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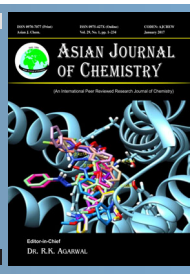

Comparison of Chemical Composition of Root and Rhizosphere Soil Extracts of *Tagetes patula* L.: GC-MS Analysis

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The chemical composition of root and rhizosphere soil extracts of plant of *Tagetes patula* L. was investigated by GC-MS for the identification of allelochemicals. The dried and powdered root material was subjected to Soxhlet extraction using methanol. The rhizosphere soil around the plant was also extracted with methanol. GC-MS analysis of the roots and rhizosphere soil extracts led to identification of 25 and 27 compounds, respectively. The major compounds identified in methanol root extract were 5-hydroxymethylfurfural (21.81 %), butanoic acid, 2,2-dimethyl-3-oxo-methyl ester (11.71 %), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (12.68 %), 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (8.50 %). However in rhizosphere soil extract dimethylsulfoxonium formylmethylide (6.32 %), 2-phenylthieno[2,3-b]thiophene (9.34 %), hexadecanoic acid (16.64 %), α-terthienyl (5.81 %), stigmasterol (5.32 %), γ-sitosterol (5.71 %) were present as major compounds.

Keywords: GC-MS, Rhizosphere soil, Root extracts, *Tagetes patula*.

INTRODUCTION

Tagetes patula L. belonging to the genus Tagetes, family Asteraceae is native to Central America, but it is found throughout the world as an important ornamental plant [1]. It is an annual plant, 40 cm of height that grows at 1500-2500 m above the sea level. In India, it is cultivated as a floriculture crop and capitula are sold loose or used in garlands for social and religious purposes [2]. It has been used in folk medicine to treat colics, diarrhoea, vomit, fever, skin diseases and hepatic disorders. Allelochemicals are toxic plant metabolites or their products that are released into the microenvironment [3]. *T. patula* produces several compounds including essential oils that are biologically active [4] and thus potentially allelopathic. This species is known as a rich source of biological active compounds known as natural thiophenes whose activity is enhanced by irradiation with long wavelength ultraviolet light [5-7]. Thiophenes exhibit substantial biological activities such as nematicides, insecticides, antibiotics and fungicides [8-11], under irradiation whereas their toxicity is much reduced in darkness. *T. patula* has been commonly used in phytochemical investigations due to the presence of several thiophenes. The oils of normal and hairy root cultures were found to contain sulfur-containing thiophene structures. Four thiophenes as major components, 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT), 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH), 5-(4-acetoxy-1-butynyl)-2,2-bithienyl (BBTOAc) and 2,2,5,2-terthienyl (α-T), occur in different concentrations in every part of *T. patula* [12]. Thiophenes and two benzofurans (dihydro-euparin and euparin) were identified in the root exudates collected from the undisturbed rhizosphere of *T. patula* [13]. The objective of the present study was to compare the compounds present in the rhizosphere soil and root extracts of *T. patula* which are responsible for the allelopathic effect.

EXPERIMENTAL

The roots of the *T. patula* were collected in the month of March from the field of the Punjab Agricultural University, Ludhiana. The roots were dried under shade and powdered using electrical grinder.

Soxhlet extraction: 50 g of dried and powdered plant material was subjected to Soxhlet extraction method using 250 mL of methanol as solvent. The extraction was carried out for 8 h. A yellowish brown solution was obtained. The methanol extract was concentrated using rotary vacuum pump. 5 g of yellowish brown oil was obtained. The process was carried out several batches to collect the methanol root extract. The extract was stored at 4 °C in a freezer for further studies. The percentage yield was calculated.

Preparation of rhizosphere soil extract: The plants of *Tagetes patula* were carefully uprooted. The roots with soil were taken in polythene bags and fiercely shaken off rhizosphere

soil. The rhizosphere soil of *T. patula* (25 g) was dipped overnight in methanol and extracted thrice (3 × 50 mL). Methanol soil extract was pooled, filtered and concentrated in vacuum to near dryness using a rotary evaporator. The extract was stored at 4 °C in a freezer for further studies. The percentage yield was calculated.

Gas chromatography-mass spectrometry (GC-MS) analysis: The hexane extract was analyzed using GC-MS (QP2010 Plus, Shimadzu, Japan), equipped with an Rtx-5 MS capillary column (30.0 m × 20 mm i.d., 0.25 µm film thickness). The injector was maintained at 250 °C and operated in split injection mode with the split valve closed for 1 min. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50 °C for 2 min, raised to 180 °C at 3 °C/min, then to 280 °C at 10 °C/min. The interface temperature was 260 °C and the ionization mode was electron impact (70 eV). The mass selective detector was operated in the scan mode between 40 and 600 *m/z*. Data acquisition was started 3.0 min after injection. MS parameters used were; Ionization voltage (EI) 70 eV, peak width 2s, mass range 40-600 amu and detector voltage 1.5 V. Peak identification was carried out by comparison of the mass spectra with mass spectra data available on database of NIST08, WILEY8, Perfumery and Flavour and Fragrance libraries [14].

RESULTS AND DISCUSSION

The yield of the methanol root extract and rhizosphere soil extract was 6 and 2 % respectively. Table-1 depicts the compounds present in root and rhizosphere soil extracts analyzed by GC-MS. Root extract mainly comprised of thiophenes (21 %), fatty acid (8 %), fatty acid ester (16 %) and steroids (4 %) whereas rhizosphere soil extract contained thiophenes (17 %), fatty acid (32 %), fatty acid ester (3 %) and steroids (15 %).

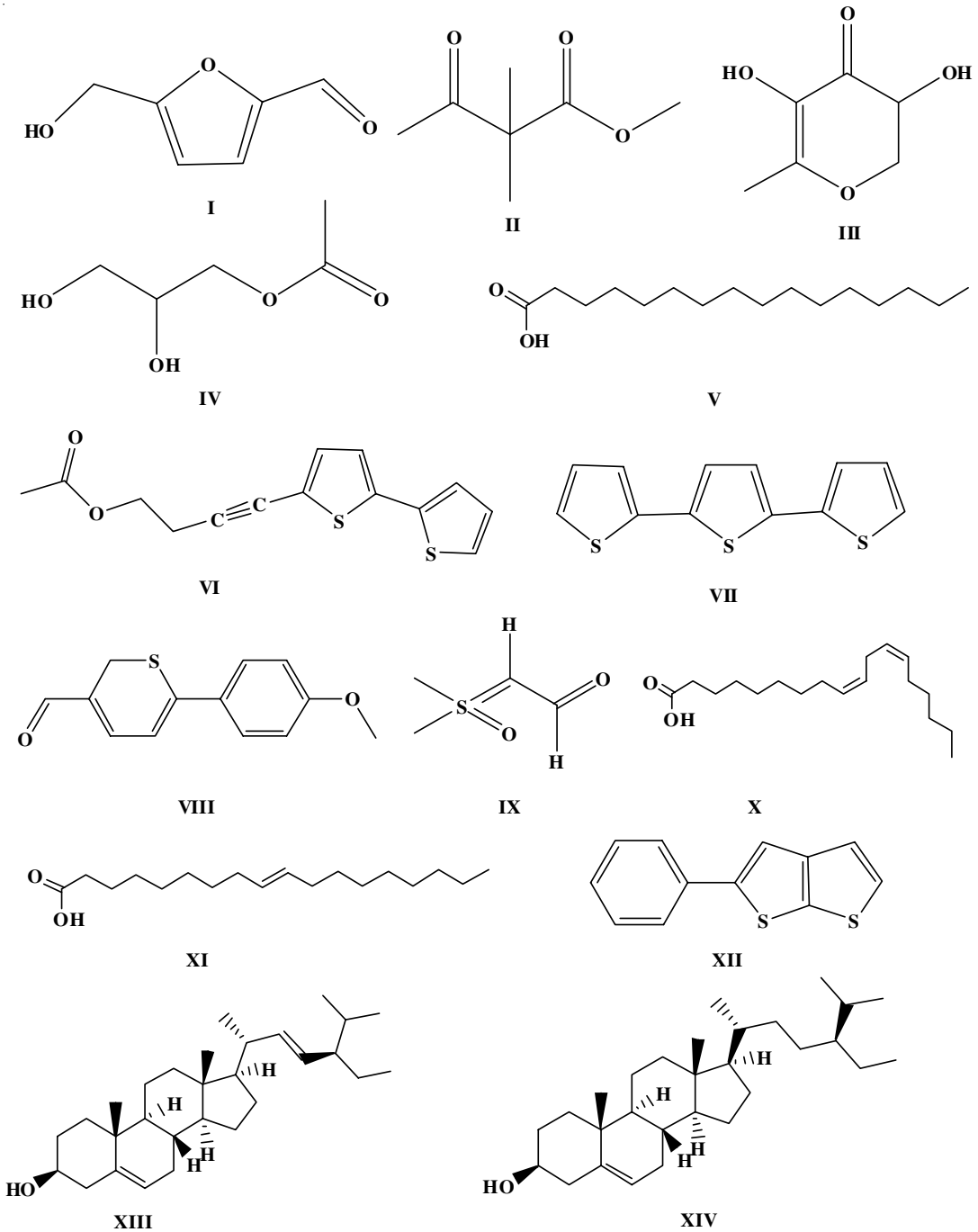
TABLE-1
CHEMICAL COMPOSITION OF THE ROOT AND RHIZOSPHERE SOIL EXTRACTS

S. No.	Compounds	Retention time (min)	Area (%)	
			Root extract	Rhizosphere soil extract
1	2-Furaldehyde	3.340	2.87	0.75
2	Dimethylsulfoxoniumformylmethylide	3.441	–	6.32
3	2-Hydroxy-2-methyl-4-pentanone	3.495	0.83	–
4	2-Furylmethanol	3.675	3.80	–
5	1,3,6-Trioxocane, 2-methyl	4.062	–	1.45
6	Butanoic acid, 2,2-dimethyl-3-oxo-, methyl ester	4.138	11.71	–
7	Dihydroxyacetone	4.454	–	3.35
8	1,2-Cyclooctanedione	5.044	1.69	–
9	2-Propanone, 1-phenyl	5.139	0.31	–
10	Ethanol, 2,2'-oxybis	5.971	–	4.39
11	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	6.368	1.24	–
12	4-Oxopentanoic acid	8.226	0.57	–
13	2-Furancarboxylic acid, methyl ester	9.370	0.83	–
14	2,5-Anhydro-1,6-dideoxyhexo-3,4-diulose	9.505	0.54	–
15	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	11.442	12.68	1.31
16	3-Tert-butyl-2,5-furandione	11.856	1.59	–
17	5-Hydroxymethylfurfural	14.371	21.81	5.62
18	1,2,3-Propanetriol, 1-acetate	15.302	5.23	–
19	Tetradecanoic acid	31.300	–	1.36
20	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]	33.341	–	0.65
21	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	33.913	–	2.16
22	Pentadecanoic acid	33.998	–	1.02
23	2-Phenylthieno[2,3-b]thiophene	35.442	4.98	9.34
24	Hexadecanoic acid, methyl ester	35.691	1.06	–
25	*cis*-9-Hexadecenoic acid	36.319	–	1.74
26	Hexadecanoic acid	36.714	4.20	16.64
27	1-Cyclohexene-1-carboxylic acid, 4-(1,5-dimethyl-3-oxohexyl)-, methyl ester,[r-(r*,r*)]	37.935	–	1.21
28	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	39.829	1.29	–
29	9,12-Octadecadienoic acid (Z,Z)	40.782	2.41	4.11
30	*cis*,*cis*,*cis*-7,10,13-Hexadecatrienal	40.913	0.80	–
31	Octadec-9-enoic acid	40.944	–	5.84
32	Octadecanoic acid	41.530	–	2.17
33	2H-Thiopyran-3-carboxaldehyde, 6-(4-methoxyphenyl)	42.311	3.14	–
34	α-Terthienyl	42.794	3.86	5.81
35	5-(4-Acetoxy-1-butynyl)-2,2'-bithienyl	46.534	8.50	1.95
36	1,2-benzenedicarboxylic acid	51.990	–	1.03
37	Tetracosyl acetate	53.822	–	1.01
38	Stigmasterol	67.462	1.52	5.32
39	γ-Sitosterol	69.536	1.63	5.71
40	Methyl commate A	71.387	–	4.81
41	24(S)-Ethyl-3α,5α-cyclocholest-22(E)-en-6-one	71.921	–	2.46

Methanol root extract: GC-MS analysis of the methanol root extract led to identification of 25 compounds representing 99 % of the total extract (Fig. 1). The major compounds identified were 5-hydroxymethylfurfural (I, 21.81 %), butanoic acid, 2,2-dimethyl-3-oxo-methyl ester (II, 11.71 %), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (III, 12.68 %), 1,2,3-propanetriol, 1-acetate (IV, 5.23 %), hexadecanoic acid (V, 4.20 %). The major thiophenes compounds present were 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (VI, 8.50 %), α-terthienyl (VII, 3.86 %) and 2H-thiopyran-3-carboxaldehyde, 6-(4-methoxyphenyl) (VIII, 3.14 %).

Rhizosphere soil extract: Twenty seven compounds were identified by GC-MS analysis of the rhizosphere soil representing 99 % of the total extract (Fig. 2). The major compounds were hexadecanoic acid (V, 16.64 %), dimethylsulfoxonium formylmethylide (IX, 6.32 %), 5-hydroxymethylfurfural (I, 5.62 %), 9,12-octadecadienoic acid (Z,Z)-(X, 4.11 %), octadec-9-enoic acid (XI, 5.09 %), The major thiophenes components present were 2-phenylthieno[2,3-b]thiophene (XII, 9.34 %) and α-terthienyl (VIII, 5.81 %). Stigmasterol (XIII, 5.32 %) and γ-sitosterol (XIV, 5.71 %) were the major steroidal components present in rhizosphere soil extract of *T. patula.*

The composition of the methanol root extract and rhizosphere soil mainly consisted of thiophenes, fatty acid, fatty acid ester and steroidal components. The root and rhizosphere soil extract showed variation in the chemical composition as well as in amount of similar compounds. The methanol root extract showed abundance in fatty acid ester whereas rhizosphere root



Structures of the major compounds in root and rhizosphere soil extracts

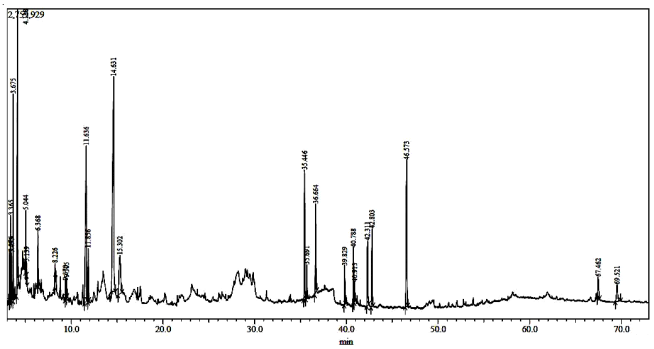


Fig. 1. Chromatogram of methanol root extract

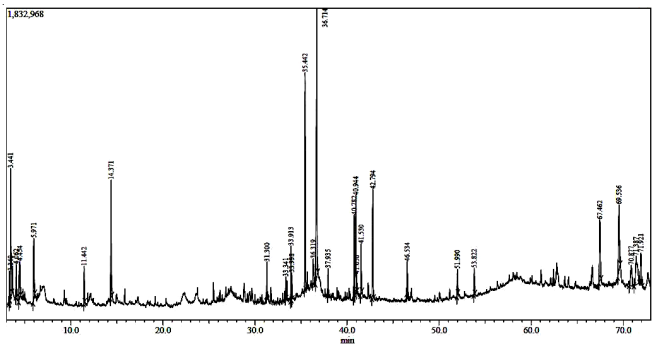


Fig. 2. Chromatogram of rhizosphere soil sample

extract consists mainly of fatty acid. The rhizosphere soil extract consisted of steroidal components as major quantities but as minor quantity in methanol root extract. Total thiophenes present were more in root extract but the rhizosphere soil contained higher amount of 2-phenylthieno[2,3-b]thiophene and α-terthienyl as compared to root extract. 5-(4-Acetoxy-1-butynyl)-2,2'-bithienyl present in higher concentraton in root extract as compared to rhizosphere soil extract. Monoterpenes and sesquiterpenes were not present in root as well as in rhizosphere soil extract. These result are in consonance with earlier studies on volatile compounds in flowers and roots of intact plants and *in vitro* hairy root cultures of *T. patula* investigated by GC and GC-MS. The essential oil of flowers was found to have a higher content of total sesquiterpenes and monoterpenes and negligible amount of sulfurated thiophene compounds. However the volatile components in normal, hairy and intact root oil consisted of large quantities of thiophenes [15].

Among the compound identified, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one present as major compound in root extract was known to possess antioxidant property [16] and as a novel potent aroma compound in a dairy product, Ryazhenka kefir [17], but present as minor compound in rhizosphere soil extract. 2-Phenylthieno[2,3-b]thiophene was present in more concentration rhizosphere soil extract than root extract. It is a derivative of thieno[2,3-*b*]thiophenes representing a class of heterocyclic compounds. 2-Phenylthieno[2,3-b]thiophene was not previously reported in any tagetes species. α-Terthienyl and 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl were the previously reported compounds present in abundance in the roots of *T. patula* with strong nematicial activity [18]. Octadec-9-enoic acid present in rhizosphere soil extract but absent in root extract reported as protein tyrosine phosphatase1B (PTP1B) inhibitor [19]. Hexadecanoic acid present as major compound in rhizosphere soil extract was known to possess antibacterial and antifungal activity [20,21]. Steroidal components stigmasterol and γ-sitosterol also known to possess biological activity [22,23].

Conclusion

In the present study 25 and 27 compounds have been identified from methanolic root extract and rhizosphere root extract of *T. patula* by gas chromatogram mass spectrometry (GC-MS) analysis. GC-MS showed the presence of thiophenes, fatty acid, fatty acid ester and steroidal components. It could be concluded that *T. patula* contained various bioactive compounds, which may be responsible for the medicinal and health care activity. The compounds leached by the roots of the plant reported in present study may have allelopathic potential.

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