Research Article

GC-MS Analysis of the Phytoconstituents in the Methanolic Extract of Leaves of *Elaeocarpus sphaericus Roxb.*

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ABSTRACT

Elaeocarpus sphaericus (syn. E. ganitrus) or Rudraksha is a well known medicinal herb of the Indian traditional system of medicine, which is used to treat various ailments. Plant has its importance not only for medicinal purposes but also for spiritual purpose. Our aim in this study was to analyze phytoconstituents in the methanolic extract of leaves of *Elaeocarpus sphaericus* through GC-MS profiling. Leaves of the plant were dried in the shade and coarsed to make a fine powder. Extraction was done through ASE (accelerated solvent extraction) at high pressure & room temperature, using methanol as solvent. After evaporation in Rota vapor, powder was lyophilized in lyophillizer. Lyophillized powder was analyzed using GC-MS methodology. Total 53 compounds were identified using the NIST library. Four compounds 9, 12, Octadecadien-1-ol (Linolyl alcohol), (12.30%), stigmost-5-En-3-ol (11.04%), Pentadecanoic acid (7.4%) and Methyloctadeca-9, 12, Deonate (5.56%) were present as major compounds in the methanolic extract of Rudraksha. Compounds identified, have their importance in pharmaceutical and industrial purposes, therefore the extract prepared through ASE used for sourcing of these compounds of natural origin.

Keywords: Rudraksha, *Elaeocarpus sphaericus,* GC-MS analysis, Methanolic extract, Leaf.

INTRODUCTION

Elaeocarpus sphaericus belongs to family *elaeocarpaceae* which has about 360 species. Distribution of these species occurs throughout the Australia, East Asia, Malaysia and the Pacific Islands. Asian region alone contains 120 species and out of this 25 species are reported in India.[1] *Elaeocarpus sphaericus* (Rudraksha) plant has tremendous importance because various parts of the plant are used in the treatment of stress, anxiety, hypertension, arthritis and liver diseases and skin diseases.[2,3] Wearing a Rudraksha bead is helpful in controlling blood pressure & stress due to its electromagnetic property.[4]

Various Indolizidine alkaloids including elaeocarpiline, isoelaeocarpiline, epielaeocarpiline, epiisoelaeocarpiline, epialloelaeocarpiline, alloelaeocarpilline, elaeocarpidine and pseudo isoelaeocarpilline alkaloids was isolated from the leaves of Rudraksha.[5-7] A non aromatic indolizidine alkaloid such as rudrakine was isolated from *E. sphaericus*.[8] HPTLC analysis has shown the presensce of quercetin & kampferol in the extract prepared from the seed of *Elaeocarpus sphaericus*.[9-10]

GC-MS technique is the most commonly used technique for the identification and quantification of the active constituents from the various compounds. GC-MS technique is a tool which has the perfect combination of liquid chromatography and mass spectrometry. Due to its high resolution capacity, sensitivity, good reproducibility and low cost characteristics technique is highly convenient for analyzing plant metabolomics. Technique is convenient for analyzing qualitatively and quantitatively multi component mixture as volatile compounds, fatty acids, lipids, non polar components and alkaloids. Separation of the sample done with the help of GC in a column where analytes get separated on the basis of the differences in their boiling points and the differences in distribution between stationary phase and mobile phase. Identification of the compounds done with the help of MS where compounds get ionized and separated on the basis of mass to charge ration and generate electric signals to give the 3D information of the compound.[11-12]

GC-MS analysis of leaves of *E. serratus* and essential oil from the leaves of *Elaeocaprus sphaericus* have been reported previously but there is no report on the GC-MS analysis of leaves extract of *E. sphaericus*.[13,14] Therefore in this study we have analyzed the methanolic extract of leaves of *E. sphaericus* prepared through ASE (Accelerated solvent extraction) system. ASE operates at room temperature and high pressure therefore phenolics do not degrade & extracts are supposed to maintain high diversity & concentrations of phenolics.

MATERIALS AND METHODS

Collection of plant

The leaves were collected from the Graphic Era University Campus, Clement town, Dehradun (Uttarakhand). Taxonomical authentication was done by Dr. Rakesh Mohan Painuly, Lecturer, Department of Botany, HNBGU University. Herbarium was submitted in the Deparment of Botany, HNBGU University, Voucher specimen number is GUH 20720.

Preparation of the extract

Accelerated solvent extraction (ASE) system was used for the extraction of plant. ASE was assembled with a solvent

controller unit (ASE350, DIONEX, and Corporation, Sunnyvale, CA, USA).[15] Leaves part of the plant was used for the extraction. The leaves were dried in the shade for the period of a one week. Dried leaves were crushed to powder. Leaf powder with silica powder in equal ration were put in the cell of ASE. Silica powder helps in the saturation of the plant, which helps in the accession of phytoconstituents. Methanol was used as solvents for the extraction. Extraction was done at the 25°C temperature and pressure of 1500 psi. After extraction of the extract, evaporation of the solvent was done in rota vapor (Rota Vapor124, Bucchi, and Flawil, Switzerland). Evaporated extract were lyophilized in the lyophilizer. Lyophillized extract was stored at the 4°C for further use.

GC-MS analysis

GC-MS analysis was performed at University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi in GCMS QP2010 ultra system, which was interfaced with a mass spectrometer. The GC-MS system had a elite-1 fused silica capillary column, having a dimension of 30 × 0.25 mm Id × 0.25 µm df, composed of 5% diphenyl 95% dimethyl poly siloxane, operated in the electron impact mode with ionization energy of 70eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min with an injection volume of 2 µl and having split ration 10:1.

RESULTS AND DISCUSSION

Phytoconstituents were detected with the help of database of National Institute Standard and Technology (NIST library), which had standards for more than 62,000 compounds. The Spectrum of the unknown was compared with the spectrum of known. The name, molecular weight, and structure of the components of the test materials were ascertained. Identification of the compounds were done on the basis of peak area, retention time (RT) and molecular formula. Total 53 compounds were analyzed through GC-MS analysis, which were presents in the methanolic extract of the *E. sphaericus. Roxb* (Figure1).

Phytoconstituents with their peak area, area%, retention time, name, molecular weight/molecular formula are presented in Table 1. The range of RT was from the 3.157 to 36.68 minutes. Most prevailing compounds were, 9-12-octadecadien-1-ol (Linoleyl alcohol) (12.30%), Stigmost-5-En-3-ol (11.04%), Pentadecanoic acid (7.40%) and Methyloctadeca-9, 12 Denoate (5.56%).

GC-MS analysis of plant has shown presence of 53 compounds. 9, 12-Octadecadienoic acid (Z, Z)-(Linoelic acid) is a major demanding product of cosmetic industry due to its anti-inflammatory, acne reductive, and moisture retaining properties.[16-18]

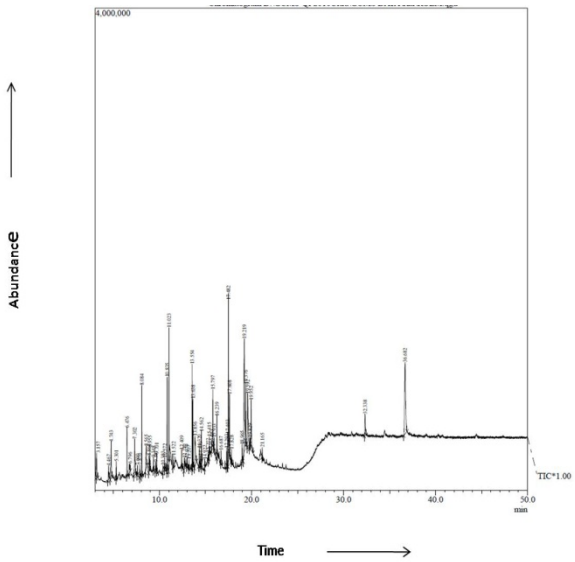

Figure 1: Total ion chromatogram of Methanolic extract of Rudraksha (*Elaeocarpus sphaericus*).

Pentadecanoic acid which is used as marker for butter fat consumption.[19] Stigmast-5-en-3-ol can be used as antidiabetic agent as it shown an insulin-like effect and also stimulates glucose transportation, compound has much interested by researchers due to its ability to reduce Benign prostatic hyperplasia (BPH) and blood cholesterol level.[20-23]

CONCLUSION

Several organic compounds have been identified in the leaves of *E. sphaericus* which have tremendous pharmacological and industrial applications. ASE can be implied for extraction and sourcing of these useful compounds.

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Disclosure of Statement: An Indian Patent entitled, 'A phytochemical rich herbal extract and the process of extraction thereof.' Application No. 1625/DEL/2014, has been filed for the extract in the study.

Table: 1: Compounds identified in methanolic extracts of *E. sphaericus* through GC-MS analysis

Peak#	Retention Time	Area%	Name	Molecular Weight/ Molecular formula.
1	3.157	1.84	Dl-Glyceraldehyde dimer	180.16 G/mol $C_6H_{12}O_6$
2	4.467	1.01	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	144.12 G/mol $C_6H_8O_4$
3	4.743	2.86	2h-Pyran-2,6(3h)-Dione	112.08 G/mol $C_5H_4O_3$
4	5.301	0.90	D-Limonene	136.24 G/mol $C_{10}H_{16}$
5	6.476	1.79	1,6-Octadien-3-ol, 3,7-dimethyl-(Linalyl propionate)	210.312G/mol $C_{13}H_{22}O_2$
6	6.796	0.94	Benzeneethanol	122.164 G/mol $C_8H_{12}O$
7	7.302	2.84	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-one	144G/mol $C_6H_8O_4$
8	7.600	0.36	Acetic Acid(Phenylmethyl Ester)	150.17G/mol $C_9H_{10}O_2$
9	7.870	0.42	Terpinen-4-ol	154.24 G/mol $C_{10}H_{18}O$
10	8.084	2.95	Dodecane	170.33 G/mol $C_{12}H_{26}$
11	8.565	2.25	6-Octen-1-Ol, 3,7-Dimethyl- (Citronellol)	156.26 G/mol $C_{10}H_{20}O$
12	8.864	0.70	2,3-Dihydroxypropyl Acetate	134.130 G/mol $C_5H_{10}O_4$
13	8.955	1.03	3-Methyl-2-butenoic acid, decyl ester	240 G/Mol $C_{15}H_{28}O_2$
14	9.348	0.56	2-Propenal, 3-Phenyl-	132 G/mol C_9H_8O
15	9.587	0.36	Phenol, 5-Methyl-2-(1-Methylethyl)-(Thymol)	150 G/mol $C_{10}H_{14}O$
16	9.701	0.55	1,6-Octadien-3-ol, 3,7-dimethyl-, formate (Linalool)	182.2G/mol $C_{11}H_{18}O_2$
17	10.383	0.65	Undecane, 4,4-dimethyl-	184.36 G/mol $C_{13}H_{28}$
18	10.572	0.35	2,6-Octadien-1-Ol, 3,7-Dimethyl-, Acetate, (Z)-	196.28 G/mol $C_{12}H_{20}O2$
19	10.835	2.83	Geranyl acetate	196.29 G/mol $C_{12}H_{20}O_2$
20	11.023	3.98	Tetradecane	198.39 G/mol $C_{14}H_{30}$
21	11.522	0.48	Bicyclo[7.2.0]Undec-4-Ene, 4,11,11-Trimethyl-(Caryophyllene)	204.35 G/mol $C_{15}H_{24}$
22	12.409	0.54	7-Isopropenyl-4a-Methyl-1-Methylenedeca(β-Silene)	204.35 G/mol $C_{15}H_{24}$
23	12.710	0.80	4-Cyclopropylmethylbenzonitrile	157.23 G/mol $C_{11}H_{11}N$
24	12.979	0.59	Nonadecane, 9-Methyl-	282.54 G/mol $C_{20}H_{42}$
25	13.207	0.33	2-Bromotetradecane	277.28 G/mol $C_{14}H_{29}Br$
26	13.554	3.13	Heptadecane	240.47 G/mol $C_{17}H_{36}$

27	13.638	2.31	1,2-Benzenedicarboxylic Acid, Diethyl Ester	222.24 G/mol $C_{12}H_{14}O_4$
28	13.856	3.56	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxy (Quinic acid)	192.16 G/mol $C_7H_{12}O_6$
29	14.320	0.76	Methyl (3-Oxo-2-Pentylcyclopentyl)Acetate (Dihydrojasmonate)	226.31 G/mol $C_{13}H_{22}O_3$
30	14.439	0.67	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-Octahydro-	$C_{15}H_{26}O$ 222.36 G/mol
31	14.562	1.00	1-(4-isopropylphenyl)-2-methylpropyl acetate	$C1_5H_{22}O_2$ 234 G/mol
32	14.922	0.30	3-Methyl-4-(2,6,6-Trimethyl-1-Cyclohexen-1-yl	206G/mol $C_{14}H_{22}O$
33	15.277	0.24	Pentadecane, 8-Hexyl-	296.57G/mol $C_{21}H_{44}$
34	15.415	0.82	Octanal, 2-(Phenylmethylene)-	216.31G/mol $C_{15}H_{20}O$
35	15.663	0.23	Benzoic Acid, Phenylmethyl Ester	212 G/mol $C_{14}H_{12}O2$
36	15.797	2.51	Tricosane	324.62 G/mol $C_{23}H_{48}$
37	15.933	0.46	9-Octadecenoic Acid (Z)-	282 G/mol $C18H_{34}O_2$
38	16.239	1.42	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	278G/mol $C_{20}H_{38}$
39	16.687	0.66	3,7,11,15-Tetramethyl-2-hexadecen-1-ol(Phytol)	296.53 G/mol $C_{20}H_{40}O$
40	17.293	0.67	1,4-Dioxacyclohexadecane-5,16-dione	256.337 G/mol $C_{14}H_{24}O_4$
41	17.403	0.93	Methyl Ester Of 3-(3,5-Di-Tert-Butyl-4-Hydroxyphenyl-propionic acid	292.41 G/mol $C_{18}H_{28}O_3$
42	17.482	7.40	Pentadecanoic acid	242.40 G/mol $C_{15}H_{30}O_2$
43	17.608	2.25	1,2-Benzenedicarboxylic Acid, Dibutyl Ester (Di butyl Pthalate)	278.34 G/mol $C_{16}H_{22}O4$
44	17.828	0.36	Eicosane	282.55G/mol $C_{20}H_{42}$
45	18.965	0.83	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[(phytol)	296.53 G/mol $C_{20}H_{40}O$
46	19.219	12.30	9,12-Octadecadien-1-Ol (Linoleyl alcohol)	266.46 G/mol $C_{18}H_{34}O$
47	19.376	2.79	Octadecanoic acid (Stearic acid)	284.48G/mol $C_{18}H_{36}O_2$
48	19.592	5.56	Methyl Octadeca-9,12-Dienoate (Methyl lineolate)	294.47 G/mol $C_{19}H_{34}O_2$
49	19.829	0.41	9,12-Octadecadienoic acid (Z,Z)-	280.445 G/mol $C_{18}H_{32}O_2$
50	19.952	3.07	9,12-Octadecadienoic acid (Z,Z)-	280.445 G/mol $C_{18}H_{32}O_2$
51	21.165	0.48	1-Phenanthrenecarboxylic Acid, 7-Ethenyl	316.47 G/mol $C_{21}H_{32}O_2$
52	32.338	1.92	2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl-3 (Vit E, αtocopherol)	530.77 G/mol $C_{33}H_{54}O_5$
53	36.682	11.04	Stigmast-5-En-3-Ol	414.71 G/mol $C_{29}H_{50}O$

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