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Short Communication

Phytochemical Characterization of Rhizome, Fruit, Leaf and Callus of *Rheum emodi* Wall. using GC-MS

Ruchi Singh*, Preeti Chaturvedi

ABSTRACT

Background: *Rheum emodi* is an immensely useful medicinal herb of Himalaya having remarkable antidiabetic and anticancerous activities reported mainly from rhizomatous portion of the plant. The present study reports Gas Chromatographic- Mass spectroscopic characterization of both the conventionally used part i.e., the rhizome as well as other parts of *R. emodi* including callus to exploit the nonconventional parts for future use. **Objective:** To explore the bioactive constituents in the methanol extract of rhizome, fruit, leaf and callus of *R. emodi*. **Methods:** Phytochemical characterization of the plant extracts was performed by using GC-MS QP 2010 Plus. Various constituents were identified after matching their mass fragmentation pattern with data available in GC-MS library of National Institute of Standards Technology (NIST) and Wiley Registry of Mass Spectral Data's, New York (Wiley). **Results:** Total of 95 bioactive compounds were obtained in methanol extract of *R. emodi* out of which rhizome, fruit, leaf and callus revealed 31, 38, 27 and 47 compounds respectively. Two anthraquinones, chrysophanol (43.97%) and physcion (3.23%) were obtained from rhizome whereas fruit possessed only physcion (4.66%). **Conclusion:** The present findings characterizes and helps to supplement the chemical profile of the plant for its futuristic role in nutritional, pharmaceutical and therapeutic industries.

Key words: *Rheum emodi,* Rhizome, Fruit, Leaf, Callus, GC-MS analysis.

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INTRODUCTION

Rheum emodi Wall. (*Polygonaceae*) commonly known as Indian Rhubarb is an important medicinal herb of Himalayan region widely distributed in China, Nepal, Bhutan and India. It is distributed in the temperate and subtropical regions in India from Kashmir to Sikkim at an altitudinal range from 2800-3000 m.[1-2] The plant is used throughout the world for managing various ailments such as jaundice, headache, migraine, paralysis, sciatica, asthma, diarrhea, cancer and liver disorders etc.[3] The rhizome of *R. emodi* is the major source of anthraquinones which include emodin, emodin glycoside, chrysophanol, chrysophanol glycoside, physcion, aloe-emodin and rhein.[4] These anthraquinones are widely used in dyeing textile, paints, cosmetics, foods, imaging devices as well as its derivatives show various pharmacological activities *viz*., antiarthiritic, anti-inflammatory, antifungal, antiviral, antibacterial, anti-diabetic, laxative and neuro protective effects.[5] Besides, these anthraquinones of *R. emodi* are also responsible for anticancerous activity. Emodin and aloe-emodin inhibited the proliferation of human gastric cancer cell line MKH45;[6] rhein induced apoptotic cell death in human colon cancer cell line COLO 32DM;[7] physcion is used in treating human cervical cancer and chrysophanol induced necrosis in human liver cancer cells.[8-9] Different phytoconstituents present in *R. emodi* exhibited antioxidant activities as well.[10]

With increasing side effects of synthetic drugs, plant derived therapeutics become more promising. Rising demand of plant-based medicines necessitates screening of their bioactive constituents for novel compounds of therapeutic importance. A detailed literature review on the plant under investigation has shown that so far, there are no published reports on the chemical components of different parts of *R. emodi* other than the rhizome. The present study focuses on chemical characterization of the important Himalayan herb *viz*., *R. emodi* for isolation and identification of its important chemical constituents from both conventionally used part i.e., rhizome as well as other parts of the plant.

MATERIALS AND METHODS

Collection of Plant samples

The plant material (Rhizome and fruits) of *R. emodi* was collected from Bagheswar district (29°51'0"N 79°46'0"E, 1,004m) of Uttarakhand in the month of March, 2016. Rhizomes were grown under controlled environment facility available in College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, for further studies.

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Leaf explants obtained from the pot grown plants (3 months old) were surface sterilised with 1% bavistin and 0.1 % $HgCl_2$ and inoculated on Murashige and Skoog medium supplemented with 3% sucrose, 5.0 µM NAA, 10.0 µM BAP and 0.8 % agar. Inoculated explants were placed in culture room with 16 h photoperiod supplied by cool white fluorescent tube lights at 25 ± 2°C for callus induction. 30 days harvested callus was used for sample preparation.

Preparation of crude extract

The plant samples (Rhizomes, fruits, leaves and callus) were air dried at room temperature and grinded separately to fine powder. 2g fine powder of each sample was subjected to extraction in 250 ml of methanol using Soxhlet apparatus (Khera Instruments Pvt. Ltd., Delhi, India). The methanol extract was evaporated in a rotary evaporator (U-Tech, Star Scientific Instruments, Delhi, India) at 70°C to obtain solid mass. The solid mass was collected and stored at 4°C for further use. The extracted samples was redissolved in methanol for GC-MS analysis.

GC-MS analysis

The Gas Chromatography-Mass Spectroscopic (GC-MS) analysis of methanol extract was performed by using GC-MS (QP2010 Plus, Gas Chromatograph Mass spectrometer, SHIMADZU, USA.) equipped with Rxi ®-5Sil MS capillary GC column (5% phenyl 95% dimethyl polysiloxane) with 30 m length, 0.25mm dia. and 0.25µm film thickness. Flow rate of mobile phase (Carrier gas: He) was set at 1.21 ml/min. Temperature (Oven temperature) was set at 80°C subsequently raised to 250°C at 5°C/min and injected with 1µl volume. Samples dissolved in methanol were run fully at range of 40-700 m/z.

Identification of compounds

The bioactive compounds obtained by GC-MS were identified after matching their mass fragmentation pattern with data available in GC-MS library of National Institute of Standards Technology (NIST) and Wiley Registry of Mass Spectral Data's, New York (Wiley). The relative percentage of each constituent was expressed as percentage with peak area normalization.

RESULTS AND DISCUSSION

The gas chromatograms of rhizome, leaf, fruit and callus of *R. emodi* confirmed the existence of numerous interesting compounds with different retention times. The compounds were identified through mass spectrometry attached with GC. The identified compounds and their molecular formula, molecular weight, peak area (%) are given in Table 1; nature of the compounds and their biological activities are given in Table 2. Overall, 95 bioactive compounds were obtained in methanol extract of different parts of *R. emodi* out of which rhizome, fruits, leaf and callus extracts revealed 31, 38, 27 and 47 peaks respectively. The GC-MS chromatograms of rhizome, fruits, leaves and callus are shown in Figure 1-4. Some of these compounds are unique to a specific plant part while others occurred as common compounds as shown by venn diagram in Figure 5. There are only 2 compounds that are common to all extracts whereas rhizome, fruits, leaf and callus possessed 09, 16, 16 and 18 unique compounds respectively. 16 compounds were common in rhizome and callus. In fruit and callus, 15 compounds were similar whereas in fruit and rhizome 14 compounds were common. Leaf and callus, however showed only 10 common compounds. Rhizome, fruit and leaf showed only one common compound viz., myristyl palmitate whereas rhizome, leaf and callus extracts showed methyl palmitate as a common compound.

Major bioactive compounds (%) of rhizome are: Chrysophanol (43.97), Bis (2-ethylhexyl) phthalate (21.38), Flavidin (5.09), Beta-D-Glucopyranose, 1,6-anhydro-(3.38), Physcion (3.23), Guanosine (3.16), n-Hexadecanoic acid (2.81), Diosgenin (2.65) and β-sitosterol (2.37).

Methanol extract of fruits revealed 38 bioactive compounds (%) out of which Bis (2- ethylhexyl) phthalate(24.41), Beta-D-Glucopyranose, 1,6-anhydro-(15.41), n-Hexadonic acid (7.41), cis-9-Hexadecenal (7.28), Physcion (4.66), Pyrogallol (3.84), γ-sitosterol (3.77), Myristyl palmitate (2.66), Palmitoleyl oleate (2.44), (Z)-Decyl icos-9-enoate (2.04), Patchoulol (2.01) and Lupeol (1.94) are the major compounds.

Likewise, leaf extract of *R. emodi* revealed 28 compounds(%) out of which Bis (2- ethylhexyl) phthalate (24.69), Methyl palmitate (12.82), 6-Octadecenoic acid, methyl ester, (Z)-(9.93), Diosgenin acetate (6.98), Diosgenin (6.84), 9,12-Octadecadienoic acid (Z,Z)-(4.47), β-sitosterol (3.55), Squalene (3.21) and 7, beta-hydroxydiosgenin (1.37), are the major compounds. *In vitro* grown callus extract of *R. emodi* in particular, revealed maximum number of compounds (47). Bis (2- ethylhexyl) phthalate (19.19), γ-sitosterol (9.86), Trans-2,3-dimethylthiane (9.02), Methyl palmitate (8.82), 4H-Pyran-4-one, 2,3-dihydro3,5-dihydroxy-6-methyl-(8.23), Guanosine (7.96), 4-Methyl-1-octene (3.21), Acetin, 1-mono (3.17), 2-Hydroxycyclopentadecanone (3.06), Diosgenin (2.14) and Stigmasta-3,5-dien-7-one(1.13) are the major compounds of the callus extract.

GC-MS analysis of crude extracts of different parts of *R. emodi* revealed presence of several bioactive compounds having strong biological activities such as antibacterial, antifungal, anti-inflammatory, antidiabetic, anticancerous and other prophylactic activities. Besides, the different extracts showed presence of several important anthraquinones like Chrysophanol and Physcion that are known to possess anticancer activity. The other anticancer compounds present in the extracts are 9-Octadecenoic acid(Z)-, methyl ester, Diosgenin, 7, beta-Hydroxydiosgenin, Lupeol and Pyrogallol. γ-sitosterol, β-sitosterol and Diosgenin are the important anti-diabetic compounds reported in the extracts. Lupeol, Neophytadiene, Stigmasta-3, 5-dien-7-one are anti-inflammatory compounds whereas Squalene, Vitamin E, Hexadecanoic acid, methyl ester are antimicrobial compounds reported in the extract (Table 2).[11-36]

Squalene, an important triterpene having antioxidant, immunostimulatory and anticancer properties was found in all the extracts of *R. emodi* except rhizome with maximum area in leaf (3.21%). Present study revealed that leaf of *R. emodi* can also be used as a potential source of diosgenin as rhizome, leaf and callus possessed 2.65%, 6.84% and 2.14% diosgenin respectively. Conventionally, diosgenin is obtained mainly from *Dioscorea* sp.[37] and is used in pharmaceutical industries for preparing various steroidal drugs against cancer, inflammation, hypercholesterolemia and several types of infections. γ-sitosterol, an important antidiabetic compound was also obtained in fruit (3.77%) and callus (9.86%) extracts of *R. emodi*. The present study reports for first time, the presence of important anticancerous compounds *viz*., pyrogallol (3.84%) and physcion (4.66%) in fruits of the plant. Squalene, diosgenin, γ-sitosterol, pyrogallol and physcion are reported imperative anticancerous and antidiabetic compounds that are found to be present in nonconventional parts of *R. emodi* rather than the conventionally used rhizome. Hence, other parts of the plant can also be used for medicinal purpose relinquishing the dependence on underground rhizome.

CONCLUSION

R. emodi is an important medicinal plant showing presence of several anticancer and antidiabetic compounds. *R. emodi* possess plethora of anthraquinones. Physcion, an important anticancer anthraquinone till now reported only in rhizome of *R. emodi* was also present in the fruits. Along with the anthraquinones, other secondary metabolites *viz.,* fatty acids, triterpenes, phytosterols, steroids, phenols, essentials oils were also present in different parts of *R. emodi*. These secondary metabolites are

Table 1: Phytoconstituents identified in methanol extract of different parts of *R. emodi* by GC-MS.

S.No.	Compounds	Formula	Molecular weight (g/mol)	Rhizome (Area %)	Fruit (Area %)	Leaves (Area %)	Callus (Area %)
1	Guanosine	$C_{10}H_{13}N_5O_5$	283.244	3.16	-	-	7.96
2	Beta-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162.141	3.38	15.41	-	1.53
3	Hexadecanoic acid, methyl ester	$C_7H_{14}O_5$	270.457	0.98	0.99	-	0.46
4	2,6,10-Dodecatrienal, 3,7,11-trimethyl	$C_{15}H_{24}O$	220.350	-	-	1.16	-
5	Beta.-Amyrone	$C_{23}H_{34}O_4$	372.505	-	-	-	0.22
6	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	2.81	7.41	-	-
7	Isopropyl palmitate	$C_{19}H_{38}O_2$	298.511	0.51	1.08	-	0.40
8	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	0.18	-	12.82	8.82
9	Eicosane	$C_{20}H_{42}$	282.556	-	0.61	-	-
10	Cholesterol chloroformate	$C_{28}H_{45}ClO_2$	449.12	-	-	2.07	-
11	Diosgenin acetate	$C_{29}H_{44}O_4$	456.66	-	-	6.98	1.26
12	Trans-2,3-dimethylthiane	$C_7H_{14}S$	130.24	-	-		9.02
13	Dicyclohexyl phthalate	$C_{20}H_{26}O_4$	330.424	-	-	0.81	-
14	3-Bromocholest-5-ene	$C_{27}H_{45}Br$	449.51	0.28	1.55	-	0.42
15	Physcion	$C_{16}H_{12}O_5$	284.267	3.23	4.66	-	-
16	Diosgenin benzoate	$C_{34}H_{46}O_4$	518.727	1.07	-	-	-
17	Cholesta-4,6-dien-3-ol,	$C_{27}H_{44}O$	384.64	0.59	1.69	-	1.31
18	Diosgenin	$C_{27}H_{42}O_3$	414.63	2.65	-	6.84	2.14
19	Myristyl palmitate	$C_{30}H_{60}O_2$	452.808	0.29	1.43	0.46	-
20	Trisdibutylphenyl phosphite	$C_{42}H_{63}O_3P$	646.92	0.31	1.34	-	1.02
21	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410.68	0.42	-	-	1.13
22	7.beta.-Hydroxydiosgenin	$C_{29}H_{46}O$	410.686	0.34	-	-	-
23	Cholestenone	$C_{27}H_{44}O$	384.64	0.29	-	-	0.28
24	Tetracosamethyl-cyclododecasiloxane,	$C_{24}H_{72}O_{12}Si_{12}$	889.84	1.27	0.76	-	0.22
25	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662.93	0.76	-	-	2.93
26	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	741.54	0.75	-	-	-
27	Silane, 2-butenylmethoxymethylphenyl-	$C_{12}H_{18}OSi$	206.35	-	0.42	-	-
28	Carvone	$C_{10}H_{14}O$	150.221	-	0.88	-	0.28
29	2-Hydroxycyclopentadecanone	$C_{15}H_{28}O_2$	240.38	-	-	-	3.06
30	Stearic acid	$C_{18}H_{36}O_2$	284.48	-	-	-	0.98
31	4 [(Trimethylsilyl)oxy]cyclohexanol	$C_9H_{20}O_2Si$	188.342	-	0.99	-	-
32	Patchoulol	$C_{15}H_{26}O$	222.372	-	2.01	-	-
33	Neophytadiene	$C_{20}H_{38}$	278.515	-	0.58	1.99	0.66
34	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	593.232	-	0.32	-	-
35	Tetracosyl acetate	$C_{26}H_{52}O_2$	396.70	0.51	-	-	-
36	Heptadecane	$C_{17}H_{36}$	240.475	-	0.32	-	-
37	3-(3-Methylbutyl)thiophene-1,1-dioxide	$C_9H_{14}O_2S$	186.0	-	-	-	0.34
38	Silicone oil			-	0.66	-	-
39	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{34}O_2$	294.479	0.18	1.11	-	-
40	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.564	-	0.44	-	0.39
41	Squalene	$C_{30}H_{50}$	410.73	-	1.12	3.21	0.19
42	Hexacosane	$C_{26}H_{54}$	366.71	-	1.08	-	-
43	Tetratetracontane	$C_{44}H_{90}$	619.204	-	0.41	-	-
44	Triphenyl phosphate	$C_{18}H_{15}O_4P$	326.28	0.36	-	-	-
45	Dicyclohexyl phthalate (Isocarnosol)	$C_{20}H_{26}O_4$	330.424	1.09	-	-	-
46	1H-purin-6-amine	$C_{12}H_{10}FN_5$	243.245	-	1.19	-	-
47	Gamma-sitosterol	$C_{29}H_{50}O$	414.718	-	3.77	-	9.86
48	Lupeol	$C_{30}H_{50}$	426.729	-	1.94	-	-

Continued...

Table 1: Cont'd.

S.No.	Compounds	Formula	Molecular weight (g/mol)	Rhizome (Area %)	Fruit (Area %)	Leaves (Area %)	Callus (Area %)
49	Digitoxigenin-4-en	$C_{23}H_{32}O_4$	372.505	-	-	-	0.39
50	Pentadecanoic acid, isopropyl ester	$C_{18}H_{36}O_2$	284.48	-	-	1.15	-
51	(Z)-Decyl icos-9-enoate	$C_{30}H_{58}O_2$	450.78	-	2.04	-	-
52	1,2,4-Trimethyl-3-nitrobicyclo [3.3.1] nonan-9-one	$C_{12}H_{19}NO_3$	225.288	-	-	0.49	-
53	Methyl 3-oxoheptanoate	$C_8H_{14}O_3$	158.197	-	-	-	1.21
54	2,6-di-tert-butyl-4-methylphenol	$C_{15}H_{24}O$	220.35	-	-	-	0.34
55	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.424	-	-	0.78	-
56	Phthalic acid, heptyl trans-hex-3-enyl ester	$C_{21}H_{30}O_4$	346.46	-	-	1.21	-
57	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.44	0.15	0.25	4.74	1.25
58	Phthalic acid, cyclobutyl isobutyl ester	$C_{16}H_{20}O_4$	276.332	-	-	0.73	0.38
59	1-Octadecyne	$C_{18}H_{34}$	250.47	-	-	0.95	-
60	cis-9-Hexadecenal	$C_{16}H_{30}O$	238.415	-	7.28	-	-
61	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296.48	-	-	9.93	-
62	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.434	-	-	2.67	0.55
63	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666.38	-	0.84	-	-
64	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	$C_{18}H_{26}O_3$	290.39	-	-	2.15	-
65	β-sitosterol	$C_{29}H_{50}O$	414.718	2.37	-	3.55	-
66	4-Methyl-1-octene	C_9H_{18}	126.239	-	-	-	3.21
67	Vitamin E	$C_{29}H_{50}O_2$	430.71	-	0.49	1.16	0.16
68	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.126	-	-	-	1.18
69	2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)	$C_{10}H_{14}O$	150.22	-	-	2.45	-
70	7, beta-Hydroxydiosgenin	$C_{27}H_{42}O_4$	430.0	-	-	1.37	0.24
71	Cyclohexanol, 3,5-dimethyl-	$C_8H_{16}O$	128.215	-	-	0.53	-
72	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O$	390.564	21.38	24.41	24.69	19.19
73	Acetin, 1-mono	$C_5H_{10}O_4$	134.13	-	-	-	3.17
74	Carvacrol	$C_{10}H_{14}O$	150.221	-	-	-	0.15
75	Sequirin C	$C_{17}H_{18}O_5$	302.32	0.67	-	-	-
76	Flavidin	$C_{15}H_{12}O_3$	240.258	5.09	-	-	-
77	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.177	-	-	-	1.78
78	Pyrogallol	$C_6H_6O_3$	126.111	-	3.84	-	-
79	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	-	-	-	0.15
80	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	0.74	0.60	-	0.43
81	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	-	-	-	0.33
82	1-Octadecanol	$C_{18}H_{38}O$	270.50	-	-	-	0.08
83	1-Chlorooctane	$C_8H_{17}Cl$	148.674	-	-	1.00	-
84	Palmitoleyl oleate	$C_{34}H_{64}O_2$	504.884	-	2.44	-	-
85	Methyl stearate	$C_{19}H_{38}O_2$	298.511	-	-	1.99	-
86	cis-1,2-Cyclohexanedimethanol	$C_8H_{16}O_2$	144.211	-	-	1.47	-
87	Myristyl palmitate	$C_{30}H_{60}O_2$	452.808	-	2.66	-	-
88	22,23-Methylene-cholesterol	$C_{28}H_{46}O$	398.67	-	-	-	0.47
89	alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.71	0.26	-	-	-
90	Cyclopropa[5,6]-33-norgorgostan-3-ol, 3',6-dihydro-,(3.beta.,5.beta.,6.alpha.,22.xi.,23.xi.)	$C_{30}H_{50}O$	426.71	-	-	-	0.28
91	1,2-benzenedicarboxylic acid, dicyclohexyl ester	$C_{20}H_{26}O_4$	330.42	-	-	-	1.31
92	Methyl margarate	$C_{18}H_{36}O_2$	284.484	-	0.34	-	-
93	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.125	-	-	-	8.23
94	Stigmasterol	$C_{29}H_{48}O$	412.70	-	-	-	0.44
95	Chrysophanol	$C_{15}H_{10}O_4$	254.241	43.97	-	-	-

Table 2: Major bioactive compounds present in different parts of *R. emodi* and their uses.

S.No.	Compounds	Nature	Biological activities	References
1	Guanosine	Purine nucleoside	Antiprotozoal	Avila *et al.* 1987.[11]
2	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	Antibacterial and antifungal	Chandrasekaran *et al.* 2011.[12]
3	9-Octadecenoic acid (Z)-, methyl ester	Linoleic acid ester	Anti-cancer activity	Yu *et al.* 2005.[13]
4	9,12-Octadecadienoic acid (Z, Z)-	Fatty acid	Anti-inflammatory and antiarthritic	Jones 2002.[14]
5	Diosgenin	Phytosteroid sapogenin	Antidiabetic, reduces high blood pressure, prevent cardiovascular disease, colon cancer, in oral contraceptives manufactures.	Deshpande and Bhalsing 2014.[15]
6	7, beta-hydroxydiosgenin		Free radical scavenging, Anti-diabetic, anticancer.	Delazar *et al.* 2010;[16] Balogun *et al.* 2013.[17]
7	Squalene	Triterpene	Antioxidant, antibacterial, anticancerous, anti-tumor, immunostimulant, lipooxygenase-inhibitor.	Reddy and Couvreur 2009;[18] Raman *et al.* 2012.[19]
8	Vitamin E		Antimicrobial, antioxidant, antiinfertility, anti-inflammatory, antispasmodic.	Suriyavathana and Indupriya 2014.[20]
9	γ-Sitosterol	Triterpenoid	Antidiabetic	Balmurugan *et al.* 2011.[21]
10	Lupeol (Farganasterol)	Triterpene	Anti-inflammatory, anti-cancer.	Nguemfo *et al.* 2009;[22] Saleem *et al.* 2008.[23]
11	9-Octadecenoic acid (z)-, 9-hexadecenyles	Triterpene	Anti-inflammatory activity, Anti-cancer.	Geetha 2001;[24] Saleem 2009.[25]
12	Neophytadiene	Sesquiterpenoids	Anti-inflammatory, antipyretic, antimicrobial, analgesic.	Carretero *et al.* 2008;[26] Mendiola *et al.* 2008.[27]
13	Stigmasterol	Phytosterol	Thyroid inhibitory, antiperoxidative and hypoglycemic effects	Panda *et al.* 2009.[28]
14	Chrysophanol	Anthraquinone	Anti-cancer (Colon and liver cancer).	Lee *et al.* 2011;[29] Lu *et al.* 2010.[8]
15	Physcion	Anthraquinone	Anti-cancer (cervical cancer)	Wijesekara *et al.* 2014;[9] Pan *et al.* 2016.[30]
16	Stigmasta-3,5-dien-7-one	Steroid	Anti-inflammatory	Park *et al.* 2016.[31]
17	β-sitosterol	Sterol	Anti-diabetic	Karan *et al.* 2012;[32] Gupta *et al.* 2011.[33]
18	Pyrogallol	dihydroxy phenol	Anti –cancer (human lung cancer).	Yang *et al.* 2009[34]; Han and Park 2017;[35] Mitsuhashi *et al.* 2008.[36]

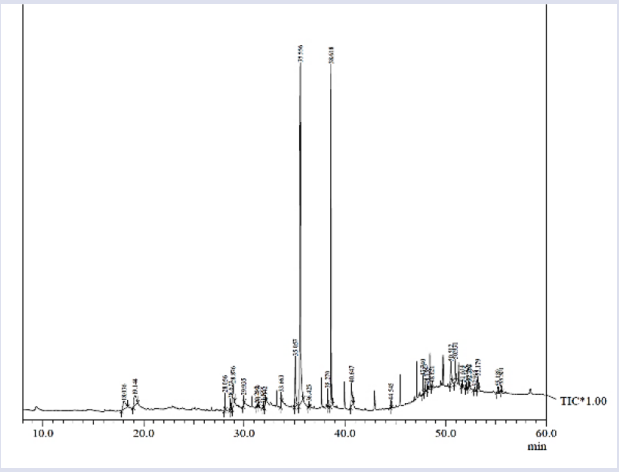

Figure 1: GC-MS chromatogram of rhizome extract of *R. emodi*.

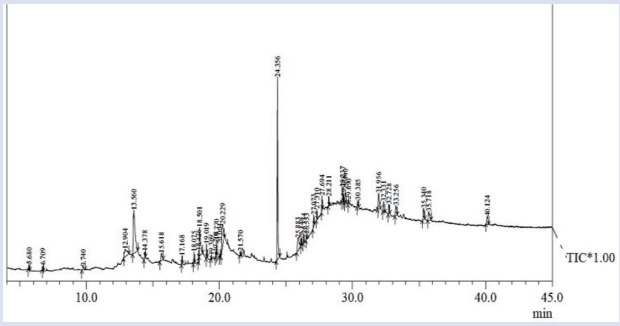

Figure 2: GC-MS chromatogram of fruit extract of *R. emodi*.

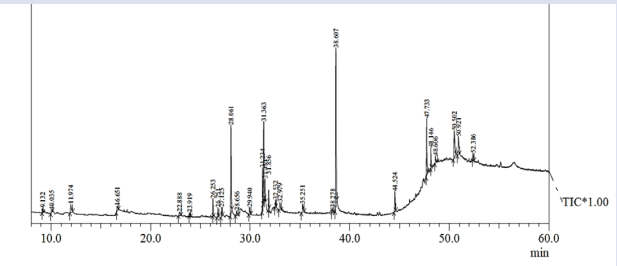

Figure 3: GC-MS chromatogram of leaf extract of *R. emodi*.

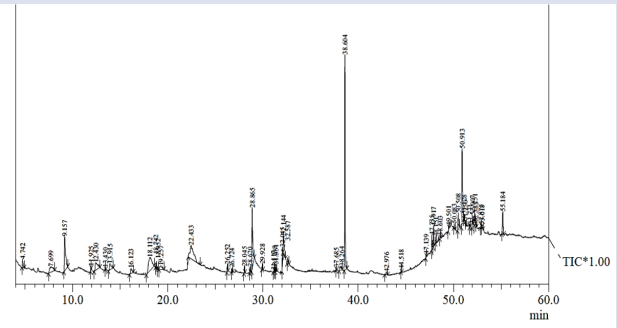

Figure 4: GC-MS chromatogram of callus extract of *R. emodi*.

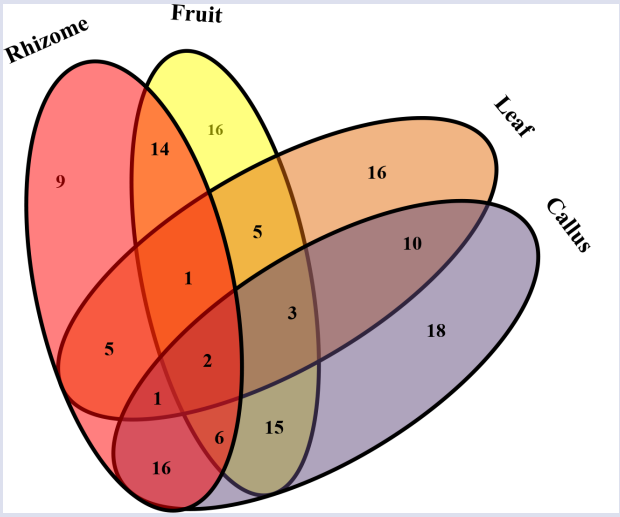

Figure 5: Venn diagram represented the unique and common compounds in different parts of *R. emodi*.

known to possess anticancerous, anti-diabetic, anti-inflammatory, antimicrobial properties. Presence of diosgenin in rhizome, leaf and callus of *R. emodi* indicates that these parts of the plant as well as *in vitro* grown callus can be used as an alternative source of this important anticancerous and antidiabetic compound. The findings of the present study provide complete GC-MS profile of the plant giving possible alternative sources of important bioactive compounds supplementing nutritional and pharmaceutical applications of the plant.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectroscopy; **NAA:** Napthalene acetic acid; **BAP:** 6-Benzylaminopurine; **MS:** Murashige and Skoog; **NIST:** National Institute of Standards Technology.

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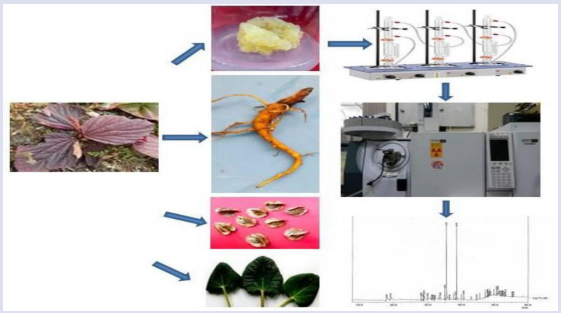
GRAPHICAL ABSTRACT



SUMMARY

- Gas Chromatographic-mass spectroscopic characterization of methanol extracts of rhizome, aerial parts and callus of *R. emodi* yielded 95 bioactive compounds which are known to possess antibacterial, antifungal, anti-inflammatory, antidiabetic, anticancerous and other prophylactic activities. Two anthraquinones (anti-cancerous compound) *viz.*, chrysophanol (43.97%) and physcion (3.23%) were obtained from rhizome part whereas fruit possessed only physcion (4.66%).

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