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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF BIOACTIVE COMPONENTS FROM THE RHIZOME EXTRACT OF *NARDOSTACHYS JATAMANSI* DC

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ABSTRACT

In this study, the bioactive compounds of *Nardostachys jatamansi* have been evaluated using gas chromatography-mass spectrometry (GC-MS). The chemical composition of the rhizome of *N. jatamansi,* petroleum ether, chloroform, and ethanol extract was investigated using agilent 7890 GC-MS instrument. *N. jatamansi* extract contains 61 compounds, i.e., actinidine (11.2%), indane (28.7%), aristolene (7.2%), gurjuenen (5.5%), valencene (8.9%), globulol (8.2%), betapatachoulene (8.4%), etc. Actinidine and indane are the major compounds in the ethanol extract along with few minor compounds.

Keywords: Chloroform extract, Gas chromatography-mass spectrometry analysis, *Nardostachys jatamansi.*

INTRODUCTION

Nardostachys jatamansi DC is an important plant of the family Valerianaceae found growing from Himalayan regions to southwest China and has been used as crude drugs in traditional medicines. It is commonly known as Indian spikenard. It is an erect perennial herb, 10-60 m height with long stout, woody rootstock found in the alpine the Himalayas from Punjab to Sikkim and Bhutan at an altitude of 3000-5000 m. The bitter tonic obtained from the rhizomes of the *Jatamansi,* also called Bhutajata, Jatahilaa, Thapaswini can be used as a neuroprotective, sunscreen, stimulant, antispasmodic, repellent, antipyretic, antioxidant, as well as to treat herpes infection, leprosy, various neuropsychiatric illnesses, and excessive thirst. The plants are known to be rich in sesquiterpenes [14], which have been found to exhibit antimalarial, antinociceptive, and cytotoxic activities, as well as to enhance the action of nerve growth factor [15-17]. It also has other ayurvedic applications such as in complexion, kidney stones, jaundice, removes blood impurities, spasmodic hysteria and other nervous convulsive ailments, heart palpitations, nervous headache, flatulence, epilepsy, convulsions, respiratory and digestive diseases, skin diseases, typhoid, gastric disorders.

The plant is reported for hypolipidemic effects in triton-induced hyperlipidemic rats, for inhibitory activity of methanolic and water extracts against acetyl cholinesterase, anticonvulsant, and neurotoxicity profile in rats, as well as protective effect in rat cerebral ischemia [4-6]. Comparative study of the volatile compounds in the *N. jatamansi* DC and *N. chinesis* was done from the commercially available oil. However, the detailed study of phytochemicals in different extracts of *N. jatamansi* was not done. Hence, the objective of the present study is to identify the phytochemical constituents with the gas chromatography-mass spectrometry (GC-MS) technique.

METHODS

Plant material and extraction

Rhizomes of *N. jatamansi* were purchased from local shops of Udupi, Karnataka, India and authenticated. The dried rhizomes were pulverized to powder in a mechanical grinder. The dried powder was weighed and subjected to Soxhlet extraction procedure in the increasing order of their polarity of solvents, i.e. petroleum ether, chloroform, and ethanol. The extract was concentrated under reduced pressure using rotary evaporator. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS analysis

GC combined with mass spectroscopy is a preferable methodology for routine analysis of compounds. 1 Ul of petroleum ether extract, chloroform extract, and ethanol extract was injected into the GC-MS instrument.

GC analyzes were carried out on an Agilent 7890 instrument equipped with a hydrogen flame ionization detector and HP-5 capillary column (30 m × 0.32 mm × 0.25 µm, J and W Scientific). Nitrogen was used as carrier gas at a flow rate of 1 mL/minute. Initially, the column temperature was maintained at 60°C for 2 minutes, followed by a temperature gradient from 60°C to 120°C at 2°C/minutes and held constant for 5 minutes at 120°C, then raised to a temperature of 150°C at 1°C/minutes and finally to a temperature of 200°C with a 5°C/minutes rise and maintained for 10 minutes at 200°C. The injector and detector temperatures were maintained at 250°C and operated in split mode (split ratio 1:10). GC-MS was performed on an Agilent 5975C mass selective detector interfaced with an Agilent 7890A gas chromatograph. GC-MS analyzes were performed under similar conditions using an HP-5-MS capillary column (30 m × 0.32 mm × 0.25 µm, J and W Scientific).

Thin-layer chromatography was performed on silica gel G-coated plates (0.25 mm for analytical) developed with hexane (system I), hexane–ethyl acetate (98.5:1.5; system II), dichloromethane (system III), and dichloromethane–methanol (98.5:1.5; system IV). Compounds were visualized by spraying with a solution of 3.0% anisaldehyde, 2.8% H_2SO_4, and 2% acetic acid in ethanol followed by heating for 1-2 minutes.

Identification of compounds

Interpretation of the mass spectrum GC-MS was conducted using the database of National Chemical Laboratory (NCL), Pune. The spectrums of the unknown compounds were compared with the spectrum of known compounds stored in the NCL library.

RESULT AND DISCUSSION

GC-MS analysis of the phytochemicals present in different extracts (pet ether, chloroform, and ethanol) of *N. jatamansi* showed the presence of 61 compounds. The active principles with their retention time (RT) probability, R-match, and relative percentages and compound name are presented in the Tables 1-3. The GC-MS chromatogram of the petroleum

Table 1: Compounds detected in the petroleum ether extract of *N. jatamansi*

Serial number	Rt	Probability	R-match	Percent	Compound
1	7.359	39.7	835	0.22	3-Methylbutanoic acid
2	10.899	15.3	920	0.054	(-)-β-Pinene
3	13.634	11.9	801	0.016	1,8-Cineol
4	26.569	3.07	837	0.11	3-Methyl-3-hexen-2-ol
5	26.962	9.41	765	0.024	Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene-
6	27.078	2.71	884	0.018	Bicyclo|5.1.0|octane, 8-(1-methylethylidene)-
7	28.097	3.87	800	1.18	Beta-pinenoxide
8	28.594	40.4	870	0.22	Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene-
9	29.316	8.86	796	0.113	D-Verbenone
10	29.628	9.58	879	0.08	Carveol
11	30.072	5.2	769	0.049	7'-Oxaspiro|cyclopropane-1,4'-tricyclo|3.3.1.0 (6,8)|nonan-2'-one|
12	30.75	18.5	812	0.017	(+)-β-Guaiene
13	30.888	18.5	866	0.037	(R)-(+)-Limonene
14	32.345	10.3	854	0.221	τ-Gurjunene
15	33.018	17.9	914	0.18	(-)-β-Elemene
16	33.636	9.52	851	0.099	(-)-α-Gurjunene
17	33.805	6.9	918	1.672	β-Maaliene
18	34.191	12.3	934	1.163	(+)-9-Aristolene
19	34.664	202	32.8	0.178	β-Vatirenene
20	34.916	26.7	953	7.917	(+)-1[10]-Aristolene or (+)-Calarene
21	35.016	11.6	884	0.176	Eudesma-3,7[11]-diene
22	35.257	53.6	972	2.691	Seychellene
23	35.35	20.8	928	0.314	α-Guaiene
24	35.586	9.25	920	5.466	(-)-α-Gurjunene
25	35.954	39.6	916	2.569	α-Patchoulene
26	36.292	15.1	950	0.444	(+)-Aromadendrene
27	36.419	22.3	928	3.961	(-)-α-Gurjunene
28	36.565	12.1	743	0.478	5β,7βH,10α-Eudesm-11-en-1α-ol
29	37.527	6.43	912	1	τ-Gurjunene
30	37.705	14.6	843	0.636	(E)-β-Ionone
31	37.981	26.3	953	8.887	(+)-Valencene
32	38.246	5.71	873	0.469	Seychellene
33	38.566	25.7	819	2.05	8-Camphenemethanol
34	39.06	28.5	910	3.677	Eudesma-3,7[11]-diene
35	39.448	36.3	920	0.272	(-)-β-Cadinene
36	39.572	4.78	843	0.479	Globulol
37	40.438	5.42	856	0.548	β-Vatirenene
38	41.15	3.34	785	0.611	Thujopsene
39	41.77	21.6	835	0.671	2-Butenal, 2-methyl-4-[2,6,6-trimethyl-cyclohexen-1-yl]-
40	42.034	8.58	900	4.367	Seychellene
41	42.284	9.25	865	1.433	Isoledene
42	42.727	7.4	859	2.156	Cubenol
43	43.039	3.51	876	1.259	(-)-Alloaromadendrene
44	44.309	6.93	863	6.067	(+)-Aromadendrene
45	45.388	4.16	892	8.402	β-Patchoulene
46	46.121	74.7	946	4.679	(+)-Valeranone or 1[2H]-Naphthalenone, octahydro-4a, 8a-dimethyl-7-[1-methylethyl]-,|4aR-[4aα,7β,8aα]|-
47	48.202	15.2	947	2.014	Valerenal
48	50.27	18.6	865	0.773	2,4a-Methanonaphthale-7[4aH]-one, 1,2,3,4,5,6-hexahydro-1,1,5,5-tetramethyl-,[2s-cis]-
49	50.538	10.1	840	8.197	Globulol
50	50.759	35.1	782	0.934	Methylhinokiate
51	51.231	39.1	747	1.898	Butanal, 3-hydroxy-2-methyl-4-|4-t-butyl|-
52	51.617	12	955	1.233	9-Acetyl-2,6-dimethyl-10-hydroxybicyclo|4.4.0|deca-1,4-dien-3-one
53	52.546	3.8	826	2.755	6-Methyl-5-[1-methylethylidene]-3,6,9-decatrien-2-one
54	53.603	25.7	823	0.493	6-[1-Hydroxymethyl-vinyl]-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
55	53.981	5.35	828	0.275	2[1H] Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-[1-methylethenyl]-
56	54.151	7.21	832	0.221	Phenol, 2-methoxy-4-[1-propenyl]-
57	55.241	5.87	868	0.224	Verrucarol
58	59.412	21.3	722	0.982	3-[6,6-Dimethyl-5-oxohept-2-enyl]-cycloheptanone
59	60.902	22.1	861	0.73	4,4-Dimethyl-3-[3-methylbut-3-enylidine]-2-methylenebicyclo|4.1.0|heptane
60	61.194	21.7	873	1.133	Dehydroaromadendrene
61	67.263	4.91	822	0.807	1,4-Butanediol, 1-phenyl-

RT: Retention time, *N. jatamansi: Nardostachys jatamansi*

Table 2: Compounds detected in the chloroform extract of *N. jatamansi*

Serial number	Rt	Probability	R-match	Percent	Compound
1	8.287	98.5	978	16.589	1,1,2,2-Tetrachloroethane
2	10.871	85.5	866	0.262	Pentachloroethane
3	13.513	20.1	760	0.868	1,1,2-Trichloro-2-methylpropane
4	13.863	62.1	767	0.27	2,3-Dichloro-2-methylbutane
5	15.655	98.9	979	3.413	1,1,1,2,2,2-Hexachloroethane
6	17.194	1.45	919	0.37	4-Chloro-2-[chloromethyl]-1-butene
7	18.888	96.4	927	2.859	1,1,2,3,3-Pentachloropropane
8	25.009	57.6	679	2.855	1,1,2,2,3,3-Hexachloropropane
9	29.528	33.1	861	2.22	(S)-(-)-Actinidine
10	30.066	5.25	780	2.715	1-Isopropenyl-3-propenyl-cyclopentane
11	32.28	11.3	903	1.166	β-Patchoulene
12	32.616	27.5	784	0.297	4-Iodo-2-oxaadamantan-6-one
13	32.828	7.18	774	0.143	Bicyclo|4.1.0|heptane, 7-bicyclo|4.1.0|hept-7-ylidene
14	34.161	17.1	818	0.478	β-Guaiene
15	34.594	3.48	853	0.532	(-)-Alloaromadendrene
16	34.689	6.91	813	1.037	Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene-
17	34.853	7.5	775	3.824	8-Camphenemethanol
18	35.009	28.7	875	1.171	β-Vatirenene
19	35.187	31.6	943	1.973	Seychellene
20	35.903	5.47	854	2.272	Viridiflorol
21	36.358	5.48	870	2.233	Cyperene
22	37.093	2.63	804	0.267	α-Selinene
23	37.195	11.5	793	0.167	(+)-β-Cedrene
24	37.427	7.38	889	0.86	τ-Gurjunene
25	37.494	8.22	856	0.918	(+)-Aromadendrene
26	37.649	21.8	922	0.867	Seychellene
27	37.846	13.7	842	4.78	(±)-Cadinene
28	38.115	60.9	731	2.04	Dehydrocyclolongifolene oxide
29	39.436	37.6	962	1.404	15-Methyltricyclo|6.5.2[13,14].0[7.15]|pentadeca-1,3,5,7,9,11,13-heptene
30	39.923	33.8	806	0.396	4,5,9,10-Dehydroisolongifolene
31	40.158	4.1	703	0.82	(+)-Longifolene
32	40.604	11	786	0.587	Nerolidol-epoxyacetate
33	41.736	14.1	806	0.459	2-Butenal, 2-methyl-4-[2,6,6-trimethyl-1-cyclohexen-1-yl]-
34	42.699	8.77	760	4.431	Caryophyllene oxide
35	42.86	12.6	813	0.828	1-Nonadecene
36	43.005	18.4	731	0.863	Bicyclo|4.2.0|oct-1-ene, 2-methyl-7-endo-phenyl
37	44.215	14.2	834	4.095	τ-Himachalene
38	44.356	5.71	866	3.282	Isoledene
39	45.293	3.64	876	1.421	Seychellene
40	45.415	12.9	855	0.716	β-Vatirenene
41	46.028	42.9	923	3.385	(+)-Latamansone or (+)-Valeranone
42	46.629	2.96	912	2.726	(+)-Valencene
43	48.039	6.08	909	2.674	β-Patchoulene
44	48.655	3.25	900	3.763	9-Acetyl-2,6-dimethyl-10-hydroxybicylco|4.4.0|deca-1,4-dien-3-one
45	48.973	8.03	817	0.839	Murolan-3,9[11]-diene-10-peroxy
46	50.111	8.17	792	0.849	2,2,7,7-Tetramethyltricyclo|6.2.1.0 (1,6)|undec-4-en-3-one
47	50.71	4.51	860	0.475	Verrucarol
48	51.555	19	774	1.535	1-Chloro-7-heptadecene
49	59.474	2.28	869	1.212	1-Eicosene
50	70.653	14.9	824	5.795	4-Allylphenol

RT: Retention time, *N. jatamansi: Nardostachys jatamansi*

Table 3: Compounds detected in the ethanol extract of *N. jatamansi*

Serial number	Rt	Probability	R-match	Percent	Compound
1	10.271	44	907	3.737	n-Propylbenzene
2	10.622	26.3	937	27.892	m-Ethyltoluene
3	10.927	15.8	940	2.08	1,3,5-Trimethylbenzene
4	11.399	19.2	927	8.213	p-Ethyltoluene
5	12.032	22.4	946	9.219	1,3,5-Trimethylbenzene
6	13.371	16.9	879	1.467	1,2,4-Trimethylbenzene
7	13.962	17.6	928	28.787	Indane
8	29.426	14.1	938	11.729	(±)-Actinidine
9	46.023	4.19	850	1.957	(+)-Valeranone
10	59.532	73.7	816	1.897	Ethylpalmitate
11	65.522	23.4	784	1.092	Ethyllinoleate
12	65.749	11.1	795	1.929	Ethyloleate

RT: Retention time, *N. jatamansi: Nardostachys jatamansi*

ether extract, chloroform extract, ethanol extract is shown in the Figs. 1-3 respectively.

About 61 compounds are found in the petroleum ether extract. The results revealed that valencene (8.8%), globulol (8.2%), beta-patacholene (8.4%), aristolene (7.9%), and alpha-gurjunene (5.4%) were found as major components in petroleum ether extract. A total of 50 compounds found in chloroform extract in which tetrachloroethane (16.6%), allylphenol (5.8%), and valeranone (3.4%) are the major compounds. 11 compounds are reported from ethanol extract out of which m-ethyl toluene (27.9%), indane (28.8%), and actinidine (11.8%) are major compounds.

The bioactive compounds of *N. jatamansi* have been evaluated using GC-MS. The chemical composition of the *N. jatamansi* was investigated using aglient 7890 GC-MS instrument. It has been reported that secondary metabolites exert a wide range of biological activities on physiological systems. Among the identified chemicals globulol, gurjunene, and aristolene have a role in antioxidants and antimicrobial activities. Similarly, the presence of alkaloids such as indane and actinidine in the ethanol extract of *N. jatamansi* are reported to have a role in the antidiabetic and anticancer activity. In traditional medicine, the rhizome of *N. Jatamansi* used to treat epilepsy, hysteria, coryza, convulsions, internal colic, palpitations of the heart, and mental disorders. Thus, this type of GC-MS analysis is the fruitful step toward understanding active principles in medicinal plants, and this type of study will be helpful for further

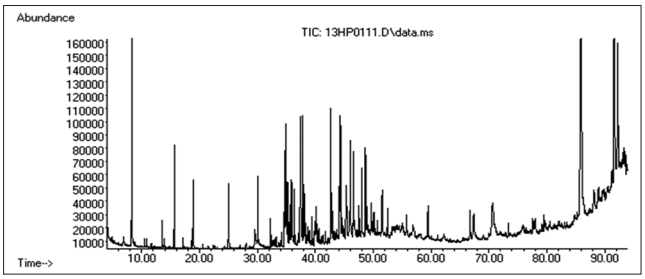

Fig. 1: Gas chromatography-mass spectrometry chromatogram of the petroleum ether extract of the *Nardostachys jatamansi*

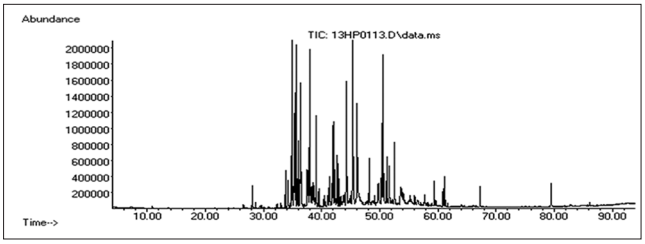

Fig. 2: Gas chromatography-mass spectrometry chromatogram of the chloroform extract of the *Nardostachys jatamansi*

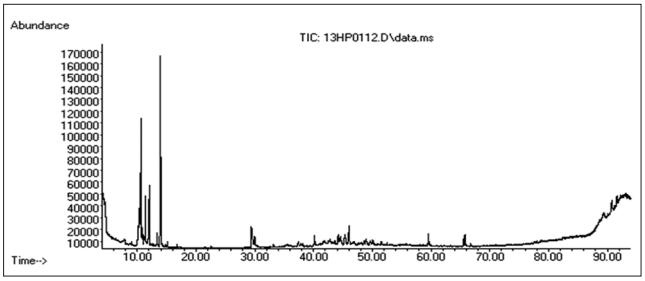

Fig. 3: Gas chromatography-mass spectrometry chromatogram of the ethanol extract of the *Nardostachys jatamansi*

detailed study and isolation of active components present in the plant extract.

CONCLUSION

The quality of herbal medicine can be evaluated by the construction of chromatographic fingerprints. In the present study, 60 compounds have been identified from the rhizome of *N. jatamansi* DC by GC-MS analysis. There for GC-MS method is a direct and fast analytical approach for identification of terpenoids and steroids and only a few grams of plant material is required [18]. The presence of various bioactive compounds with different biological activity such has anti-inflammatory, antipyretics, antibacterial, antifungal, skin conditioning have been identified, justifies the use of the whole plant for various ailments by traditional practitioners.

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