RESEARCH ARTICLE

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ajwain (Trachyspermum ammi) Seed Extract

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ABSTRACT

The objectives of this research were to determine the chemical composition of the extract of *Trachyspermum ammi* L. seeds by using gas chromatography-mass spectrometry analysis (GC-MS).

The GC-MS is a matchless method for the study and measuring quantity of organic volatile and semi-volatile compounds. Gas chromatography is employed to separates mixtures into individual components employing a temperature-controlled capillary column. Mass spectrometry is utilized to recognize a variety of components from their mass spectra. In the present study, volatile/ semi-volatile compounds present in *Ajwain* seed extract were analyzed. *Ajwain* seed extract is extracted by soxhlet extraction method and then analyzed by GC-MS. Total of nine compounds were found and quantified in this study.

The major bioactive compounds in *Ajwain* seed extract are 3,5-dimethylanisole (83.19%), 6-octadecenoic acid, methyl ester, (Z)-, 7-octadecenoic acid, methyl ester (7.42%), and 2-cyclohexyl-2,5-cyclohexadiene-1,4-dione, 4-oxime (3.01%).

Keywords: Analysis, Extract, Gas chromatography-mass spectrometry (GC-MS), Trachyspermum ammi.

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INTRODUCTION

T. ammi is commonly known as *Ajwain*; it belongs to the family Apiaceae,¹ it is herbaceous plant, the herb is widely grown in dry and semi-dry regions.² *Ajwain* is perennial up to 90 cm, the stem is striated, it contains 16 flowers; flowers actinomorphic, white, male, and bisexual; corolla five, petals bilobed; stamens five, alternating with the petals.³

Trachyspermumammi is found all in various regions, such as, Iran, Pakistan, Afghanistan, and India, as well as, europe, while it is indigenous to Egypt.⁴ Seeds or fruits of *Ajwain* are used for medical and nutritional purposes.⁵ *Ajwain* has an assortment of pharmacological action, and it is utilized as a part of phytomedicine around the globe for treated paralysis, tremor, and palsy as well as other neural disorders in the field of neurology.⁶ It also, correct the auditory weakness.⁷ *Ajwain* is used in the treatment of cough and gastrointestinal disorders, such as, nausea, reflux, vomiting, abdominal cramps, and loss of appetite.^{6,8}

Therefore, the present research is conducted to investigate the phytochemical constituents of *T. ammi* L. seeds, using the GC-MS technique.

MATERIALS AND METHODS

Collection of Plant Materials

The *T. ammi* (*Ajwain*) seeds were purchased from Aurangabad, Maharashtra, India. The selected seeds were identified and authenticated in the Department of Botany, Maulana Azad College, Aurangabad (MS).

Preparation of Seed Extract

The seeds of *T. ammi* (*Ajwain*) were grinded to powder using an electrical grinder. The required quantity of the powder of *T. ammi* was weighed, transferred to soxhlet apparatus at 50 to 55°C, treated with mixture from methanol and chloroform (50/50%). The extraction continues until the color disappeared to get an extract. Then, the extract was filtered by using Whatman no. 1, and supernatant was dried by water bath. After that, the extracts 1 μ L of the sample of the solutions was employed in GC-MS for analysis of different compounds.^{9,10}

GC-MS of T. ammi seeds

The GC-MS analysis of extract *T. ammi* seeds was performed using Thermo Scientific triple quadrupole GC-MS (Trace 1300 GC, TSQ 8000 triple quadrupole MS) equipped with TG 5MS ($30 \times 0.25 \mu$ m ID $\times 0.25 \mu$ m df). For GC-MS detection, helium gas was used as a carrier gas at a constant flow rate of 1 mL/min, and an injection volume of 1 μ L was employed. The injector temperature was maintained at 250°C, the ionsource temperature was 250°C, and the oven temperature was programmed from 230°C. The oven temperature was maintained at 50°C isothermal at 280°C, mass spectra transfer line temperature.¹¹

RESULTS AND DISCUSSION

The GC-MS chromatogram analysis of the extract of *T. ammi* seeds (Figure 1) showed nine peaks, which indicates the presence of nine phytochemical constituents. The chemical compounds, molecular formula, and molecular weight were as shown in Table 1. The major phytochemical constituents are 3,5-dimethylanisole at RT (7.00), 6-octadecenoic acid, methyl ester, (*Z*)-, 7-octadecenoic acid, methyl ester at RT (13.67), and 2-cyclohexyl-2,5-cyclohexadiene-1,4-dione,4-oxime at RT (13.92).

From GC-MS analysis, extract of *T. ammi* seeds contains biological activities compounds such as a-cymene act as

antioxidant, anti-inflammatory, antitumor,^{12,13} ç-terpinene has the antitrypanosomal activity,¹⁴ n-hexadecanoic acid (palmitic acid) is reported to be an antioxidant, nematicide, and a pesticide,¹⁵ 3-carene act as general anesthetic,¹⁶ 3,5-dimethylanisole act as antimicrobial, anti-inflammatory, and antioxidant,¹⁷ cis-13-octadecenoic acid act as antiinflammatory, antiandrogenic, anticancer, preservative, and hypocholesterolemic,¹⁸ trans-13-octadecenoic acid act as an acidifier, arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity, inhibit the production of uric acid, urine acidifier, anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, anti leukotriene-D4, hypocholesterolemic,¹⁹

Thus, GC-MS analysis of *Ajwain* seed extract is the first step towards understanding the active compounds in this plant,



Figure 1: GC-MS profile of extract of T. ammi seeds

S. No.	RT	Area %	Compound name	Molecular formula	Molecular weight
1	4.04	0.48	o-cymene	C ₁₀ H ₁₄	134
			benzene,1methyl3(1methylethyl)	$C_{10}H_{14}$	134
			benzene,1ethyl2,4dimethyl	$C_{10}H_{14}$	134
2	4.45	0.72	ç-terpinene	$C_{10}H_{16}$	136
			cyclohexene, 1methyl4(1methylethylidene)	$C_{10}H_{16}$	136
			3-carene	$C_{10}H_{16}$	136
3	7.00	83.19	3,5-dimethylanisole	$C_9H_{12}O$	136
4	12.25	0.73	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
			l-(+)-ascorbic acid 2,6dihexadecanoate	C ₃₈ H ₆₈ O	652
			estra-1,3,5(10)-trien17áol	$C_{18}H_{24}O$	256
5	13.58	1.17	8,11-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
			7,10-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
6	13.67	7.42	6-octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296
			7-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
7	13.92	3.01	2-cyclohexyl-2,5-cyclohexadiene-1,4-dione,4-oxime	$C_{12}H_{15}NO_2$	205
8	14.23	1.28	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl], methyl ester	$C_{22}H_{38}O_2$	334
			8,11-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
9	14.34	2.01	trans-13-octadecenoic acid	C ₁₈ H ₃₄ O ₂	282
			cis-vaccenic acid	$C_{18}H_{34}O_2$	282
			cis-13-octadecenoic acid	$C_{18}H_{34}O_2$	282

Table 1: GC-MS analysis of bioactive components of extract of T. ammi seeds

Table 2: Reported biological activities of compounds present in Ajwain seed extract										
S. No.	Compound name	Area %	Molecular formula	Molecular weight	RT (min)	Reported bioactivity	Structure			
1	a-cymene	0.48	$C_{10}H_{14}$	134	4.04	Antioxidant, anti- inflammatory, and antitumor				
2	c-terpinene	0.72	$C_{10}H_{16}$	136	4.45	Antitrypanosomal	Ķ			
3	n-hexadecanoic acid (palmitic acid)	0.73	$C_{16}H_{32}O_2$	256	12.25	Antioxidant, nematicide, and a pesticide	······································			
4	3-carene	0.72	$C_{10}H_{16}$	136	4.45	General anesthetic	\times			
5	3,5-dimethylanisole	83.19	$C_9H_{12}O$	136	7.00	Antimicrobial, anti- inflammatory, and antioxidant				
6	cis-13-octadecenoic acid	2.01	$C_{18}H_{34}O_2$	282	14.34	Anti-inflammatory, anticancer, and hypocholesterolemic	<u>}</u>			
7	trans-13-octadecenoic acid	2.01	$C_{18}H_{34}O_2$	282	14.34	Arachidonic acid inhibitor, inhibits the production of uric acid, anti-inflammatory, cancer preventive, anemiagenic, insectifuge, and flavor	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			

and such a study would be helpful for a more detailed study. Further investigation into the pharmacological importance of *T. ammi* and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medicinal plants (Table 2).

CONCLUSION

In the present study, nine compounds from seeds extract of *T. ammi* (*Ajwain*) were identified by GC-MS analysis. The biological activities of each of the identified phyto components range from antimicrobial, antioxidant, anti-inflammatory, antitumor, antitrypanosomal, and anticancer. The research findings have shown that the seeds of *Ajwain* are extensively rich in secondary metabolites. The plant seeds have a high potential for a vast number of bioactive compounds, which justified its use for various ailments by traditional practitioners. These findings have provided a scientific basis to the ethnomedical usage of the plant. However, isolation of the individual phytochemical constituents, subjecting it to biological activity and toxicity profile, will give fruitful results.

AUTHORS' CONTRIBUTIONS

BA carried out the experiments and analysis, MH participated in interpretation of results, drafted the manuscript, and also helped in the experiments and analysis. AK and JP carried out the characterization of compounds. All authors have read and approved the final manuscript.

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