Research Article

GC-MS ANALYSIS OF BIOACTIVE PHYTOCONSTITUENTS FROM *RUMEX VESICARIUS* L.

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ABSTRACT

Phytochemical constituents are responsible for medicinal activity of plant species. *Rumex vesicarius* is one of the medicinally important plants belonging to the family Polygonaceae and used in the treatment of cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative. In the present study the methanolic extract of *Rumex vesicarius* has been subjected to GC-MS analysis. Sixty chemical constituents have been identified. The major chemical constituents are n-Hexadecanoic acid (17.87%), 2(4H)-Benzofuranone, 5,6,7,7A-Tetrahydro-6-H (11.44%), 1,3-Butanedione, 1-Phenyl- (24.79%), triphenyl-Stibine (20.32%), 9,10-Anthracenedione, 1,8-dihydroxy-3-methy (31.04%) and Ethyl Oleate (15.17%). Medicinal potential of these compounds need further research on toxicological aspects to develop safe drug.

Key Words : *Rumex vesicarius* L., GC-MS analysis, Phytochemicals, flavonoid.

INTRODUCTION

Rumex vesicarius L. is a wild edible annual plant, belongs to family Polygonaceae, commonly known as “Bladder dock”. *Rumex vesicarius* L. has many important medicinal uses such as treatment of tumors, hepatic diseases, bad digestion, constipation, calculi, heart troubles, pains, diseases of the spleen, hiccup, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea. The plant also used as cooling, laxative, stomachic, tonic, analgesic, appetizer, diuretic, astringent, purgative, antispasmodic and antibacterial agents. The genus *Rumex* was also possess important biological activities, like antimicrobial, anti-inflammatory, antidiarrhoeal and antiviral. In the present study crude flavonoid extracts of the plant parts in methanolic solvents were analyzed by GC-MS technique to study the major and minor phyto-constituents of the aerial edible vegetative parts of the plant.^{1,2}

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plant free from disease was collected from the hills of Jaipur. *Rumex vesicarius* (voucher no. is RUBL 21074) was authenticated by Herbarium, Department of Botany, Rajasthan University, Jaipur, Rajasthan, India. The plant parts were washed thoroughly 2-3 times with running water and once with sterile distilled water. Leaf, stem and flower material was then air-dried on sterile blotter under shade.

Sample Preparation for GCMS Analysis

About 5g of powdered material of plant was taken in a clean, flat-bottomed glass container and soaked in 25mL of 80% methanol. The container with its content was sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whattman filter paper. The filtrate (methanolic extract) obtained for the plant was evaporated under ceiling fan and in a water bath until dried.

Gas Chromatography and Mass Spectroscopy (GC-MS)

The Gas chromatography-Mass spectrometry (GC-MS) analysis of the extracts was performed using a GC-MS. The GCMS analysis was conducted at JNU, Delhi. The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with using Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280 °C at 1 to 40°C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 ml/min). 2µl of respective diluted samples was manually injected in the split less mode³, with split ratio and with mass scan of 50-600 amu. Total running time of GC-MS is 45min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalisation. Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) and the Wiley library for mass spectra, having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations. Studying of medicinal plants also facilitates to comprehend plant toxicity and also helps to protect human and animals from natural poisons. Hence the present study was undertaken to find out the bioactive compounds present in some plant extracts by using Gas Chromatography and Mass

Spectroscopy. The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentrations (peak area %) are presented separately in Table 1-3 and the GC-MS chromatogram with peak area of each extract is also given Figure 1-3.

Totally 60 constituents was identified in the present study from all the three crude extract of plant part (stem, leaf and flower). Crude flavonoid extract of leaf part recorded highest number of (23) phytoconstituents, following the stem extract with (21) phytoconstituents, while in flower extract lower number of (16) phytoconstituents was observed which includes mainly major constituents. More than 10 constituents were commonly present in all the three extracts.

The crude flavonoid extract of leaf showed 23 constituents, the major constituent were n-Hexadecanoic acid (17.87%) , 2(4H)-Benzofuranone, 5,6,7,7A-Tetrahydro-6-H (11.44%) and Bis(2-ethylhexyl) phthalate (11.52%) in along with minor constituents were also reported (Table 1). The GC-MS chromatogram with peak area has shown in Figure 1.

The crude flavonoid extract of stem showed 21 constituents (Table 2), the major constituent were 1,3-Butanedione, 1-Phenyl- (24.79%), triphenyl- Stibine (20.32%), n-Hexadecanoic acid (12.6%) and bis(2-ethylhexyl) phthalate (5.59%) along with major constituents, minor constituents were also recorded. The GC-MS chromatogram with peak area was given in Figure 2.

The crude flavonoid extract of flower revealed 16 constituents (Table 3), the major constituents were 9,10-Anthracenedione, 1,8-dihydroxy-3-methy (31.04%), Ethyl Oleate (15.17%), Bis(2-ethylhexyl) phthalate (12.06%) and 3-Buten-2-one, 4-hydroxy-4-phenyl (4.99%) along with major constituents, minor constituents were also reported. The GC-MS chromatogram with peak area was presented in Figure 3.

The various phytochemicals which are common in the plant part, contributes to the medicinal activity of the plant (Tables 4).

Table 1: Phytochemicals identified in the Flavonoid extract of Leaf of *R. vesicarius* by GC-MS

S.No.	R. Time	Area %	Name	Molecular Formula	Molecular Weight
1.	6.042	0.99	4-(2-Methoxyethyl)phenol	C ₉ H ₁₂ O ₂	152
2.	7.684	3.66	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150
3.	9.812	1.09	Ethyl 5-oxo-2-pyrrolidinecarboxylate	C ₇ H ₁₁ NO ₃	157
4.	15.063	3.33	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans-	C ₁₆ H ₁₆	208
5.	15.37	11.44	2(4h)-Benzofuranone, 5,6,7,7a-tetrahydro-6-h	C ₁₁ H ₁₆ O ₃	196
6.	17.992	17.87	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256
7.	18.37	3.44	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284
8.	20.388	4.83	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238
9.	20.62	2.81	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308
10.	20.686	7.72	Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310
11.	20.996	2.02	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312
12.	22.178	0.79	Triphenylstibine	C ₁₈ H ₁₅ Sb	352
13.	23.491	2.61	Hexanedioic acid, bis(2-ethylhexyl) ester	C ₂₂ H ₄₂ O ₄	370
14.	24.312	2.39	Isoxazole, 5-chloro-4-(2-phenylethyl)-	C ₁₁ H ₁₀ ClNO	207
15.	24.707	0.9	Oxalic acid, 3,5-difluorophenyl tetradecyl ester	C ₂₂ H ₃₂ F ₂ O ₄	398
16.	25.567	11.52	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390
17.	25.726	1.72	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	C ₂₂ H ₂₀ OS	332
18.	29.969	1.39	2-methylhexacosane	C ₂₇ H ₅₆	380
19.	30.502	1.02	Squalene	C ₃₀ H ₅₀	410
20.	31.384	2.02	Eicosane, 3-methyl-	C ₂₁ H ₄₄	296
21.	32.177	1.11	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-tri	C ₂₇ H ₄₆ O ₂	402
22.	35.196	2.06	Vitamin E	C ₂₉ H ₅₀ O ₂	430
23.	39.3	2.4	Stigmast-5-en-3-ol, (3.β.)-	C ₂₉ H ₅₀ O	414

Table 2: Phytochemicals identified in the Flavonoid extract of Stem of *R. vesicarius* by GC-MS

S. No.	R. Time	Area %	Name	Molecular Formula	Molecular Weight
1.	9.155	24.79	1,3-Butanedione, 1-phenyl-	C ₁₀ H ₁₀ O ₂	162
2.	13.478	1.05	2-Buten-1-one, 3-amino-1-phenyl-	C ₁₀ H ₁₁ NO	161
3.	15.061	1.23	(2-Phenylcyclobutyl) benzene	C ₁₆ H ₁₆	208
4.	15.34	0.88	2(4h)-Benzofuranone, 5,6,7,7a-tetrahydro-6-h	C ₁₁ H ₁₆ O ₃	196
5.	18.009	12.6	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256
6.	18.366	2.48	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284
7.	19.082	0.98	Hexadecanoic acid, trimethylsilyl ester	C ₁₉ H ₄₀ O ₂ Si	328
8.	19.686	0.8	Dibenzoylmethane	C ₁₅ H ₁₂ O ₂	224
9.	20.398	2.78	Oleic Acid	C ₁₈ H ₃₄ O ₂	282
10.	20.61	1.78	n-Propyl 9,12-octadecadienoate	C ₂₁ H ₃₈ O ₂	322
11.	20.675	3.97	Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310
12.	20.991	1.25	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312
13.	22.214	20.32	Stibine, triphenyl-	C ₁₈ H ₁₅ Sb	352
14.	23.488	1.11	Hexanedioic acid, bis(2-ethylhexyl) ester	C ₂₂ H ₄₂ O ₄	370
15.	24.308	1.04	Benzonitrile, m-phenethyl-	C ₁₅ H ₁₃ N	207
16.	25.561	5.59	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390
17.	27.107	4.35	2,4-dibenzoyl-2,4-diamino-Nitrobenzol	C ₂₀ H ₁₅ N ₃ O ₄	361
18.	34.575	0.44	Stigmast-5-en-3-ol, oleate	C ₄₇ H ₈₂ O ₂	678
19.	35.186	2.09	Vitamin E	C ₂₉ H ₅₀ O ₂	430
20.	37.884	0.68	Stigmasterol	C ₂₉ H ₄₈ O	412
21.	39.278	2.63	Stigmast-5-en-3-ol, (3.β.)-	C ₂₉ H ₅₀ O	414

Table 3: Phytochemicals identified in the Flavonoid extract of Flower of *R. vesicarius* by GC-MS

S.No.	R. Time	Area %	Name	Molecular Formula	Molecular Weight
1.	9.142	4.99	3-Buten-2-one, 4-hydroxy-4-phenyl-	C ₁₀ H ₁₀ O ₂	162
2.	16.249	0.79	3,7,11-trimethyl-2,6,10-dodecatrienyl acetat	C ₁₇ H ₂₈ O ₂	264
3.	18.37	3.46	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284
4.	19.082	0.93	Hexadecanoic acid, trimethylsilyl ester	C ₁₉ H ₄₀ O ₂ Si	328
5.	20.617	5.65	Ethyl (9Z,12Z)-9,12-octadecadienoate	C ₂₀ H ₃₆ O ₂	308
6.	20.683	15.17	Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310
7.	20.996	2.25	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312
8.	22.178	7.98	Triphenylstibine	C ₁₈ H ₁₅ Sb	352
9.	23.057	31.04	9,10-anthracenedione, 1,8-dihydroxy-3-methy	C ₁₅ H ₁₀ O ₄	254
10.	23.491	3	Hexanedioic acid, bis(2-ethylhexyl) ester	C ₂₂ H ₄₂ O ₄	370
11.	25.563	12.06	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390
12.	27.105	1.66	2,4-dibenzoyl-2,4-diamino-Nitrobenzol	C ₂₀ H ₁₅ N ₃ O ₄	361
13.	30.504	3.18	Squalene	C ₃₀ H ₅₀	410
14.	34.224	0.95	Cholesta-4,6-dien-3-ol, (3.β.)-	C ₂₇ H ₄₄ O	384
15.	35.007	1.31	Cholesterol	C ₂₇ H ₄₆ O	386
16.	39.296	2.94	Stigmast-5-en-3-ol, (3.β.)-	C ₂₉ H ₅₀ O	414

Table 4: Activity of phyto-components identified in the methanolic extracts of the whole plant of *Rumex vesicarius* by GC-MS

S.No.	Compound Name	Nature	Activity
1	Hexadecanoic acid, ethyl ester	Ester compound	Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor ⁴
2	Benzoic acid, 4-ethoxy-,ethyl ester	Ester compound	Antifungal ⁵
3	Ethyl oleate	Ethanolic fatty acid oleic	Antibacterial ⁶
4	Octadecanoic acid, ethyl	Stearic acid estre	No activity reported ⁷
5	Squalene	triterpene	Antioxidant ^{8,9,10}
6	Stigmast-5-EN-3-OL	steroidal	Antiinflammatory, anti-pyretic, antiarthritic, anti-ulcer ¹¹
7	2-Methoxy-4-Vinylphenol	Phenolic	Antimicrobial, Antioxidant, Anti inflammatory, Analgesic ¹²
8	Vitamin E	lipid	Antioxidant ^{13,14,4}
9	Linoleic acid ethyl ester	Linoleic acid ester	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Inssectifuge ¹⁴
10	Oleic Acid	Oleic acid	Antiinflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge, Flavor ¹⁵

Figure 1: GC-MS Chromatogram of *Rumex vesicarius* L. Leaf extract Figure 2: GC-MS Chromatogram of *Rumex vesicarius* L. Stem extract Figure 3: GC-MS Chromatogram of *Rumex vesicarius* L. Flower extract

DISCUSSION

Hexadecanoic acid, ethyl ester is a ester compound of fatty acid and possess various activity like Antioxidant, Flavor, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor. 2-methoxy-4-vinylphenol is a phenolic compound having Antimicrobial, Antioxidant, Anti inflammatory, Analgesic activities.

Squalene and Vitamin E both show antioxidant activity and prevents the propagation of free radical reaction but squalene is triterpene in nature and vitamin E is a lipid soluble fatty acid. Squalene is also a antitumor agent.

Ethyl oleate, Benzoic acid, 4-ethoxy and 2-methoxy-4-vinylphenol compounds with antimicrobial properties. Stigmast-5-EN-3-OL, Linoleic acid ethyl ester and Oleic Acid compounds are with antiinflammatory properties. The presence of various bioactive compounds justifies the use of the whole plant recommended for phytopharmaceutical importance.

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