ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Studies on Volatile component of *Tagetes patula*

HumairaSiddiqi[1], Shaheen Faizi[1] ,Samina Bano[1], Sumayya Saied [2], Shaista Naz[2], Erum Zahir[2]

1.H.E.J. Research Institute of Chemistry, International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, pakistan
2.Department of Chemistry, University of Karachi, Karachi, Pakistan

Manuscript Info ***Abstract***

Manuscript History:

Received: 12 January 2015
Final Accepted: 22 February 2015
Published Online: March 2015

Key words:

T. patula, G.C/G.C.M.S. studies, pesticidal activity

***Corresponding Author**

Dr.Humaira Siddiqi

Analysis of the petroleum ether extracts of different parts of *T. patula* resulted in the identification of thirty eight (38) compounds by G.C/G.C.M.S. studies which include hydrocarbons, fatty acis, fatty acid esters, thiophenes, terpenes and steroids. The initial results of pesticidal activity against *Sitophylusoryzae* showed that this plant is also a good source of pesticidal agents.

INTRODUCTION

Tagetespatula is a member of the marigold section of the Asteraceea family. Various work had been reported on its essential oil composition (Szarka et al., 2007,2006), (Dhingra et al., 1956), (Hethelyi et al., 1987), (Stojanova et al., 2000), (Krishna et al., 2002), (Rayalak et al., 2003), (Marothi et al., 2004), (Sagar et al., 2005).Terpenes, mainly (*Z*) and (*E*) ocimenone along with limonene, caryophyllene, piperitone and piperitenone were the major chemical compounds of the essential oil obtained from capitula and leaves of *Tagetespatula*(Romagnoli et al., 2005). Recently attention has been focused on the biological activity of essential oil of *T.patula*(Rondon et al., 2006), (Dutta et al., 2007).

Tagetespatula was credited with nematicidal properties. Plantation of *T. patula* in alternate rows with potatoes is said to help in controlling the root-knot of potatoes. It also suppresses weed growth and is usefull as green manere. This plant contains various thiophenes derivatives including α-terthienyl (Szarka et al., 2007). It was investigated that α -terthienyl was potentially use-full phototoxic insecticide and it was a model phototoxin for plant-insect interaction studies (Didyk et al., 2007).
Various nematicidal, weed suppressive effect and insecticidal work had been previously reported on *Tagetespatula* and other species. Due to high degree of chemo diversity observed within the essential oil and relation of biological activity with chemical composition; in the present studies the petroleum ether extracts of different parts of *T.patula*have been investigatedthrough G.C/G.C.M.S.studies and pesticidal activity of different extracts were also carried out.

Material and Methods

Plant Extraction

Fresh, dried, uncrushed orange red flowers (750 g) of *Tagetespatula* collected from the campus of University of Karachi in 2003 were extracted twice with petroleum ether (P.E). After petroleum ether extractions flowers were extracted twice with methanol. The petroleum ether and methanolic extract afforded residues JFP (5 g) and JFM (liquidish) after evaporation in *vacuo*. The residue JFM was partitioned between distilled water and P.E to get petroleum ether fraction. The aqueous layer was further extracted with $CHCl_3$ (three times) EtOAc (six times) and BuOH (four times). Each phases (P.E, $CHCl_3$, EtOAc except BuOH) were washed with water and dried with anhydrous sodium sulphate and after evaporation of these fractions JFM-P (14 g), JFM-C (2 g), JFM-EA (14 g), JFM-Bu (10 g) were obtained.
The aques phase after butanol extraction was freeze dried giving a residue JFMaq which was treated with methanol affording, methanol soluble (JFMaqM) and insoluble JFM aq MI fractions. In another experiment to separate polysaccharides from JFMaq MI, it was dissolved in 10 ml of water and added drop by drop into 250 ml of methanol; thereby two fractions JFMaq MIM (methanol soluble) and JFMaq MII (methanol insoluble) were obtained.
The components of all the petroleum ether extracts of different part of the plant *e.g.;* flowers, seeds, roots and whole plant (JFP, JSP, JRP, JWP) and pet ether extract (JFP) and pet ether phase (JFM-P) were analyzed by G.C/G.C.M.S.spectra.

SEEDLINGS Extraction

Three different sizes of *T. patula* seedlings (**I** = 11 cm, 7 g, **II** = 13 cm, 7 g, **III** = 21 cm, 9 g) were extracted with petroleum ether (P.E) at room temperature. The P.E extracts were freed of the solvent in*vacuo* giving the residues JSdPI, JSdPII, JSdPIII respectively. The marc which were obtained after filtration of P.E was extracted with ethyl acetate and then with methanol which were evaporated under reduce pressure giving ethyl acetate (JSdEAI, JSdEAII, JSdEAIII) and methanol (JSdMI, JSdMII and JSdMIII) extracts. The JSdPI, JSdPII and JSdPIII were examined through G.C./G.C.M.S.analysis.

Pesticidal Activity

Sitophylusoryzae were reared in the laboratory under controlled conditions. The temperature was maintained between 28-31^oC and H.R.as 60%±5. For the treatment of Sitophylusoryzae different concentration of JFM-aqMI, JFM-aqM and JFMaqMIM.at different time intervals (Table 4) were used. Filter paper impregnation method was adopted. After 24hours of treatment mortality percent was noted.

G.C/ G.C.M.S. studies

All samples were analysed by GC-FID and GC-MS analysis. GC-FID was carried out using a Shimadzu GC-9A, fitted with a (30 mx 053 mm, 05µm thickness) using SPB-5 column. Oventemperature programming: 50^oC (5mm), 50-235^oC (5^oC min^{-1}, and 235^oC (35 min). Nitrogen was used as carrier gas at a flow rate of 30 ml min^{-1}, sample injection was carried out with a split ratio of 35:1at a temperature of 240^oC, the FID temperature was 220^oC. G.C.M.S.. analyses were carried out using a HP-5 column (30 m x 0.320 mm film thicknesses) fused silica column was used with oven temperature programming 50 ^oC (2 min), 50-250 ^oC (5 ^oC min^{-1}), and 250 ^oC . Helium was used as carrier gas at a flow rate of 1.8 ml min^{-1}. Sample injection was carried out with a split ratio of 35:1 at a temperature of 25 ^oC.

For G.C.M.S.detection, an electron ionization system was used with ionization energy of 70 ev. Each sample was then analyzed three times with GC-FID to obtain the percentage concentration of each constituent. All samples were injected manually and split after dilution (1/100 in methanol). The mass fragmentation pattern was compared with the reported mass fragmentation pattern of those of other essential oils of known composition, with pure compound and with those in NIST mass spectra libraries (http.//webbook.nistgov.chemistry). The percentage composition of each component was calculated with the 100% method using chromatogram peak areas.

Result and Discussion

Identification of chemical constituents through G.C. / G.C.M.S.

The composition of the petroleum ether extracts of flowers, root, seeds and aerial part (without flowers) is mentioned in Table 1. For identification of compounds, the MS fragmentation pattern was compared with NIST data base and reported fragmentation pattern because most of the compounds are likely present in the literature (Krishna et al., 2002), (Romagnoli et al., 2005). These studies revealed that the root extract comprised of fatty acid esters (48.81%), hydrocarbons (29.3%) and monoterpenes (11.1%). The main components of root were methyl palmitate (28.2%), methyl 9, 12, 15-octadecatrienoate (16.9%), nonacosane (11.3%) and hentriacontane (15.0%). Small amount of triatriacontane (3.0%) methylligneocerate (3.7%), tagetone (3.7%) and *Z* and *E*ocimenone (3.7% each) were also present (Table 1).

The seed extract contained methyl palmitate (22.5%) nonacasone (14.0%) and hentriacontane (11.7%) as main components. Beside this, fatty acids tetradecanoic acid (4.6%) and hexadecanoic acid (9.3%), methyleugenol (4.6%) and thiophene (α-terthienyl (7.03%) were present. The other component methyl 9, 12-octadecadienoate, triacosane, heptacosane, tritriacontane and methyllignocerate were present in reasonable amount in seed extract. It is of interest to note that this extract did not contain monoterpenes (Table 1).

The extracts of aerial part more or less have the same composition profile as of root and seeds. It comprised of fatty acid methyl ester (67.25%), hydrocarbon (11.4%) fatty acid (5.5%), monoterpene (5.4%) and thiophenes (5.5%). It differed from root and seeds by the presence of 5-[4-acetoxy-1-butenyl]-2,2′-bithiophene (5.5%) and two alkynes [1-pentadecyne (6.0%) and 1-eicosyne (6.0%)].

The table 2 showed the composition of two different petroleum ether extracts of flowers. These extract containdthiophenes, hydrocarbons, fatty acids, aromatic acid, methyl esters and terpenes.

Table-3 highlighted the comparision among the extracts of three different sizes of seedlings (JSdPI, JSdPII, and JSdPIII). JSdPI has only two fatty acids, palmitic acid and octadecanoic acid (44.4%). Both JSdPII and JSdPIII contained fatty acids (42%, 21.6%), fatty acids methyle ester (17%, 11.6%) and hydrocarbons (41%, 42%).

Pesticidal activity

In the previous investigation the pesticidal activity of different extracts) of all parts of *Tagetespatula* against store grain pest was evaluated to find out which part produced high biocidal response.
The extract JFP (non polar) and JFM were effective against *Sitophylusoryzae.* From the methanolic extract (JFM) five different phases (JFM-P, JFM-C, JFM-EA, JFM-Bu and JFM-aq) have been obtained of which JFM-P and JFM-aq were found to be toxic while JFM-C and JFM-Bu have no toxic effect(Tabassum et al., 2006), G.C/G.C.M.S.studies revealed that petroleum ether extract of flowers contains thiophenes,hydrocarbon ,fatty acids and their esters (Table 2) while isolation studies showed that polar extract contains highly hydroxylated aliphatic and aromatic compounds (Faizi et al., 2011). Recent studies suggested that flavonoids can act as potential grain protectants *via* contact, oviposition deterrent and ovicidal action. The use of flavonoids can be a useful and sustainable strategy for the protection of stored grains from pests(Salunke et al., 2005). Flavonoids and thiophenes are botanical insecticides which are contributing to the pesticidal activity of JFP, JFM-EA and JFM. Other compounds may also play their role in this activity.
In recent studies three different fractions (JFMaqMI, JFMaqMII and JFMaqMIM were obtained by solvent separation of JFMaq. Among these JFMaqMI had not toxic effect while all the rest fractions have toxicity against *Sitophylusoryzae*(Table 4). The present investigation showed clearly that most of the extracts, phases and fractions significantly possess promising pesticidal activity against stored grain pest *Sitophylusoryzae.*

Table 1: The chemical composition of the petroleum ether extract of different parts of*Tagetespatula*.

S.No	Name of compounds	M.Formula	M.Weight	Seeds Realtive conc. (%)	Root Realtive conc. (%)	Stem +Leaves Realtive conc. (%)
1	*Z*-Ocimenone	$C_{10}H_{14}O$	150	-	3.7	-
2	*E*-Ocimenone	$C_{10}H_{14}O$	150	-	3.7	2.7
3	Tagetone	$C_{10}H_{16}O$	152	-	3.7	2.7
4	Methyl eugenol	$C_{11}H_{14}O_2$	178	4.6	-	-
5	1-Pentadecyne	$C_{15}H_{28}$	208			60
7	Tetradecanoic acid	$C_{14}H_{28}O_2$	288	4.6	-	-
8	5´-Methyl-5-(3-but-1-ynyl)-2,2´bithiophene	$C_{13}H_{10}S_2$	230	-	-	-
9	α-Terthienyl	$C_{12}H_8S_3$	248	7.03	-	-

10	Hexadecanoic acid	$C_{16}H_{36}O_2$	256	9.3	-	-
11	Methyl palmitate	$C_{16}H_{34}O_2$	270	22.5	28.2	27.7
12	5-[4-Acetoxy-1-butenyl]-2,2´-bithiophene	$C_{14}H_{12}S_2O_2$	276	-	-	5.5
13	1-Eicosyne	$C_{20}H_{38}$	278	-	-	6.0
14	Ethyl palmitate	$C_{18}H_{36}O_2$	284	-	11.3	5.1
15	Methyl 9,12,15-Octadecatrienoate	$C_{19}H_{32}O_2$	292	-	16.9	26.3
16	Methyl 9,12-Octadecadienoate	$C_{19}H_{34}O_2$	294	7.03	-	-
17	9,12-Eicosadienoic acid	$C_{20}H_{36}O_2$	308	-	-	5.5
18	Triacosane	$C_{23}H_{48}$	324	4.6	-	-
19	Cycloletracosane	$C_{24}H_{48}$	336			4.15
20	Methyl behenitate	$C_{23}H_{46}O_2$	354	-	-	4.15
21	Heptacosane	$C_{27}H_{56}$	380	4.6	-	-
22	Methyl lignocerate	$C_{25}H_{50}O_2$	382	4.6	3.7	2.7
23	Nonacosane	$C_{29}H_{60}$	408	14.0	11.3	2.7
24	Methyl cerinitate	$C_{27}H_{54}O_2$	410	-	-	1.3
25	Hentriacontane	$C_{31}H_{64}$	436	11.7	15.0	2.7
26	Tritriacontane	$C_{33}H_{68}$	464	4.7	3.0	-

Table 2: The chemical composition of the petroleum ether extract and fraction of*Tagetespatula* flowers.

S.No	Name of compounds	M.Formula	M. Weight	JFM-PE Realtiveconc . (%)	JF-P Realtive conc. (%)
1	4,4-Dimethyl-2-pentene	C_7H_{14}	98	1.96	-
2	2-Methyl decane	$C_{11}H_{24}$	156	0.98	-
3	Decanoic acid	$C_{10}H_{20}O_2$	172	7.35	-
4	Methyl eugenol	$C_{11}H_{14}O_2$	178	2.45	3.65
5	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methyl ethenyl)-4-(1-methylethylidine	$C_{15}H_{24}$	204	-	3.2
6	4-Pentyl benzoic acid	$C_{13}H_{18}O_2$	206	1.96	-
9	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	-	4.05
10	Methyl pentadecanoate	$C_{16}H_{32}O_2$	256	3.92	-
11	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	-	9.0
12	α-Terthienyl	$C_{12}H_8S_3$	248	9.8	4.5
13	Octadecanoic acid	$C_{18}H_{36}O_2$	284	-	6.75
14	Methyl palmitate	$C_{17}H_{34}O_2$	270	49	27.0
15	Ethyl palmitate	$C_{17}H_{36}O_2$	284	-	-
16	Methyl 9,12-Octadecanoate	$C_{19}H_{34}O_2$	294	24.5	-
17	Stigmasterol	$C_{29}H_{48}O$	412	-	2.7
18	β-Sitosterol	$C_{29}H_{50}O$	414	-	11.25
19	Tochopherol	$C_{28}H_{45}O_2$	416	-	2.75
20	α or β-Amyrine	$C_{30}H_{50}O$	426	4.9	9.0
21	Vitamin E	$C_{29}H_{50}O_2$	430	-	4.5

Table 3: The chemical composition of the petroleum ether extracts of different sizes of seedling of *Tagetespatula*.

S.No.	Name of compounds	M. Formula	M. Weight	Seedling JSdPI Realtive conc.(%)	Seedling JSdPII Realtive conc.(%)	Seedling JSdPIII Realtive conc.(%)
1	Palmitic acid	$C_{16}H_{32}O_2$	256	55.5	21.6	33.6
2	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	44.4	-	8.4
4	Methyl behenitate	$C_{23}H_{46}O_2$	354	-	-	8.4
5	Heptacosan	$C_{27}H_{56}$	380	-	-	60.7
6	Methyl lignocerate	$C_{25}H_{50}O_2$	382	-	11.06	8.4
7	Nonacosane	$C_{29}H_{60}$	408	-	10.05	17
8	Hentriacontane	$C_{31}H_{64}$	436	-	8.17	17

Table 4: Mortality mean of fractions of JFMaq against*Sitophylusoryzae* at differtent time of intervals.

S.No		Doses in µg/cm^2	Mortality		Mean%	
1	JFMaqMI		24 hrs	48hrs	72hrs	96hrs	120hrs
		471.40	0	-	-	-	-
		928.80	70	-	-	-	-
		1885.60	90	-	-	-	-
2	JFMaqMII						
		9.88	45	15	-	10	20
		19.64	95	15	-	20	30
		29.46	100	65	-	50	50
		39.28	100	80	-	80	60
3	JFMaqMIM						
		157.13	15	15	10	-	-
		314.26	25	25	10	-	-
		471.40	30	30	25	-	-
		628.53	35	45	35	-	-

References

Dhingra, S. N. and Dhingra, D. R. (1956): Essential oil of *Tagetespatula*. Perfum. Essent. Oil Record., 47: 391-394.

Didyk, N. P. and Mashkovska, S. P. (2007): Allelopathy: new concepts and methodology, evaluation of weed suppressive effect of allelochemicals of ornamental marigold species. Taylor & Francis.

Dutta, B. K., Kamarkar, S., Naglot, A., Aich, C. J. and M. Begum. (2007): Anticandidial activity of some essential oils of a mega biodervisity hotspot in India. Mycos., 50(2):121-124.

Faizi, S., Dar, A., Siddiqi, H., Naqvi, S., Naz, A. and Bano, S.(2011): Bioassay guided isolation of antioxidant agents with analgesic properties from flowers of *Tagetespatula*. Pharm. Biol., 49(5): 516-525

Hethelyi, E., Danos, B., Tetenyi, P. and Juhasz, G. (1987): Phytochemical studies on Tagetes species: Infra specific difference of the essential oil in *T. minuta* and *T. tenuifollia*. Herba Hung., 26(2-5): 145-158.

Krishna, A., Gopal, R., Mallavarapu, S.K. (2002): Volatile oil constituents of the capitula, leaves and shoots of Tagetespatula L. J. Essent. Oil Res., 14: 433-436.

Marothi, M., Piccogtia, R., Boavati, B. and Marathi, I. (2004): Characterization and yield evaluation of essential oils from different Tagetes species. J. Essent. Oil Res., 16: 440-444.

Rayalak, R. S. and Jase, J. (2003): Chemical polymorphism in Asteraceae. Pak. J. Scient.Indust. Resear., 46(5):327-332 .

Romagnoli, C., Bruni, R., Andreotti, E., Rai, M. K., Vicentini, C. B. and Mares D. (2005): Chemical characterization and antifungal activity of essential oil of capitula from wild Indian Tagetespatula L. Protoplasma., 225: 57-65.

Rondon, M., Velasco, J., Hernands, J. and Pechanda, M. (2006): Chemical composition and antibacterial activity of the essential oil of T.patula. Rev.Latinoamericana de Quimica., 34(1-3): 32-36.

Sagar, V. D., Naik, S. N., Rout, P. and Rao,Y. R. (2005): Composition of essential oils of *T.patula* growing in the northern India. J. Essent. Oil, Res., 17(4): 446-448.

Salunke, B. K., Kotkar, H. M., Mendki, P. S., Upasani, S. M. and Maheshwari V. L. (2005): Efficacy of flavonoids in controlling Callosobruchuschinensis (L.) (Coleoptera: Bruchidae), a post-harvest pest of grain legumes. Crop Protection., 24: 888–893.

Stojanova, A. and Primova, T. (2000): Effect of mineral fertilization on the essential oil composition of *Tagetespatula* from Bulgaria., J. Essent. Oil Res., 12(5): 609-612.

Szarka, S., Hethelyi, E. and Balvanyos, I. (2006): GC and GC-MS studies on the essential oil and thiophene from *T.patula* L. Chromatogr., 63(supple): S67-S73.

Szarka, S., Hethelyi, E. and Balvanyos, I. (2007): Essentiol oil constitute of intact plant and in vitro culture of *T.patula* L. J. Essent. Oil, Res., 19(1): 65-68.

Tabassum, R., Rao,S., Gabol, K. and Faizi,S. (2006) : Efficacy of Jaffery plant (*Tagetespatula*) against rice weevil *Sitophilus Oryzae* .Pakistan J. Entomol., 21 (1&2): 5-9.