



Updated Detailed Review of *Trachyspermum Ammi*: Composition, Applications and Pharmacological Profile

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Abstract: *Trachyspermum Ammi* (T. Ammi) is a traditional medicinal plant belonging to the family Apiaceae. It, also known as Ajacine or Ajwain, is an herb with Egyptian origins. It is distributed all over India and mainly cultivated in Rajasthan and Gujarat. This review is designed to discuss the updated pharmacological activities of T. Ammi, its phytochemistry, herbal formulations, and patents filed on its novel formulations and their actions. Until now, no such review has discussed the above mentioned particulars on the same platform. The review aims to provide the updated traditional and pharmacological activities of *T. Ammi*, along with its phytochemistry, herbal formulations, and microwave-assisted extraction method. The microwave-assisted extraction method is less time-consuming, cost-effective, and gives more extractive value than other methods. We have also added the latest novel patents of the T.ammi. It is an annual plant with tall growth that is fragrant and bears white blooms and little brownish berries, small, grey, bitter, and peppery. The seeds or fruit of this plant are the parts that are used most frequently. Ajwain's fruits have 5% essential oil in them. Ancient researchers highly influenced the use of this herb. The essential oil brings on Ajwain's aroma and flavor. Ajwain is a potent traditional medicine that is frequently used to cure a variety of illnesses in both humans and animals. To include literature evidence to support the article's theme, a search was performed on five electronic databases, including PubMed, Scopus, Web of Science, Embase, and Google Scholar, by using specific keywords.

Keywords: *Trachyspermum Ammi*, Herbal formulations, Phytoconstituents, Patents, Microwave-Assisted Extraction.

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I. INTRODUCTION

Ayurveda has extensively used *Trachyspermum Ammi*, also known as Ajacine or Ajwain, a herb with Egyptian origins and belongs to the Apiaceae family¹. The annual herbaceous plant family Apiaceae includes the highly prized medicinal plant Ajwain (*Trachyspermum Ammi*)². It is an annual plant with tall growth that is fragrant and bears white blooms and little brownish berries, small, grey, bitter, and peppery. Ajwain seeds have a softer flavour when cooked. The seeds or fruit of this plant are the parts that are used most frequently. It resembles caraway or cumin seeds. It has numerous green stems and branches, tiny feather-like leaves, and four to twelve rays of flower heads, and each head has six to sixteen blooms^{3,4}. The bitter and pungent taste of brown, seed-like fruits of *T. ammi* is processed as nutraceuticals for medical and condiment reasons^{5,6}. Alcohols, aldehydes, ketones, acids, and esters are among the oxygenated derivatives of monoterpenes and sesquiterpenes and make up most of the essential oil's volatile constituents⁷. 2.0–4.4% of the seeds' weight is dark oil. Numerous phytochemicals in *T. ammi* have been discovered to contain thymol (35%–60%). Thyme contains -pinene with a high concentration of salt propisochlor (50%–55%) and -pinene (30%–35%), a non-thymol fraction. The entire plant also contained glycosides, resins, starch, inorganic acids, phenolic compounds, amino acids, protein,

coumarins, phlobatannins, carbohydrates, vitamins, minerals, tannins, carotenoids, alkaloids, steroids, saponins, and flavonoids, in addition to volatile compounds^{8,9}. The fruit's water-soluble components, including one monoterpenoid, five novel monoterpenoid glucosides, two glucosides from aromatic compounds, and two glucides, have also been identified⁵. It is commonly grown in dry and semi-dry areas with much salt in the soil. It involves glabrous and minutely pubescent properties and can grow up to 90cm. Although native to Egypt, Ajwain is widely grown and sold in Iran, Afghanistan, Pakistan, India, and Europe^{1,5,10}. The Indian states where it is grown are Bihar, Gujarat, Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, and West Bengal. The seeds contain 2-4.4% of Ajwain oil, a brownish oil¹⁰. The fruit has traditionally been administered to treat stomach issues like flatulence, indigestion, colic, and diarrhoea. Other pharmacological and biological effects include nematocidal, anti-helminthic, anti-filarial, insecticidal, anti-inflammatory, anti-cancer, anti-inflammatory, antiseptic, hypotensive, hypolipidemic, antihypertensive, antispasmodic, anti-lithiasis, diuretic, and antitussive effects^{2,6,11,12}. Recent studies have found the plant's antioxidant, antiviral, antifungal, nematocidal, inflammatory, analgesic, hepatoprotective, antiepileptic, antifever, and viability of spermatogonia stem cells in vitro and wound healing capabilities^{4,8,13}. Noval patents granted on *Trachyspermum Ammi* are listed in Table I.

Table I: Patents granted on *Trachyspermum Ammi*

Country	Inventor	Patent file no.	Invention	References
South Korea	Steel Wire Iron Seo Hwajin Soren Pole	KRI01241180B1 KRI01241180B1	Essential oil from <i>Trachyspermum Ammi</i> having a spermicidal effect and uses thereof	14
Australia	Ganga Raju, Gokaraju Rama Raju, Venkata Kanaka, Venkata Krishna Raju Alluri, and Kiran Bhupathiraju Trimurtulu Golakoti, Krishanu Sengupta, Ranga Raju Gokaraju	AU2019200802A1	Synergistic dietary supplement compositions for enhancing physical performance	15
United States	Esther Pons, Cedric Rousseau, Krishanu Sengupta, Philippe Ragot	US20200390696A1	Edible Product Comprising Reconstituted Plant Material	16
United States	John Turner	US9532593B2	Herbal smoking blend	17
United States	Mariya Grega, Drew L. Lichtenstein, Vivek Kuttappan, Jeffery Escobar Monestel, Mercedes Vazquez-Anon, Graciela B. Arhancet, Matthew Mahoney, Scott Long	US9801845B2	Antimicrobial compositions and uses thereof	18
United States	Jeyaganesh DEVARAJ, Md. Zishan Akhter, and Pranay RANJAN	US20220408734A1	Hybrid solvents and fabrics for antimicrobial application	19
European Patent Office	Yoshikazu Yonei, Masayuki Yagi, Hiroshige Kawai, and Masako Shoshihara	EP2949362B1	Ages-degrading agent and use thereof	20
United States	Sebastian Hoffmann, Philippe Desbordes, Pierre-Yves Coqueron, Ulrike Wachendorff-Neumann, Pierre CRISTAU, and Peter Dahmen	US10130095B2	Fungicidal compositions of pyrazolecarboxylic acid alkoxyamides	21
WIPO (PCT)	Sean EVANS and Donna EVANS	WO201500064A1	Composition for treating pain and/or inflammation comprising eugenol and beta-caryophyllene	22
United States	Gregory Brian LEE	US20200397711A1	Microparticle compositions for treatment of infection or disease, methods of making the same, and	23

			methods of treating subjects with microparticle compositions	
WIPO (PCT)	Samuel Guilds, John Coulthurst, and Seabrook, Jr.	WO2006068759A2	Liposomes containing phytochemical agents and methods for making and using the same	24
United States	David Scharich, Matthew Alan WARD, Brian Lee ORWAT, and III	US20170348449A1	Method of disinfecting a thermal control unit	25
WIPO (PCT)	Ibrahim Abou-Nameh	WO2009135049A1	Methods and compositions of plant micronutrients	26
WIPO (PCT)	Ibrahim Abou-Nameh	WO2010126794A1	Methods for increasing growth and yield of plants with methionine compounds	27

Table I shows the latest novel patents granted by various authorities of different countries on formulations and activities of T. Ammi. All the inventors of different countries representing the particular invention, along with the patent number, title of invention, granting country, and authority, have been listed in this table I. The chemical composition of Ajwain oil with different major constituents including thymol²⁸, γ -terpinene, and p-cymene^{2,29}carvone, limonene, and dillapiole and carvacrol². Fruit extract showed antispasmodic and antihypertensive effects by preventing contraction caused by K⁺. Additionally, the extract showed

protective efficacy in mice against toxicity brought on by CCl₄ and paracetamol^{8,13}. In the traditional medical system, *Trachyspermum Ammi* treats headaches, neurological conditions, joint discomfort, and rheumatoid arthritis. It also reduces inflammation¹³. Bean bug species *Riptortus clavatus* nymphs and adults (male and female) were strongly repulsed by the essential oils from ajowan plants. Thymol and carvacrol, two of the components found in the essential oil of the ajowan plant, had the strongest anti-bean insect repellent effects³⁰(Fig. 1).



Fig.1: *Trachyspermum Ammi* Sprague fruits

2. PLANT PROFILE

It is grown in Afghanistan, India, Iran, Iraq, and Pakistan and originates in Egypt^{1,10,31}. The Indian states where it is grown are Bihar, Gujarat, Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, and West Bengal³². Taxonomical classification is listed in Table 2.

Kingdom	Plantae
Subkingdom	Tracheobionta, Vascular Plant
Division	Magnoliopsida- Flowering plant
Phylum	Spermatophyta, Seed Plant
Subphylum	Angiospermae, Seed Plant
Species	Ammi
Family	Apiaceae
Genus	Trachyspermum
Order	Apiales
Class	Magnoliopsida- Dicotyledons
Common name	Ajwain
Taxonomic rank	Taxon

Trachyspermum Ammi of the Apiaceae family is a medicinal spice of great value. The roots are naturally diuretic, and these extracts have excellent aphrodisiac properties³⁴. The seeds have 2-4.4% of Ajwain oil, a brown oil. Thymol, the major ingredient in this oil, is used to treat bronchial diseases, anorexia, and digestive system issues³⁵. The oil has fungicidal, antibacterial, and anti-aggregatory properties. Ajwain is a potent traditional medicine that is frequently used to cure a variety of illnesses in both humans and animals³⁶. It treats diarrhetic dyspepsia and flatulence. Ajwain seeds are

stinging and bitter and have digestive, anthelmintic, carminative, and laxative properties³⁷. It also has anti-inflammatory and antioxidant properties and relieves stomach problems, aches, and ulcers. The essential oils in the seeds have around 50% of the thymol's potent anti-spasmodic and anti-fungal activities. Furthermore, toothpaste and fragrances contain thymol³⁸. The effect of planting months on different constituents of *Trachyspermum Ammi* is listed in Table 3.

Table 3: Effect of planting months on different constituents of *Trachyspermum Ammi*^{4,10}

Sowing time	(%) Composition					
	Major components				Minor components	Essential oil (kg/ha)
	Thymol	γ -terpinene	p-cymene	β -pinene		
October	61.9	20.16	11.79	1.93	4.27	17.0
November	55.00	24.00	9.07	7.05	7.08	15.82
December	54.13	19.05	9.04	3.39	14.39	14.07
January	42.78	27.35	9.10	14.04	6.73	13.98
February	24.56	15.01	6.65	39.17	14.61	13.21
March	30.69	12.52	4.28	5.62	46.89	10.42

Table 3 shows the potential effect showing period (months) on the percentage composition of some of the major components of *T. ammi*, including thymol, γ-terpinene, p-cymene, and β-pinene. Besides this, the table also depicted the effect on minor components and essential oil composition.

3. BOTANICAL DESCRIPTION

Ajwain is mostly grown in arid and semiarid areas where the soil is highly salty^{5,8}. Ajwain is a 60-90 cm tall perennial

shrub³⁹. The stalk is striped, and the flowers are actinomorphic, white, male, and bisexual, with five corollas, bilobed petals, and five stamens, alternating with petals; the lower ovary has a knob-like stigma; the fragrant fruit is cordate and ovoid, and it is a Cremona carp with persisting stylopodium. 7 pairs of lateral leaflets and a terminal leaflet comprise a leaf pin⁴⁰. The fruit has two grey, compressed, oval mericarps about 1.7 mm and 2 mm long. Each mericarp has five ridges and six vittae, typically independent, principal ridges⁴¹. Vernacular names of *T. Ammi* are in Table 4.

Table 4: Vernacular names of *Trachyspermum Ammi*: - ^{3, 42-44}

Language	Vernacular names
Sanskrit	Yamini, Yaminiki, Yaviniki, Dipyaka
Assamese	Jain
Arabic	Kamun Mulki, Al-Yunan
English	Bishop's weed, Carom, falsly lovage seeds, ajwan seed, Ethiopian cumin
Hindi	Ajwain, Spairkai
Bengali	Yamani, Yauvan, Yavan, Javan, Yavani, , Jain, Jowan
China	Xi Ye Cao Guo Qin
Armenian	Hounastan
Dutch	Ajwan
Farsi	Nanava
Thai	Chilan
Kashmiri	Kath
Gujrati	Ajma, Ajmo, Yavan, Javain
Kannada	Oma, Yom, Omu
Korean	Ayowan
Malayalam	Oman
Nepali	Javano
Punjabi	Lodhar
Marathi	Onva
Urdu	Azwain Desi
Oriya	Juani
Tamil	Omam
Telugu	Vamu
Persian	Nankhah, Zenyan
Turkish	Misiranason
Singhalese	Assamodum

4. MICROSCOPIC CHARACTERS

The flexible part of the fruit displays the creation of two hexagonal bonds joined together by carpophores, epicarps made up of one layer of extended table cells, mesocarps made up of long to polygonal cells with various vittophores, and carpophores veins⁴⁵. Very lengthy cells, the integument, the slow-moving coffin fashioned like a cell, small embryo-filled cells, small, spherical oil molecules—all of them are made up of cells with thin, polygonal walls—make up the endosperm⁴⁶. When the powder is examined under a microscope, it reveals the presence of endosperm cell clusters and oil globules⁴⁷.

5. PHYTOCHEMICAL STUDIES

Investigations of ajwain seeds have shown that they include mineral matter (7.1%) that contains calcium, phosphorus, iron, and nicotinic acid, as well as fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, and flavones³. Thymol is the main component (between 35% and 60%) of the 2% to 4% brownish essential oil produced by ajwain fruits⁴⁸. Paracymene, α -terpinene, α - and β -pinenes, dipentene, β -terpinene, and carvacrol are present in the nonthymol fraction. The plant also contains trace quantities of camphene, myrcene, and α -3-carene. Alcoholic extracts have a saponin that is extremely hygroscopic. The fruits contain a yellow, crystalline flavone, a steroid-like compound, 6-O- β -glucopyranosyloxythymol, glucoside, and 25% oleoresin that contains 12% volatile oil (thymol, γ -terpinene, para-cymene, and α - and β -pinene). Carvone (46%), limonene (38%), and dillapiole (9%) are the main components of *T. ammi*'s oil⁴⁹.

6. PHARMACOGNETIC PROFILE

The plant can be identified for quality and purity of the plant medication which can be standardized with a pharmacognostic profile. A detailed analysis of the drug's organoleptic and physicochemical properties was published by Hardel Danendra Kumar et al. in 2013. The colour of the drug powder is light brown, and the taste is pleasant, while its order is characteristic. The foreign matter is 2.4%w/w, loss on drying is 4.7 \pm 0.29 %w/w, pH of 1% is 3.23 \pm 0.09 w/v; Total ash is 8.6 \pm 0.29%, Acid insoluble ash is 0.49 \pm 0.02%, Water soluble extractive is 42 \pm 0.32, Alcohol soluble extractive 17.9 \pm 0.80, Angle of repose 50 \pm 0.1126, and Carr's index is 27 \pm 1.724⁵⁰.

6.1. Qualitative Phytochemistry

Terpenes, Fixed Oils, Terpenes, and Glycosides are all present in crude drug powder. Seed ethanol extract reveals that reducing sugar, tannins, and glycoside is present. An extract of ethanol and petroleum ether reveals the presence of amino acids, proteins, sterols, terpenes, glycosides, and alkaloids⁵². Katasani Damodar et al., have done a detailed phytochemical study on the seed's methanol, acetone, hexane, and chloroform extract. They found the presence of different metabolites in different solvents. Carbohydrates are present in the methanol, acetone, and chloroform but absent in the hexane; reducing sugar is present in methanol and acetone and absent in chloroform and hexane; Monosaccharide is present in methanol and absent in remaining solvent; Tannins is present in acetone solvent extract and absent in remaining solvent, Terpenes is present

in methanol, hexane, chloroform and absent in acetone, Alkaloids are present in methanol, hexane, acetone and absent in chloroform extract, Anthraquinones are present in methanol and acetone. At the same time, it is absent in the chloroform and hexane extract; Cardiac Glycoside is present in the methanol and acetone solvent extract and in the chloroform and hexane⁵¹.

6.2. Quantitative phytochemistry

According to an examination of ajwain seeds, they contain mineral matter (7.1%), which includes calcium, phosphorus, iron, and nicotinic acid, as well as fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, and flavones. Thymol is the majority (35% to 60%) of the 2% to 4% brownish essential oil that the Ajwain fruits produce. The plant also contains trace amounts of camphene, myrcene, and α -3-carene⁵². The nonthymol fraction (thymene) contains paracymene, γ -terpinene, α - and β -pinenes, dipentene, α -terpinene, and carvacrol. Alcoholic extracts have a saponin that is extremely hygroscopic. A glucoside, a yellow, crystalline flavone, and a compound that resembles a steroid that also contains 6-O- β -glucopyranosyloxythymol have been extracted from the fruits, along with a 25% yield of oleoresin that contains 12% volatile oil (thymol, γ -terpinene, paracymene, and α - and β -pinene). *Trachyspermum Ammi*'s main oil components are carvone (46%), limonene (38%), and dillapiole (9%)⁵³.

7. ESSENTIAL OIL EXTRACTED WITH THE HELP OF MICROWAVE-ASSISTED EXTRACTION

Regarding the quantity and quality of the finished product, the essential oil (EO) extraction process is crucial. Microwave-assisted extraction (MAE), a new extraction method with low energy consumption, high efficiency, short process time, and minimal environmental effect, was used to extract the ajowan EO. The microwave-assisted extraction of ajowan EO and to simulate and improve EO production and chemical profile. The extraction time most significantly influenced the yield and composition, followed by the microwave power. Short extraction times (80 min) and high irradiation powers (1.37 W/g) maximized thymol content, but p-cymene and γ -terpinene content were adversely linked with the above mentioned factors. In contrast to settings that maximize p-cymene or thymol and γ -terpinene, extended extraction durations (160 min) and high irradiation power (1.37 W/g) are more favourable for yield. Higher quantities of thymol and equivalent yields were guaranteed by microwave-assisted extraction compared to traditional hydro distillation operating at the same extraction time. It is interesting to note that the extraction duration influences hydro distillation in a manner like microwave-assisted extraction (MAE). Microwave-assisted extraction has the tremendous benefit of adaptability since its settings may be accurately modified, even though hydro distillation can produce results equivalent to MAE⁵⁴.

8. CHEMICAL CONSTITUENTS OF T. AMMI

Till now, fibers (11.9%), glycosides, tannins, moisture (8.9%), carbohydrates (24.6%), protein (17.1%), fat (21.1%), saponins, and flavones are the phytoconstituents of Ajwain that have been identified so far. Other components (7.1%) include iron, phosphorus, calcium, thiamine, riboflavin, iodine, cobalt,

manganese, nicotinic acid, and copper⁵⁵. Saponin is derived in huge amounts in the alcoholic extract process⁵⁶. Ajwain is popular for the brownish essential oil of the family Apiaceae⁵⁷. Nowadays, Ajwain's aroma and flavor are brought on by the essential oil. Ajwain's fruits have 5% essential oil in them. Nonetheless, some researchers say essential oil outputs can reach 9%, a significant amount⁵⁸. Thymol, carvacrol, γ -terpinene, cymene, and limonene typically make up 35% to 60% of the essential oil in Ajwain, Paracymene, Gamma-terpinene, Alpha-pinene, Styrene, Beta pinene, α -terpinene, Betaphyllanderene Delta-3-carene, carvacol, and terpinene-4-ol are all present in the non-thymol part⁵⁹. Dillapiole (8.9%), carvone (46.2%), and limonene (38.1%) are all present in the oil part. Petroselinic acid, linoleic acid, oleic acid, palmitic acid, and petroselinic acid were all identified from the fruits of Ajwain²⁸. Recently, 6-hydroxy carvacrol 2-O-D-Glucopyranoside and 3,5-Dihydroxytoluene 3-O-D-Galactopyranoside were identified as new glycosyl components from the fruit of ajwain⁶⁰. The fruits of Ajwain contain a chemical called 6-O-glucopyranosyloxy thymol, which resembles a steroid. New

monoterpenoid glucosides include monoterpenoid and 7-tetrol (2S, 6Z) 7, 7-Dimethyloct-3 (10) 1, 2, 6, and 7-Tetrol -ene 6-Hydroxythymol and 1-O-D-glucopyranoside 3-O- β -D-Glucopyranoside these are the Ajwain fruit's water-soluble extract; 2-Methyl-3-Buten-2-ol- β -D-Glucopyranoside Glucide and Benzyl-D-Glucopyranoside in particular (3R) A brand-new aromatic chemical glucoside are 2-hydroxy methyl butane-1,2,3,4-tetrol⁴¹ Nucleosides like uridine and adenosine were also isolated from Ajwain fruits along with other glucosides like 1-Deoxypentitol and 1-Deoxy-L-Erythritol⁶¹. Ancient researchers highly influenced the use of this herb. It exhibited various therapeutic properties and was known to have various chemical properties. This paper reviews the clinical applications of this herb and other pharmacological properties of current medicine. Thymol has antioxidation properties by preventing chelating metal ions and free radicals, and boosting endogenous antioxidants, whether they are dependent on or not⁶². Additionally, the non-phenolic monoterpene terpinene exhibits antioxidant effects⁶³. The chemical composition of *Trachyspermum Ammi* by GC-MS is listed in Table 5.

Table 5: Chemical composition of *Trachyspermum Ammi* by GC-MS^{1,3,64}

Composition	IUPAC	Retention Time
Sabinene	4-Methylidene-1-propan-2-ylbicyclo [3.1.0] hexane	10.43
β -Pinene	6,6-Dimethyl-4-methylidenebicyclo [3.1.1] heptane	10.76
B -Myrcene	7-Methyl-3-methylideneocta-1,6-diene	11.32
Verbenene	6,6-dimethyl-4-methylidenebicyclo[3.1.1]hept-2-ene	11.54
Menthatriene	1-methyl-4-prop-1-en-2-ylcyclohexa-1,3-diene	11.78
Foeniculin	1-(3-methylbut-2-enoxy)-4-[(E)-prop-1-enyl]benzene	12.17
o-Cymene	1-methyl-2-propan-2-ylbenzene	12.65
p-Cymene	1-Methyl-4-propan-2-ylbenzene	12.82
α -Pinene	4,6,6-Trimethylbicyclo [3.1.1] hept-3-ene	13.34
γ -Terpinene	4-Methyl-1-(1-methylethyl)-1,4-cyclohexadiene	13.71
Isobornyl isobutyrate	[(1R,2R,4R)-1,7,7-trimethyl-2 bicyclo[2.2.1]heptanyl] 2-methylpropanoate	14.45
Umbellulone	4-methyl-1-propan-2-ylbicyclo[3.1.0]hex-3-en-2-one	16.83
Tertridecanal	Tetradecane	20.14
Thymol	5-Methyl-2-propan-2-ylphenol	21.86
Davanone	2-(5-ethenyl-5-methyloxolan-2-yl)-6-methylhept-5-en-3-one	23.34
Myristiain	4-methoxy-6-prop-2-enyl-1,3-benzodioxole	27.46
Thymol hydroquinone	2-methyl-5-propan-2-ylbenzene-1,4-diol	34.54
Manool	(3R)-5-[(1S,4aS,8aS)-5,5,8a-trimethyl-2methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3 methylpent-1-en-3-ol	37.19
Thymyl acetate	(5-methyl-2-propan-2-ylphenyl) acetate	41.56
Ionone	(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	42.34
Silphine	(1R,5S,8R,11R)-5,7,7,11 tetramethyltricyclo[6.3.0.0 ^{1,5}]undec-2-ene	45.34
Cedrane	(1S,2R,5S,7S,8R)-2,6,6,8-tetramethyltricyclo[5.3.1.0 ^{1,5}]undecane	49.56

9. PHYTOCONSTITUENTS IN DIFFERENT PLANT PARTS

• Seeds

Seed of T.ammi contains the phytoconstituents α -phellandrene, β -pinene, Cis-myrtanol, γ - terpinene, o-carene, Ot-pinene, p-cumin-7-ol, p- mentha-1,3,8 triene, Thymol, Carbohydrate, Glycosides, Saponins, Calcium, Flavones, Fiber, Moisture, Fat, Protein, Mineral. The pharmacological activities are Hepatoprotective, Antibacterial, Antioxidant,

Antihypotensive, Abortifacient, Estrogenic, Insecticidal, and Antiulcer⁴⁴.

• Fruits

Fruits of T. Ammi contains 3, 5-Dihydroxytoluene 3-O- β -DGalactopyranoside (gycosal compound), 1-(3-isopropyliden-2,2-dimethyl cyclopropyl) isopropanol (Nonterpenoids), Alpha-pinene, Alpha-terpinene, Beta pinene, Beta phellandrene, Carvacrol, Delta-3- carene, Gamma-terpinene, Paracymene, Styrene, Terpinene-4-ol (Non-thymol), 2-

Methyl-3-Buten-2ol- β -D-Glucopyranoside, 7-Dimethyloct3(10)-ene-1, 2, 6, 7-Tetrol 1-O- β -D-Glucopyranoside and 6-Hydroxythymol 3-O- β -D-Glucopyranoside having the pharmacological activities of anti-filarial, antifertility, antipyretic, analgesic, anti-inflammatory⁴⁴.

10. APPLICATIONS OF AJWAIN IN TRADITIONAL PERSIAN MEDICINE AND MEDIEVAL

- This plant is frequently used in conventional medical programs in various specialties and pharmacies. Ajwain has long been well-known in Persian Traditional Medicine (TPM) for its traditional purposes⁶⁵.
- Ajwain seeds are a popular and effective component of the treatment used by Iranian laborers. Ajwain is hot, dry, and acidic to a third degree when it comes to temperature⁶⁶.
- Oral use of seeds is helpful in paraplegia, tremors, and paralysis, as well as other neurological disorders in the brain⁶⁷.
- Persian physicians have also applied eye and ear drops made of Ajwain seeds to control infections and correct hearing loss⁶⁸.
- Ajwain is believed to be useful for coughing, dysphonia, and pleurisy in the respiratory system. The fruit was frequently used to treat anorexia, nausea, vomiting, reflux, and other stomach and liver conditions⁶⁹. They are known to have invigorating and stimulating effects and be helpful for gastrointestinal issues. Ajwain was allegedly used as an anti-helminthic and treatment for different naturally occurring poisonous substances⁷⁰. Also, it is thought that drinking wine helps to break up calculi and stones. Moreover, Iranian workers believe the seeds have aphrodisiac, galactagogic, and diuretic properties⁷¹.
- The topical use of Ajwain as a cosmetic has left the skin with a yellowish tinge. Moreover, it was a component of drugs for pityriasis, leukoderma, and honey in all cases of

ecchymosis⁷². It has been suggested in the field of toxicology that washing the injured region with a decoction of Ajwain seeds lessens the agony brought on by a scorpion bite⁷³. Moreover, it was utilised to lessen the negative symptoms of opiate withdrawal.

- Moreover, Ajwain is described as a strong analgesic and anti-inflammatory. Thus, it is only applied to the afflicted region when combined with egg white or honey. Persian doctors use ajwain to treat persistent fever and complaints. Ajwain seeds may be used to make hydrosol and oil, which have medical uses⁷⁴. Ajwain hydrosol coupled with Cinnamon and Borage was strongly advised as a main therapeutic remedy for the treatment of paralysis, paralysis, tremors, and neurological illnesses such as chronic pain and neuropathic pain, which are listed in the medical manuscripts and Persian Medical literatures⁵⁵.

11. PHARMACOLOGICAL PROFILE

The fruit has traditionally been used to treat intestinal problems such as flatulence, indigestion, colic, galactagogue, stomach, carminative, expectorant, and diarrhea. Recent studies have linked the plant to additional pharmacological and biological effects, including effects on wound healing, hepatoprotection, antiepileptic, antiseptic, amoebiasis, antibacterial, fever, and inflammation. Oil-fried seeds and a mild soup made from the plant's seeds were also used as galactagogues to treat diarrhoea⁴. The fruit powder and the plant's aqueous extract, high in thymol, had a dose-dependent anti-helminthic effect²⁸. The seeds of Ajwain are bitter and act as an anthelmintic, carminative, laxative, and stomachic. It also treats stomach ulcers, stomach aches, and piles. Fruit extract confirmed antispasmodic and antihypertensive effects by preventing contraction caused by K⁺. Many herbal formulations of T. ammi are available in the market; some are listed in Table 6.

Table 6: Herbal Formulations of *Trachyspermum Ammi*

S. No.	Herbal formulations	Uses	Manufacturers	Ref.
1	Ajwain oil (<i>Trachyspermum Ammi</i> 100% Natural Essential Oil)	Used in the treatment of osteoarthritis, cervical spondylitis, rheumatoid arthritis, back discomfort, joint pain, and morning stiffness and useful in aromatherapy.	Salvia Cosmeceuticals	⁷⁵
2	Ammi visnaga	useful in leucoderma, spasms of the uterus, kidney stones, and as a diuretic.	Dr. Willmar Schwabe India Pvt. Ltd.	⁷⁶
3	BrijBooti Ajwain Moti	Used in treating osteoarthritis, cervical spondylitis, rheumatoid arthritis, back discomfort, joint pain, and morning stiffness.	Birju Mahavir Pvt Ltd	⁷⁷
4.	Hingvastak Powder	Boosts the body's capacity to absorb and assimilate nutrients while lubricating the intestines for easier disposal. Stimulates the digestive fire and appetite.	Shivamastu	⁷⁸

Table 6 shows some of the marketed herbal formulations of T. Ammi, along with its uses and the name of the manufacturers. Additionally, the extract showed protective efficacy against toxicity caused by CCl₄ and paracetamol in mice. Catecholamines from the adrenal medulla may contribute to ACTH stress-related relief or an increasing rise in the adrenal gland is accompanied by an increase in catecholamines and intracellular cyclic AMP⁸ release. The actions of Ajwain as per Ayurveda are listed in Table 7.

Terms	Actions
Abhiyantar pachansansthana	It helps the digestive system and is also used to eliminate parasites.
Sansthana karam wahay	It is utilized locally as a Vedna sansthapak analgesic or painkiller.
Sawashana Sansthana	It lessens the amount of Kapha (lubrication) in the human body.
Raktavaha Sansthana	Improves blood circulation
Prajanana-Sansthana	It has important fertility-enhancing qualities.
Mutravaha Sansthana	It facilitates urination.

Table 7 depicts the different nomenclature used in Ayurveda to define the respective action of T. Ammi on the body.

12. PHARMACOLOGICAL ACTIVITIES

12.1. Analgesic and Antinociceptive Effects

Using the Tail-flick Analgesiometer Device, in vivo research was conducted to investigate Ajwain's analgesic and antinociceptive efficacy. Research has demonstrated that after 2 hours of medication administration, ethanolic release considerably lengthens Tail-Flick Latency (TFL). Formalin testing has also been used in experimental research to evaluate the antinociceptive effects of Ajwain hydroalcoholic extract with morphine sulphate⁷⁹. The results demonstrated that in the anxious and recent stages, Ajwain release had an antinociceptive impact⁸⁰. Ajwain essential oil was the subject of research akin to this one, and it was discovered that it performed substantially better in the most recent stage of formalin testing, probably because it contains the chemical thymol⁸¹. Also, the analgesic impact of essential vegetable oils on the temperature of neuropathic feet was examined in a randomized controlled experiment with placebo control. The results showed that Ajwain essential oil significantly reduced foot burns compared with a placebo⁸².

12.2. Antibacterial and Antifungal Activities

The impact of fungal diseases on public health is significant and currently under-researched, and they are an increasing global issue⁸³. The therapeutic benefits and antibacterial abilities of *Trachyspermum Ammi* are well established⁸⁴. In vitro, Gram-positive, Gram-negative, and fungal development are all inhibited by the essential oil of the T. ammi fruit^{2,85}. To test the effectiveness of Ajwain antibacterial agents, aqueous and acetone extracts were tested against *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* Typhi *Salmonella*, the agarreus strain of *Shigella flexnericus*, *Enterococcus faecalis*, and *Staphylococcus agarregus*⁸⁶. Studies have shown that the release of acetone shows more activity when compared to aqueous extraction. The ethanolic extract of Ajwain has antibacterial activity against eight types of *Helicobacter pylori*. And Ajwain methanolic extract has shown anti-bacterial activity against 11 species at 2 mg/source in efficient agar distribution. Measured using the Diameter of Inhibition Zones (DIZ). DIZ was over 15 mm against *Staphylococcus epidermidis* and *Staphylococcus aureus*, 10mm - 14 mm against *Bacillus pumilus* and *Pseudomonas aeruginosa*, and 7-9 mm against *Escherichia coli*, *Bordetella bronchiseptica*, and *Klebsiella pneumoniae*. On the other hand, *Pseudomonas fluorescens* and *Micrococcus luteus* were found to be inactive^{87,88}. The main chemical constituents of methanolic T. ammi extract were thymol 48.96%, p-cymene 23.73%, and gamma-terpinene 15.98%. Since Ajwain may have a high concentration of Carvacrol or Thymol in its total oil, the said

phenolic compounds are reported to be bacteriostatic or bactericidal agents depending on how concentrated Ajwain showed a positive effect on antifungal activity⁷⁵. *Curvularia ovoidea* and *Aspergillus Niger* at 5000 ppm as a low blockade. It also showed the positive effect of the antimicrobial activity of *Trachyspermum Ammi* Oil Against *Enterococcus faecalis* Biofilm Formed on Tooth Substrate⁸⁹. The antibacterial activities of T. ammi seed extracts were higher in methanolic and ethanolic extracts than in aqueous extract. Even at the lowest concentration, Ajwain methanolic extract exhibits a significant anti-fungal effect, suggesting that Ajwain extract has a greater potential for anti-fungal activity⁹⁰. Several T. ammi extracts and essential oils have demonstrated their antifungal and antibacterial properties against various bacteria that cause food spoilage and antibiotic-resistant bacteria^{1,91,92}.

12.3. Insecticidal Assessment

Endophytes have been found to provide host plants with several advantages, including improved nutrient uptake and defense against predators, pests, and abiotic stressors⁹³. Alpha-glucosidase inhibitory activity of isolated endophytes from the T. ammi plant was assessed⁹⁴. T. ammi has been shown to have insecticidal activity against *Sitophilus zeamais*⁶⁴. Since isolate AZ-9 showed the highest activity against α -glucosidase, it was determined that it was *E. spinifera*. *Exophiala* is a saprophytic fungi isolated from oligotrophic hot and humid settings like restrooms, steam rooms, and dishwashers rich in hydrocarbons⁴. T. ammi endophytic fungi were examined for alpha-glucosidase inhibitory activities. Maximum inhibition (96%) was seen in isolate AZ-9, which morphological and molecular evidence determined to be *Exophiala spinifera*. *Spodoptera litura* (Fab.) was fed a diet containing an insecticide to test the insecticidal activity of the inhibitor. Significant larval mortality and emerging adult abnormalities were the results of it. Additionally, a decrease in the activity of digestive enzymes was seen in-vivo. A nutritional examination of *S. litura*'s food utilization parameters demonstrated the harmful effect of the AZ-9 inhibitor. *S. litura*'s relative growth and consumption rates were significantly lower. Essential Oils' Insecticidal Activity Against Adult *P. interpunctella*. When the adults of *P. interpunctella* were exposed to caraway (T. ammi), the mortality values significantly increased by the rising essential oil concentration. The mortality values reached 30% and 70% when the adults were exposed to 6 μ L/L air concentrations of T. ammi, respectively, and all the adults were killed by 14 μ L/L air or higher concentration. The essential oil from T. ammi effectively killed adult *P. interpunctella* insects⁶⁴.

12.4. Anthelmintic Activity

Bovine filarial *Setaria digitata* worms were used as the test subject for an in vitro anti-filaria assay using Ajwain methanolic extract. The extracted crude was subjected to flash chromatography to prepare the directed bioassay classification. Both the active fraction and the raw extraction underwent HPLC analysis. By using both worm motility and MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] reduction assays, the raw extract and active component have demonstrated substantial action against the adult *S. digitata*. The MS, H-NMR, and IR analysis systematically identified a separate functional target as a phenolic monoterpene. The in vivo anti-filaria activity of the substance was next examined against the human filarial worm *Brugia malayi*. The results show that *B. malayi* is resistant to vivo macrofilaricidal action and female worm sterility. *Haemonchus contortis* in sheep and *Ascaris lumbricoides* in humans are considered in the Ajwain Anthelmintic research. The outcomes were brought about by preventing parasites' energy metabolism from working properly by allowing ATPase activity. Ajwain was also reported to have cholinergic activity and peristaltic gut movement. This fact may help in the expulsion of intestinal parasites and contribute to their anthelmintic activity. The presence of thymol in *T. ammi* is important for its anthelmintic activity⁹⁵. Thymol has been shown to interfere with the energy metabolism of parasites by influencing the activity of membrane intrinsic proteins such as ATPases and the permeability of membranes, which results in the discharge of energy sources⁹⁶. Thymol binding to membranes may increase the curvature and polarity of the membrane surface. On the other hand, it can activate and stimulate the binding of some synthetic anthelmintics to the gamma-aminobutyric acid A-receptor (GABAA-R), increasing the effects of these compounds⁴.

12.5. Antiplatelet Activity

The dried ethereal extract of Ajwain is used to conduct the antiplatelet function⁹⁷. Ajwain seeds thereby prevented platelet aggregation by arachidonic acid, collagen, and epinephrine in an in vitro investigation using human blood samples⁹⁸.

12.6. Anti-inflammatory Effects

Ajwain was also tested to show an anti-inflammatory effect. n-hexane, chloroform, methanol, and aqueous extract content have an in vivo anti-inflammatory effect. A plethysmometer was used to measure the size of normal hind paws to assess the anti-inflammatory activity on male Wistar rats weighing 200–225 g kept in usual conditions. After administering the doses for 30 minutes, carrageenan (1%) was subcutaneously injected into the right paw of each rat. A plethysmometer measured the paw size immediately after the carrageenan injection. *T. ammi*'s anti-inflammatory properties were tested on rats at 500 and 1000 mg/kg doses. The edoema caused by carrageenan is a two-phase reaction;

- Kinin, histamine, and serotonin production mediate the primary phase.
- The secondary phase is linked to prostaglandin synthesis.

T. ammi seed extracts have anti-inflammatory effects through various peripheral and central mechanisms (potentiating in the neurotransmission of GABA), including by inhibiting

endogenous substances like leukotrienes and prostaglandins, the main mediators in inflammation. Plant extracts revealed a percentage inhibition of *T. ammi* at a dose of 500 mg/kg; n-hexane showed a more anti-inflammatory effect than chloroform and methanol plant extract. At a 1000 mg/kg dose, n-hexane showed greater anti-inflammatory activity than methanolic and chloroform extracts regarding *T. ammi* percentage inhibition¹³. By preventing IL-1 and IL-8 from being secreted by a bronchial epithelial cell line, it has shown in vitro anti-inflammatory tendency^{99,60}.

12.7. Antitussive and Bronchodilatory Effects

In old medieval manuscripts, the effects of Ajwain antitussive have been documented. In this regard, measuring the quantity of cough generated was used to examine the reported clinical effects of aerosols associated with two different concentrations of macerated and aqueous extracts of Ajwain seeds and codeine, carvacrol, and saline⁹⁹. The results show that both concentrations of Ajwain seeds significantly reduce the number of coughs, which may be due to their potent anti-inflammatory properties¹⁰⁰. Similar investigations have demonstrated the inhibition of Histamine (H1) receptors isolated from Guinea-pig tracheal chains by Ajwain release and essential oils¹⁰¹. Another study examined the bronchodilatory properties of various important Ajwain ingredients in the respiratory field. The findings suggested that the quantity of carvacrol in essential oils may be responsible for their calming and bronchodilator effects¹⁰². An investigation determined if the decocted extract of Ajwain had bronchodilatory effects on asthmatic patients' airways¹⁰³. According to the findings, compared to the concentrated impact of theophylline being used, the emission has an almost bronchodilatory effect on asthmatic airways¹⁰⁴.

12.8. Diuretic and Anti-lithiasis Activity

Ethnopharmacological accounts claim that Ajwain has diuretic and anti-lithiasis properties¹⁰⁵. As a result, studies involving humans were conducted, and Ajwain seeds were taken from milk and administered orally to participants who had urine incontinence for nine days. The results against a pure calcium oxalate stone are good⁷. The ingredients in ajwain, including thymol, carvacrol, and flavonoids, are thought to have diuretic qualities¹⁰⁶. Ajwain extract has been found in animal studies to enhance salt and potassium excretion and urine production. To assess Ajwain's diuretic effects on people, more study is necessary¹⁰⁷. The most typical type of kidney stone, calcium oxalate, has been demonstrated to be prevented by substances found in Ajwain. According to rat research, Ajwain extract decreased kidney stone development by lowering the concentrations of calcium, oxalate, and phosphate in urine¹⁰⁸.

12.9. Antihyperlipidemic Properties

Another proven function of Ajwain is its antihyperlipidemic properties. In vivo, research reveals that Ajwain powder seeds are very effective in lipid profile and can reduce the amount of LDL-cholesterol, total lipids cholesterol, and triglycerides. In addition, seed extraction reduces the atherogenic index and increases HDL-cholesterol levels in albino rabbits. Compared to methanolic extract, petroleum ether extract showed more significant results in increasing HDL cholesterol levels and decreasing LDL cholesterol levels. Additionally, petroleum ether extract effectively

decreased the atherogenic index⁶². The significant anti-hyperlipidaemic effects of the plant's methanol and aqueous extracts are present in rat models with triton-induced hyperlipidemia. The study showed that both extracts at dosages of 3 g/kg and 5 g/kg considerably decreased levels of total cholesterol, triglycerides, and low-density lipoprotein while significantly increasing the blood concentration of high-density lipoprotein¹⁰⁹.

12.10. Detoxification Activity

The release of aflatoxin by Ajwain can seeds supports traditional related reports. Thus, in a randomized controlled trial, Ajwain seed extract showed a significant decrease in aflatoxin G1¹¹⁰. Thymol, one of the essential oils found in Ajwain, is said to have a cleansing impact on the body². Thymol is a naturally occurring antibacterial and antioxidant chemical that has been demonstrated to enhance liver function and aid in removing toxins from the body. Moreover, it could improve digestion by easing the bloating, gas, and other problems with digestion that could result from a buildup of toxins in the body¹¹¹.

12.11. Antioxidant Properties

Free radicals are produced in an organism due to the oxidation of biomolecules such as proteins, lipids, nucleic acids, and carbohydrates. Free radicals are the main initiator of the development of many degenerative diseases. Antioxidants neutralize these free radicals, preventing cell deterioration. Polyphenols are antioxidants that prevent the body's cells and chemical constituents from oxidative and reactive atom damage. The essential oil extracted from T.ammi has high antioxidant activity and is recommended for nutraceutical and pharmacological uses^{2,112}. Hexachlorocyclohexane-induced oxidative stress and toxicity in vivo studies have been used to investigate Ajwain extract's antioxidant and ameliorative abilities. Considering the findings, dietary Ajwain extract can lessen the toxicity caused by hepatic free radical stress. The effect of reactive oxygen species (ROS) and free radicals produced during drought stress^{29,113}. Thymol is also an antioxidant and anti-inflammatory, reducing CRP (C-reactive protein) levels, IL-1, IL-6, TNF-, TNF-, and MMP9 (matrix metalloproteinase 9). The antioxidant activity can be estimated by three different methods, namely FRAP, DPPH, and ABTS⁶⁴. The T. ammi oil sample showed significant concentration-dependent antioxidant activity by reducing the DPPH free radical with an IC50 value of 36.41 g mL. In contrast, ascorbic acid's IC50 value was determined to be 28.09 g mL. It was discovered that the oil functions as an antioxidant and has hydrogen-donating properties similar to those of ascorbic acid. The concentration of the extract plus ascorbic acid (5.0-100 µg mL) had a greater scavenging effect¹¹⁵. A cheap approach frequently used to assess the antioxidative ability of different natural compounds with plant origins is the DPPH assay¹¹⁶.

12.12. Antiulcer Activity

Ajwain has been used to treat intestinal diseases, diarrhea, abdominal pain, and ulcer¹¹⁷. Using different models of lesions, Ajwain ethanolic extract led to a significant reduction in wound index in veterinary treatment and showed wound protection in all models. The herb *Trachyspermum Ammi* for its ability to treat ulcers. Various ulcer rat models were used for the investigation. The ulcer index was significantly

reduced after pre-treating the animals with the plant's ethanolic extract at 100 mg/kg and 200 mg/kg. It significantly decreased the ulcerative lesions, suggesting that *Trachyspermum Ammi* has potent anti-ulcer properties¹¹⁸.

12.13. Antihypertensive Activity

The antihypertensive and antispasmodic properties of Ajwain have been investigated. An aqueous-methanolic extract of seeds reduced arterial blood pressure in models of sedated animals, according to similar research¹¹⁹. Also, the isolated rabbit aorta and jejunum preparations inhibited K-shrinkage when using Ajwain extract. These results support Ajwain's antihypertensive and antispasmodic properties¹²⁰.

12.14. Digestive Stimulant Activity

Traditional healers suggest the cure as a stimulant. Ajwain's ability to raise bile acid, stomach acid, and digestive enzyme activity has been established. The length of the meal may be shortened as well⁴². Ajwain increases pancreatic lipase and amylase activity as an enzyme modulator, which may promote digestive function¹²¹.

12.15. Viability of Spermatogonia Stem Cells in Vitro

Mice male laboratory (3-6 days old) were used, and testis tissue was processed enzymatically in two phases to yield spermatogonia stem cells. The mice's harvested testes were washed in phosphate-buffered saline (PBS)¹¹³. 10, 20, and 30 µL of the T. ammi essential oil were added to the cell cultures. The culture media were changed every other day and kept in a 35°C, 5% CO2 humid environment. Using an inverted microscope and ImageJ software, the diameter and number of colonies were counted and analysed⁸. A hemocytometer was used to count the cells.92.8% of the cells collected from the mouse testes expressed the stimulated by retinoic acid gene 8(Stra8)¹²². The diameter and quantity of colonies developed much higher in the oil treatments than in the control group, especially 20 µL of oil. Oxidative stress and Reactive oxygen species (ROS) can damage cell membranes, enzymes, structural proteins, and nucleic acids. Antioxidants can counteract these negative effects¹²³. After two weeks of therapy, analysis of gene expressions revealed that T. ammi oil affected the gene expressions in spermatogonia cells. The expression of the Bcl2 and BAX genes was changed to decrease cell apoptosis. In the presence of the oil, spermatogonia cell markers Plzf and ID-4 were overexpressed. After being subjected to oil, the expression of ID-4 and Plzf increased, whereas the expression of c-Kit decreased⁸. The main component of the studied oil was thymol, which positively affected the spermatogonia cells in the medium. Thymol has antioxidation properties by preventing chelating metal ions and free radicals and boosting endogenous antioxidants, whether dependent on or independent of enzyme⁶². Additionally, the non-phenolic monoterpene -terpinene has antioxidant effects⁶³. However, glioblastoma cells treated with thymol developed apoptosis due to Ca²⁺ being released from the endoplasmic reticulum¹²⁴. At lesser quantities, thymol and carvacrol both exhibited antioxidant action. According to their concentration, phenolic substances frequently exhibit pro- and anti-oxidant activities¹²⁵. Better results were obtained when 20 µL of the oil was used to treat the spermatogonia cells, indicating the significance of

concentration in preserving spermatogonia cells using *T. ammi* oil⁵.

12.16. Spermicide activity

An in vitro investigation was done to investigate the spermicidal properties of Ajwain essential oil. The results showed that the volatile oil had potent spermicidal action. As a result, the oil may be used as a natural contraceptive¹²¹. Human spermatozoa's membrane and DNA were harmed by *T. ammi* essential oil, which also severely inhibited motility. The findings of decreased viability, acrosomal state, nuclear chromatin status, altered cell surface shape, and loss of viability revealed that exposure to *T. ammi* essential oil severely impaired the cytoplasmic membrane, leading to an imbalanced metabolism¹²⁶.

12.17. Toxicity and teratogenicity

In their survey conducted in 1987, several districts of Uttar Pradesh (India) reported using 14 indigenous medicinal herbs, including *Trachyspermum Ammi*. Of the 155 women in the fertile phase, 50 of the 75 pregnant women surveyed in the village of Kallipuschium, Lucknow district, reported to have used *T. ammi* seed for an abortion. Since the herb was not completely effective, there was a chance that it could result in congenital abnormalities⁶³. Ajwain may be dangerous to consume while pregnant because it is teratogenic in rat fetuses¹²². Based on teratogenicity seen in rat fetuses, ten plants, including *T. ammi*, posed a significant risk of potential human fetotoxicity⁶³.

12.18. Estrogenicity

The National Dairy Research Institute in India⁶³ investigated the estrogenic content of *T. Ammi*. The dry Ajwain seed's total phytoestrogen concentration was at 473 ppm. The herb comes in second place on the list of plants tested for total phytoestrogen concentration. It should be noted that the plant has historically been employed as a galactagogue¹²⁷.

12.19. Antidiabetic Activity and Diabetic Neuropathy

Streptozotocin (45 mg/kg i.p.) was used to cause diabetes in Wistar rats, which was followed by 12 weeks of treatment-induced diabetic neuropathy. To comprehend the neuropathic protection of thymol in STZ-treated Wistar rats, biomarkers were examined. The effects of thymol extracted from *Trachyspermum Ammi* at doses of 10 and 20 mg/kg on streptozotocin-induced diabetes and diabetic neuropathy in Wistar rats have been studied. At this dosage, the blood glucose level was seen to be declining. The stimulation of insulin secretions from beta cells, which increase glucose metabolism and regenerate the surviving cells, must be the reason for the decrease in blood glucose levels. Diabetic neuropathy, a common consequence of prolonged diabetes conditions, is treated using the protective actions of the isolated molecule thymol. People with diabetes who suffer from nerve damage and peripheral neuropathy report losing their ability to feel pain¹²⁸. A delayed reaction in diabetic control group animals due to extensive nerve damage and loss of perception caused by prolonged diabetes. A progressive healing activity was observed in treated groups, particularly in groups receiving 20 mg/kg of thymol. However, the diabetic groups tended to have high sensitivity

responses in nerves, which delayed responses during week 12 due to severe nerve damage¹⁰⁰.

12.20. Anti-microbial

The ability of *Trachyspermum Ammi* to fight pathogens like *Klebsiella pneumonia*, *E. coli*, and *Staphylococcus aureus* was collected from urine cultures of patients hospitalized with urinary tract infections. The study showed that the plant's essential oil was active against *E. coli* at a minimum inhibitory concentration (MIC) of 100 ppm, whereas the highest MIC against *Klebsiella pneumonia* was 250 ppm¹²⁹. *Trachyspermum Ammi* has good antimicrobial effects on gram-negative microbial strains. The plant's ethanol extract was efficient against the gram-negative bacterium *Pseudomonas sp.*, while the acetone extract displayed strong antibacterial potential against *Escherichia coli*. The anti-viral potential of *Trachyspermum Ammi*'s methanolic extract against the Hepatitis C Virus (HCV) protease in in-vitro research. The extract was found to be a strong inhibitor of the tested virus⁵⁵. The anti-fungal effects of the *Trachyspermum Ammi* flavonoid extract on proinflammatory biomarkers such as tumor necrosis factor (TNF-) and interleukin-18 (IL-18)³⁷. Essential oils and their corresponding fractions were tested for their antifungal properties against nine different strains of fungi from the American Type Culture Collection (ATCC), including *Candida krusei* (ATCC 6258), *Candida albicans* (ATCC 10261), *Candida parapsilosis* (ATCC 4344), *Candida glabrata* (ATCC 90030), *Aspergillus fumigatus* (ATCC (CBS 14.65)), *Aspergillus flavus* (ATCC 64025). Thymol, carvacrol, *r*-cymene, and *g*-terpinene are present in *Trachyspermum Ammi* essential oil and are responsible for their antibacterial properties. A lower concentration of *Trachyspermum Ammi* essential oil (0.3 mg/mL) may prevent the growth of some strains of *Staphylococcus aureus*. *Trachyspermum Ammi* essential oil has fungistatic properties against the *Candida* and *Aspergillus* species at low concentrations (0.0625 to 1 mL/mL)¹³⁰.

12.21. Anti-amnesic

A 10-day in-vivo experiment using mouse models to assess the anti-amnesic effects of treating with *Trachyspermum Ammi* seed powder. Scopolamine, electroshock, and alprazolam were used to create amnesia. Several variables were calculated throughout the trial, including serum cholesterol, brain monoaldehyde (MDA), brain-reduced glutathione (GSH), and brain nitrite. The step-down latency of the Passive avoidance paradigm (PAP) and the object identification task's (ORT) discrimination index significantly increased, together with the rise in brain glutathione (GSH) levels, a significant decrease in the brain's MDA level, AChE activity, and nitrite level¹³¹.

12.22. Anti-Candida activity

The most typical cause of human fungal infections, which can range from superficial mucosal infections to widespread, potentially fatal infections, is *Candida albicans*³³. The majority of disseminated mycoses are candidiasis-related. The fungus *Candida albicans* is the one that results in the most invasive and opportunistic fungal illnesses. It was discovered that an Ajwain methanol extract was efficient against *C. albicans*. Aromatic water (AW) was tested for its antifungal properties against 16 common *Candida* strains, including *C. tropicalis*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. dubliniensis*, and *C.*

parapsilosis. The phenolic monoterpenes found in AW have antifungal properties. In addition to its antibacterial characteristics, this plant has a pleasant scent and antioxidant qualities. There is evidence that *T. ammi* AW's EO has anti-candida activity and is high in phenolic monoterpenes¹³². These monoterpenes cause changes in the cytoplasmic membrane's permeability, which leads to the leakage of ions and intracellular components and the development of their antibacterial properties³³. Moreover, it has been observed that thymol and carvacrol have a fungicidal effect by preventing the synthesis of ergosterol and disrupting membrane integrity. Once *T. ammi* AW was administered to the mice, candidal colonisation decreased, and the healing process began in the necrotic tissue. It also reduces inflammation and hyperemia. Carvacrol and T, thymol, which are the two primary components of the EO of AW, have anti-inflammatory or antioxidant properties⁴². The presence of phenolic monoterpenes in the EO of AW is responsible for the significant antifungal activity of AW, as evidenced by the eradication of *C. albicans* in the afflicted tissue. AW's antibacterial and antioxidant properties significantly impacted inflammation and started the necrotic tissue's healing process. To make topical herbal treatments for *Candida albicans* infections, *T. ammi* extract can be employed⁶³. The *Trachyspermum Ammi* seed extract demonstrated stronger antifungal effectiveness, with maximal inhibition zones against *C. albicans* measuring 38.3 mm and 31.3 mm, respectively¹³³.

12.23. Anti-Rheumatic Potential

An autoimmune inflammatory disease called rheumatoid arthritis (RA) is characterised by synovial hyperplasia, which erodes bones and cartilage, leads to joint dysfunction, and increases the risk of mortality¹³⁴. Two crucial inflammatory mediators involved in RA and other inflammatory illnesses are prostaglandins and nitric oxide (NO)¹³⁵. Thymol has anti-inflammatory characteristics and lowers matrix metalloproteinase 9 (MMP9) levels, IL-1, IL-6, TNF-, TNF-, and CRP (C-reactive protein)⁸³. Biogenic selenium nanoparticles (SeNPs) produced from *Trachyspermum Ammi* demonstrated anti-rheumatic and immunomodulatory effects. 25 mice were used to test the toxicity of these selenium nanoparticles (SeNPs)¹³⁶. Compared to healthy mice, the tested doses of SeNPs exhibited no observable adverse effects on the kidney, serum, liver, and spleen biochemical markers. The SeNPs treatment decreased the disease's severity, as shown by decreased paw edema and decreased lymphocytic cellular infiltration in the histological findings. SeNPs also showed a dose-independent improvement in the redox state of the inflamed synovium by greatly increasing the activity of antioxidant enzymes concerning the arthritic controls¹³⁷.

12.24. Antispasmodic Activity

Trachyspermum Ammi Essence (TAE) can suppress a strong spasmolytic and anti-spasmodic effect in an isolated rat's ileum. Ileal smooth muscle significantly relaxed when exposed to the ethanolic and aqueous extracts. Compared to aqueous extracts, TAE showed a stronger inhibitory effect on Ach-induced contractions. Several mechanisms mediate the relaxation of the gastrointestinal smooth muscle. The blocking action on excitatory pathways occurs through cholinergic and histaminergic pathways³³.

12.25. Anti-viral

The Apiaceae plant species *T. ammi*'s seed oil demonstrated exceptional antiviral activity¹³⁸. Plaque assay in vitro was used to quantify the Japanese encephalitis virus (JEV) titer, and a plate-reduction neutralization test (PRNT) was used to measure the antiviral activity of Ajwain oil. According to their research, Ajwain oil shows antiviral action in vitro against (JEV)^{139,140}.

13. CONCLUSION

The current review was designed to provide insight into the pharmacological activities, phytoconstituents, novel patent, herbal formulation, and Microwave-assisted extraction of very useful and excellent Sprague fruits, *T. Ammi*. The microwave-assisted extraction process is less time-consuming, cost-effective, and gives more extractive value than other methods. *Trachyspermum Ammi* exhibited various pharmacological properties that may be attributed to its various pharmacologically active phytoconstituents. The broad range of pharmacological activities of *T. Ammi* includes nematocidal, anti-helminthic, anti-filarial, insecticidal, anti-inflammatory, anti-cancer, anti-rheumatic, anti-inflammatory, antiulcer, antiemetic, antihypertensive, antihyperlipidemic, antispasmodic, anti-candida, anti-lithiasis, antidiabetic, diuretic, analgesic, antitussive, antiviral., etc. Glycosides, resins, starch, inorganic acids, phenolic compounds, amino acids, protein, coumarins, phlobatannins, carbohydrates, vitamins, minerals, tannins, carotenoids, alkaloids, steroids, saponins, flavonoids are the different class of constituents present in the *T. ammi*. Thymol is a major constituent with antioxidation properties, as evidenced by reduced chelate formation and free radical generation. Along with this, it also boosts the formation of endogenous antioxidants. The toxicity profile states that *T. ammi* might be dangerous for pregnant women because its teratogenic potential was reported in rat fetuses. Besides the aforementioned toxic potential in pregnancy, *T. ammi* is a very useful fruit having a wide range of active pharmacological agents with broad pharmacological profiles.

14. AUTHORS CONTRIBUTION STATEMENT

Mr. Kamal Nabi carried out the literature survey and wrote the manuscript. Mr. Imanshu aided in writing. Mr. Saswat Swarup did the formatting and provided the necessary inputs related to Ayurveda. Ms. Deepika Bhatia and Dr. Manisha Bhatti have done the language editing and formatting job. Dr. Lovedeep Singh has conceptualized and designed the idea.

15. CONFLICT OF INTEREST

Conflict of interest declared none.

16. LIST OF ABBREVIATIONS

Essential oil (EO), Microwave-assisted extraction (MAE), Diameter of Inhibition Zones (DIZ), Japanese encephalitis virus (JEV), Plate-reduction neutralization test (PRNT), *Trachyspermum Ammi* Essence (TAE), Selenium nanoparticles (SeNPs), Nitric oxide (NO), Rheumatoid arthritis (RA), Aromatic water (AW), Passive avoidance paradigm (PAP), object identification task's (ORT), Matrix metalloproteinase 9 (MMP9), C-reactive protein (CRP), Acetylcholinesterase activity (AChE), Tumor necrosis factor (TNF), Hepatitis C

Virus (HCV), Minimum inhibitory concentration (MIC), Reactive oxygen species (ROS), Minimum essential medium alpha (MEM α), Phosphate buffered saline (PBS),

Streptozotocin (STZ), Gamma-aminobutyric acid A-receptor (GABAA-R), Tail-Flick Latency (TFL).

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