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Chemical composition of the essential oils of *Tagetes patula* L. during different phenological stages

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ABSTRACT

The present study aims to investigate the detailed essential oil profiling of *Tagetes patula* L. at different stages of plant growth i.e. pre-flowering stage, full flowering and seed settings stages. The essential oils were obtained through hydro-distillation and analyzed by GC and GC/MS for the identification of their chemical constituents. The essential oil yield (%) from fresh aerial parts of *T. patula* were 0.06%, 0.06% and 0.08% (v/w) at pre flowering, full flowering and seed setting stages, respectively. A total of 38 components were identified, representing 89.65%, 91.94% and 90.84% in pre flowering, full flowering and seed setting stages, respectively. Chromatographic analysis (GC and GC/MS) has shown that all the oils of *T. patula* were dominated by monoterpene hydrocarbons (39.81-45.08%) followed by oxygenated monoterpenes (27.57-31.66%), sesquiterpene hydrocarbons (7.72-10.52%), oxygenated sesquiterpenes (6.63-7.57%) while diterpenes (1.39-1.68%) and oxygenated diterpenes (0.93-1.55%) were found in the lesser amounts. The major constituents in the oils were z-(β)-ocimene (12.76-20.29%), α-terpinolene (11.01-14.79%), piperitenone (6.46-11.96%), limonene (6.62-7.57%) and propanedinitrile dicyclohexyl (5.17-5.85%). Maximum content of z-(β)-ocimene (20.29%) was found at full flowering stage, while α-terpinolene (14.79%) and piperitenone (11.96%) at pre-flowering stage. The study concluded that however the oils were qualitatively similar but differed quantitatively during different growth stages.

KEY WORDS: *Tagetes patula*, Essential oil, GC/MS, z-(β)-ocimene, α-terpinolene.

1. INTRODUCTION

Tagetes L. commonly known as Marigold, is a native to Mexico and other temperate regions of America. It is also introduced in the tropics and subtropics region of the world as an important ornamental plant (Adams, 2017; Anonymous, 2003). The genus *Tagetes* comprises approximately 40-50 species throughout the world (Arctander, 1961). Five species have been introduced in India; *T. patula* L. (French marigold), *T. erecta* L. (African marigold), *T. minuta* L. (*Tagetes glandulifera* Schrank), *T. lucida* Cav. (Sweet-scented marigold) and *T. tenuifolia* Cav. (Striped marigold) (Armas, 2012). Commercially, Tagetes essential oil is produced from *T. patula* and *T. minuta* in some countries such as France, Australia and Kenya and also produced in India to a small extent (Adams, 2017). It is commercially cultivated in India as a floriculture crop (Bhattacharyya, 2010; Chkhikvishvili, 2016). The plant is herbaceous, branched, glabrous, annual and bushy. Its height reaches 25-100 cm with a tap root (Erdogrul, 2002). The flowers and leaves of marigold are used as folk medicine in the treatment of colics, diarrhea, vomiting, fever, skin and hepatic disease (Faizi, 2008; Garg, 1999). In India, a well-known perfume "Attar Genda" is obtained by the distillation of flower tops of *T. patula* but it is weakly demanded outside of its country of origin because of its weak natural odor than the essential oil (Hassanpouraghdam, 2011). Essential oils and extracts of *T. patula* are reported as a source of biological activities such as antioxidant, antimicrobial, antifungal, anti-inflammatory, antibacterial and antipyretic (Garg, 1999; Jabeen, 2016; Kashif, 2015; Krishna, 2002; Kuddus, 2012; Lim, 2014; Lawrence, 1985; Mares, 2002). Phytochemical studies of *T. patula* revealed the presence of secondary metabolites such as alkaloids, tannins, phenolic compounds and steroids, flavonoids, resins and fatty acids (Marotti, 2004; Martinez, 2009; Munhoz, 2014; Negi, 2013). The major constituents of essential oils of *T. patula* identified in different regions are limonene, terpinolene and (Z)-β-ocimene in the flowers from India (Prakash, 2012), piperitone and piperitenone in the oil from flowers of *T. patula* grown in India (Mares, 2002), α-terthienyl and pentatriacontane constituents are found in flowers of *T. patula* from Venezuela (Romagnoli, 2005), β-caryophyllene, (E)-β-ocimene dihydro, terpinolene and (Z)-β-ocimene in the flowers from Italy, terpinolene, (E)-β-ocimene dihydro and limonene in the oils from leaves of *T. patula* grown in Italy (Rondon, 2006), pipritone, trans-β-ocimene and terpinolene from the aerial parts grown in Venezuela (Garg, 1999). The present study aims to investigate the detailed essential oil profiling of *Tagetes patula* at different stages of plant growth.

2. MATERIALS AND METHODS

Plant material: The aerial parts (300 gm each) of *T. patula* were collected at three phenological stages; pre flowering stage (T1), full flowering stage (T2) and seed setting (T3) from the experimental field of Centre for Aromatic Plants (CAP), Selaqui, Dehradun, Uttarakhand in the month of November, December, 2017 and January, 2018 respectively.

Isolation of the essential oil: Fresh aerial parts of *T. patula* of different growth stages (phenological stages) were separately hydrodistilled for 4 hours using a Clevenger apparatus. The oil yield (%v/w) was estimated on a fresh weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulphate and kept in cool and dark place (at 4°C) until analysis.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) analysis: Gas chromatography analysis of the oils were performed by Agilent Technology (6890 model) gas chromatograph equipped with HP-5 fused silica column (30 m × 0.32 mm, 0.25 µm film thickness) and flame ionization detector (FID). Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 210°C and 230°C, respectively. The column oven temperature was programmed at 60°C to 220°C with an increase at the rate of 3°/min. The injection volume was taken as 0.2µL.

GC/MS analysis of the oils were carried out on a Perkin Elmer mass spectrometer (Claurus 500) coupled to a Perkin Elmer Claurus 500 gas chromatograph with a 60 m × 0.32 mm, 0.25 µm film thickness column (Rtx5). Helium was used as the carrier gas (flow rate 1 mL/min). The oven temperature program was ranged from 60° to 220° at the rate of 3°/min. Other conditions kept same as described under GC.

Identification of compounds: The identification of constituents was done on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes (C_9–C_{24}) under the same experimental conditions, co-injection with standards (Sigma Aldrich USA), MS library search (Wiley/NIST/Pfleger) and by comparing with the MS literature data (Adams, 2017).

3. RESULTS AND DISCUSSION

The yield of essential oils (%) from fresh aerial parts of *T. patula* was 0.06%, 0.06% and 0.08% (v/w) at pre flowering, full flowering and seed setting stages, respectively. The percent composition of the essential oils is presented in table-1. A total of 38 components were identified, representing 89.65-91.94% of the whole composition. All the oils of *T. patula* were dominated by monoterpene hydrocarbons (39.81-45.08%) followed by oxygenated monoterpenes (27.57-31.66%), sesquiterpene hydrocarbons (7.72-10.52%) and oxygenated sesquiterpenes (6.63-7.57%), while diterpenes (1.39-1.68%) and oxygenated diterpenes (0.93-1.55%) were found in the lesser amounts. In all the oils, Z-(β)-ocimene (12.76-20.29%) was found as the major constituent. Z-(β)-ocimene detected in maximum amount at full flowering stage (20.29%), while it was found comparatively lesser at pre flowering (12.76%) and seed setting stage (18.06%). α-Terpinolene, the second major compound was detected in appreciable amounts, ranged from 11.01-14.79%. The amount of α-terpinolene was maximum (14.79%) at pre flowering stage, while it decreased at full flowering (11.90%) and seed setting stage (11.01%). Similarly, the amount of piperitenone in the oil of *T. patula* was observed to be maximum (11.96%) at pre-flowering stage and found minimum (6.46%) when harvested at seed setting stage. It was observed that limonene and propanedinitrile dicyclohexyl were present in appreciable amount ranging from 6.62-7.57% and 5.17-5.85%, respectively. The other minor compounds such as (Z)-ocimenone (4.02-4.18%), (Z)-tagetone (3.36-3.45%), (E)-β-ocimene (1.85-2.49%), (E)-tagetone (1.52-2.44%) and borneol (0.72-2.44%) were also detected in the oils.

Table.1. Essential oil composition (%) of *Tagetes patula* during different phenological stages

S. No.	Compounds	$RI^{Lit.}$	Composition (%)			Identification methods
			Pre Flowering	Full Flowering	Seed Setting	
1	α-Pinene	932	0.38	0.37	0.68	RI, MS, Co-inj
2	Sabinene	969	0.07	0.07	0.08	RI, MS
3	β-Pinene	974	0.6	0.72	1.1	RI, MS
4	Myrcene	991	0.08	0.1	0.1	RI, MS
5	α-Phellandrene	1002	0.28	0.4	0.45	RI, MS
6	p-Cymene	1020	0.36	0.28	0.22	RI, MS
7	Limonene	1029	7.57	6.62	6.68	RI, MS, Co-inj
8	(Z)-β-Ocimene	1032	12.76	20.29	18.06	RI, MS, Co-inj
9	(E)-β-Ocimene	1044	1.85	2.49	2.11	RI, MS
10	Dihydrotagetone	1046	1.07	1.84	1.67	RI, MS
11	Artemisia ketone	1056	0.65	0.84	0.92	RI, MS
12	α-Terpinolene	1086	14.79	11.9	11.01	RI, MS
13	(E)-Tagetone	1139	1.52	2.42	2.16	RI, MS
14	(Z)-Tagetone	1148	3.45	3.39	3.36	RI, MS
15	Borneol	1155	0.72	1.09	2.44	RI, MS
16	Terpinen-4-ol	1174	0.82	0.12	0.11	RI, MS

17	P-Cymen-8-ol	1179	0.99	1.33	1.63	RI, MS
18	α-Terpineol	1186	0.68	0.72	0.66	RI, MS
19	(Z)-Ocimenone	1226	4.18	4.02	4.11	RI, MS
20	(E)-Ocimenone	1235	1.77	1.38	1.02	RI, MS
21	Carvone	1239	0.53	0.65	0.67	RI, MS
22	Piperitone	1249	2.64	2.63	2.44	RI, MS
23	Piperitenone	1340	11.96	7.72	6.46	RI, MS
24	trans-Caryophyllene	1417	3.46	5.67	6.09	RI, MS, Co-inj
25	β-Humulene	1436	0.62	0.49	0.79	RI, MS
26	(z)-β-Farnesene	1440	1.38	1.79	1.37	RI, MS
27	γ-Gurjunene	1475	0.67	0.42	0.43	RI, MS
28	Germacrene-D	1484	0.34	0.38	0.36	RI, MS
29	(E)-β-Ionone	1487	1.75	1.26	1.68	RI, MS
30	Bicyclogermacrene	1500	1.25	1.3	1.48	RI, MS
31	(Z)-Nerolidol	1531	0.48	0.43	0.72	RI, MS
32	(E)-Nerolidol	1561	0.63	0.28	0.82	RI, MS
33	Caryophyllene oxide	1582	0.34	0.57	0.08	RI, MS
34	Farnesyl acetate	1913	0.27	0.18	0.35	RI, MS
35	Neophytadiene	1749	1.39	1.68	1.51	RI, MS
36	Propanedinitrile, dicyclohexyl	1769	5.85	5.17	5.47	RI, MS
37	Phytol	1942	0.68	0.31	0.53	RI, MS
38	Isophytol	1946	0.82	0.62	1.02	RI, MS
	Monoterpene hydrocarbons		39.81	45.08	42.16	
	Oxygenated monoterpenes		31.66	27.57	27.66	
	Sesquiterpene hydrocarbons		7.72	10.05	10.52	
	Oxygenated sesquiterpenes		7.57	6.63	7.44	
	Diterpenes		1.39	1.68	1.51	
	Oxygenated diterpenes		1.50	0.93	1.55	
	Total identified (%)		**89.65**	**91.94**	**90.84**	
	Oil Yield (%)		**0.06**	**0.06**	**0.08**	

Note:*Retention Indices, comparison with RILit.; MS, comparison with mass spectra from libraries and literatures; Co-inj., comparison with retention time of standard compounds; $RI^{Lit.}$, retention indices (literature).

Main compounds reported in the previous studies on *Tagetes patula* essential oils from different countries are shown in table-2. A previous study on *T.patula* from Lucknow, India reported that the essential oils of capitula and shoot oils were characterized by higher amount of Z-(β)-ocimene i.e. 19.9% and 13.7%, respectively (Sagar, 2005). Similarly, report on inflorescence essential oil of *T. patula* from Merida Venezuela also indicated higher contribution of Z-(β)-ocimene (15.5%) while Z-ocimenone (8.7%) and piperitenone (8.4%) were also present in an appreciable quantity (Salehi, 2018). α-Terpinolene (14.59%) was found as the major compound in flower essential oil of *T. patula* from Gopeshwar, Uttarakhand (Lawrence, 1985). In another report from India, *T. patula* essential oil was found to be largely composed of limonene (24.5%), terpinolene (12.1%), Z-(β)-ocimene (10.4%), carvone (8.6%) and β-caryophyllene (7.4%) (Prakash, 2012). The composition of flower and leaf essential oil of *T. patula* from Bologna, Italy has been previously studied. The major components in flower essential oil were β-caryophyllene (18.2%), (E)-β-ocimene dihydrotagetone (15.0%), terpinolene (14.6%), and Z-(β)-ocimene (11.8%) and in leaf essential oil were terpinolene (22.6%), (E)-β-ocimene dihydrotagetone (16.5%), limonene (13.4%) and piperitone (7.2%) (Rondon, 2006). *T. patula* essential oil from Aragua state, Venezuela has been reported to contained β-terthienyl (43.1%), pentatriacontane (23.9%) and 2-hexyl-1-decanol (7.8%) as major components (Romagnoli, 2005). Piperitone (33.77%), trans-β-ocimene (14.83%) and terpinolene (13.87%) were reported as major constituents in the essential oil from aerial parts of *T. patula* grown in Venezuela (Rondon, 2006). GC/MS analysis of leaf and flower essential oil of *T. patula* from Gurugram Haryana, India showed that limonene (12.9%) and piperitone (11.9%) as predominant compounds in leaf essential oils and trans-sesquisabinene hydrate (12.5%), p-cymen-8-ol (11.0%) and piperitone (10.6%) in the flower essential oil (Chkhikvishvili, 2016). From Northwest Iran, hydrodistilled volatile seed oil composition of commonly growing ornamental *T. patula* was analyzed for its constituents by GC/MS. In other previous report, the principle constituents of the volatile oils were (E)-caryophyllene (44.6%) and caryophyllene oxide (14.8%) (Martinez, 2009). The present study revealed that harvesting of the crop at flowering stage is optimum time to get higher ocimene content. However, there are no major variations in the chemical composition of the oils observed.

Table.2. Main constituents (>5%) in the essential oils of *Tagetes patula* from different countries.

S. No.	Major Constituents	Plants parts	Location/ country	References
1	Limonene (24.5%); Terpinolene (12.1%); (Z)-β-Ocimene (10.4%); Carvone (8.6%); β-Caryophyllene (7.4%)	Flowers (Inflorescence)	Lucknow, India	Prakash, 2012
2	(Z)-β-Ocimene (19.9%); β-Caryophyllene (15.1%); (Z)-Tagetenone (12.4%); (E)-Tagetenone (10.4%)	Capitula	Lucknow, India	Sagar, 2005
3	Terpinolene(16.2%); (Z)-Tagetone (13.0%); Piperitenone (12.5%); Piperitone (10.2%)	Leaves	Lucknow, India	Sagar, 2005
4	(Z)-β-Ocimene (13.7%); Terpinolene (12.0%); β-Caryophyllene (10.5%); Limonene (6.8%)	Shoots	Lucknow, India	Sagar, 2005
5	Piperitone (24.74%); Piperitenone (22.93%); Terpinolene (7.8%)	Flowers	Madhya Pradesh, India	Mares, 2002
6	α-Terthienyl (43.1%) Pentatriacontane (23.9%); 2-hexyl-1-Decanol (7.8%)	Flowers	Aragua State, Venezuela	Romagnoli, 2005
7	β-Caryophyllene (18.2%); (E)-β-Ocimene (15.0%); Terpinolene (14.6%); (Z)-β-Ocimene (11.8%)	Flowers	Bologna, Italy	Rondon, 2006
8	Terpinolene (22.6%); (E)-β-Ocimene (16.5%); Limonene (13.4%); Piperitone (7.2%)	Leaves	Bologna, Italy	Rondon, 2006
9	(E)-Caryophyllene (44.6%); Caryophyllene oxide (14.8%)	Seeds	Northwest Iran	Martinez, 2009
10	Piperitone (33.77%); trans-β-Ocimene (14.83%); Terpinolene (13.87%); β-Caryophyllene (9.56%); Limonene (7.78%)	Aerial parts	Venezuela	Garg, 1999
11	β-Ocimene (22.11%); α- Terpinolene (14.59%); trans-Caryophyllene (12.69%); Z-Ocimenone (9.14%); Limonene (7.72%)	Flowers	Chamoli, India	Lawrence, 1985
12	β-Caryophyllene (18.6%); δ-Elemene (16.9%); (Z)-β-Ocimene (11.8%); Terpinolene (6.9%); (Z)-Ocimenone (6.4%)	Capitula	Lucknow, India	Bhattacharyya, 2010
13	Limonene (12.9%); Piperitone (11.9%); Dihydrotagetone (8.1%)	Leaves	Haryana, India	Chkhikvishvili, 2016
14	p-Cymen-8-ol (11.0%); Piperitone (10.6%); Piperitenone (8.1%); Limonene (6.2%); Dihydrotagetone (6.2%)	Flowers	Haryana, India	Chkhikvishvili, 2016
15	Limonene (13.6%); (Z)-β-Ocimene (8.3%); β-Caryophyllene (8.0%); Piperitone (6.1%); p-Cymen-8-ol (5.4%)	Aerial parts	Haryana, India	Chkhikvishvili, 2016
16	Terpinolene (20.9%); Piperitenone (14.0%); E-Ocimenone (9.5%); Limonene (8.4%); Z-β-Ocimene (8.1%); Piperitone (7.8%)	Leaves	Venezuela	Salehi, 2018
17	β-Caryophyllene (23.7%); Terpinolene (15.6%); Z-β-Ocimene (15.5%); Z-Ocimenone (8.7%); Piperitenone (8.4%)	Inflorescence	Venezuela	Salehi, 2018

18	E-β-Ocimene (31.0-43.3%); Dihydrotagetone (3.0-22.0%); Z-Tagetone (4.8-10.7%); (Z)-Tagetenone (4.8-10.3%)	Aerial parts	Egypt	Soule, 1996
19	E-β-Ocimene (40.4-69.8%); (Z)-Tagetenone (6.9-21.6%); Dihydrotagetone (5.3-17.7%); Z- Tagetone (1.3-12.4%)	Aerial parts	South Africa	Soule, 1996
20	Dihydrotagetone (11.9-48.1%); E-β-Ocimene (16.6-35.3%); (Z)-Tagetenone (8.1-32.5%); (E)-Tagetenone (8.1-32.5%); Z-Tagetone (18.6-27.2%)	Aerial parts	India	Soule, 1996

4. CONCLUSION

The current study concluded that the composition of the essential oils of *Tagetes patula* at pre flowering, full flowering and seed setting stages were qualitatively similar but differed quantitatively during different growth stages, however, there are no major variations in the chemical composition of the oils observed. Harvesting of the crop at flowering stage is optimum time to get higher ocimene content. Our findings will be very useful for the aromatic crop cultivators for promoting *T. patula* as an essential oil crop for cultivation in Uttarakhand region of India.

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