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Screening of secondary phytometabolite of hydro-distilled essential oil from fresh flower and leaves of African marigold (*Tagetes erecta* L.)

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ABSTRACT

African marigold (Tagetes erecta L.) is known for its high therapeutic value besides its ornamental uses. Herbs are considered to be the backbone of traditional medicines from ancient days and about 80% of the world's population depends on traditional medicines for curing various ailments. With this background, the present investigation was conducted on screening of secondary phytometabolites of hydro-distilled essential oil from fresh flowers and leaves of African marigold (Tagetes erecta L.) with the help of some phytochemical tests FTIR and DART mass spectrometry. Six phytochemicals were identified after preliminary phytochemical tests in all the samples. FTIR results revealed presence of major functional groups viz., phenols alkanes, aromatic, alchohols etc. The Direct Analysis in Real Time (DART) analysis elucidated the presence of verbenone, piperitenone, ocimenone, galic acid, camphor, terpinolene, á-pinene, thujene, sabinene, α-terpinolene and piperitone, etc., as the main components of the alkaloids, terpenes and phenolic compounds recorded by FTIR analysis in the marigold oil.

Keywords : Tagetes erecta, Marigold oil, Secondary phytometabolite, Hydro-distillation, FTIR and DART- MS.

INTRODUCTION

Marigold (*Tagetes sp.)* belonging to family Asteraceae, is a medicinal and ornamental plant with high therapeutic value (Dixit *et al.,* 2013), essential oil (Doman *et al.,* 2000) and has proved to be an effective nematicide (Gutierrez *et al.,* 2006), cosmetic (Farjana *et al*., 2009), food additive (Nandita *et al*., 2012), for pest control (Farjana, 2009) etc. The essential oil of genus *Tagetes* are effective antibiotic, antimicrobial, antiparasitic, antiseptic, antispasmodic (Chowdhury *et al.,* 2009), etc. Additionally, the decoctions of the leaves of *T. erecta* and *T. patula* have been traditionally used as antimalarial and as febrifuge (Rasoanaivo *et al*., 1992). Preliminary phytochemical analysis with *Tagetes erecta* proved that the plant is highly rich in alkaloids, phenolic compounds, flavanoids, salicylic acid, terpenes etc (Devika *et al*., 2012) although most of the reports regarding medicinal values of marigold are from wild marigold *T. minuta. (*Hadjiakhoondi *et al.,* 2005 and Chamorro *et al.,* 2008).

Hydro- distillation or steam distillation is the most widely utilized physical method for extracting essential oils from botanicals (Whish *et al.,* 1996) to identify their chemical constituents and elucidate their structural compounds (Hussain *et al.,* 2007) further. Fourier Transform Infrared spectroscopy (FTIR) is a high-resolution analytical technique which along with Direct Analysis in Real Time (DART) coupled to the AccuTOF atmospheric pressure ionization mass spectrometer permits high resolution, exact mass measurements of gases, liquids and solids (Cody *et al.,* 2005) which help in identification of phytometabolite constituents of plant. African marigold (*Tagetes erecta* L.) has gained importance since it is cultivated on a large scale as an ornamental and is sold even as a loose flower. Since *Tagetes* species *per se* are reported as a rich source of bio-colour, pigments and bioactive molecules which may be exploited in the food and pharmaceutical industry, post harvest processing of African marigold for its oil, extracted from its flower as well as its plant parts may enhance value of the crop multifold. This could evolve methods for waste management of the flowers and leaves which are generally discarded after their use in temple, marriage decorations etc. However, limited information is available regarding oil extraction from marigold (*Tagetes ecreta* L.) and its phytochemical screening. Therefore, the present study was planned with the objective to profile the secondary phytometabolites in hydro-

distilled essential oil from fresh flowers and leaves of African marigold *Tagetes erecta L.* by Fourier Transform Infrared Spectroscopy and Direct Analysis in Real Time mass spectrometry.

MATERIALS AND METHODS

Collection of sample

Fresh flowers and leaves were collected at full bloom stage in December, 2014 from the Horticulture Research Farm of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Lucknow, U.P.

Hydro-distillation (extraction of essential oil)

A known quantity of both Fresh flowers and leaves of marigold (*Tagetes erecta* L.) were subjected to hydro-distillation for 4 hours using a Clevenger apparatus. The oil extracted was dried over anhydrous sodium sulphate and stored in small sealed tubes at low temperature for FTIR and DART-MS analysis.

Phytochemical tests

The samples were tested for several phytochemicals viz. Terpenoids (Chloroform test) Protein (Biuret test) Steroids, flavonoids (Alkaline reagent test), Alkaloids, Quinones, gylcosides phenols, triterpinoids, Coumarins, carbohydrates, tannin and for saponins (Froth test) using standard procedures as quoted by Hossein and Pezhhanfar (2015) to identify the phytochemical.

Qualitative screening of Hydro-distilled extracts

Screening by FTIR analysis

FTIR can be utilized to identify some of the functional groups present in a solid, liquid or gaseous sample. In the present study, the functional groups of the oil sample are analyzed by using Fourier transform infrared spectroscopy (NicoletTM 6700, Thermo scientific: USA), with a scan range from 400 to 4000 cm-1. The Infrared spectra were reported as % transmittance. The functional groups present in the essential oil were determined by comparing the vibration frequencies in wave numbers of the sample spectrograph from library of the system and previous literature related to FTIR studies.

Direct Analysis in Real Time (DART)

The fresh flower oil sample was subjected in front of DART-MS coupled with JEOL-AccuTOF LMS-T100LC Mass spectrometer. Dry helium gas at 4 LPM flow rate was used for ionization at 350^0C. Data acquisition was from m/z 50.0 to 200.0. The orifice was set at 28 V and spectra were collected. *In silico* identification of oil components by comparison of their *m/z* values with those of a computer library and published literature was done.

RESULTS AND DISCUSSION

Preliminary screening of phytochemical .has revealed the presence of certain metabolites Table 1. The qualitative analysis carried out revealed the presence of the phytonutrients namely terpenoids, alkaloids, flavonoids, quinones carbohydrates, tannins and coumarins,. The identification of the above compounds supports the use of these oils in traditional medicine as these compounds have valuable antifungal, antibacterial and anti-inflammatory properties (Hassanshshian *et al.*, 2014; Gilani *et al.*, 2005).

Carbohydrate was identified by the formation of red colour in both the oil samples of *Tagetes* Yellow colour development after the addition of sodium hydroxide indicated the presence of flavonoid in both samples. The bioactive compound, alkaloid was identified promptly in both the samples which indicated that *Tagetes erecta* can be used to cure ear inflammation, anthelminthic and carminative. (Alan and Miller, 1996). Presence of green colour in sample after adding 10% ferric chloride indicates the presence of phenols. Coumarins test showed a positive result in both samples of the plants where Yellow colour developed after the addition of 10% sodium hydroxide to the each sample. Negative results were obtained for various other phytochemical tests such as protein steroids,saponin, glycoside,triterpinoids in both samples.

Identification of the functional groups present in sample using FT-IR Spectroscopy

The FTIR spectroscopic analysis is made based on percentage of transmittance and wave numbers. The bonds and the wave numbers (cm^{-1}) of prominent peaks of the major constituents obtained from spectra are described in Table 2. The analysis of essential oil of fresh flowers and leaves from African marigold has showed the existence of various secondary phytometabolites (Fig, 1 A &

B). The essential oil from fresh flowers shows major peaks primarily at 3361.7 cm^{-1}, 2958.1 cm^{-1}, 2924.0 cm^{-1}, 2863.2 cm^{-1}, 2734.0 cm^{-1}, 2674.6 cm^{-1}, 1629.6 cm^{-1}, 1461.7 cm^{-1}, 1379.3 cm^{-1}, 1342.9 cm^{-1}, 1296.4 cm^{-1}, 1247.0 cm^{-1}, 1135.6 cm^{-1}, 1060.9 cm^{-1}, 885.2 cm^{-1} and 724.2cm^{-1} and the essential oil from fresh leaves shows major peaks primarily at 3375.6cm^{-1} 2958.3 cm^{-1}, 2924.6 cm^{-1}, 2865.1 cm^{-1}, 1670.5 cm^{-1}, 1625.0 cm^{-1}, 1459.9cm^{-1}, 1377.8cm^{-1}, 1295.4cm^{-1}, 1219.8cm^{-1}, 1140.0cm^{-1}, 1062.1cm^{-1} ,864.3cm^{-1} and 723.1 cm^{-1}. The fresh flowers oil showed the maximum number of peaks as compared to the fresh leaves oil. The fundamental components in a sample may be identified depending on the fingerprint characters of the peaks positions, shapes and intensities (*Chen et al*., 2001)**.** Thus, in the fresh flowers oil, the peak at 3361.7 cm^{-1} was assigned to the N-H stretching vibration while that in the range of 2990-2650 cm^{-1} is mainly attributed to the stretching vibration of C-H. In addition, the peak at 1629.6 cm^{-1} is assigned to the N-H bend vibration which indicates that some amide compounds existed in the hydro-distilled essential oil of fresh flower from African marigold.. The alkane peaks at 1461.7 and 1379.3 cm^{-1} and the peak situated at 1342.9 cm^{-1} assigned to N-O Symmetric stretching. The peak at 1296.4 and 1247.0 cm^{-1} are due to C-O stretching vibrations. The peak at 1135.6 and 1060.9 cm^{-1} are due to C-N stretching vibrations. The aromatics were present at the range of 890-750 cm^{-1} (Dutta et al.,2014**)** The peak at 3375.6 cm^{-1} in fresh leaves oil was assigned to the N-H Stretching indicates the presence of amide functional group. The peak at 2958.3 cm^{-1}, 2924.6 cm^{-1} and 2865.1 cm^{-1} was due to C-H Stretching which clearly indicates the presence of alkanes in the leaf oil. The peak at 1140.0cm^{-1} and 1062.1 cm^{-1} was indicates the existence of aliphatic amines. In the samples studied, the aromatics were present at the 864.3 cm^{-1} and 723.1 cm^{-1}. Similarly samples studied have shown a major absorption in the wave length range of polyphenols (1700-600 cm^{-1}) thus indicating their potential nutraceutical value (Gorinstein *et al.,* 2010).

Screening of phytometabolites by DART-MS

The phytometabolite components present in hydro-distilled essential oil of fresh flowers from African marigold was subjected to further analysis by DART-MS and correlate the results obtained by FTIR spectroscopy. A representative DART-MS spectrogram and various constituent phytometabolites of hydro-distilled essential oil from fresh flowers and leaves of African marigold is given in Table 2 and 3.& Figure 2 and 3 respectively. The constituents were identified by matching their mass spectra with those recorded in literature. In fresh flower the peak at *m/z* 136 best match with α-pinene, terpinolene, *thujene*, sabinene, β-ocimene, limonene (R.I % 4).The peak at *m/z* 149 could be due to piperitenone, ocimenone, (R.I % 10). However, they have the same molecular formula; a distinction could not be made. The peak at *m/z* 153 could be due to piperitone or camphor (R.I % 13), while in fresh leaves oil,the peak with major relative intensity was 391 which could be due to Flavoxate having (R.I % 91), the peak at *m/z* 318 was best match with Quercetagetin with relative intensity 7%. The peak at 274 could be due to 2-Acetoxyfuranoelemene (R.I % 12), The results correspond with another study where the plant.of *T. erecta* have been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallate. (Farjana, 2009). Since some of the terpenes constituents in the sample have corresponding molecular weight, it was not possible to distinguish those using DART-MS alone. Presence of certain naturally occurring monoterpenoids and sesquiterpenoids in the sample was also observed in contrast to study of (Bashir et al., 2008). Besides this, the peak of other terpenes was observed at *m/z*, 151, 165, 167 and 169 corresponding to piperonal, verbenone, thymol, carvone, carvacrol ,furomyrcenol, trans dihydrocarvone epoxide, a- campholenic acid and galic acid respectively. 8 hydroxylinalool, cis or trans linalool xide and transpinocarvyl formate showed peak at *m/z* 170 and 180 respectively which corresponded to terpenes reported at similar *m/z* values known. (http://massfinder.com/wiki/Terpenoids_Library_List). The peak at *m/z* 183, 185 and 196 best match with citronellyl formate, (R.I % 7) n-nonyl acetate (R.I % 0.5) and lanalyl

Table 1: Preliminary phytochemical screening of hydro-distilled essential oil from fresh flowers of African marigold (*Tagetes erecta* L.)

Phytochemical tests	Fresh flower		Fresh leaf	
Terpinoids	+	Red brown colour	+	Red brown colour
Protien	-	No change	-	No change
Steroids	-	No change	-	No change
Flavonoid	+	Yellow colour	+	Yellow colour
Alkaloid	+	Green colour	+	Green colour
Quinones	+	Red colour	-	No change
Glycosides	-	No change	-	No change
Phenols	+	Green colour	-	No change
Triterpinoids	-	No change	-	No change
Coumanins	+	Yellow colour	+	Yellow colour
Carbohydrates	+	Red colour	+	Red colour
Tannin	+	Blue colour	-	No change
Saponin	-	No change	-	No change

***(+) = Present and (-) = Absent**

Table 2 : FTIR analysis of hydro-distilled essential oil from fresh flowers and leaves of African marigold (*Tagetes erecta* L.)

S. No.	Frequency (cm^{-1})	Fresh flower	Fresh leave	Bond	Functional group
1.	3390-3300	3361.7	3375.6	N-H Stretch	1*, 2* amines, amides
2.	2990-2950	2958.1	2958.3	C-H Stretch	alkanes
3.	2950-2900	2924.0	2924.6	C-H Stretch	alkanes
4.	2880-2850	2863.2	2865.1	C-H Stretch	alkanes
5.	2750-2700	2734.0	-	H-C=O: C-H Stretch	aldehydes
6.	2690-2650	2674.6	-	H-C=O: C-H Stretch	aldehydes
7.	1690-1650	-	1670.5	C=O Stretch	Carbonyls (general)
8.	1650-1600	1629.6	1625.0	N-H Bend	1* amines
9.	1490-1450	1461.7	1459.9	C-H bend	alkanes
10.	1390-1350	1379.3	1377.8	C-H rock	alkanes
11.	1350-1300	1342.9	-	N-O Symmetric stretch	Nitro compounds
12.	1300-1250	1296.4	1295.4	C-O Stretch	carboxylic acid
13.	1250-1200	1247.0	1219.8	C-O stretch	carboxylic acid
14.	1150-1100	1135.6	1140.0	C-N Stretch	Aliphatic amines
15.	1090-1050	1060.9	1062.1	C-N Stretch	Aliphatic amines
16.	890-850	885.2	864.3	C-H “oop”	aromatic
17.	730-700	724.2	723.1	C-H rock	alkanes

Table-3. Exact Mass data from the DART –MS of hydro-distilled essential oil from fresh flowers and leaves of African marigold (*Tagetes erecta* L.)

S. No.	Molecular weight	M/Z from fresh flowers	M/Z from fresh leaves	Molecular formula	R.I. (%)	Remarks
1.	97	97.08	-	$C_6H_{10}O$	1.5%	(E)-2-Hexenal
2.	109	109.08	-	C_7H8_O	12%	o-Cresol
3.	114	114.11	114.11	$C_7H_{14}O$	4%	n-Heptanal, 2Heptanone
4.	123	123.14	-	$C_8H_{10}O$	4%	p-Methylanisol, 4-Ethylphenol
5.	135	135.14	136.04	$C_{10}H_{16}$	4%	Terpinolene, *Thujene*, sabinene, á-terpinolene, á pinene, â-ocimene,limonene
6.	139	139.18	-	$C_{10}H_{18}$	0.5%	Tran-pinane, Cis pinane
7.	149	149.12	-	$C_{10}H_{14}O$	10%	Piperitenone, Ocimenone, umbellulone
8.	151	151.13	151.14	$C_{10}H_{14}0$	90%	Piperonal, verbenone, thymol, carvone, carvacrol
9.	153	153.15	-	$C_{10}H_{16}O$	13%	Piperitone, Camphor
10.	165	165.12	-	$C_{10}H_{14}O_2$	0.5%	Furomyrcenol
11.	167	167.12	167.13	$C_{10}H_{16}O_2$	10%	Trans Dihydrocarvone epoxide, a-Campholenic acid
12.	169	169.14	-	$C_7H_6O_5$	5%	Galic acid
13.	170	170.14	-	$C_{10}H_{18}O_2$	1%	8 Hydroxylinalool, cis or TransLinalool oxide
14.	180	180.13	-	$C_{11}H_{16}O_2$	1%	TransPinocarvyl formate
15.	183	183.12	-	$C_{13}H_{28}$	7%	Citronellyl formate, d- undecanoloide
16.	185	185.15	-	$C_{10}H_{14}0$	0.5%	n-nonyl acetate
17.	196	196.20	-	$C_{12}H_{20}O_2$	1%	Linalyl acetate, Geranyl acetate
18.	207	-	207.18	$C_{13}H_{20}O_2$	0.6	Trans and Cis Carvyl propionate
19.	218	-	218.24	$C_{13}H_{14}O_3$	2	Hydroxytremetone
20.	246	-	246.28	$C_{17}H_{26}O$	2	Avocadynofuran, Amberone
21.	262	-	262.26	$C_{17}H_{26}O_2$	1.5	4bAcetoxygymnomitr3(15)-ene
22.	274	-	274.31	$C_{17}H_{22}O_3$	12	2-Acetoxyfuranoelemene
23.	279	-	279.27	$C_{18}H_{30}O_2$	11	6b-Acetoxyeudesm-4(15)-en-7bol
24.	296	-	296.31	$C_{19}H_{36}O_2$	3	n-Heneicosane (C21), Methyl oleate
25.	297	-	297.31	$C_{19}H_{38}O_2$	3	Methyl stearate
26.	318	-	318.35	$C_{15}H_{10}$	7	Quercetagetin
27.	319	-	319.34	$C_{19}H_{28}O_4$	0.3	4b,5b-Diacetoxygymnomitr-3(15)-ene
28.	346	-	346.40	$C_{22}H_{34}O_3$	0.6	11-b-Hydroxykauren-15-a-yl- acetate
29.	362	-	362.38	$C_{15}H_{22}O_{10}$	1.5	Catalpol
30.	391	-	391.34	$C_{24}H_{25}NO_4$	98	Flavoxate
31.	392	-	392.35	$C_{25}H_{28}O_4$	25	Glabrol

A **B**

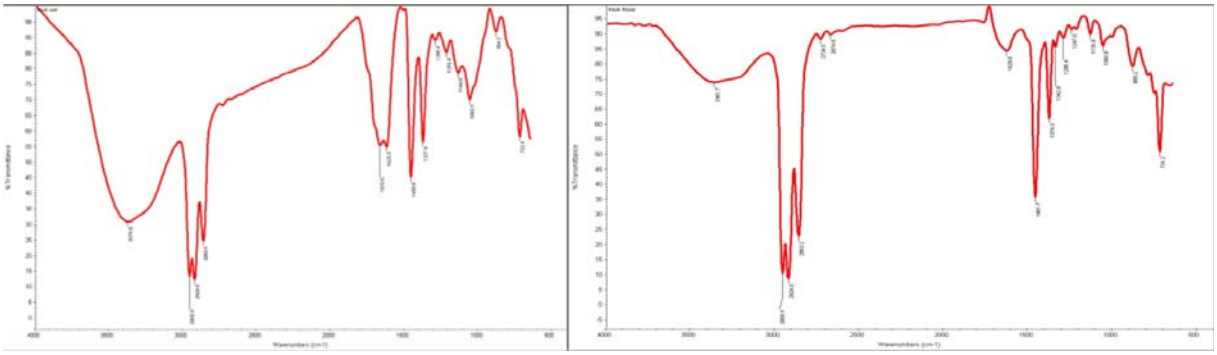

Fig 1: FT-IR Transmittance spectrum of hydro-distilled essential oil from fresh flowers (A) and fresh leaves (B) of African marigold (*Tagetes erecta* L.)

A **B**

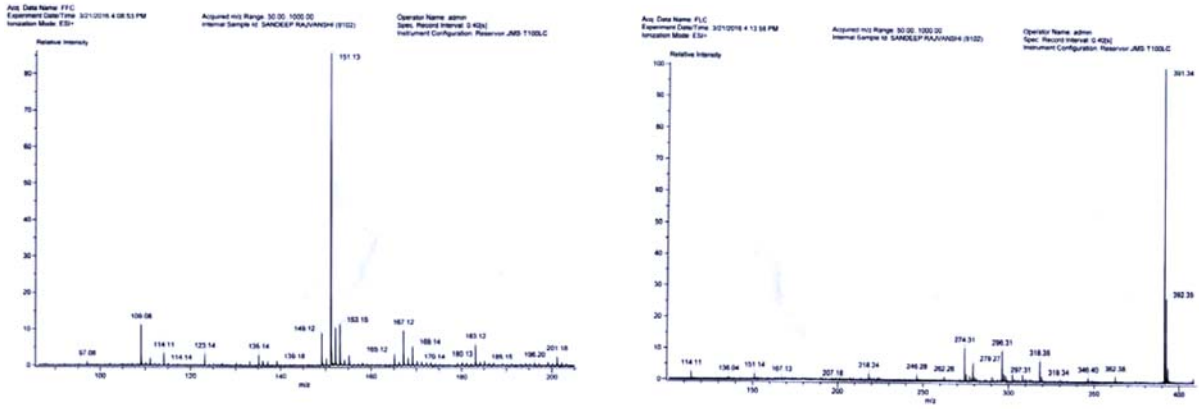

Fig 2 : DART-MS spectrogram of hydro-distilled essential oil from fresh flowers (A) and fresh leaves (B) of African marigold (*Tagetes erecta* L.)

acetate (R.I % 1) .respectively. It is not easy to identify and differentiate all the components independently based on their mass spectral data. Thus, it is suggesting, that all FTIR and DART-MS data should correlated in future with the detailed GC-MS and HPLC analysis of the sample.

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