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Chemometric Profile of Root Extracts of *Rhodiola imbricata* Edgew. with Hyphenated Gas Chromatography Mass Spectrometric Technique

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Abstract

Rhodiola imbricata Edgew. (Rose root or Arctic root or Golden root or Shrolo), belonging to the family Crassulaceae, is an important food crop and medicinal plant in the Indian trans-Himalayan cold desert. Chemometric profile of the n-hexane, chloroform, dichloroethane, ethyl acetate, methanol, and 60% ethanol root extracts of *R. imbricata* were performed by hyphenated gas chromatography mass spectrometry (GC/MS) technique. GC/MS analysis was carried out using Thermo Finnigan PolarisQ Ion Trap GC/MS MS system comprising of an AS2000 liquid autosampler. Interpretation on mass spectrum of GC/MS was done using the NIST/EPA/NIH Mass Spectral Database, with NIST MS search program v.2.0g. Chemometric profile of root extracts revealed the presence of 63 phyto-chemotypes, among them, 1-pentacosanol; stigmast-5-en-3-ol, (3β,24S); 1-teracosanol; 1-henteracontanol; 17-pentatriacontene; 13-tetradecen-1-ol acetate; methyl tri-butyl ammonium chloride; bis(2-ethylhexyl) phthalate; 7,8-dimethylbenzocyclooctene; ethyl linoleate; 3-methoxy-5-methylphenol; hexadecanoic acid; camphor; 1,3-dimethoxybenzene; thujone; 1,3-benzenediol, 5-pentadecyl; benzenemethanol, 3-hydroxy, 5-methoxy; cholest-4-ene-3,6-dione; dodecanoic acid, 3-hydroxy; octadecane, 1-chloro; ethanone, 1-(4-hydroxyphenyl); α-tocopherol; ascaridole; campesterol; 1-dotriacontane; heptadecane, 9-hexyl were found to be present in major amount. Eventually, in the present study we have found phytosterols, terpenoids, fatty acids, fatty acid esters, alkyl halides, phenols, alcohols, ethers, alkanes, and alkenes as the major group of phyto-chemotypes in the different root extracts of *R. imbricata*. All these compounds identified by GC/MS analysis were further investigated for their biological activities and it was found that they possess a diverse range of positive pharmacological actions. In future, isolation of individual phyto-chemotypes and subjecting them to biological activity will definitely prove fruitful results in designing a novel drug.

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Introduction

To identify and evaluate the therapeutic potential of medicinal herbs, isolation of active components and structural elucidation of these compounds is very essential in medicinal chemistry and natural product research. In recent years a lot of attention has been given towards the study of organic compounds from medicinal herbs and to elucidate their pharmacological activities. Numerous extraction techniques and analytical systems like spectrophotometry, capillary electrophoresis, high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), gas chromatography (GC) with flame ionization detection (FID), gas chromatography/mass spectrometry (GC/MS) have been developed for the analysis and characterization of active compounds from medicinal plants. GC/MS has become an ideal technique for qualitative and quantitative analysis of volatile and semi-volatile compounds of plant origin. It has the unique combination of a perfect separation system (GC) with the excellent identification and confirmation technique (MS) which has made it the best suited analytical system for plant compound characterization. Additionally, for rapid extraction and precise analysis of these active phyto-compounds, the experimental design should also be optimized to obtain enhanced recoveries, low solvent consumption, and reduced extraction time [1–5].

Rhodiola imbricata Edgew. (Rose root/Arctic root/Golden root/Shrolo), belonging to the family Crassulaceae, is an important food crop and medicinal plant in the high altitude region of Indian trans-Himalayan cold desert. It is a popular medicinal plant in Pakistan, Nepal, India, Tibet, China, and many other countries and is widely used as food and traditional medicine around the world. A number of metabolites like phenylpropanoids, phenylethanol derivatives, flavanoids, terpenoids, and phenolic acids have been found in good quantity from these *Rhodiola* species and extracts of these plant species, particularly those from roots, have been shown to possess pharmacological activities. A survey of the literature showed that *Rhodiola* species influence a number of

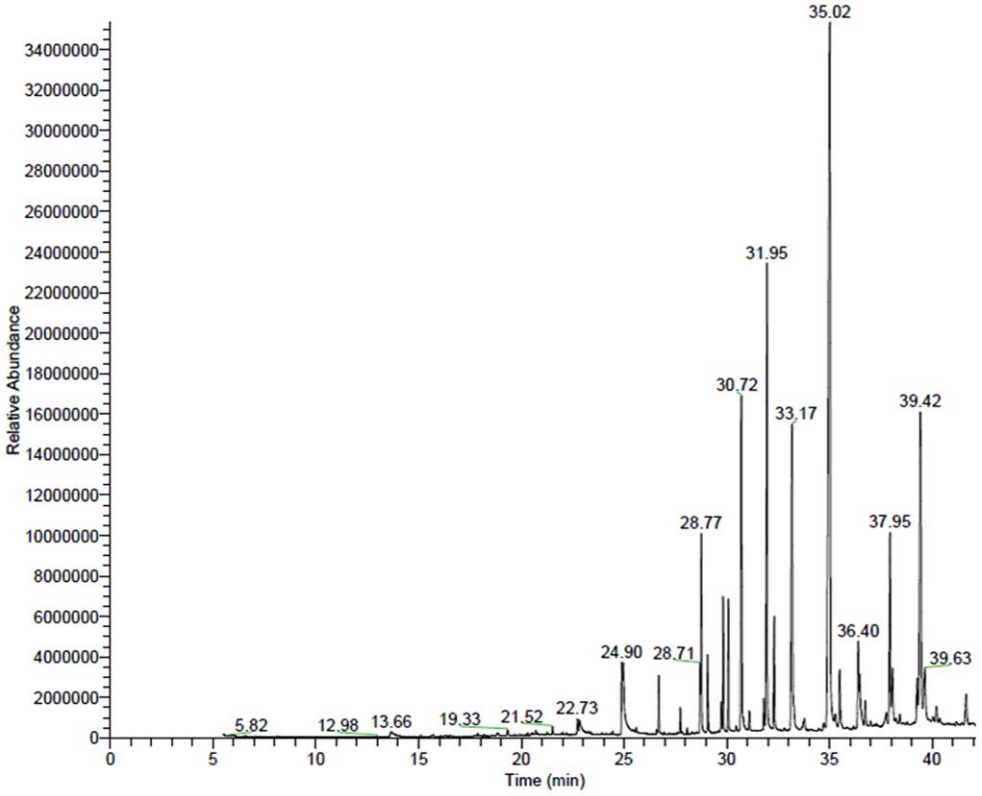

Figure 1. GC/MS chromatogram of n-hexane root extract of *R. imbricata*.
doi:10.1371/journal.pone.0052797.g001

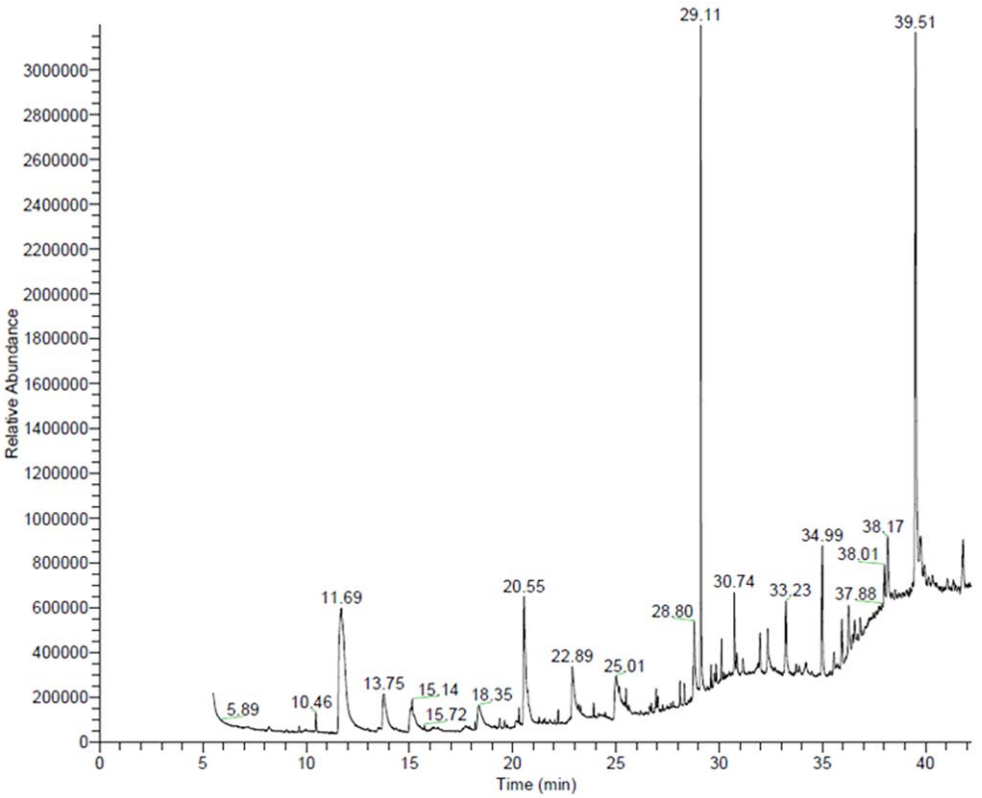

Figure 2. GC/MS chromatogram of chloroform root extract of *R. imbricata*.
doi:10.1371/journal.pone.0052797.g002

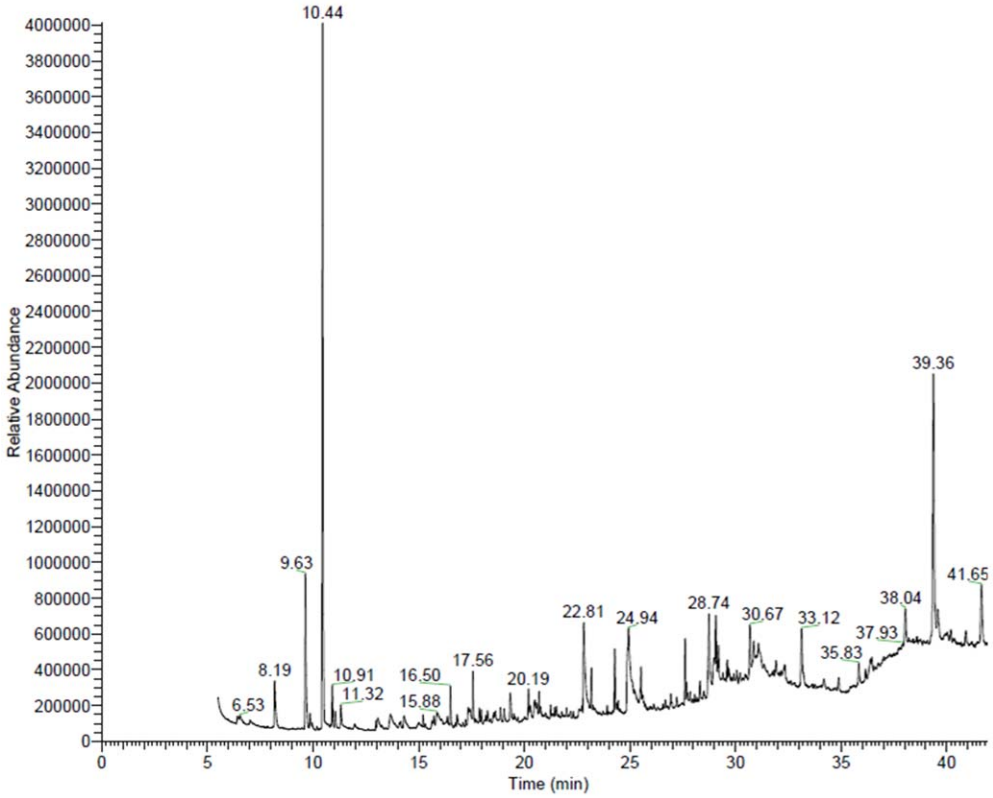

Figure 3. GC/MS chromatogram of dichloroethane root extract of *R. imbricata*.
doi:10.1371/journal.pone.0052797.g003

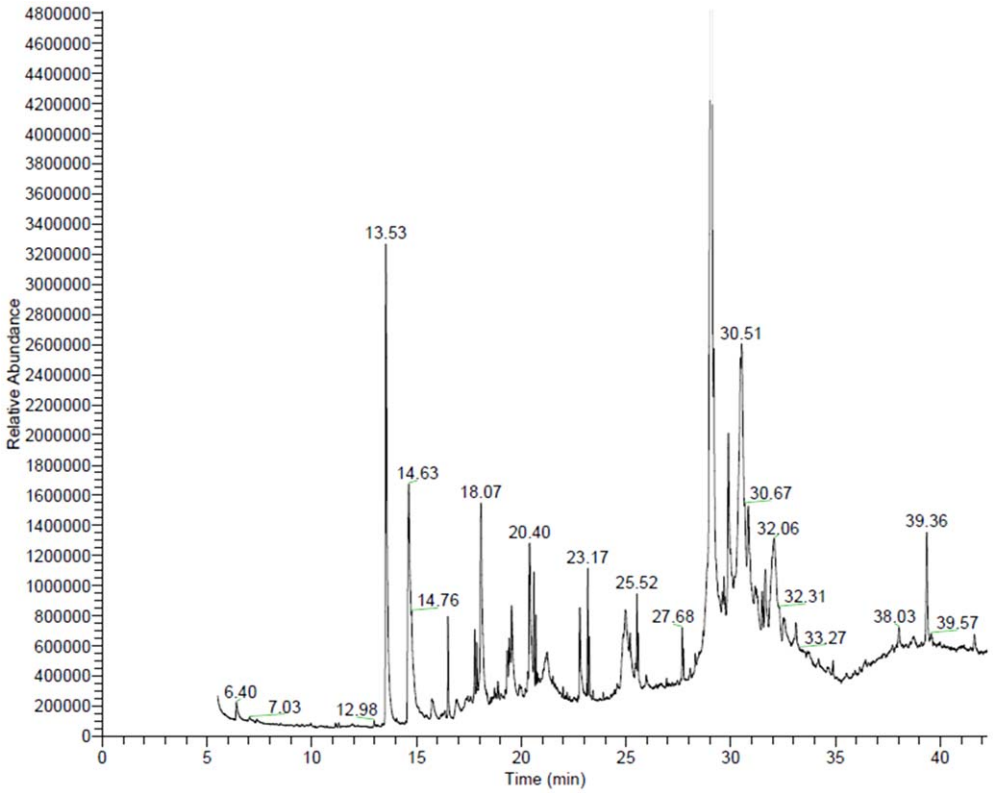

Figure 4. GC/MS chromatogram of ethyl acetate root extract of *R. imbricata*.
doi:10.1371/journal.pone.0052797.g004

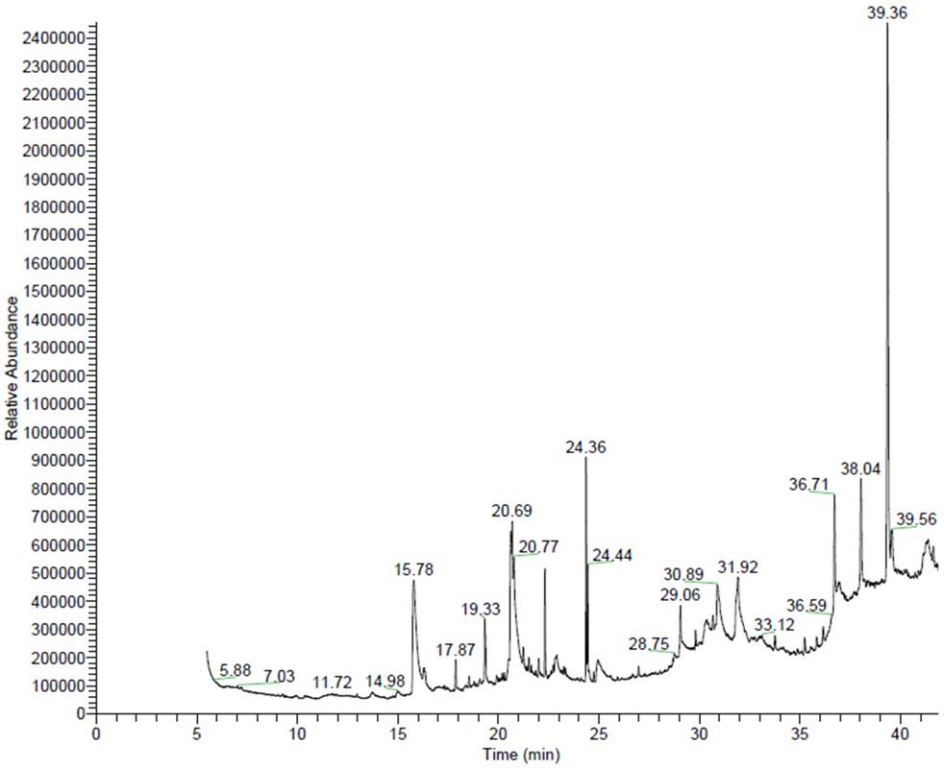

Figure 5. GC/MS chromatogram of methanol root extract of *R. imbricata*.
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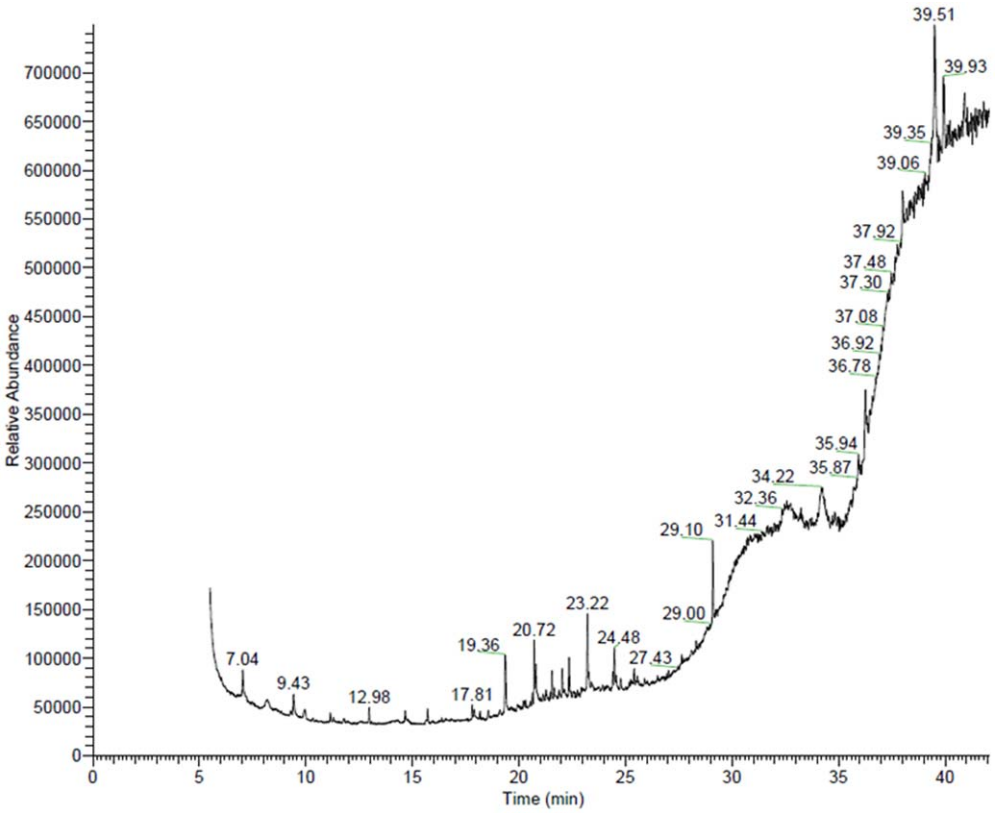

Figure 6. GC/MS chromatogram of 60% ethanol root extract of *R. imbricata*.
doi:10.1371/journal.pone.0052797.g006

Table 1. Phyto-chemotypes identified in the n-hexane root extract of *R. imbricata* by GC/MS.

S. No.	Peak RT (min)	Peak area	Peak area (%)	Compound detected	Hit	SI	RSI	Prob	CAS No	Mol. Formula	Mol. Wt.
1	13.66	3489208	0.46	3-Methoxy-5-methylphenol	1	838	874	72.98	3209-13-0	$C_8H_{10}O_2$	138
2	22.84	9266063	1.16	Hexadecanoic acid	1	801	842	73.13	57-10-3	$C_{16}H_{32}O_2$	256
3	24.91	34516141	4.16	Ethyl linoleate	1	839	876	17.81	544-35-4	$C_{20}H_{36}O_2$	308
4	26.69	6791237	0.9	1-Tetratetracontane	1	827	829	10.27	7098-22-8	$C_{44}H_{90}$	618
5	27.74	3769798	0.48	1-Pentatriacontane	1	823	838	11.01	630-07-9	$C_{35}H_{72}$	492
6	28.77	30375043	3.66	1-Hentriacontane	1	848	855	14.33	630-04-6	$C_{31}H_{64}$	436
7	29.07	9600422	1.2	Bis(2-ethylhexyl) phthalate	3	855	866	18.65	117-81-7	$C_{24}H_{38}O_4$	390
8	29.82	20564432	2.51	1-Tricosanol	4	807	823	6.18	05-01-3133	$C_{23}H_{48}O$	340
9	30.08	16467246	1.99	Eicosen-1-ol, cis-9	1	810	847	10.25	112248-30-3	$C_{20}H_{40}O$	296
10	30.7	56408242	7.01	17-Pentatriacontene	1	799	800	7.41	6971-40-0	$C_{35}H_{70}$	490
11	31.95	74961756	9.23	1-Tetracosanol	2	804	821	12.41	506-51-4	$C_{24}H_{50}O$	354
12	33.31	16764245	2.12	13-Docosen-1-ol, (Z)	1	798	820	9.17	629-98-1	$C_{22}H_{44}O$	324
13	33.17	68435403	8.53	1-Hentetracontanol	1	848	870	37.4	40710-42-7	$C_{41}H_{84}O$	592
14	35.01	229932016	28.21	1-Pentacosanol	1	812	823	31.85	26040-98-2	$C_{25}H_{52}O$	368
15	35.5	12157246	1.49	1,30-Triacontanediol	1	769	786	7.65	36645-68-8	$C_{30}H_{62}O_2$	454
16	36.4	29211487	3.47	1-Heptacosane	3	781	825	10.3	593-49-7	$C_{27}H_{56}$	380
17	36.74	5914760	0.73	α-Tocopherol-β-D-mannoside	1	814	865	57.26	CID 597057	$C_{35}H_{60}O_7$	592
18	37.95	54630744	6.4	13-Tetradecen-1-ol acetate	5	743	801	4.89	56221-91-1	$C_{16}H_{30}O_2$	254
19	38.08	6914671	0.9	Campesterol	1	771	794	53.27	474-62-4	$C_{28}H_{48}O$	400
20	39.39	107745880	13.4	Stigmast-5-en-3-ol, (3β,24S)	1	848	855	45.49	83-47-6	$C_{29}H_{50}O$	414
21	39.62	5803953	0.71	Stigmastanol	1	720	732	58.69	19466-47-8	$C_{29}H_{52}O$	416
22	41.64	10789041	1.28	Stigmast-4-en-3-one	1	699	874	18.43	1058-61-3	$C_{29}H_{48}O$	412

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Table 2. Phyto-chemotypes identified in the chloroform root extract of *R. imbricata* by GC/MS.

S. No.	Peak RT (min)	Peak area	Peak area %	Compound detected	Hit	SI	RSI	Prob	CAS No	Mol. Formula	Mol. Wt.
1	11.69	9872792	14.64	Methyl tri-butyl ammonium chloride	1	792	797	56.37	56375-79-2	$C_{13}H_{30}ClN$	235
2	13.75	2364627	4.16	3-Methoxy-5-methylphenol	1	808	858	67.78	3209-13-0	$C_8H_{10}O_2$	138
3	15.14	1927265	2.93	1,3-Benzenediol, 5-methyl	1	708	865	22.77	504-15-4	$C_7H_8O_2$	124
4	18.35	1520584	2.62	Benzenemethanol, 3-hydroxy-5-methoxy	1	811	860	85.91	30891-29-3	$C_8H_{10}O_3$	154
5	20.55	5041042	7.97	7,8-Dimethylbenzocyclooctene	1	770	849	30.04	99027-76-6	$C_{14}H_{14}$	182
6	22.89	2762363	4.13	Hexadecanoic acid	1	749	813	60.75	57-10-3	$C_{16}H_{32}O_2$	256
7	25.01	3042142	4.75	Ethyl linoleate	4	736	854	17.03	544-35-4	$C_{20}H_{36}O_2$	308
8	28.8	2066650	3.21	Benzene sulfonic acid, 4-amino-3-nitro	4	582	652	2.29	616-84-2	$C_6H_6N_2O_5S$	218
9	29.11	7589941	11.5	Bis(2-ethylhexyl) phthalate	4	793	806	9.51	117-81-7	$C_{24}H_{38}O_4$	390
10	30.12	670458	1.13	Eicosen-1-ol, cis-9	1	810	847	10.25	112248-30-3	$C_{20}H_{40}O$	296
11	30.74	2150315	3.38	17-Pentariacontene	1	799	800	7.41	6971-40-0	$C_{35}H_{70}$	490
12	31.98	1129772	1.86	1-Tetracosanol	2	804	821	12.41	506-51-4	$C_{24}H_{50}O$	354
13	33.23	1665168	2.54	1-Hentetracontanol	1	848	870	37.4	40710-42-7	$C_{41}H_{84}O$	592
14	34.99	2475797	3.82	1-Pentacosanol	8	616	763	0.8	26040-98-2	$C_{25}H_{52}O$	368
15	36.83	898525	1.31	α-Tocopherol	3	571	702	11.35	59-02-9	$C_{29}H_{50}O_2$	430
16	38.17	2267712	3.94	Campesterol	1	682	779	20.92	474-62-4	$C_{28}H_{48}O$	400
17	39.51	16343303	24.29	Stigmast-5-en-3-ol, (3β,24S)	1	798	823	43.31	83-47-6	$C_{29}H_{50}O$	414
18	41.82	1236875	1.82	Stigmast-4-en-3-one	1	540	625	8.51	1058-61-3	$C_{29}H_{48}O$	412

doi:10.1371/journal.pone.0052797.t002

Table 3. Phyto-chemotypes identified in the dichloroethane root extract of *R. imbricata* by GC/MS.

S. No.	Peak RT (min)	Peak area	Peak area %	Compound detected	Hit	SI	RSI	Prob	CAS No	Mol. Formula	Mol. Wt.
1	8.19	1187393	1.95	Eucalyptol	2	800	829	51.1	470-82-6	$C_{10}H_{18}O$	154
2	9.64	2910856	4.73	Thujone	1	835	845	23.35	546-80-5	$C_{10}H_{16}O$	152
3	10.44	11188301	0.85	Camphor	1	837	854	25.13	76-22-2	$C_{10}H_{16}O$	152
4	10.91	742204	1.22	Borneol	1	866	878	28.4	464-45-9	$C_{10}H_{18}O$	154
5	11.32	552901	0.91	β-fenchyl alcohol	1	765	836	10.05	470-08-6	$C_{10}H_{18}O$	154
6	13.06	631861	1.02	Benzenethiol, 4-chloro	1	599	670	34.81	106-54-7	C_6H_5ClS	144
7	13.66	898266	1.47	3-Methoxy-5-methylphenol	1	759	851	50.29	3209-13-0	$C_8H_{10}O_2$	138
8	15.88	2126709	3.27	Methanol, (4-carboxymethoxy) benzoyl	1	694	746	16.43	80099-44-1	$C_{10}H_{10}O_5$	210
9	16.5	571000	0.94	Phenol, 2,4-bis(1,1-dimethylethyl)	2	842	865	22.66	96-76-4	$C_{14}H_{22}O$	206
10	17.36	885243	1.44	1-Chloro-2,4-dimethoxybenzene	1	635	756	24.81	7051-13-0	$C_8H_9ClO_2$	172
11	17.56	926151	1.49	Linalyl isovalerate	1	751	812	14.87	50649-12-2	$C_{15}H_{26}O_2$	238
12	19.33	953645	1.58	1-Dotriacontane	1	787	803	45.91	544-85-4	$C_{32}H_{66}$	450
13	20.49	975679	1.66	Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)	1	661	835	45.49	7507-89-3	$C_9H_{10}O_4$	182
14	22.81	4154976	6.55	Hexadecanoic acid	1	801	855	70.5	57-10-3	$C_{16}H_{32}O_2$	256
15	23.18	1089256	2.18	Oleic acid	1	772	798	49.32	112-80-1	$C_{18}H_{34}O_2$	282
16	24.28	1364685	2.11	Bacteriochlorophyll-c-stearyl	1	755	767	13.97	CID5367801	$C_{52}H_{72}MgN_4O_4$	840
17	24.94	6245279	9.95	Ethyl linoleate	1	783	883	10.73	544-35-4	$C_{20}H_{36}O_2$	308
18	27.61	1330635	2.16	Hexanedioic acid, bis(2-ethylhexyl) ester	1	695	773	29.62	103-23-1	$C_{22}H_{42}O_4$	370
19	28.74	2480706	3.96	Benzene sulfonic acid, 4-amino-3-nitro	6	590	655	1.81	616-84-2	$C_6H_6N_2O_5S$	218
20	29.1	5070293	8.15	1,3-Dimethoxybenzene							
21	33.12	1747914	2.74	1-Hentetracontanol	4	675	828	4.63	40710-42-7	$C_{41}H_{84}O$	592
22	38.04	2440601	3.88	Campesterol	1	652	759	13.55	474-62-4	$C_{28}H_{48}O$	400
23	39.36	9725435	15.42	Stigmast-5-en-3-ol, (3β,24S)	1	814	853	63.99	83-47-6	$C_{29}H_{50}O$	414
24	40.9	366122	0.6	Stigmast-3,5-dien-7-one	1	512	760	71	2034-72-2	$C_{29}H_{46}O$	410
25	41.65	1869647	2.84	Stigmast-4-en-3-one	1	628	856	22.86	1058-61-3	$C_{29}H_{48}O$	412

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physiological functions including neurotransmitter levels, central nervous system activity, and cardiovascular function. It is being used to stimulate the nervous system, decrease depression, enhance work performance, eliminate fatigue, and prevents high-altitude sickness. Most of these effects have been ascribed to constituents such as salidrosides (rhodiolosides), rosavins, and p-tyrosol. Many pharmacological studies on *R. imbricata* have demonstrated that this plant exhibits cardioprotective, anti-inflammatory, antistress, dermal wound healing, and adaptogenic activities. It has also been found to possess antioxidant, antiaging, immuno-stimulant, radioprotective, and anticarcinogenic properties [6–24]. All these reports validate its use in traditional system of medicine.

However, the phytochemistry of the most important plant part having the medicinal and therapeutic potential, the root of *R. imbricata* has not been studied in considerable details. Hence, aim of the present investigation was to identify and quantify the chemotypes extracted successively in different solvents such as n-hexane, chloroform, dichloroethane, ethyl acetate, methanol, and 60% ethanol, from roots of *R. imbricata* from trans-Himalayan cold desert of Ladakh, India, by hyphenated GC/MS technique.

Materials and Methods

Chemicals

n-Hexane, chloroform, dichloroethane, ethyl acetate, methanol, ethanol, and water CHROMASOLV HPLC grade and all other chemicals used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Ethics statement

All necessary permits were obtained for the described field studies. The permit was issued by Dr. B. Balaji (IFS), Divisional Forest Officer, Leh Forest Division, Jammu & Kashmir, India.

Plant materials and extraction

R. imbricata roots were collected from the trans-Himalayan region (Chang-La Top, altitude = 5330 m above mean sea level, Indus valley, Ladakh) of India in the month of October, 2011 after the period of senescence, with the prior permission from the local authorities. The plant roots were washed thoroughly and cut into small pieces and shade dried at room temperature for 15 days. Then they were finely powdered and used for extraction. The root powder (20 gm) was taken for the sequential extraction in six solvent systems with increasing polarity *viz*. n-hexane, chloroform, dichloroethane, ethyl acetate, methanol, and 60% ethanol by Soxhlet apparatus (Borosil GlassWorks Limited, Worli, Mumbai, India) at 40°C. The extracted fractions were concentrated under

Table 4. Phyto-chemotypes identified in the ethyl acetate root extract of *R. imbricata* by GC/MS.

S. No.	Peak RT (min)	Peak area	Peak area %	Compound detected	Hit	SI	RSI	Prob	CAS No	Mol. Formula	Mol. Wt.
1	10.11	18961417	4.16	3-Methoxy-5-methylphenol	1	880	881	78.08	3209-13-0	$C_8H_{10}O_2$	138
2	14.63	17158116	8.4	1,3-Benzenediol, 5-methyl	1	909	929	70.69	504-15-4	$C_7H_8O_2$	124
3	16.5	1721777	0.85	Phenol, 2,4-bis(1,1-dimethylethyl)	1	860	887	30.17	96-76-4	$C_{14}H_{22}O$	206
4	17.78	1213849	0.61	1-Dodecanol, 3,7,11-trimethyl	1	696	721	3.99	6750-34-1	$C_{15}H_{32}O$	228
5	18.07	11340896	5.75	Benzenemethanol, 3-hydroxy-5-methoxy	1	866	868	75.89	30891-29-3	$C_8H_{10}O_3$	154
6	19.54	7034263	3.44	Phenol, 3,5-dimethoxy acetate	7	636	829	4.41	23133-74-6	$C_{10}H_{12}O_4$	196
7	20.4	7323033	3.57	7,8-Dimethylbenzocyclooctene	1	803	870	51.6	99027-76-6	$C_{14}H_{14}$	182
8	20.6	4129209	2.12	Eicosen-1-ol, cis-9	1	750	771	5.19	629-96-9	$C_{20}H_{40}O$	296
9	21.22	6328149	2.95	α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	1	720	759	37.89	597-12-6	$C_{18}H_{32}O_{16}$	504
10	22.8	3545343	1.84	Hexadecanoic Acid	1	795	837	71.82	57-10-3	$C_{16}H_{32}O_2$	256
11	23.17	2659100	1.34	Oleic acid	1	779	794	11.53	112-80-1	$C_{18}H_{34}O_2$	282
12	24.97	9179686	4.46	Dodecanoic acid, 3-hydroxy	1	674	706	35.46	1883-13-2	$C_{12}H_{24}O_3$	216
13	25.52	2261098	1.14	Bacteriochlorophyll-c-stearyl	1	722	739	6.88	CID5367801	$C_{52}H_{72}MgN_4O_4$	840
14	27.68	1352685	0.72	17-Pentatriacontene	1	720	737	46.41	6971-40-0	$C_{35}H_{70}$	490
15	29.08	76996880	27.61	1,3-Dimethoxybenzene	2	712	767	11.52	151-10-0	$C_8H_{10}O_2$	138
16	30.51	29595091	16.9	1,3-Benzenediol, 5-pentadecyl	1	664	797	23.45	3158-56-3	$C_{21}H_{36}O_2$	320
17	32.06	11993388	5.75	Cholest-4-ene-3,6-dione	1	710	773	32.12	984-84-9	$C_{27}H_{42}O_2$	398
18	39.36	4575754	2.12	Stigmast-5-en-3-ol, (3β,24S)	1	757	821	54.07	83-47-6	$C_{29}H_{50}O$	414
19	41.64	684162	0.32	Stigmast-4-en-3-one	1	508	755	16.81	1058-61-3	$C_{29}H_{48}O$	412

doi:10.1371/journal.pone.0052797.t004

Table 5. Phyto-chemotypes identified in the methanol root extract of *R. imbricata* by GC/MS.

S. No.	Peak RT (min)	Peak area	Peak area %	Compound detected	Hit	SI	RSI	Prob	CAS No	Mol. Formula	Mol. Wt.
1	13.73	224745	0.43	3-Methoxy-5-methylphenol	1	730	810	72.09	3209-13-0	$C_8H_{10}O_2$	138
2	15.78	5548712	11.07	Ethanone, 1-(4-hydroxyphenyl)	1	884	910	60.39	99-93-4	$C_8H_8O_2$	136
3	17.87	345721	0.66	1-Dodecane	1	699	751	16.38	112-40-3	$C_{12}H_{26}$	170
4	19.33	1157289	2.21	1-Dotriacontane	1	777	785	14.73	544-85-4	$C_{32}H_{66}$	450
5	20.69	8405167	17.01	Octadecane, 1-chloro	1	734	738	22.3	386-33-2	$C_{18}H_{37}Cl$	288
6	22.32	905705	1.73	Hexadecanoic acid, methyl ester	1	793	866	61.21	112-39-0	$C_{17}H_{34}O_2$	270
7	22.89	1901742	3.67	Hexadecanoic acid	4	660	762	11.87	57-10-3	$C_{16}H_{32}O_2$	256
8	24.36	1592833	3.99	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	5	807	833	6.93	3443-82-1	$C_{21}H_{38}O_4$	354
9	24.44	821776	1.74	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	2	804	825	32.86	18465-99-1	$C_{21}H_{36}O_4$	352
10	24.96	1495805	3.35	Ethyl linoleate	10	681	835	2.99	544-35-4	$C_{20}H_{36}O_2$	308
11	29.06	1614826	3.57	1,3-Dimethoxybenzene	34	471	675	0.33	151-10-0	$C_8H_{10}O_2$	138
12	30.91	3109377	5.92	Ascaridole	1	619	700	57.05	512-85-6	$C_{10}H_{16}O_2$	168
13	31.92	3533697	7.23	Unknown	-	-	-	-	-	-	-
14	33.77	174824	0.56	δ-Tocopherol	1	667	763	89.78	119-13-1	$C_{27}H_{46}O_2$	402
15	36.71	4419092	8.42	α-Tocopherol	1	731	853	46.36	59-02-9	$C_{29}H_{50}O_2$	430
16	38.04	2615057	4.98	Campesterol	1	726	810	24.4	474-62-4	$C_{28}H_{48}O$	400
17	39.36	11245680	21.91	Stigmast-5-en-3-ol, (3β,24S)	1	813	850	67.6	83-47-6	$C_{29}H_{50}O$	414
18	41.38	902689	1.55	Stigmast-4-en-3-one	45	395	709	0.74	1058-61-3	$C_{29}H_{48}O$	412

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vacuum and reduced pressure (BUCHI Rotavapor R-205, BUCHI Labortechnik AG CH-9230, Flawil, Switzerland) at 40°C by circulation of cold water using thermostat maintained at 4°C in order to minimize the degradation of thermolabile compounds. The dry extracts were then stored in a −80°C freezer till further analysis.

Preparation of sample for GC/MS analysis

The 25 mg of concentrated n-hexane, chloroform, dichloroethane, ethyl acetate, methanol, and 60% ethanol root extracts were redissolved in the respective solvents, vortexed properly and filtered through 0.22 µm syringe filter (Millipore Corp., Bedford, MA, USA). One microlitre aliquot of the sample solution was injected into the GC/MS MS system for the requisite analysis.

Instrumentation and chromatographic conditions

GC/MS analysis was carried out on a Thermo Finnigan PolarisQ Ion Trap GC/MS MS system comprising of an AS2000 liquid autosampler (Thermo Finnigan, Thermo Electron Corporation, Austin, TX, USA). The gas chromatograph was interfaced to a mass spectrometer instrument employing the following conditions *viz*. Durabond DB-5 ms column (30 m×0.25 mm×0.25 µm),

Table 6. Phyto-chemotypes identified in the 60% ethanol root extract of *R. imbricata* by GC/MS.

S. No.	Peak RT (min)	Peak area	Peak area %	Compound detected	Hit	SI	RSI	Prob	CAS No	MF	MW
1	19.37	258681	5.69	1-Dotriacontane	1	769	805	39.82	544-85-4	$C_{32}H_{66}$	450
2	20.72	276232	5.44	Heptadecane, 9-hexyl	1	696	719	24.93	55124-79-3	$C_{23}H_{48}$	324
3	21.56	62567	1.23	Dibutyl phthalate	5	799	875	5.32	84-74-2	$C_{16}H_{22}O_4$	278
4	22.36	115053	2.27	Hexadecanoic acid, methyl ester	1	624	700	20.11	112-39-0	$C_{17}H_{34}O_2$	270
5	23.22	296422	5.84	Unknown	-	-	-	-	-	-	-
6	24.48	194215	3.83	Unknown	-	-	-	-	-	-	-
7	25.41	56168	1.11	Unknown	-	-	-	-	-	-	-
8	29.1	181582	3.58	Bis(2-ethylhexyl) phthalate	2	697	786	11.81	117-81-7	$C_{24}H_{38}O_4$	390
9	34.21	644518	12.7	Unknown	-	-	-	-	-	-	-
10	39.51	1832781	35.53	Unknown	-	-	-	-	-	-	-
11	39.95	550771	10.86	Unknown	-	-	-	-	-	-	-
12	40.92	604576	11.92	Unknown	-	-	-	-	-	-	-

doi:10.1371/journal.pone.0052797.t006

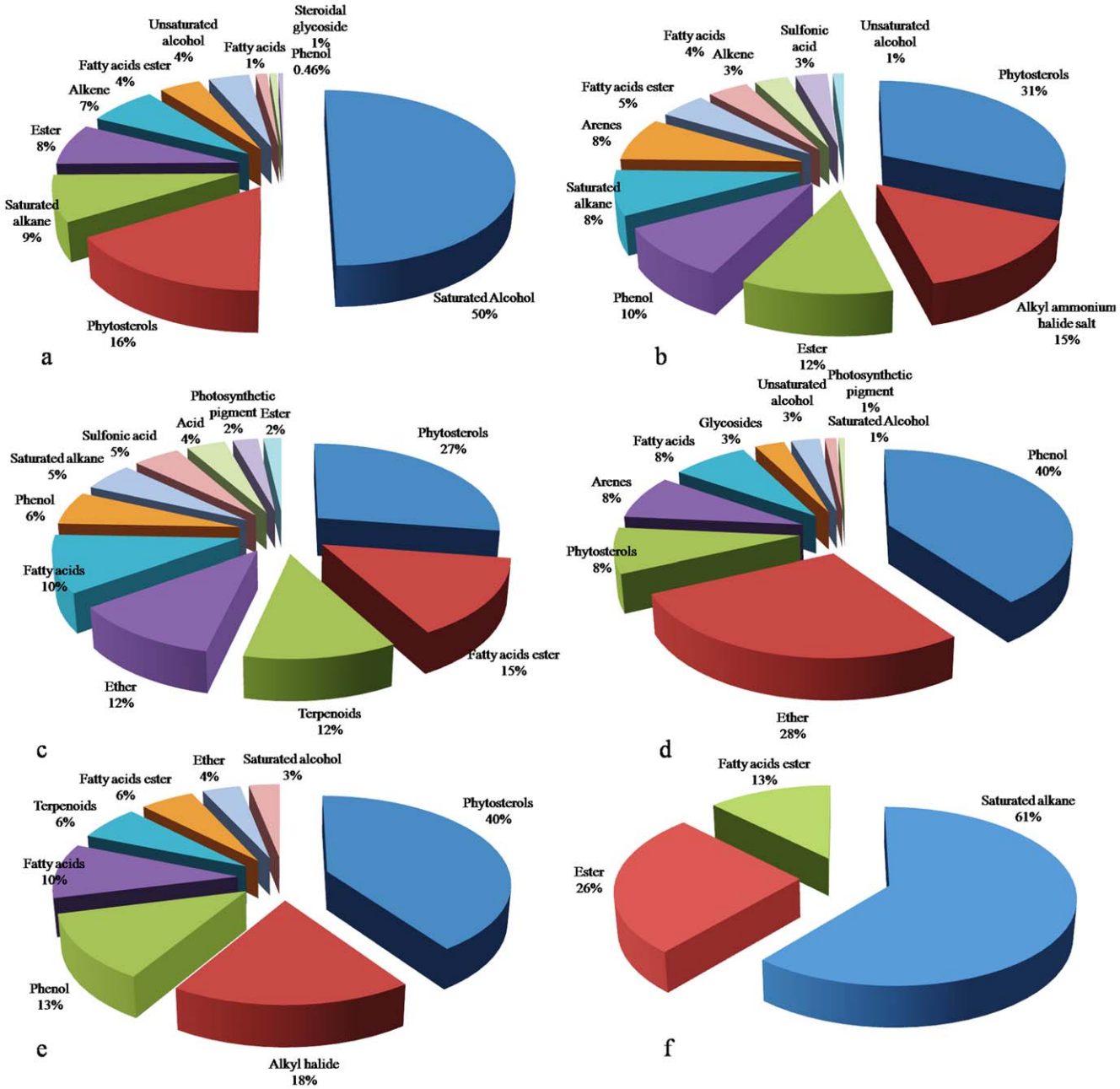

Figure 7. Estimation of major phytochemical groups in different root extracts of *R. imbricata*, a) n-hexane extract, b) chloroform extract, c) dichloroethane extract, d) ethyl acetate extract, e) methanol extract, f) 60% ethanol extract.
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operating in electron impact [electron ionisation positive (EI^+)] mode at 70 eV, helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min, an injection volume of 0.5 EI was employed (split ratio of 10:1), injector temperature 280°C, and transfer line temperature 300°C. The oven temperature was programmed from 50°C (isothermal for 2 min), with gradual increase in steps of 10°C/min, to 300°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 s, and full mass scan range from 25 m/z to 1000 m/z. The data acquisition was performed on Finnigan Xcalibur data acquisition and processing software version 2.0 (ThermoQuest, LC and LC/MS Division, San Jose, California, USA).

Identification of components

Interpretation of mass spectrum of GC/MS was done using the NIST/EPA/NIH Mass Spectral Database (NIST11), with NIST MS search program v.2.0g [National Institute Standard and Technology (NIST), Scientific Instrument services, Inc., NJ, USA]. 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.

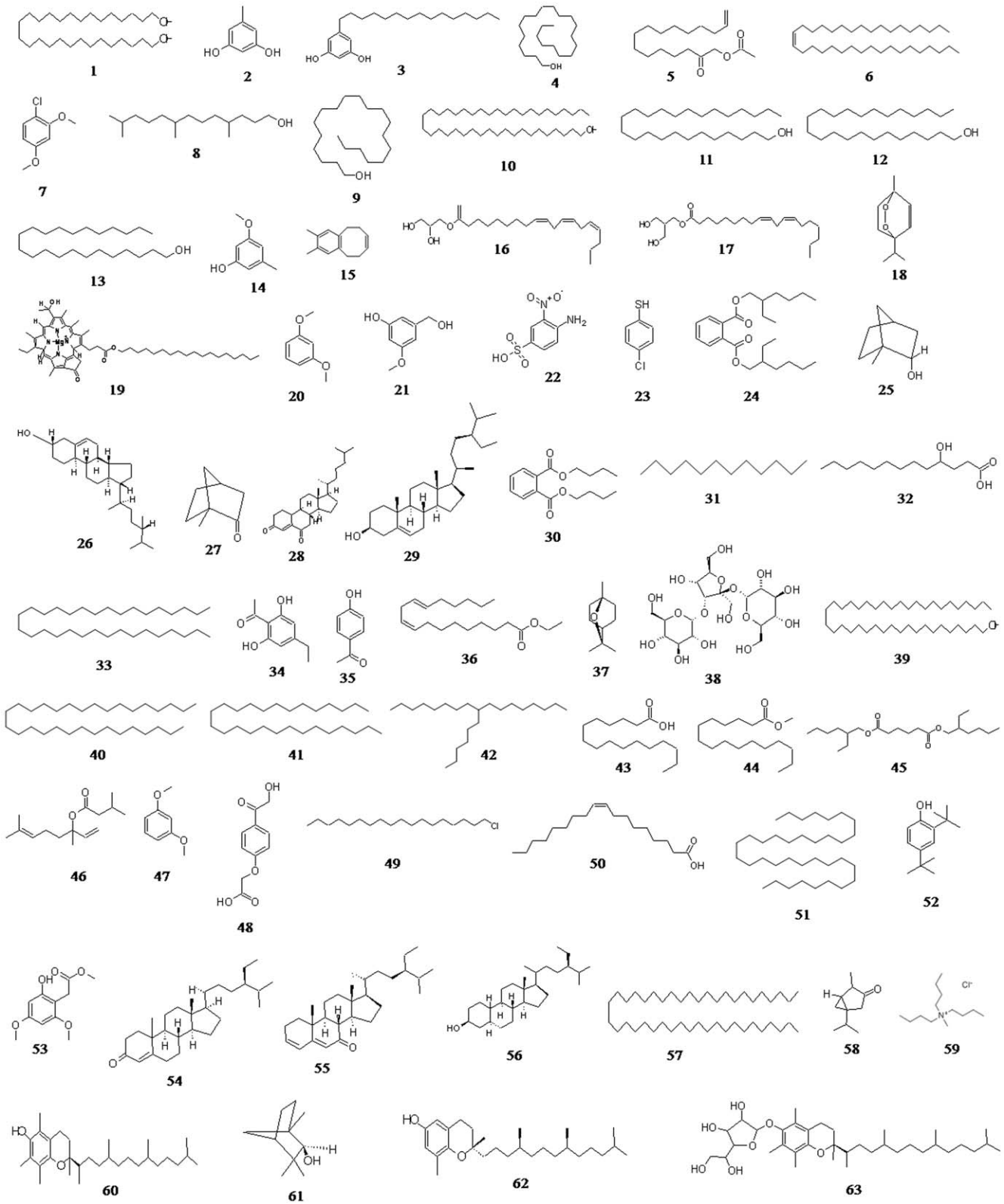

Figure 8. Phyto-chemotypes identified in different root extracts of *R. imbricata*. 1: 1,30-triacontanediol; **2:** 1,3-benzenediol, 5-methyl; **3:** 1,3-benzenediol, 5-pentadecyl; **4:** 13-docosen-1-ol, (Z); **5:** 13-tetradecen-1-ol acetate; **6:** 17-pentatriacontene; **7:** 1-chloro-2,4-dimethoxybenzene; **8:** 1-dodecanol, 3,7,11-trimethyl; **9:** eicosen-1-ol, cis-9; **10:** 1-hentetracontanol; **11:** 1-pentacosanol; **12:** 1-tetracosanol; **13:** 1-tricosanol; **14:** 3-methoxy-5-methylphenol; **15:** 7,8-dimethylbenzocyclooctene; **16:** 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z); **17:** 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester; **18:** ascaridole; **19:** bacteriochlorophyll-c-stearyl; **20:** benzene, 1,3-dimethoxy; **21:** benzenemethanol, 3-hydroxy-5-methoxy; **22:** benzene sulfonic acid, 4-amino-3-nitro; **23:** benzenethiol, 4-chloro; **24:** bis(2-ethylhexyl) phthalate; **25:** borneol; **26:** campesterol; **27:** camphor; **28:** cholest-4-ene-3,6-dione; **29:** stigmast-5-en-3-ol, (3β,24S); **30:** di-butyl phthalate; **31:** 1-dodecane; **32:**

dodecanoic acid, 3-hydroxy; **33:** 1-dotriacontane; **34:** ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl); **35:** ethanone, 1-(4-hydroxyphenyl); **36:** ethyl linoleate; **37:** eucalyptol; **38:** α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl; **39:** 1-hentetracontanol; **40:** 1-hentriacontane; **41:** 1-heptacosane; **42:** heptadecane, 9-hexyl; **43:** hexadecanoic acid; **44:** hexadecanoic acid, methyl ester; **45:** hexanedioic acid, bis(2-ethylhexyl) ester; **46:** linalyl isovalerate; **47:** 1,3-dimethoxybenzene; **48:** methanol, (4-carboxymethoxy)benzoyl; **49:** octadecane, 1-chloro; **50:** oleic acid; **51:** 1-pentatriacontane; **52:** phenol, 2,4-bis(1,1-dimethylethyl); **53:** phenol, 3,5-dimethoxy acetate; **54:** stigmast-4-en-3-one; **55:** stigmast-3,5-dien-7-one; **56:** stigmastanol; **57:** 1-tetratetracontane; **58:** thujone; **59:** methyl tri-butyl ammonium chloride; **60:** α-tocopherol; **61:** β-fenchyl alcohol; **62:** δ-tocopherol; **63:** α-tocopherol-β-D-mannoside.
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Results

GC/MS chromatograms of n-hexane (Fig. 1), chloroform (Fig. 2), dichloroethane (Fig. 3), ethyl acetate (Fig. 4), methanol (Fig. 5), and 60% ethanol (Fig. 6) root extracts of *R. imbricate* as per the experimental procedure discussed above, showed various peaks indicating the presence of different chemotypes in the respective extracts.

GC/MS chemometric profile

n-Hexane root extract. The n-hexane root extract revealed the presence of 22 different chemotypes which were characterized and identified (Table 1, Fig. 1) by comparison of their mass fragmentation patterns with the similar in NIST database library. Of these 22 chemotypes, 1-pentacosanol (28.21%), stigmast-5-en-3-ol, (3β,24S) (13.40%), 1-teracosanol (9.23%), 1-henteracontanol (8.53%), 17-pentatriacontene (7.01%), and 13-tetradecen-1-ol acetate (6.40%) were found to be major constituents whereas 1-hentriacontane (3.66%), 1-heptacosane (3.47%), 1-tericosanol (2.51%), 13-docosan-1-ol, (Z) (2.12%), eicosen-1-ol, cis-9 (1.99%) 1,30-triacontanediol (1.49%), stigmast-4-en-3-one (1.28%), bis(2-ethylhexyl) phthalate (1.20%), hexadecanoic acid (1.16%), 1-tetrateracontane (0.90%), campesterol (0.90%), α-Tocopherol-β-D-mannoside (0.73%), stigmastanol (0.71%), 1-pentatriacontane (0.48%), and 3-methoxy-5-methylphenol (0.46%) were found to be present in trace amount.

Chloroform root extract. GC/MS chemometric profile of chloroform root extract showed the presence of 18 different chemotypes (Table 2, Fig. 2). Amongst these, stigmast-5-en-3-ol, (3β,24S) (24.30%), methyl tri-butyl ammonium chloride (14.64%), bis(2-ethylhexyl) phthalate (11.50%), 7,8-dimethylbenzocyclooctene (7.97%), ethyl linoleate (4.75%), 3-methoxy-5-methylphenol (4.16%), and hexadecanoic acid (4.13%) were found to constitute major amount while, campesterol (3.94%), 1-pentacosanol (3.82%), 17-pentariacontene (3.38%), benzene sulfonic acid, 4-amino-3-nitro (3.21%), orcinol (2.93%), benzenemethanol, 3-hydroxy, 5-methoxy (2.62%), 1-hentetracontanol (2.54%), 1-tetracosanol (1.86%), stigmast-4-en-3-one (1.82%); and α-tocopherol (1.31%), and eicosen-1-ol, cis-9 (1.13%) were found to be present in trace quantity.

Dichloroethane root extract. GC/MS chemometric profile of dichloroethane root extract illustrated the presence of 25 different chemotypes (Table 3, Fig. 3). Among these, camphor (17.78%), stigmast-5-en-3-ol, (3β,24S) (15.42%), ethyl linoleate (9.95%), 1,3-dimethoxybenzene (8.15%), hexadecanoic acid (6.55%), and thujone (4.73%) were present in major amount, whereas, benzene sulfonic acid, 4-amino-3-nitro (3.96%), campesterol (3.88%), methanol, (4-carboxymethoxy) benzoyl (3.27%), stigmast-4-en-3-one (2.84%), 1-hentetracontanol (2.74%), oleic acid (2.18%), bis(2-ethylhexyl) adipate (2.16%), bacteriochlorophyll-c-stearyl (2.11%), eucalyptol (1.95%), ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl) (1.66%), 1-dotriacontane (1.58%), linalyl isovalerate (1.49%), 3-methoxy-5-methylphenol (1.47%), 1-chloro-2,4-dimethoxybenzene (1.44%), borneol (1.22%), 4-chlorothiophenol (1.02%), phenol, 2,4-bis(1,1-dimethylethyl) (0.94%), fenchyl alcohol (0.91%), and stigmast-3,5-dien-7-one (0.60%) were found to be present in trace.

Ethyl acetate root extract. Nineteen different chemotypes were identified in ethyl acetate extract (Table 4, Fig. 4). Amongst these 19 chemotypes, 1,3-dimethoxybenzene (27.61%), 1,3-benzenediol, 5-pentadecyl (16.90%), 3-methoxy-5-methylphenol (10.11%), 1,3-benzenediol, 5-methyl (8.40%), benzenemethanol, 3-hydroxy, 5-methoxy (5.75%), cholest-4-ene-3,6-dione (5.75%), and dodecanoic acid, 3-hydroxy (4.46%) were found to constitute major amount, whereas, 7,8-dimethylbenzocyclooctene (3.57%) 3,5-dimethoxyphenyl acetate (3.44%), α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl (2.95%), stigmast-5-en-3-ol, (3β,24S) (2.12%), eicosen-1-ol, cis-9 (2.12%), hexadecanoic acid (1.84%), oleic acid (1.34%), bacteriochlorophyll-c-stearyl (1.14%), phenol, 2,4-bis(1,1-dimethylethyl) (0.85%), 1-pentatricontene (0.72%), 1-dodecanol, 3,7,11-trimethyl (0.61%), and stigmast-4-en-3-one (0.32%) were found to be present in trace.

Methanol root extract. The methanol root extract revealed the presence of 18 different chemotypes (Table 5, Fig. 5). Among the identified chemotypes, stigmast-5-en-3-ol, (3β,24S) (21.91%), octadecane, 1-chloro (17.01%), ethanone, 1-(4-hydroxyphenyl) (11.07%), α-tocopherol (8.42%), ascaridole (5.92%), and campesterol (4.98%) were found to present in major amount, while, linolein, 2-mono (3.99%), hexadecanoic acid (3.67%), 1,3-dimethoxybenzene (3.57%), ethyl linoleate (3.35%), 1-dotriacontane (2.21%), linolein, 1-mono (1.74%), methyl palmitate (1.73%), stigmast-4-en-3-one (1.55%), 1-dodecane (0.66%), δ-tocopherol (0.56%), and 3-methoxy-5-methylphenol (0.43%) were found to be present in trace.

60% Ethanol root extract. GC/MS chemometric profile of 60% ethanol root extracts illustrated the presence of 12 different chemotypes (Table 6, Fig. 6). Amongst the identified chemotypes, dotriacontane (5.69%), and heptadecane, 9-hexyl (5.44%) were found to be present in major amount, whereas, bis(2-ethylhexyl) phthalate (3.58%), hexadecanoic acid, methyl ester (2.27%), and dibutyl phthalate (1.23%) were found to be present in trace.

Discussion

We have conducted the present investigation to identify the major volatile and semivolatile components in the root of *R. imbricata*. The presence of various bioactive compounds justifies the use of the plant by traditional practitioners of 'Amchi' system of medicine in trans-Himalayan Ladakh region. Also, extensive pharmacological studies were conducted by different researchers with the plant root extracts [6–23] and the results were very promising to justify the use of this plant as therapeutic agent.

However, the phytochemical profiling of the plant root still remains to be unexplored and to the best of our knowledge, this is the first ever study of its kind on the GC/MS chemometric profiling of the root extracts. In medicinal chemistry, it is very essential to ascertain the chemotyping of medicinal plant parts that are responsible for its numerous pharmacological properties and by this technique we may be able to scientifically determine and validate the traditional uses, pharmacological activities, and therapeutic potential of these plant parts. Profiling of metabolites

Table 7. Distribution of phyto-chemotypes in different root extracts of *R. imbricatea*.

Phyto-chemotypes	Root extracts					
	n-Hexane	Chloroform	Dichloroethane	Ethyl acetate	Methanol	60% Ethanol
1,30-Triacontanediol	√	–	–	–	–	–
1,3-Benzenediol, 5-methyl	–	√	–	√	–	–
1,3-Benzenediol, 5-pentadecyl	–	–	–	√	–	–
13-Docosen-1-ol, (Z)	√	–	–	–	–	–
13-Tetradecen-1-ol acetate	√	–	–	–	–	–
17-Pentatriacontene	√	√	–	√	–	–
1-Chloro-2,4-dimethoxybenzene	–	–	√	–	–	–
1-Dodecanol, 3,7,11-trimethyl	–	–	–	√	–	–
Eicosen-1-ol, cis-9	–	–	–	√	–	–
1-Hentetracontanol	√	–	√	–	–	–
1-Pentacosanol	√	√	–	–	–	–
1-Tetracosanol	√	√	–	–	–	–
1-Tricosanol	√	–	–	–	–	–
3-Methoxy-5-methylphenol	√	√	√	√	√	–
7,8-Dimethylbenzocyclooctene	–	√	–	√	–	–
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	–	–	–	–	√	–
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	–	–	–	–	√	–
Ascaridole	–	–	–	–	√	–
Bacteriochlorophyll-c-stearyl	–	–	√	√	–	–
Benzene, 1,3-dimethoxy	–	–	–	√	√	–
Benzenemethanol, 3-hydroxy-5-methoxy	–	√	–	√	–	–
Benzene sulfonic acid, 4-amino-3-nitro	–	√	√	–	–	–
Benzenethiol, 4-chloro	–	–	√	–	–	–
Bis(2-ethylhexyl) phthalate	√	√	–	–	–	√
Borneol	–	–	√	–	–	–
Campesterol	√	√	√	–	√	–
Camphor	–	–	√	–	–	–
Cholest-4-ene-3,6-dione	–	–	–	√	–	–
Stigmast-5-en-3-ol, (3β,24S)	√	√	√	√	√	–
Di-butyl phthalate	–	–	–	–	–	√
1-Dodecane	–	–	–	–	√	–
Dodecanoic acid, 3-hydroxy	–	–	–	√	–	–
1-Dotriacontane	–	–	√	–	√	√
Ethanone, 1-(2,6-dihydroxy-4-methoxyphenyl)	–	–	√	–	–	–
Ethanone, 1-(4-hydroxyphenyl)	–	–	–	–	√	–
Ethyl linoleate	√	√	√	–	√	–
Eucalyptol	–	–	√	–	–	–
α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	–	–	–	√	–	–
1-Hentetracontanol	–	√	–	–	–	–
1-Hentriacontane	√	–	–	–	–	–
1-Heptacosane	√	–	–	–	–	–
Heptadecane, 9-hexyl	–	–	–	–	–	√
Hexadecanoic acid	√	√	√	√	√	–
Hexadecanoic acid, methyl ester	–	–	–	–	√	√
Hexanedioic acid, bis(2-ethylhexyl) ester	–	–	√	–	–	–
Linalyl isovalerate	–	–	√	–	–	–

Table 7. Cont.

Phyto-chemotypes	Root extracts					
	n-Hexane	Chloroform	Dichloroethane	Ethyl acetate	Methanol	60% Ethanol
1,3-Dimethoxybenzene	–	–	√	–	–	–
Methanol, (4-carboxymethoxy)benzoyl	–	–	√	–	–	–
Octadecane, 1-chloro	–	–	–	–	√	–
Oleic acid	–	–	√	√	–	–
1-Pentatriacontane	√	–	–	–	–	–
Phenol, 2,4-bis(1,1-dimethylethyl)	–	–	√	√	–	–
Phenol, 3,5-dimethoxy, acetate	–	–	–	√	–	–
Stigmast-4-en-3-one	√	√	√	√	√	–
Stigmast-3,5-dien-7-one	–	–	√	–	–	–
Stigmastanol	√	–	–	–	–	–
1-Tetratetracontane	√	–	–	–	–	–
Thujone	–	–	√	–	–	–
Methyl tri-butyl ammonium chloride	–	√	–	–	–	–
α-Tocopherol	√	√	–	–	√	–
β-Fenchyl alcohol	–	–	√	–	–	–
δ-Tocopherol	–	–	–	–	√	–
α-Tocopherol-β-D-mannoside	√	–	–	–	–	–

√ Present; – Absent.

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in plant extracts permits the complete phenotyping of genetically or environmentally adapted plant systems and such investigations draw on simple extraction procedures that have been shown to be very robust and have permitted broad range of high-throughput applications in plant metabolomics. [25–27].

The major phytochemical groups in n-hexane, ethyl acetate, and 60% ethanol extracts were saturated alcohol (50%), phenols (40%), and alkanes (61%) respectively. On the other hand, phytosterols were the major group in chloroform (31%), dichloroethane (27%), and methanol (40%) extracts. The total of various volatile and semi volatile groups present in different root extracts of *R. imbricata* had the following distribution order: phytosterols (122%), alkanes (83%), phenols (69.46%), esters (48%), ethers (44%), fatty acid esters (43%), fatty acids (33%), terpenoids (18%), arenes (16%), alkyl ammonium halide salt (15%), alkenes (10%), sulfonic acid (8%), unsaturated alcohols (8%), organic acids (4%), saturated alcohols (4%), glycosides (3%), photosynthetic pigments (3%), steroidal glycoside (1%). The order of extraction capacities of different polarity solvents for phytosterols, phenols, fatty acids, alkanes, esters, fatty acid esters, ethers, unsaturated alcohols, arenes, terpenoids, alkenes, sulfonic acid, photosynthetic pigment, and saturated alcohols was as follows:

1. Phytosterols: methanol (40%), chloroform (31%), dichloroethane (27%), n-hexane (16%), ethyl acetate (8%)
2. Phenols: ethyl acetate (40%), methanol (13%), chloroform (10%), dichloroethane (6%), n-hexane (0.46%)
3. Fatty acids: dichloroethane (10%) = methanol (10%), ethyl acetate (8%), chloroform (4%), n-hexane (1%)
4. Alkane: 60% ethanol (61%), n-hexane (9%), chloroform (8%), dichloroethane (5%)
5. Esters: 60% ethanol (26%), chloroform (12%), n-hexane (8%), dichloroethane (2%)
6. Fatty acid esters: dichloroethane (15%), 60% ethanol (13%), methanol (6%), chloroform (5%), n-hexane (4%)
7. Ethers: ethyl acetate (28%), dichloroethane (12%), methanol (4%)
8. Unsaturated alcohols: n-hexane (4%), ethyl acetate (3%), chloroform (1%)
9. Arenes: chloroform (8%) = ethyl acetate (8%)
10. Terpenoids: dichloroethane (12%), methanol (6%)
11. Alkenes: n-hexane (7%), chloroform (3%)
12. Sulfonic acid: dichloroethane (5%), chloroform (3%)
13. Photosynthetic pigment: dichloroethane (2%), ethyl acetate (1%)
14. Saturated alcohols: methanol (3%), ethyl acetate (1%)

The steroidal glycoside, alkyl ammonium halide salt, organic acids, and glycoside were found only in n-hexane (1%), chloroform (15%), dichloroethane (4%), and ethyl acetate (3%), respectively. Eventually, in the present study we have found phytosterols, terpenoids, fatty acids, fatty acid esters, alkyl halides, phenols, alcohols, ethers, alkanes, and alkenes as the major group of phytochemotypes in the different root extracts of *R. imbricate* (Fig. 7, Table 7). All these compounds identified by GC/MS analysis (Fig. 8) were further investigated for their biological activities [28] and most of them were found to possess a diverse range of positive pharmacological actions (Table 8).

Most of the pharmacological studies were conducted with the aqueous, ethanol, and hydro-alcoholic root extracts of this plant and it was found to have numerous biological activities such as anti-stress, adaptogenic, anti-hypoxic, immune-stimulatory, anti-cancer, cytoprotective, radioprotective, anti-hemolytic, anti-inflammatory, and wound healing potential [6–23]. Our investigations conclude that the compounds present in the ethanol and water extracts have the potential to perform these functions.

Table 8. Biological activities of active principles present in different root extracts of *R. imbricate*.

Phyto-chemotypes	Biological activity
Eicosen-1-ol, cis-9	Antimalarial, antifungal, antioxidant
1-Tricosanol	Antibacterial, antifungal
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	5-Alpha-reductase inhibitor, antiMS, antiacne, antialopecic, antianaphylactic, antiandrogenic, antiarteriosclerotic, antiarthritc, anticoronary, antieczemic, antifibrinolytic, antigranular, antihistaminic, antiinflammatory, antileukotriene-D4, antimenorrhagic, antiprostatitic, cancer-preventive, carcinogenic, comedolytic, hepatoprotective, hypocholesterolemic, immunomodulator, insectifuge, metastatic, nematicide, propecic
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, antiarthritic, anticoronary, insectifuge
Ascaridole	Analgesic, ancylostomicide, anthelmintic, antiflatulent, antimalarial, carcinogenic, carminative, fungicide, nematicide, pesticide, plasmodicide, sedative, transdermal, trypanocide, vermifuge
Borneol	(-)-Chronotropic, (-)-inotropic, allelochemic, analgesic, antiacetylcholine, antibacterial, antibronchitic, antiescherichic, antifeedant, antiinflammatory, antiotitic, antipyretic, antisalmonella, antispasmodic, antistaphylococcic, antiyeast, CNS-stimulant, CNS-toxic, candidicide, choleretic, flavor; fungicide, hepatoprotective, herbicide, herbicide, inhalant, insect-repellent, insectifuge, irritant, myorelaxant, nematicide, perfumery, pesticide, sedative, tranquilizer
Campesterol	Antioxidant, hypocholesterolemic
Camphor	Allelopathic, analgesica, anesthetic, antiacne, antidiarrheic, antidysenteric, antiemetic, antifeedant, antifibrositic, antineuralgic, antipruritic, antiseptic, antispasmodic, CNS-stimulant, cancer preventive, carminative, convulsant, cosmetic, counterirritant, decongestant, deliriant, ecbolic, emetic, epileptigenic, expectorant, fungicide, herbicide, insect-repellent, insectifuge, irritant, nematicide, occuloirritant, P450-2B1-inhibitor, pesticide, respirainhibitor, respirastimulant, rubefacient, stimulant, transdermal, verrucolytic, vibriocide
Stigmast-5-en-3-ol, (3β,24S)	Androgenic, angiogenic, anorexic, antiadenomic, antiandrogenic, antibacterial, anticancer (breast), anticancer (cervix), anticancer (lung), antiedemic, antiestrogenic, antifeedant, antifertility, antigonadotrophic, antihyperlipoproteinaemic, antiinflammatory, antileukemic, antilymphomic, antimutagenic, antiophidic, antioxidant, antiprogestational, antiprostaglandin, antiprostatadenomic, antiprostatitic, antipyretic, antitumor (breast), antitumor (cervix), antitumor (lung), antiviral, apoptotic, artemicide, cancer-preventive, candidicide, caspase-8-inducer, estrogenic, febrifuge, gonadotrophic, hepatoprotective, hypocholesterolemic, hypoglycemic, hypolipidemic, pesticide, spermicide, ubiquiot, ulcerogenic
Di-butyl phthalate	Antimicrobial, Antifouling
Dodecanoic acid, 3-hydroxy	Flavor
Eucalyptol	Anesthetic, anthelmintic, antibacterial, antihalitosic, antiseptic, antitussive, decongestant, expectorant, hypotensive, insectifuge, irritant, pesticide, vermicide
α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	Preservative
Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor
Hexadecanoic acid, methyl ester	Antioxidant, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor, hypocholesterolemic
Linalyl isovalerate	Fragrance
Oleic acid	5-Alpha-reductase-inhibitor, allergenic, anemiagenic, antialopecic, antiandrogenic, antiinflammatory, antileukotriene-D4; cancer-preventive, choleretic, dermatitigenic, flavor, hypocholesterolemic, insectifuge, irritant, percutaneostimulant, perfumery, propecic
1-Pentatriacontane	Herbistat
Stigmast-4-en-3-one	Antiprostatitic
Stigmast-3,5-dien-7-one	Antifertility
Thujone	Abortifacient, anthelmintic, antibacterial, antiseptic, antispasmodic, cerebrodepressant, convulsant, counterirritant, emmenagogue, epileptigenic, hallucinogenic, herbicide, neurotoxic, perfumery, pesticide, respirainhibitor, toxic
β-Fenchyl alcohol	Antimicrobial, antioxidant, flavor
δ-Tocopherol	5-HETE-inhibitor, allergenic, analgesic, antiMD, antiMS, antiPMS, antiaggregant, antiaging, antialzheimeran, antianginal, antiarteriosclerotic, antiarthritic, antiatherosclerotic, antibronchitic, anticancer (breast), anticariogenic, anticataract, antichorea, antichoreic, anticonvulsant, anticoronary, antidecubitic, antidementia, antidermatitic, antidiabetic, antidysmenorrheic, antiepitheleomic, antifibrositic, antiglycosation, antiherpetic, antiinfertility, antiinflammatory, antiischemic, antileukemic, antileukotriene, antilithic, antilupus, antimaculitic, antimastalgic, antimelanomic, antimyoclonic, antineuritic, antineuropathic, antinitrosaminic, antiophthalmic, antiosteoarthritic, antioxidant, antiparkinsonian, antiproliferant, antiradicular, antiretinopathic, antirheumatic, antisenility, antisickling, antispasmodic, antisterility, antistroke, antisunburn, antisyndrome-X, antithalassemic, antithrombotic, antithromboxane-B2, antitoxemic, antitumor; antitumor (breast), antitumor (colorectal), antitumor (prostate), antitumor (stomach), antiulcerogenic, apoptotic, calcium-antagonist, cancer-preventive, cardioprotective, cerebroprotective, circulatory-stimulant, circulotonic, hepatoprotective, hypocholesterolemic, hypoglycemic, immunomodulator, immunostimulant, insulin-sparing, lipoxygenase-inhibitor, NO-inhibitor, ornithine-decarboxylase-inhibitor, P21-inducer, phospholipase-A2-inhibitor, protein-kinase-C-inhibitor, vasodilator

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Though, the root extracts of the plant obtained by polar solvent extraction have been investigated for their pharmacological actions in considerable detail, non polar root extracts were not studied till date. Hence, our primary objective in the present work was to find the bioactive constituents present in the non polar extraction of root of this herb. These findings will definitely usher in new directions in pharmacological and therapeutic investigations with the root extracts obtained from non polar solvent extraction such as n-hexane, chloroform, dichloroethane, and ethyl acetate.

Conclusion

In the present study, sixty three phyto-chemotypes have been identified from n-hexane, chloroform, dichloroethane, ethyl acetate, methanol, and 60% ethanol root extracts of *R. imbricata* by GC/MS analysis. It showed the existence of various bioactive principles that confirm the application of *R. imbricata* for various ailments in traditional system of medicine. However, isolation of individual phyto-chemotypes and subjecting them to biological activity will definitely give fruitful results to find a novel drug. It could be concluded that *R. imbricata* contains various bioactive phyto-chemotypes having phyto-pharmaceutical importance. However, further studies will need to be undertaken to ascertain its bioactivity, toxicity profile, effect on the ecosystem, and agricultural products.

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Author Contributions

Concise review of manuscript: MS RSC RBS. Conceived and designed the experiments: ABT PD JK MS RSC OPC RBS. Performed the experiments: ABT PD JK. Analyzed the data: ABT PD MS OPC. Contributed reagents/materials/analysis tools: ABT PD JK. Wrote the paper: ABT PD OPC.

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