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Secondary Phytometabolite Profiling of Hydro-distilled Essential Oil of Dry Flower from African marigold (*Tagetes erecta* L.) by FTIR and DART Mass Spectrometry

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ABSTRACT

African marigold (Tagetes erecta L.) is known for its high therapeutic value besides its ornamental uses. The beneficial effect of herbal medicine typically results from the combination of secondary metabolites such as glycosides, alkaloids, flavonoids, tannins, gums, etc., produced in the herbs. Thus, plants with medicinal values, their derivatives and characterized secondary phytometabolites are becoming popular as an alternative to synthetically produced allopathic systems of medicine. In the present investigation, essential oil extracted from dry flowers of African marigold (Tagetes erecta L.) by hydro-distillation was subjected to screening for its phytometabolites with the help of FTIR which revealed presence of major functional groups viz., aliphatic amines, phenols alkanes and nitro compounds etc. The samples were further subjected to mass spectral ionization technique, Direct Analysis in Real Time (DART) which elucidated the presence of piperitenone, ocimenone, umbellulone, terpinolene, α-pinene, thujene, sabinene, α-terpinolene and piperitone, etc., as the main components of the alkaloids, terpenes and phenolic compounds recored by FTIR analysis in the marigold oil.

Key words: *Secondary phytometabolite, Tagetes erecta, Marigold oil, Hydro-distillation, African marigold, FTIR and DART- MS.*

INTRODUCTION

Marigold (*Tagetes erecta L.)* native of Mexico and other warmer parts of America is a genus of herbs, which belongs to family Compositae, and has become naturalized in the tropics and subtropics[2]. The beneficial effect of herbal medicine typically results from the combination of secondary metabolites such as glycosides, alkaloids, flavonoids, tannins, gums etc produced in herbs. Thus, plants with medicinal values, their derivatives and characterized secondary phytometabolite are becoming popular as an alternative to synthetically produced allopathic systems of medicine[1]. The oil of marigold species is used in high quality perfumes and its carotenoid pigments are used in food industry.

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Further, there are reports of use of *Tagetes* oil in perfumery and as a flavoring constituent[3] from the wild marigold *Tagetes minuta* which is not readily available nor is it cultivated by farmers. *Tagetes erecta* which is large flowered African marigold is used most commonly as a winter annual and as loose flower for use in religious ceremonies, garlands, decoration in marriages etc.

The essential oil extracted from marigold is reported to be antihaemorrhagic, anti-inflammatory, antiseptic, antispasmodic, astringent, diaphoretic and emmenagogue and is valuable in aromatherapy for its powerful skin healing properties[10,12]. Additionally, the decoctions of the leaves of *Tagetes erecta* and *Tagetes patula* have been traditionally used as antimalarial and as febrifuge[11].

Hydro- distillation or steam distillation is the most widely utilized physical method for extracting essential oils from the botanical material[15]. Phytochemical studies carried out in different species of *Tagetes* from other countries have revealed the presence of flavonoids and terpenes displaying pharmacological and insecticidal properties[13]. Fourier Transform Infrared spectroscopy (FTIR) is a high-resolution analytical technique to identify chemical constituents and elucidate structural compounds[9]. Direct Analysis in Real Time (DART) has been coupled to the AccuTOF atmospheric pressure ionization mass spectrometer to permit high resolution, exact mass measurements of gases, liquids and solids[6] which help in identification of phytometabolite constituents of plant. African marigold (*Tagetes erecta* L.) is a very important flower which has gained importance since it is cultivated on a large scale and is easily available even as a loose flower. Post harvest processing of marigold for its oil, extracted from its flower as well as its plant parts may enhance value of the crop multifold since marigold is reported as a rich source of bio-colour, pigments and bioactive molecules which may be exploited in the food and pharmaceutical industry. This technology may also be used for waste management of flowers which are generally discarded after their use in temple, marriage decoration 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 screen the secondary phytometabolite in hydro-distilled essential oil from dry flower of African marigold *Tagetes erecta L.* by FTIR and DART mass spectrometry.

MATERIALS AND METHODS

Collection of sample

Flowers 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. and were subjected to air drying for 24 hours in shade, protected from direct sunlight and subsequently stored in air tight containers for further study.

Hydro-distillation (extraction of essential oil)

Dry flowers (100g) 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.

Qualitative screening of Hydro-distilled extracts

Screening by FTIR analysis

Oil extracted from the dried flowers was subjected to FTIR spectroscopy (NicoletTM 6700, Thermo scientific: USA), with a scan range from 400 to 4000 cm-1. The IR spectra of phytometabolites 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 DART-MS was recorded on a JEOL-AccuTOF LMS-T100LC Mass spectrometer having a DART (Direct Analysis in Real Time) source. The given sample was subjected as such in front of DART source.

Dry helium gas was used with 4 LPM flow rate for ionization at 350^{0}C. Data acquisition was from m/z 50.0 to 200.0. The orifice was set at 28 V and spectra were collected. The components in the oil were identified by comparison of their *m/z* values with those of a computer library and with data already published in literature.

RESULTS AND DISCUSSION

Determination of the functional groups present using FT-IR Spectroscopy

FTIR spectroscopic analysis of essential oil of dry flowers from African marigold extracted by hydro-distillation has showed the existence of various secondary phytometabolites (Fig, 1). The bonds and the wave numbers (cm-^{1}) of prominent peaks of the major constituents obtained from spectra are described in Table 1. The essential oil from dry flowers shows major peaks primarily at 3402.8 cm-^{1}, 2957.7 cm-^{1}, 2924.1 cm-^{1}, 2863.9 cm-^{1}, 2733.7 cm-^{1}, 1708.4 cm-^{1}, 1618.4 cm-^{1}, 1460.3 cm-^{1}, 1378.3 cm-^{1}, 1219.4 cm-^{1}, 1219.4 cm-^{1}, 1135.3 cm-^{1}, 1060.8 cm-^{1}, 890.8 cm-^{1}, 802.9 cm-^{1} and 724.7cm-^{1}. The fundamental components in a sample may be identified depending on the fingerprint characters of the peaks positions, shapes and intensities[5]. Thus, the peak at 3402.8 cm-^{1} is assigned to the O-H stretching vibration while that in the range of 2990-2700 cm-^{1} is mainly attributed to the stretching vibration of C-H. In addition, the peak at 1708.4 cm-1 is assigned to the C=O stretching vibration which indicates that some α, β-unsaturated aldehydes and/or ketones compounds existed in the hydro-distilled essential oil of dry flower from African marigold.. The alkane peaks at 1460.3 and 1378.3 cm-1 and the peak situated at 1219.4 cm-^{1} assigned to C –O stretching. The peak at 1135.3 and 1060.8 cm-1 are due to C-N stretching vibrations. The aromatics are present at the range of 800-890 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[8].

Elucidation of phytometabolites by DART-MS

The phytometabolite components present in hydro-distilled essential oil of dry 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 dry flowers of African marigold is given in Figure 2 and Table 2. The constituents were identified by matching their mass spectra with those recorded in literature. The peak at *m/z* 149 could be due to piperitenone, ocimenone, (R.I % 11) and peak at *m/z* 136 best match with *α* pinene, terpinolene, *thujene,* sabinene, β-ocimene, limonene (R.I % 4).However, they have the same molecular formula; a distinction could not be made. The peak at *m/z* 152 could be due to piperitone or camphor (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[7]. 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*[4]. Besides this, the peak of other terpenes was observed at *m/z,* 151, 167, 169 corresponding to piperonal, verbenone, thymol, carvone, carvacrol , a-campholenic acid and galic acid respectively. Trans-linalooloxide, citronellyl formate, d- undecanoloide, showed peak at *m/z* 172 and 183 respectively which corresponded to terpenes reported at similar *m/z* values known[14]. 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.

Table 1: FTIR analysis of hydro-distilled essential oil of dry flowers from African marigold (*Tagetes erecta* L.)

S.NO.	FREQUENCY (CM^{-1})	DRY FLOWER	BOND	FUNCTIONAL GROUP
1.	3450-3400	3402.8	O-H stretch	alcohols, phenols
2.	2990-2950	2957.7	C-H Stretch	alkanes
3.	2950-2900	2924.1	C-H Stretch	alkanes
4.	2880-2850	2863.9	C-H Stretch	alkanes
5.	2750-2700	2733.7	H-C=O: C-H Stretch	aldehydes
6.	1750-1700	1708.4	C=O Stretch	α, β- unsaturated aldehydes, ketones
7.	1650-1600	1618.4	N-H Bend	1* amines
8.	1490-1450	1460.3	C-H bend	alkanes
9.	1390-1350	1378.3	C-H rock	alkanes
10.	1250-1200	1219.4	C-O stretch	carboxylic acid
11.	1150-1100	1135.3	C-N Stretch	Aliphatic amines
12.	1090-1050	1060.8	C-N Stretch	Aliphatic amines
13.	890-850	890.8	C-H "oop"	aromatic
14.	850-800	802.9	C-H Bend (para)	aromatic
15.	730-700	724.7	C-H rock	alkanes

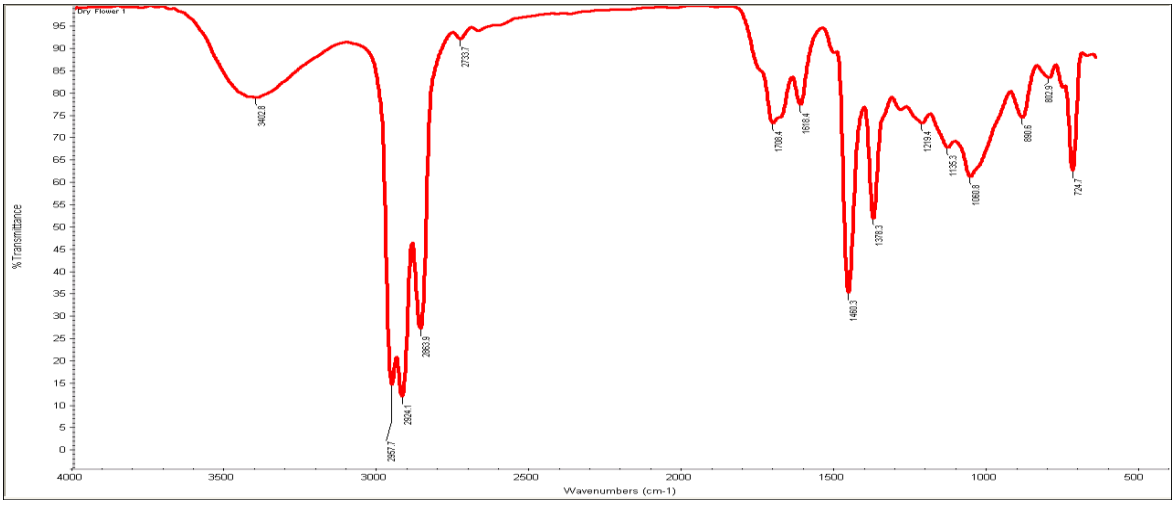

Fig 1: FT-IR Transmittanc spectrum of hydro-distilled essential oil from dry flower of African marigold (*Tagetes erecta* L.)

Table 2: Exact Mass data from the DART –MS of hydro-distilled essential oil of dry flowers from African marigold (*Tagetes erecta* L.)

S.NO.	MOLECULAR WEIGHT	MEASURED MASS	MOLICULAR FORMULA	RELATIVE INTENSITY (%)	REMARKS
1.	97	97.07	$C_6H_{10}O$	1%	(E)-2-Hexenal
2.	114	114.11	$C_7H_{14}O$	5%	n-Heptanal, 2Heptanone
3.	123	123.14	$C_8H_{10}O$	4%	p-Methylanisol, 4-Ethylphenol
4.	135	135.14	$C_{10}H_{16}$	4%	Terpinolene, *Thujene*, sabinene, α-terpinolene, α pinene, β-ocimene,limonene
6.	139	139.17	$C_{10}H_{18}$	0.5%	Tran-pinane, Cis pinane
7.	149	149.11	$C_{10}H_{14}O$	11%	Piperitenone, Ocimenone, umbellulone
8.	151	151.13	$C_{10}H_{14}0$	91%	Piperonal, verbenone, thymol, carvone, carvacrol
9.	152	152.13	$C_{10}H_{16}O$	12%	Piperitone, Camphor
10.	165	165.11	$C_{10}H_{14}O_2$	4%	Furomyrcenol
11.	167	167.13	$C_{10}H_{16}O_2$	16%	Trans Dihydrocarvone epoxide, a-Campholenic acid
12.	169	169.15	$C_7H_6O_5$	7%	Galic acid
13.	172	172.20	$C_{10}H_{20}O_2$	1%	Trans-linalooloxide
14.	181	181.12	$C_{11}H_{18}O_2$	0.5%	Geranyl formate
15.	183	183.12	$C_{13}H_{28}$	10%	Citronellyl formate, d-undecanoloide
16.	185	185.16	$C_{10}H_{14}0$	2%	n-nonyl acetate

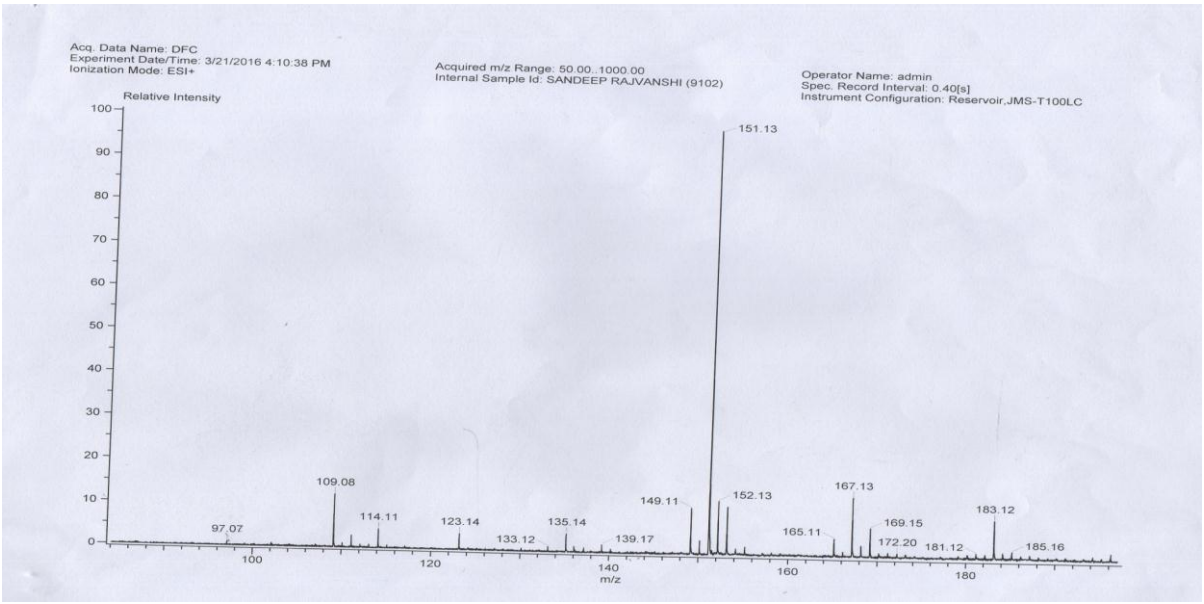

Fig. 2: DART-MS spectrogram of hydro-distilled essential oil of dry flower from African marigold (*Tagetes erecta* L.)

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