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GC-MS ANALYSIS OF BIOACTIVE COMPONENTS IN THE ETHANOLIC AND METHANOLIC EXTRACT OF *Syzygium cumini*

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ABSTRACT

In developing countries, high rate of mortality was found due to diabetes and other cardiovascular diseases etc. Plants are used worldwide as a source of many powerful drugs. The present investigation was carried out to determine the bioactive phytoconstituents (polyphenols and essential oils) in the ethanolic (ESE) and methanolic (MSE) extracts of seeds of *S. cumini* by GC-MS analysis. 34 major compounds were found in MSE such as 5,10-Dichloro-5,10-Dimethyltricyclo [7.1.0.0 (4-6)] decane (7.93%), Tetradecanoic acid (13.08%) α-Caryophyllene (4.52%), 1,10-Decandiol (19.83%), Bicyclo (4.4.O) Decane (1.37%), Octadienol (1.31%), Cadinene (0.24%), 2-Furancarboxaldehyde 5-(hydroxymethyl) (17.11%), Oxirane etc. and 25 components were found in ESE such as Nondecanoic acid (10.85%), Decahydro-4A-Methyl-1-Methylene-7-(1-methylethenyl) (6.81%), 12-Methyl-E,E-2,13-octadecadien-1-ol (19.83%) Furanone dihydroxy-2-Methyl (4.19%), and Caryophyllene oxide (1.32%), Isogeraniol (0.50%) etc. were found in less amount in ESE. Thus, we found MSE as a most potent and enriched extract than that of ESE and can be used for the treatment of various hazardous diseases.

KEY WORDS: Phytoconstituents, GC-MS Analysis, hexadecanoic acid, caryophyllene, ethanolic seed extract (ESE), methanolic seed extract (MSE)

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INTRODUCTION

Medicinal plants have been used worldwide for the treatment of various diseases principally in developing countries where infectious diseases are endemic and contemporary health amenities are inadequate[1]. *Syzigium cumini* (family, Myrtaceae) is an evergreen tropical tree, native to India, Pakistan and Indonesia and has been used as a traditional and folklore system of medicine.[2] The common names for *S. cumini* are Java plum, Black plum, Jambul and Indian Blackberry.[3] Such plants are globally distributed and have enormous diversity which may provide remedies for diabetes and aging as well as other medical and agricultural applications. The bark of the plant is astringent, sweet, refrigerant, carminative, diuretic, digestive, antihelmintic, febrifuge, constipating, stomachic and antibacterial[4]. The fruits and seeds are used to treat diabetes, pharyngitis, splenopathy, urethrorrhea and ringworm infection.[5] The leaves are antibacterial and used to strengthen the teeth and gums and used to treat diabetes, constipation, leucorrhoea, stomachache, fever, gastropathy, strangury, dermopathy and to inhibit blood discharges in the faeces.[6] Seeds of *S. cumini* plant have been used as a home remedy in Ayurveda to fight diabetes and to possess gastro-protective properties. Seeds of *S. cumini* are rich in protein, calcium and a glycoside jamboline, regulates the blood sugar level and a natural remedy for diabetes.[7] The seed powder, decoction, juice or paste of the different parts are used in traditional medicine and the plant possesses acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin[8]. Seeds are the waste product but the essential oils and extracts of seeds are of growing interest both in the industry and scientific research because of their antioxidants, antibacterial etc. activities that make them beneficial as natural additives in foods and pharmaceutical industries[9]. The quantity and quality of the extracted antioxidant polyphenolics varies owing to the difference in the polarity of the solvent used for the extraction of phenolics from natural sources.[10, 11] Based on our earlier studies *S. cumini* methanolic seed extract was found to have maximum antioxidative potenial and than that of ethanolic seed extract and was proved as an enriched source of polyphenols[12]. Taking into consideration its therapeutic value, natural antioxidants such as phenolic compounds, phytocomponents, essential oils and hydrocarbons of *S. cumini* were need to be explored. In this regard, Gas chromatography has gained widespread acceptance in various application areas, such as process control and quality control in the food industry, oil-industry, environmental and bio medical sciences.[13] The ability to analyze the full range of pesticides and many of the GC-MS pesticides makes the GC-MS the ideal system to provide confirmatory pesticide identification. So, the toxicity issues can be overcome due to food and other essentials. However, the phytochemistry of of *S. cumini* the seeds has not been studied in extensive details so far therefore, we aimed to analyze the active compounds (phytocontents) of *S. cumini* seed extracts using gas chromatography and mass spectrometry (GC-MS) because of its high sensitivity and resolving power for the separation of mixtures of *S. cumini* seed extracts.

MATERIALS & METHODS

Chemicals

Acetone, methanol, ethanol were purchased from qualigens.

Seed Collection

Seeds were collected from Noida, Uttar Pradesh, India in the month of July and were identified and authenticated by Dr. Anshu Rani, Department of Botany, Govt. P.G. College, Abu Road, Rajasthan, India. Seeds were washed thoroughly and air dried. The dried seeds were ground and the powder was collected for the preparation of ethanolic and methanolic extracts.

Preparation of organic solvent extracts of S. cumini seeds

Ethanolic and methanolic extracts of *S. cumini* seeds were prepared by using a Soxhlet apparatus. 10 g of seed powder was mixed with the 100 ml of ethanol and methanol respectively. The temperature was set at their boiling points and 10-12 cycles were run for concentrating the extracts. The rotary vacuum concentrator was used for further drying the extracts at the room temperature and the dried mass was reconstituted at the concentration of 1 mg/ml.

GC-MS analysis

The active principles of *S. cumini* seed extracts with their retention time (RT), molecular formula (MF) and molecular weight (MW) were examined by GC-MS. GC-MS analysis of the ethanolic and methanolic extracts of *S. cumini* seeds was performed using a Perkin–Elmer GC Clarus 500 system consists of an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). Helium (99.99%) was taken as carrier gas in electron impact mode at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (split ratio 10:1). An electron ionization system with an ionization energy of 70 eV was operated. The injector and the ion-source temperature was set at 250°C and 200°C respectively. The oven temperature was set at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s. The solvent delay was 0 to 2 min, and the total GC/MS running time was 32 minutes. The peak area (%) of each component was calculated.

RESULTS AND DISCUSSION

The detection of the compounds present in the ethanolic and methanolic extract was done on the basis of direct comparison of the mass spectral data and retention time those for standard compounds, and by computer matching with the Wiley 229, Nist 107, 21 Library (USA), as well as by comparison of the fragmentation patterns of the mass spectra reported in the literature (Fig-1 and 2).[14-16] The compounds were identified by comparing their retention time and covate indexes with that of literature and by interpretation of mass spectra. The basis of separation of organic components in GC-MS analysis was polarity and charge to mass ratio. The quantitative estimation of each peak was made by estimating area of the peak. The GC-MS analysis of *S. cumini* revealed the presence of phytochemical constituents such as phenolic compounds, essential oils, organic hydrocarbons etc. that could contribute the medicinal value of the plant.

Table 1
GC-MS Analysis of S. cumini methanolic seed extract (MSE)

Peak	R. Time	Area	Area %	Molecular Weight	Molecular Formula	Molecule	Application
1	9.063	458311	3.33	72	C4H80	Oxirane 2,3-Dimethyl	Pharmaceuticals
2	12.089	2355535	17.11	126	C6H6O3	2-Furancarbxaldehde, 5-(hydroxymethyl)	Pharmaceuticals
3	13.985	340430	2.47	204	C15H24	Beta Caryophyllene	Pharmaceuticals
4	14.135	623014	4.52	204	C15H24	Caryophyllene	antitumor, analgesic, antibacterial analgesic, aniinflammatory and fungicidal
5	14.819	332501	2.41	136	C10H16	1,3,6 Octatriene,3,7-dimethyl	Pharmaceutical
6	14.938	1091484	7.93	232	C12H18Cl2	5,10-Dichloro-5,10-Dimethyltricyclo [7.1.0.0(4,6)]Decane	Antibacterial, petroleum jelly
7	15.763	74621	0.54	204	C15H24	Germacrene	Flavours
8	15..970	63298	0.46	204	C15H24	2-Isopropenyl-5-Isopropyl-7,7-Dimethylbicyclo[4.1.0]Hept-3-Ene	Laboratory uses
9	16.487	33296	0.24	204	C15H24	Cadinene	Additive
10	17.031	71209	0.52	140	C9H16O	1-Methyl-2-Methylenecyclo Heptanol	Phenol
11	17.390	44804	0.33	125	C7H11NO	Bicyclo [2.2.1]Heptan-2-One	Antibacterial
12	17.839	97164	0.71	168	C10H16O2	Cyclopropane Carboxylic Acid, 3-(3-Butenyl)-2,2-Dimethyl	Laboratory uses
13	18.070	133085	0.97	220	C15H24O	Caryophyllene Oxide	antitumor, analgesic, fungicidal,antibacterial
14	18.662	188648	1.37	138	C10H18	Bicyclo(4.4.O)Decane	Petroleum jelly, antibacterial
15	18.921	16629	0.12	142	C8H14O2	2,3'-Bifuran, Octahydro	Industry
16	19.043	11429	0.08	128	C8H16O	4-Dimehylcyclohexan-1-ol	Laboratory use
17	19.123	30852	0.22	126	C8H14O	Cyclohexanemethanol,4-methylene	Laboratory use
18	19.274	323629	2.35	152	C10H16O	Beta Pinenoxid	Pharmaceutical
19	19.454	194639	1.41	124	C9H16	Cis Salvene	Laboratory use
20	19.973	542613	3.94	306	C20H34O2	8,11,14-Eicosatrieonic Acid	Laboratory use
21	20.853	41473	0.30	144	C8H16O2	Methyl Heptanoate	Perfumes, soaps, cosmetics
22	22.291	54109	0.39	172	C10H20O2	Capric Acid	Flavours, perfumes
23	22.787	270601	1.00	154	C10H18O	3-Thujanol	Medicinal
24	22.817	180405	1.31	126	C8H14O	Cis,cis-4,6-Octadienol	antibacterial
25	23.307	219081	1.59	186	C12H26O	5,9-Dimethyl 1-Decanol	Phenol
26	24.492	43629	0.32	100	C6H12O	Pentanal 2-methyl-	Laboratory uses
27	24.848	133931	0.97	270	C17H34O2	Hexadecanoic Acid Methyl Ester	Antioxidant, antibacterial
28	25.929	1801515	13.08	242	C15H30O2	Tetradecanoic Acid Methyl Ester	Antioxidant, antibacterial
29	27.687	70742	0.51	226	C14H26O2	4-Dodecen-1-ol, Acetate	Perfumes, soaps, cosmetics
30	27.768	22389	0.16	102	C6H14O	Pentanol 2-Methyl	Laboratory use
31	28.085	48679	0.35	110	C8H14	Spiropentane propyl	antibacterial
32	28.171	30711	0.11	100	C6H12O	Oxirane, 3-ethyl-2,2-dimethyl-	Laboratory uses
33	28.513	37606	0.27	284	C18H36O2	Hexadecanoic Acid Methyl Ester	Antioxidant, antibacterial
34	29.134	5376482	19.83	174	C10H22O2	1,10-Decandiol	Bactericidal

A number of phytochemicals were screened in ethanolic and methanolic extract of *S. cumini* seeds but more phytocontents were detected in methanolic extract than that of ethanolic

extract. In MSE, 5,10-Dichloro-5,10-Dimethyltricyclo [7.1.0.0(4,6)] decane (7.93%) was found as major compound followed by tetradecanoic acid (13.08%) α-caryophyllene (4.52%), 1,10-Decandiol (19.83%), Beta Caryophyllene (2.47%), Bicyclo (4.4.O) Decane (1.37%), octadienol (1.31%), cadinene (0.24%), 4-Dodecen-1-ol acetate (0.51%), 2-Furancarboxaldehyde 5-(hydroxymethyl) (17.11%), Oxirane 2,3-dimethyl (3.33%) etc. along with other minor phytoconstituents as described in table 1. Other bioactive components such as germacrene, salvene, capric acid, eicosatrienoic acid, bifuran, cyclohexanemetanol etc. were obtained in ample proportion and enhanced their pharmaceutical value. The peak intensities for phytoconstituents were shown for *S. cumini* MSE in figure 1.

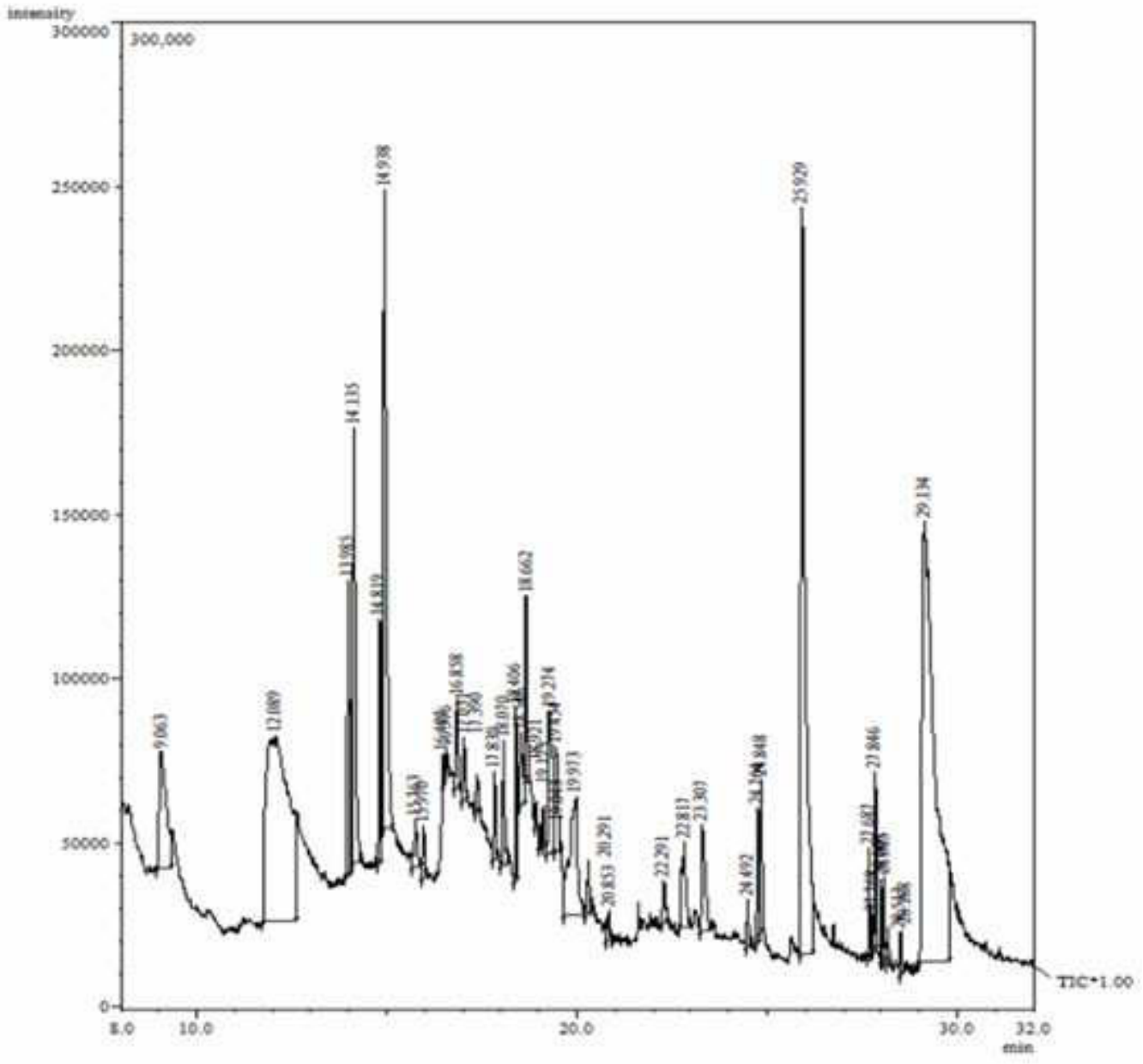

Figure 1
GC MS analysis of S. cumini methanolic seed extract (MSE)

GC-MS analysis of *S. cumini* MSE showed higher content of polyphenolic constituents than esters and oils.

Table 2
GC-MS Analysis of S. cumini ethanolic seed extract (ESE)

Peak	R.Time	Area	Area %	Molecular weight	Molecular Formula	Molecule	Application
1	9.034	1137121	4.19	100	C5H8O2	3(2H)-Furanone, Dihydroxy-2-Methyl-	Laboratory uses
2	11.893	4864853	17.94	126	C6H6O3	5-(Hydroymethyl)-2-Furaldehyde	Laboratory uses
3	13.975	1340124	4.94	204	C15H24	Bicyclo (7.2.0)Undec-4-ene, 4,11,11-Trimetyl-8-Metylene	Laboratory uses
4	14.913	1846651	6.81	136	C10H16	Decahydro 4A-Methyl-1-Methylene-7-(1-Methylethenyl)	Medicinal
5	15.713	242516	0.89	332	C20H28O4	3-Keto-isosteviol	Unknown
6	16.437	125582	0.46	204	C15H24	Germacrene	Odour
7	16.814	244366	0.90	258	C18H26O	1,3-Bis-(2-Cyclopropyl,2-Methylcyclopropyl)-Buten-2,-One-1	Antibacterial
8	16.986	191988	0.71	204	C15H24	Germacra1(10),4(15),5-Triene,	Flavours
9	17.330	122084	0.45	222	C15H26O	2,6,10-Dodecatriene-1-ol, 3,7,11-Trimethyl	Antibacterial
10	17.799	218607	0.81	208	C14H24O	13-Tetradece-11-yn-1-ol	Laboratory uses
11	18.034	233401	0.86	154	C10H18O	3- Thujanol	Medicinal
12	18.371	334918	1.23	152	C10H16O	Limonene Oxide	Flavours
13	18.489	357761	1.32	220	C15H24O	Caryophyllene Oxide	antitumor, analgesic, fungicidal, antibacterial
14	18.996	29548	0.11	142	C9H18O	5-Methyl-5 Octen-1-ol	Phenol
15	19.791	135545	0.50	154	C10H18O	Isogeraniol	Perfumes, fragrance
16	20.289	302738	1.12	168	C11H20O	10-Undecyn-1-ol	Phenol
17	22.462	26107	0.10	112	C7H12O	3-Methyl-4-Hexyn-3-ol	Phenol
18	22.787	270601	1.00	154	C10H18O	2-Methyl-3-Isobutenyl-4-Penten-2-ol	Phenol
19	23.271	335667	1.24	222	C15H26O	Guaiol	Laboratory uses
20	24.816	224708	0.83	284	C18H36O2	Hexadecanoic acid, 15-methyl-, methyl ester	Antioxidant, antibacterial
21	25.949	2942428	10.85	298	C19H38O2	Nondecanoic acid	Antioxidant
22	26.724	7911	0.03	284	C18H36O2	Heptadecanoic acid Methyl Ester	Antioxidant antibacterial
23	27.986	193472	0.71	266	C17H30O2	7,10-Hexadecanoic Acid Methyl Ester	Antioxidant
24	28.500	22645	0.08	212	C13H24O2	9-Dodecanoic Acid Methyl Ester	Antioxidant
25	29.141	5376482	19.83	280	C19H36O	12-Methyl-E,E-2,13-octadecadien-1-ol	Phenol

In ESE, Bicyclo (7.2.0) Undec-4-ene, 4,11,11-Trimethyl-8-Methylene (4.94%) was found maximum along with other phytocomponents such as Nondecanoic acid (10.85%), Decahydro-4A-Methyl-1-Methylene-7-(1-methylethenyl) (6.81%), 12-Methyl-E,E-2,13-octadecadien-1-ol (19.83%), Caryophyllene oxide (1.32%), isogeraniol (0.50%), 3(2H)-Furanone Dihydroxy-2-Methyl (4.19%) and 5-(Hydroymethyl)-2-Furaldehyde (17.11%) etc. as shown in table 2. Thujanol, limonene oxide, guaiol, 10-Undecyn-1-ol, 3-Methyl-4-Hexyn-3-ol, 2-Methyl-3-Isobutenyl-4-Penten-2-ol and other phenolic compounds were found also in moderate amount and increased the medicinal value of *S. cumini* ethanolic seed extract. The peak intensities for the phytoconstituents in S. cumini ESES were shown in figure 2.

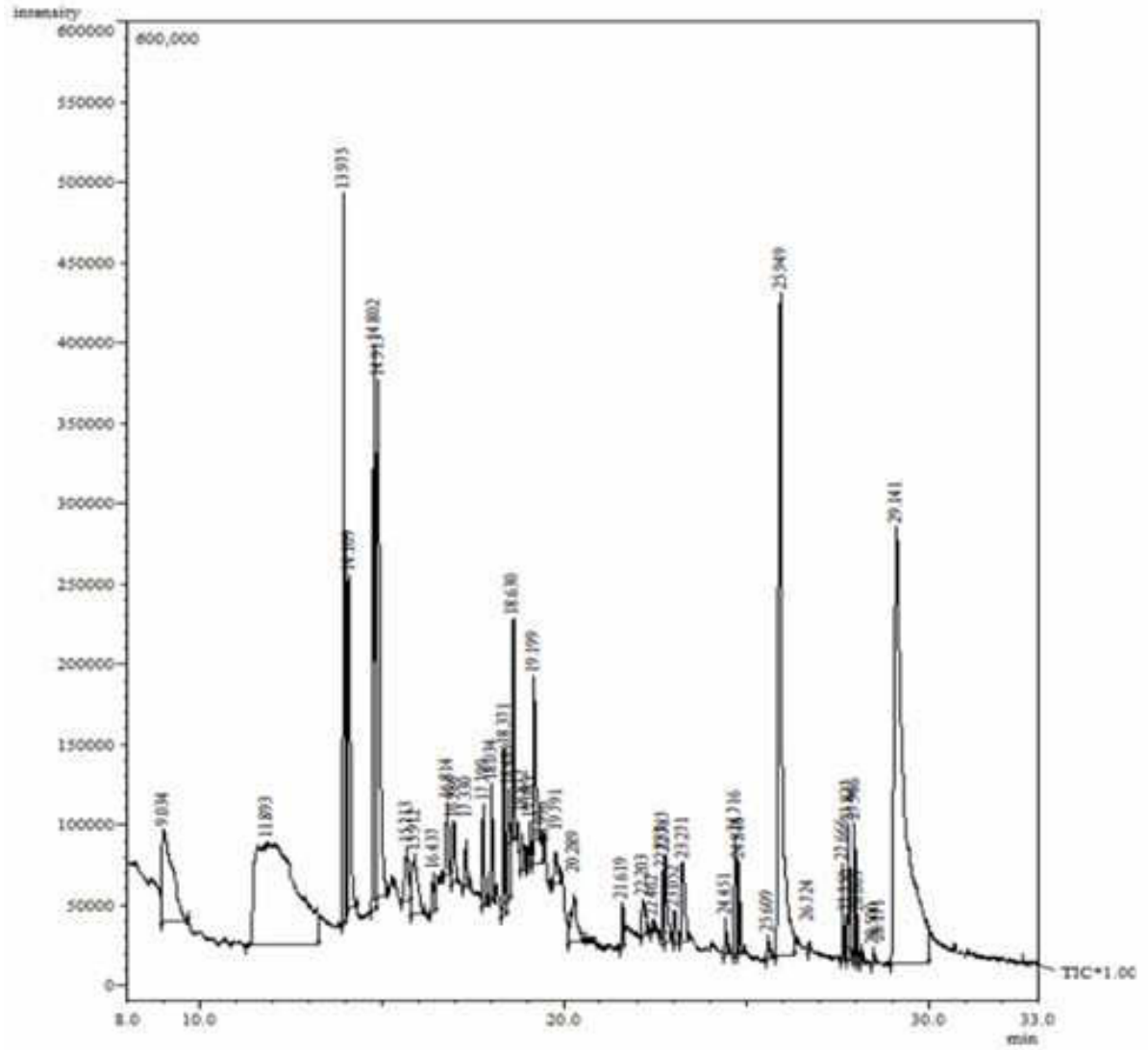

Figure 2
GC MS analysis of S. cumini ethanolic seed extract (ESE)

GC MS analysis of S. cumini ethanolic seed extract (ESE)

GC-MS analysis of *S. cumini* ESE showed higher content of esters and oils than polyphenolic constituents Major volatile and semivolatile compounds were identified in *S. cumini* ethanolic and methanolic seed extracts. Some of the phytocostituents were obtained in both of the extracts in which caryophyllene, germacrene, thujanol, hexadecanoic acid, limonene oxide etc. showed the potency of both ESE and MSE. Due to high no. of bioactive components present in MSE, proved it a more pharmacologically active extract than ESE. The inhibitory activity of *S. cumini* was observed because of presence of polyphenols, flavonoids, terpeniods, sesquiterpenoids etc. Among the identified compounds hexadecanoic acid, dodecanoic acid have the antioxidant and antimicrobial activities.[17] Furan carboxaldehyde has antimicrobial activity and used as a preservative.[18] Bifuran has anticancer activity. Dimethyltricyclyl decane has antibacterial activity and used for the formation of petroleum jelly. Caryophyllene has antitumor, analgesic, antibacterial analgesic, aniinflammatory and fungicidal activities suggesting an increase in electronegativity increases their activities.[19] All of the bioactive components obtained are used for medicinal purposes and can show a good therapeutic value in the various treatments.

CONCLUSION

Thus, in our opinion, various phytocontents were explored in *S cumini* seed extracts by GC-MS analysis and MSE was found to be an enriched extract than that of ESE. The study revealed the presence of some antimicrobial, insecticidal and valuable constituents in *S. cumini* seed extracts and confirmed their pharmaceutical importance.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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