	0.4 ± 0.1	1.5 ± 0.3	0.5 ± 0.1	1.5 ± 0.3	0.3 ± 0.1
(*E*)-ocimenone	0.5 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Piperitone	0.4 ± 0.1	–	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	t	0.2 ± 0.1	t	0.2 ± 0.1
δ-Elemene	19.8 ± 0.5	1.8 ± 0.4	19.0 ± 1.4	1.2 ± 0.3	21.0 ± 1.0	1.0 ± 0.3	22.5 ± 0.5	0.8 ± 0.4	22.3 ± 0.4
Piperitenone	0.5 ± 0.1	0.9 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
β-Caryophyllene	6.5 ± 0.3	7.9 ± 0.3	6.9 ± 0.4	7.7 ± 0.3	7.3 ± 0.4	7.5 ± 0.4	8.2 ± 0.4	9.2 ± 0.3	8.0 ± 0.3
β-Humulene	0.6 ± 0.1	0.1	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	t	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Geranyl acetone	0.9 ± 0.3	1.0 ± 0.2	0.8 ± 0.3	1.3 ± 0.2	0.3 ± 0.1	1.6 ± 0.3	0.2 ± 0.1	1.8 ± 0.3	0.2 ± 0.1
(*E*)-β-farnesene	–	0.8 ± 0.2	–	0.9 ± 0.1	0.2 ± 0.1	1.2 ± 0.2	0.3 ± 0.1	1.1 ± 0.2	0.5 ± 0.1
(*E*)-β-ionone	0.6 ± 0.2	0.1	0.5 ± 0.1	0.2 ± 0.1	1.0 ± 0.2	0.1	0.6 ± 0.1	t	0.4 ± 0.1
Bicyclogermacrene	–	t	–	0.3 ± 0.1	0.4 ± 0.1	0.1	0.1	0.2 ± 0.1	t
(*E*,*E*)-α-farnesene	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	–	0.3 ± 0.1	t	0.4 ± 0.1	–
Monoterpene hydrocarbons	60.8	78.9	61.9	73.9	55.0	78.0	53.4	74.1	52.0
Oxygenated monoterpenes	9.0	8.0	8.4	8.9	8.1	8.0	8.3	8.1	8.9
Sesquiterpene hydrocarbons	27.3	11.1	26.9	10.7	29.3	9.1	32.1	9.9	30.1
Oxygenated sesquiterpenes	0.6	0.1	0.5	0.2	1.0	0.1	0.6	t	0.4
Total	98.0	98.6	97.9	94.1	93.4	95.7	94.3	92.5	92.5

t, trace (<0.1%); RRI cal, relative retention indices calculated; RRI lit, relative retention indices literature reported.

contained fewer amounts of waxy compounds (5.4%). Interestingly, the only unidentified compound in the concrete was not detected in the absolute (methanol extract), thus the compound was non-polar in nature. The ^{1}H NMR spectra of the methanol insoluble wax comprised most of the peaks in the aliphatic region. Therefore, the unidentified compound might be a high molecular weight hydrocarbon. The detail chemical compositions of concrete and absolute are presented in Table 1.

The percentage compositions of mono- and sesquiterpene hydrocarbons in concrete (25.2%) and absolute (49.8%) were less in comparison to the essential oil (63.3%). But the yield of essential oil was poor in comparison to the pentane extract. It indicated that, quantitatively the terpene hydrocarbons in the concrete and absolute were still more in comparison to the essential oil. These terpene hydrocarbons were more volatile in distilled water; therefore, the non-polar terpenes were enriched in the essential oil. On the other hand, increase percentages of oxygenated mono- and sesquiterpenes were detected in absolute with compared to the essential oil.

The analysis of floral volatiles by SPME using PDMS/DVB/CAR fiber gave altogether an entirely different composition. The volatile compositions of live and plucked capitula in different time interval are presented in Table 2. It was observed that initially, the high volatile compounds were enriched on the fiber and slowly all the compounds were attained equilibrium with the fiber and headspace of the capitula. From Table 2, it has been decided that, the time interval of 60 min was sufficient for the compounds to attain equilibrium with the fiber. There was hardly any difference in composition of floral volatiles (plucked) analysed in 60 min and 75 min. Thus, it was considered to analyse the live capitula upto 60 min. There was somewhat dissimilar in volatile composition of live and plucked capitula. The live capitula were enriched with monoterpene hydrocarbons (73.9–78.9%); whereas plucked capitula were enriched with sesquiterpene hydrocarbons (26.9–32.1%). The plucked capitula contained improved percentage of 1,8-cineole (4.5–6.0%), γ-terpinene (3.8–4.6%), terpinolene (5.7–16.7%), (*E*,*E*)-allo-ocimene (0.5–1.0%), δ-elemene (19.0–22.5%) and (*E*)-β-ionone (0.4–1.0%); whereas living capitula were enriched with (*Z*)-β-ocimene (46.7–50.5%), (*E*)-β-ocimene (8.5–10.8%), allo-ocimene (5.8–9.9%), P-cymen-8-ol (0.5–1.0%), β-caryophyllene (7.3–9.2%), geranyl acetone (1.0–1.8%), and (*E*)-β-farnesene (0.8–1.2%). The compound like linalool was not detected in living capitula, whereas terpinen-4-ol was not detected in plucked capitula. The similar observation was reported by Mookherjee et al. (1990) that, the plucked flower contained improved percentage of linalool.

Monoterpenes were reported to function as pollinator attractant or act as defense chemicals in plants (Pichersky and Gershenzon, 2002). Recently, Kishimoto et al. (2006) reported that

(*E*)-β-ocimene and allo-ocimene were two important compounds, which showed resistance against pathogens. It was reported that, β-caryophyllene has anti-inflammatory, anti-biotic, anti-oxidant, anti-carcinogenic and local anaesthetic activities (Adorjan and Buchbauer, 2010). Moreover, these compounds were found with improved percentage in living *T. patula* capitula as compared to plucked capitula.

4. Conclusion

The improved percentage recovery of terpene hydrocarbons in the essential oil is due to their high volatility with steam but the poor recovery of essential oil in comparison to the solvent (pentane) extracts. Though, the pentane extract (concrete) gave improved yield but contained 47% of waxy components, which needs to separate from the terpenoids. 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. A major portion of waxy compounds (42%) were separated by addition of methanol and followed by chilling. The wax free absolute might be used in pharmaceutical application or in high grade perfumery. In headspace analysis, it is possible to analyse the volatile floral composition at different time interval, when capitula is attached to plant and even after plucking. The headspace analysis of living and plucked capitula gave different volatile composition. The organoleptic nature of the living capitula could be sense from long distances due to the presence of volatile monoterpenes.

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