

Original Research Article

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## Variations in Quantitative and Qualitative Composition of Essential Oils from Leaves and Flowers of French Marigold (*Tagetes patula*)

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### ABSTRACT

#### Keywords

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Essential oil content in two different plant parts (leaves and flowers) from marigold belonging to the species *Tagetes patula* were evaluated by hydro distillation and examined by gas chromatography (GC) and GC-mass spectrometry (GC-MS) at the flowering stage. The leaves parts showed richer in oil contents as compared to flowers whereas, both leaves and flowers contains very similar qualitative oil composition (compounds) differing only in the relative percentages and. The oil of leaves contained higher amounts of limonene, (Z)- $\beta$ -ocimene, (E)- $\beta$ -ocimene, terpinolene, dihydrotageton, and piperitone. Whereas, the oil of the flowers showed higher concentration of (Z)-tagetone,  $\beta$ -caryophyllene, caryophyllene oxide, piperitone oxide and germacrene-D.

### Introduction

Marigold (*Tagetes spp.*) belongs to the asteraceae family and is native of Central and South America, especially Mexico. Marigold is a well-known ornamental plant widespread all over the world because of ease in cultivation, longer blooming period and varied flowers colours, size, shape etc. There are thirty-six species accepted under genus of *Tagetes*, most widely known being *T. patula* (Neher *et al.*, 1986). *T. patula* are mostly utilized as an ornamental crop world-wide. It also provides raw material for commercial production of several kinds of useful compounds. In India, marigolds occupy an area of 66.13 Thousand hectors with

production of 603.18 Thousand MT loose flower (NHB database, 2015-16). It is grown as a major commercial loose flower crop and being used in religious and social ceremonies. Beside plant in native is traditionally utilized for human use as beverages, condiments and in folk medicines. At present crop is exploited for extraction of carotenoids pigments, which is used for food's colorants, nutritional supplements and in cosmetic industry and proving to be commercial resources of essential oils (Anonymous, 1976). Essential oils are known for their antibacterial and insecticidal properties (Piccaglia *et al.*, 1997). The *Tagete* soil has been mainly used for the compounding of high-grade perfumes. Oil content and the quantitative ratios of the oil

components within the species depends on several factors such as crop growing location, plant development stage, different parts of the plant used (Chalchat *et al.*, 1995). The aim of this study was to compare the content of essential oils extracted from leaves and flower parts of *T. patula* at flowering stage and also variation in composition of essential oil.

## Materials and Methods

The field experiment was carried out at the research farm of Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi, at 77° 12' E longitude 28°40'N latitude and at an altitude of 228.16m above the mean sea level. The experimental design was a randomized block with three replications. The materials utilized for the present study consisted of genotypes of French marigold (*Tagetes patula*). The seeds of were sown in the pro trays with soilless mixtures comprising of cocopeat: perlite: vermiculite in ratio of 3:1:1 and planted in poly house during rainy season, i.e. June during 2014. Irrigation was given daily with rose can so as to maintain proper moisture. Drenching was done with 19:19:19 @ 0.5% (5g/l) at 15 days after sowing. Transplanting of these seedlings was carried when plants reached four to five leaf stage after sowing. The seedlings were then transplanted at spacing of 45×45 cm and the standard cultural practices like hoeing, irrigation, weeding, staking and fertilizer application were followed as per the requirement of the crop.

## Isolation of essential oil

The laboratory work was carried out at ICAR-National Bureau of Plant Genetic Resources, New Delhi. Fresh leaves and flowers of *T. patula* were collected separately early morning around 5 to 7 am at full growth stage before the initiation of flower buds. Harvested

fresh leaves and flowers weighing 1kilogram were cut into small pieces and immediately subjected to hydro distillation for 3 hours using a Clevenger-type of apparatus. The extracted oils from both the parts leaves and flowers were dried over anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>) and kept in brown glass bottles at refrigerated conditions prior to GC-FID and GC/MS analysis.

## Gas chromatography and Gas chromatography/mass spectrometry

Capillary gas chromatography (GC) was carried out using an Agilent gas chromatograph 7890 A, equipped with a flame ionization detector (FID) and a non-polar HP-5MS capillary column made up of 5% phenyl methyl silicone, 95% dimethylpolysiloxane (30x 0.25 mm, 0.25 µm film thickness). Helium was used as the carrier gas at the flow rate of 1 mL/minute. The oven temperature was programmed from 60°C to 240°C at a rate of 3°C /minute with initial hold of 10 minutes at 60°C and final hold of 10 minutes at 240°C. The injector and detector temperatures were maintained at 220°C and 250°C, respectively. The sample (0.1µL) was injected neat in a split ratio (1:40) at 220°C area percentage reports obtained by GC-FID were used for quantification purposes. GC/MS analysis were carried out on an Agilent GC/MS equipped with a MSD detector 5975C and a HP-5MS capillary column (30 m length/0.25mm internal diameter: 0.25 µm film coating) under similar chromatographic conditions as mentioned above. Helium was used as the carrier gas. The mass unit conditions were ion source 250°C, ionization energy 70 eV. The acquisition mass range used was 40-400 *m/z*. The volatile constituents were identified by comparing the retention indices determined with reference to a homologous series of n-alkanes under identical experimental condition, co-injection

with that of authentic compounds (sigma) and matching mass spectral data of the peaks with mass spectra with those stored in NIST/Wiley and Adams mass spectral libraries and literature values (Adams 2001; Davies 1990). The relative amounts of individual components were calculated based on GC peak area (FID response) without using a correction factor.

### Results and Discussion

The essential oils yield of leaves and flower of *Tagetes patula* are presented in Table 1. Essential oil content in leaves ranged from (0.08-0.15 %) v/w and flowers ranged from (0.02-0.09 %) v/w respectively on fresh weight basis. On average, leaves were richer in oil contents than flowers.

The results obtained in CG and GC/MS analysis of the oils are shown in Table 2 and the components are listed in order of the retention index of the constituents. Essential oils of both leaves and flowers contained more or less the same compounds differing only in the relative percentages. Overall thirty seven compounds were identified and were characterized by large amounts of monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpenes hydrocarbons and oxygenated sesquiterpenes etc. The majority of them were monoterpenes with high percentage being oxygenated compounds.

*Tagetes patula* leaves contained higher amounts of terpinolene (13.72-21.79) %, limonene (4.89-8.83) %, (z)-β-ocimene (4.09-19.53), piperitone (2.46-19.46%) and

dihydrotagetone (0.23-3.08%). Whereas the oils of flowers had higher concentration of β-Caryophyllene (3.92-42.76%), germacrene-D (1.48-6.72%), (Z)-Tagetone (1.29-4.38), caryophyllene oxide (0.68-24.3%), and piperitone oxide (0.11-1.23). The result are in close confirmation by Sz Szarka *et al.*, (2006), oils from flower heads were rich in β-caryophyllene (53.5%) and the leaves contained terpinolene in high concentration (21.1%). Besides, Machado *et al.*, (1994) had identified β-caryophyllene, limonene, piperitone and piperitinone as main constituents in *T. patula* oil. The essential oil of the leaves, flowers and stems of *Tagete patula* was reported to contain ocimene, limonene, linalool, linayl acetate and tagetone (Dhingra and Dhingra, 1956). Recently, Prakash *et al.*, (2012) experiment conducted on the chemical compositions from the capitula of *Tagetes patula* result in identification of (Z) -α-ocimene, (E) -β-ocimene, terpinolene, (Z)-ocimenone and (E)-ocimenone.

A comparison of the compositions of the two oils showed that the oil of leaves contained higher amounts of limonene, (Z)-β-ocimene, (E)-β-ocimene, terpinolene, dihydrotagetone, and piperitone (Figure 1). Whereas, the oil of the flowers showed higher concentration of (Z)-tagetone, β-Caryophyllene, caryophyllene oxide, piperitone oxide and germacrene-D, which resemblance the composition reported by Krishna *et al.*, (2004) as leaves had high content of limonene, terpinolene and piperitone, whereas the oil of flowers had high concentration of (Z)-β-ocimene, linalool, dihydrotagetones, piperitenone, β-caryophyllene and piperitone oxide.

**Table.1** Essential oil yield<sup>a</sup> (%) of leaves and flower of *Tagetes patula*

	Fresh weight basis (Oil %)	
	Leaves	Flowers
<i>Tagetes patula</i>	0.08-0.15	0.02-0.09

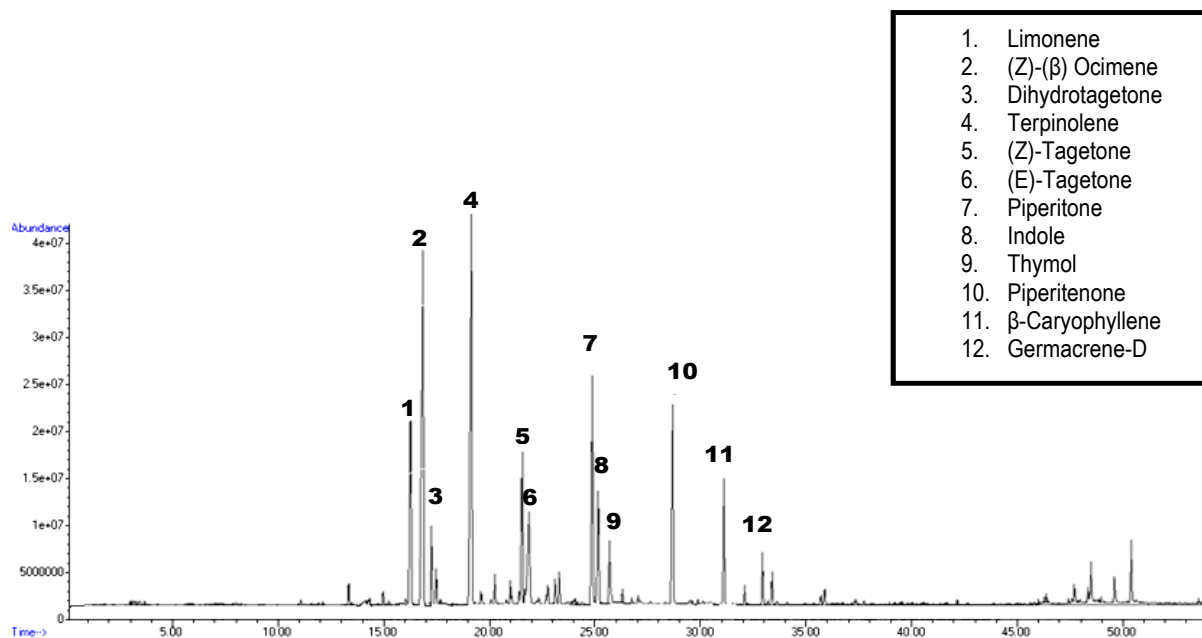
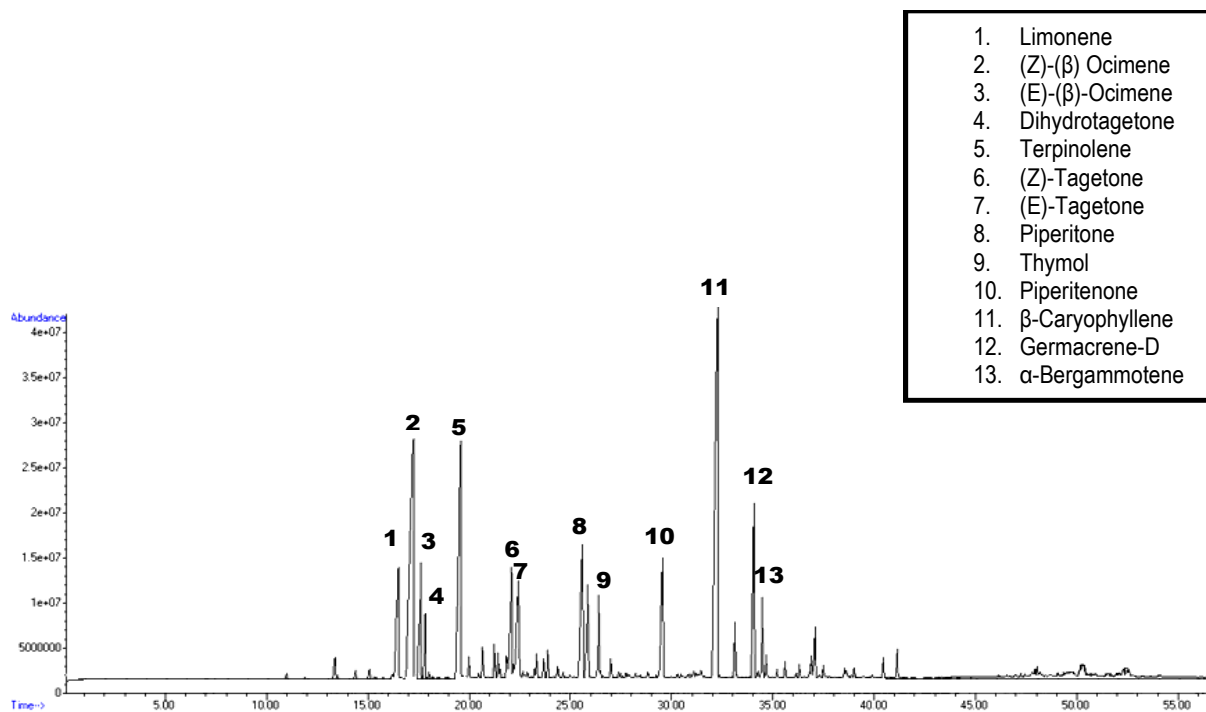
Note: <sup>a</sup> Expressed as range (%) of relative area percentage for individual compound across the *Tagetes patula*

**Table.2** Chemical composition<sup>a</sup> (%) of leaves and flower of *Tagetes patula*

SL. No	RT	Component	Flowers	Leaves
1.	10.965	$\alpha$ -Pinene	0.15-0.36	0.24-0.37
2.	13.336	Sabinene	0.22-0.73	0.28-0.81
3.	14.366	Myrcene	0.17-0.65	0.21-0.48
4.	15.033	$\alpha$ -Phellandrene	0.12-0.57	0.33-0.73
5.	16.476	Limonene	0.9-7.76	4.89-8.83
6.	16.98	(Z)-( $\beta$ ) Ocimene	3.55-16.82	4.09-19.53
7.	17.43	(E)-( $\beta$ )-Ocimene	1.08-4.65	0.79-10.56
8.	17.585	Dihydrotagetone	0.44-2.85	0.23-3.08
9.	19.51	Terpinolene	0.77-25.5	13.72-21.79
10.	19.935	Linalool	0.19-0.61	0.24-0.82
11.	20.413	P-mentha-1,3,8-triene	0.11-0.73	0.14-0.78
12.	21.171	Allo-ocimene	0.21-0.66	0.10-0.46
13.	21.415	(Z)-Ocimenoxide	0.33-3.79	0.12-4.43
14.	21.806	(E)-Ocimenoxide	0.41-2.03	0.37-2.88
15.	21.946	(Z)-Tagetone	1.29-4.38	0.70-5.88
16.	21.959	(E)-Tagetone	0.20-6.20	0.52-7.74
17.	22.791	Borneol	0.13-0.44	0.08-0.56
18.	23.228	Terpin-4-ol	0.32-0.84	0.34-0.89
19.	23.599	P-cymen-8-ol	0.42-1.37	0.33-1.88
20.	25.381	(Z)-ocimenone	2.81-7.27	0.2-9.42
21.	25.812	(E)-ocimenone	2.53-6.38	2.21-8.08
22.	26.5	Piperitone	0.44-14.9	2.46-19.46
23.	26.934	Isopiperitenone	0.22-0.95	0.13-1.31
24.	27.396	Isobornylacetate	0.15-0.21	0.08-0.23
25.	27.795	Indole	0.72-0.65	0.18-1.22
26.	27.849	Thymol	0.12-0.37	0.12-0.62
27.	29.63	Piperitenone	3.04-14.7	6.25-18.3
28.	30.26	Piperitenone oxide	0.11-1.23	0.08-0.54
29.	32.033	$\beta$ -Caryophyllene	3.92-42.7	2.23-6.65
30.	33.034	$\beta$ -Humulene	0.21-0.88	0.18-0.25
31.	33.051	(E)- $\beta$ -farnesene	0.11-2.97	0.19-0.85
32.	33.95	Germacrene-D	1.48-6.72	0.98-3.57
33.	34.181	$\alpha$ -Bergamotene	0.09-0.29	0.18-0.25
34.	34.424	Bicyclogermacrene	0.92-3.12	0.66-2.03
35.	36.278	(E)-nerolidol	0.21-0.69	0.2-0.74
36.	36.831	Spathulenol	0.33-4.29	0.03-1.42
37.	37.029	Caryophyllene oxide	0.68-24.3	0.18-0.69
		Monoterpenes hydrocarbons	6.52-32.5	11.05-32.57
		Oxygenated monoterpenes	14.51-95.0	28.38-109.29
		Sesquiterpenes hydrocarbons	6.94-57.4	4.62-14.34
		Oxygenated sesquiterpenes	1.01-28.59	0.21-2.11

Note: <sup>a</sup>Expressed as range (%) of relative area percentage for individual compound across the *Tagetes patula*: RT, retention time on HP-5 MS capillary column

Fig.1 Total-ion chromatograms of *Tagetes patula* essential oil from (a) flower (b) leaves



The results evidenced that essential oil yield percentage differ among the parts (leaves and

flowers) used. Oil content in leaves were higher than in flowers. The constituents of

essential oil of leaves contained higher amounts of limonene, (Z)- $\beta$ -ocimene, (E)- $\beta$ -ocimene, terpinolene, dihydrotageton, and piperitone. Whereas, the essential oil of the flowers showed higher concentration of (Z)-tagetone,  $\beta$ -caryophyllene, caryophyllene oxide, piperitone oxide and germacrene-D. Besides ornamental value *T. patula* has potential source for commercial exploitation of various compounds. Therefore more attention is required to thoroughly screen this species for its phytoconstituents.

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