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Trachyspermum ammi L. (Ajwain): Phytochemical composition and pharmacological applications-development and insight

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Abstract
Trachyspermum ammi L (Ajwain) is a widely recognized medicinal seed and a member of the Apiaceae family, which has been valued in both culinary and traditional medicine for its wide-ranging health benefits. It has been commonly used to aid digestion, relieve respiratory issues, ease menstrual discomfort, and support lactation. Modern research is now uncovering the scientific basis for these traditional uses and highlighting the bioactive compounds in ajwain, such as thymol, carvacrol, limonene, and p-cymene, which contribute to its medicinal effects. Evidence from preclinical and clinical studies suggests that ajwain, especially in the form of essential oil, exhibits anti-inflammatory, antimicrobial, gastroprotective, cardioprotective, and anticancer effects, often with comparable or greater efficacy than conventional drugs and with fewer adverse effects. Additionally, recent studies suggest that ajwain may have a potential role in modulating gut microbiota and contributing to integrative and preventive medicine. Despite extensive research, several challenges remain that need to be addressed, including the need for standardized extraction methods, a deeper understanding of bioavailability and pharmacokinetics, and further clinical validation. This review provides an update on current development regarding the phytochemistry, pharmacological properties, and therapeutic potential of ajwain, offering a comprehensive insight that connects traditional practices with modern scientific evidence.

Keywords: Ajwain, *Trachyspermum ammi* L., phytochemical composition, pharmacological properties, toxicity

1. Introduction
Ajwain seeds, scientifically known as *Trachyspermum ammi* (L.) Sprague (syn. *Carum copticum* L.) is a member of the Apiaceae family and has been studied extensively for its unique medicinal properties, which enrich our health and wellness. Traditionally, ajwain seeds have been used in households for centuries not only to enhance culinary flavor but also to relieve flu, menstrual pain, and digestive discomfort. Also, ajwain was used as a home remedy by wet nurses as an abortifacient and as a galactagogue to support lactation. Beyond domestic remedies, ajwain has also found a role in professional medical settings, including the use of cardiac glycosides present in the crude aqueous seed extract for the treatment of cardiac problems [1]. Locally and culturally, ajwain seeds provide a wide range of practical applications. Their carminative properties can help alleviate digestive issues such as indigestion, nausea, diarrhea, flatulence, menstrual cramps, and a poor appetite [2]. Remarkably, it has also been used to alleviate the adverse effects associated with opioid withdrawal [3]. Thymol is a significant monoterpene phenol in the essential oil of the plant with primarily anti-inflammatory, antimicrobial, gastroprotective, and hepatoprotective activities [4]. Although ajwain has been used for centuries in culinary and medicinal traditions, scientific knowledge about its complete pharmacological profile is still emerging. Much of the available research is scattered across studies that focus either on individual bioactive compounds or on selected therapeutic effects, making it difficult to gain a comprehensive understanding of the plant. In this review, we aim to integrate and critically analyze the current knowledge of ajwain, covering its traditional significance, phytochemical composition, and experimentally validated pharmacological activities.

By bringing these dimensions together, we made an effort to provide an updated and integrative perspective on Ajwain seeds that not only strengthens the scientific basis for its therapeutic applications but also highlights its emerging relevance in preventive and integrative medicine.

2. Methodology

The literature used in this paper was searched and obtained from search engines like PubMed, ScienceDirect, MDPI and Google Scholar using keywords "Ajwain", "*Carom copticum*", "*T. ammi*" as well as combinations of keywords to narrow down the search further such as "Ajwain and pharmaceutical effects", "Ajwain AND anti-cancer", "Ajwain AND traditional medicine" and "Ajwain and phytochemistry". Papers obtained were filtered to include only those from 2015 onwards for review. Ultimately, we used 53 published papers, of which 1 was a clinical trial.

3. Plant description

The plant grows to a height of 90cm and is cross-pollinated, producing clusters of small, white, 5-petaled flowers. The Ajwain seed has an acute almond shape and is brown or greyish. The most remarkable characteristic of the seed is its pungent smell, which contributes significantly to its uses in cooking and aromatherapy, found across various cultures [5].

3.1 Scientific Name and Classification

In the extensive parsley family of *Apiaceae*, consisting of approximately 3780 species, our plant of interest is *Trachyspermum ammi (L.) Sprague*, commonly known as Ajwain. The flowchart (Figure 1) below outlines a more detailed taxonomical classification order of this plant.

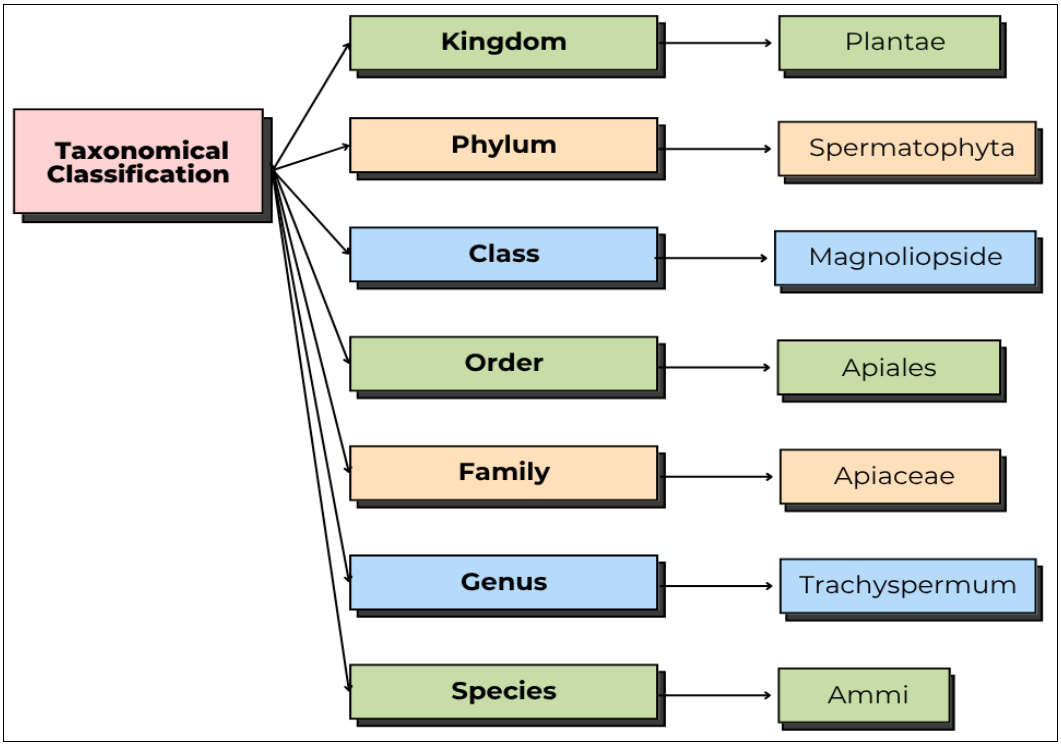


Fig 1: Scientific Taxonomical Classification [6-7]

Table 1: Ajwain in native languages

Language	Name Used	References
Sanskrit	*Yamini, Yaminiki, Yaviniki, Yavani*	6, 8
Assamese	*Jain, Joni-gutti*	6
Bengali	*Yamani, Yauvan, Yavan, Javan, Yavani, Yoyana*	6
English	*Bishop's weed*	6
Gujrati	*Ajma, Ajmo, Yavan, Javain, ayamo*	6
Hindi	*Ajwain, Jevain*	6
Kannada	*Oma, Yom, Omu, ajamoda*	6
Malayalam	*Oman, Ayanodakan*	6
Marathi	*Onva*	6
Oriya	*Juani*	6
Tamil	*Omam*	6
Telugu	*Vamu*	53
Arabic	*kamun al-mulaki, taleb el koub, Nankha*	4, 6, 52
Farsi	*nanava*	6
Korean	*ayowan*	6
Punjabi	*ajavain, lodhar*	6
Nepali	*javano*	6
Portuguese	*oregano-semente, ajowan*	6
Singhalese	*assamodum*	6
Thai	*chilan*	6
Turkish	*misiranason*	6

3.2 Synonyms
Ajwain is a prevalent ingredient in Asian and Arab households for culinary purposes. It is known by various names, including Caraway Seed, Bishop's Weed, Carom seed, Ethiopian Cumin, Wild parsley, and Carom ajwain, among others. The Latin name for this spice is *Carum copticum*, and it has also been referred to as "Zenyen" or "Nankah" in medieval Persian pharmaceutical manuscripts. In Ayurvedic practices, ajwain is known as "Admoda Arka" [6]. Below is a table (Table 1) listing the names by which ajwain is commonly referred to in other native languages.

3.3 Distribution
Ajwain seeds are usually grown and cultivated throughout Mediterranean regions, South Mexico, North-East Africa, Costa Rica, Europe, and South-West Asian countries such as Pakistan, Afghanistan, Iran, Iraq, and Baluchistan. However, they are native to the Egyptian and Indian subcontinents. In India, the major ajwain-producing states are Rajasthan, Maharashtra, Gujarat, Bihar, Madhya Pradesh, West Bengal, and Uttar Pradesh. Crops are also grown on a large scale in places such as Australia, Hawaii, the Philippines, South Africa, Sri Lanka, Southeast Asia, and Tropical America. It is used extensively in Ayurveda, Persian, Japanese, and Yunani medical systems, where many of its medicinal properties are employed for a range of illnesses and therapies [8].

3.4 Cultivation
Ajwain is an annual herb that typically flowers from October onward and can be found in flower by January. Within April, it may bear fruit and can be harvested by May or June. It is cultivated in arid lands with high salinity, pH 6.5 to 8.2, and temperatures of 15 to 25 °C. Hence, it is cultivated in the less hot months of Egypt and India [6]. The aroma of the seed and the plant itself can vary according to cultivation conditions, including soil type, temperature, humidity, extraction time, and other factors; hence, there may be differences in seeds based on the region from which they were harvested. It also plays a role in the phytochemical components of the extract, which may differ accordingly. Thymol is present in a substantial amount in Ajwain oil, ranging from 30-60%, but this may sometimes be overshadowed by other minor constituents, such as γ-terpinene and p-cymene, in seeds harvested from other regions or at different times.

4. Traditional Significance
Over the centuries, ajwain seeds have been an integral part of traditional Indian treatments for common ailments. Forty-one of these include a variety ranging from acidity, alcohol cravings, stone diseases, colic in babies, diabetes, paralysis, sexual disabilities, and even deafness, whereby simple concoctions were prepared and topically applied or orally administered to receive the desired treatment [9-10]. Not just for internal qualms, ajwain has been shown to benefit when used topically as well. It has been incorporated into medications for skin issues such as pityriasis and eczema, rheumatic soreness, and even to relieve the pain of scorpion bites, according to Persian medicine [6]. It has been used in Ayurvedic medicine to reduce ringworm infections on the skin [8]. When discussing insects, ajwain extract is used for its insecticidal and larvicidal properties, which can significantly contribute to reducing cases of malaria and yellow fever [11]. In Ayurveda, the ajwain herb is known as “Yavani,” and the extract of ajwain is referred to as “Admoda Arka” [8]. According to the Vaidya Gurus, it is used as a traditional medicine for coughs, colds, pain, headaches, heartburn, asthma, diarrhea, painful menstruation, cholera, stomach discomfort, and smooth respiratory and kidney function. According to Ayurveda manuscripts, some of the essential characteristics of ajwain help cure diseases such as Grahani (irritable bowel syndrome), Gulma (abdominal tumors), Kasa (cough), and Anaha (bloating) [6]. The various parts of the ajwain plant, like fruit, leaf, and root, are utilized for their diverse medicinal properties. The fruits of this plant possess the following properties: Tonic, anti-spasmodic, carminative, and stimulant, which are used to treat various health problems, including cholera, indigestion, diarrhea, atonic dyspepsia, colic, flatulence, bronchitis, and sore throat. The fruit is crushed into a paste, which is externally applied to alleviate colic pain. In traditional medicine, a compress made from crushed or heated fruit is used in the fomentation process and applied to the chest area to treat respiratory issues such as asthma, cough, or congestion. Furthermore, the leaf is crushed and made into a juice, which serves as an anthelmintic agent that helps the body eliminate parasitic worms. Meanwhile, the root is diuretic and carminative, relieving stomach diseases and pyretic conditions [8]. Furthermore, in addition to these medicinal practices, fumigation with ajwain powder, along with other medicinal herbs, has been found to reduce airborne bacteria and is a prescribed approach for treating bacterial infections. This is commonly practiced on infants and mothers as part of postpartum care to prevent diseases and promote healing [12].

In Persian medicine, ajwain has been a primary factor in opium withdrawal treatments. This was a problem addressed in the health care of countries such as Afghanistan, Turkey, Iran, the UK, and the US. Furthermore, in ancient Persian medicine records, ajwain was part of the list of herbal substitutes to help quit opium addiction. It was also used in neurological therapeutics in traditional Persian medicine for disorders such as paralysis, palsy, and tremors [8]. Even administered as eye and ear drops for auditory ailments and is more commonly prescribed for gastrointestinal and respiratory problems. Awain was also used to dissolve kidney stones, such as calculus, when consumed with wine. Furthermore, it was administered locally for pityriasis, leukoderma, all kinds of ecchymosis, and even used for the treatment of female genital disorders [2]. In traditional Yunani medicine, the ajwain seeds are called ‘Naankhwah’, where ‘Naan’ translates to ‘food’ and ‘Khwah’ translates to ‘desiring’, as it plays a significant role in the digestion of food. It also aids in the healing of drug addiction, such as ‘Afiyoon’ (refined or condensed opium) [9].

5. Chemical constituents
The chemical profile of ajwain reveals the compounds responsible for its extraordinary therapeutic properties. Analysis of ajwain essential oil (EO) revealed a list of flavonoids, terpenes, and phenolic compounds. The comprehensive table below (Table 2) highlights the chemicals responsible for ajwain's overall well-being and protection against ailments such as microbial infections and inflammatory conditions.

Table 2: Chemical constituents and their specifications [6, [2, 13]

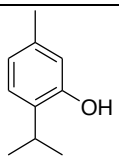
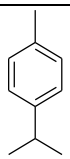
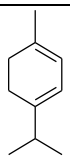
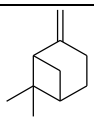
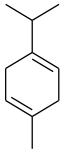
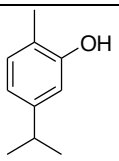
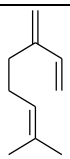
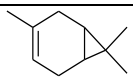
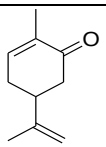
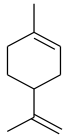
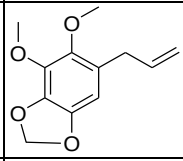
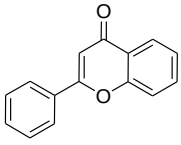
Chemical	Structure	Percentage	Effect	References
Thymol		35-60%	Anti-inflammation, Antioxidant, Anti-microbial, Anti-spasmodic	2, 3
p-cymene		50-55%	Anti-microbial	13
Terpinene		30-35%	Antioxidant	13
β-pinene		4-5%	Anti-aging	13
γ-terpinenes		30-35%	Insecticidal, Estrogenic, Antiulcer, Antioxidant, Abortifacient, Antibacterial, Antihypotensive, Hepatoprotective	6, 13
Carvacrol		7.1%	Anti-aging, Antioxidant, Anti-diabetic, Muscle relaxant, Bronchodilatory effect.	6, 13
Camphene		Found in trace amounts	Hypolipidemic	8, 14, 15
Myrcene			Antioxidant, Anti-microbial	13
3-carene			Anti-aging, Anti-microbial	13, 15
Carvone		48%	Anti-microbial, Anti-inflammatory, Anti-spasmodic, Antioxidant	6, 15
Limonene		38%	Anti-tumor, Anti-inflammation, Anti-aging, Anti-microbial	6, 13
Dillapiole		9%	Antioxidant, Anti-inflammatory, Anti-proliferative on cancer cells	6
Flavones		7.1%	Antioxidant, Antibacterial, Antihypotensive, Hepatoprotective	2, 6, 8
Zinc	-		Anti-diabetic, Anti-ulcer	13
Manganese	-		Anti-diabetic	13

Table 3: General nutrient composition [5]

Nutrient Group	Percentage
Carbohydrates	25%
Protein	17%
Fat	21%
Fibres	21%
Vitamins	
Thiamines	0.21mg/100 g
Riboflavin	0.28 mg/100 g
Nicotinic acid	2.1 mg/100g
Carotene	71 mcg/100g

6. Pharmacological effects

6.1 Antioxidant effects

The antioxidant activity of ajwain is primarily associated with the presence of oxygenated sesquiterpenes and monoterpenes, namely thymol, p-cymene, -terpinene, and -pinene compounds [16]. The ethanolic seed extract of ajwain seed, which contains phytochemicals such as alkaloids, glycosides, terpenoids, saponins, phenols, and steroids, demonstrates significant antioxidant and free radical scavenging properties. Also, the extract could serve as a natural antioxidant and a potential therapeutic agent for preventing oxidative stress-related degenerative diseases [17].

The methanolic extracts of ajwain seeds have also displayed antioxidant properties and effectively inhibited the production of hydroxyl radicals. An increase in the concentration of these extracts, as demonstrated by reducing power, indicates their ability to scavenge free radicals [18]. Ajwain seeds EO exhibited promising antioxidant efficacy in DPPH and superoxide anion scavenging assays [19].

A study has suggested that EO extracted from ajwain seeds could serve as a natural alternative to chemically synthesized preservatives used in packaged foods [20]. This is supported by the fact that high concentrations of the primary component, thymol, expressed increased levels of antioxidant activity, which can thereby prevent the rancidity of food materials. Moreover, the antioxidant effects of ajwain extract are strongly associated with the concentration of substances such as flavonoids, phenolic acids, and carotenoids, which are found in increased levels in frozen ajwain leaves compared to fresh leaves. The low temperatures were believed to disrupt the cell walls of the plant, which likely facilitated the release of carotenoid compounds, explaining the observed increase in antioxidant activity [21].

6.2 Antibacterial

As documented in multiple research articles, ajwain exhibits antibacterial properties against various bacterial species, making it applicable in the food, agriculture, and pharmaceutical industries. Across different preparations including EOs, seed extracts, and organic solvents-ajwain has consistently demonstrated strong antibacterial effects. In a 2014 study, researchers examined the growth of Listeria monocytogenes, a bacterium often found in refrigerated foods and known to cause serious illness. The results showed that the 0.3% concentration of ajwain EO with 4% NaCl supplementation had the most effective antibacterial effect, with a 2 log CFU/ml decrease over 12 days [22]. This could be attributed to thymol's ability to penetrate through the bacterial membrane and the salt's subduing effect on the enzymes within. In a 2022 study, ethanol seed extract showed a significant effect against Staphylococcus aureus, but not against gram-negative bacteria, with *Salmonella paratyphi* being the most inhibited. Their zones of inhibition were compared with those of the commonly used antibiotic Gentamicin, which is used to combat bacterial infections. The results were astounding, Gentamicin only had an inhibitory zone of 18 mm against *S. aureus*, whereas ajwain had an inhibitory zone of 28.3 mm. The same effect was observed in the case of *S. paratyphi*, where the antibiotic had an effect of 14 mm, and ajwain had an impact of 18.5 mm [23].

In another study focusing on Escherichia coli, EO concentrations of 0.03% and 0.04% had the greatest effect on bacterial culture, resulting in reductions of 6.84 and 6.73 CFU/ml, respectively. Moreover, the impact of the EO on group beef was investigated, with an antibacterial effect observed at a concentration of 0.25%. Still, the most significant impact is observed at 0.5%, accompanied by the inhibition of Shiga toxin transcription [24]. A separate study examining the antibacterial activity of Ajwain EO against *E. coli* in minced beef determined that 1.75% was the minimum concentration required to inhibit bacterial growth. However, raising the concentration to 3.25% was sufficient to inhibit the growth of all tested bacterial strains, suggesting the effectiveness of ajwain in preserving food safety and its overall antibacterial properties [25].

In another case, powdered ajwain seeds dissolved in ethyl acetate are tested against four clinically significant bacterial strains known for their antimicrobial resistance: *S. aureus, Micrococcus luteus, Pseudomonas aeruginosa, and Hafnia alvei.* The ethyl acetate extract of ajwain exhibited visible zones of inhibition against all tested bacteria, with the most pronounced effect on *H. alvei* and the least on *M. luteus*. Using organic solvents, such as ethyl acetate, shows more pronounced antibacterial activity than using water. This enhanced activity was because of the presence of thymol and carvacrol, which are found in the seeds. Notably, thymol has been reported to be effective even against multidrug-resistant bacteria that are unresponsive to third-generation antibiotics [26].

To better gauge the antibacterial effects, a study tested ajwain EO, ethyl acetate extract, ethanol extract, and aqueous extract against nine bacteria including gram-negative (*E. coli, Klebsiella pneumoniae, Salmonella typhi, Pneumococcus auroginosa, Erwinia carotovora, Agrobacterium tumefaciens*) and gram-positive (*Bacillus subtilis, Bacillus atrophoeus, S. aureus*) ones. The EO displayed a more pronounced effect than the crude extracts, with the aqueous extract having the least impact. The EO presented them with a zone of inhibition as high as 43mm in *B. atrophoeus*. Although the aqueous extract was reported to be the least effective of all the extracts, its impact is not negligible, as it is comparable to that of a regular antibiotic, inhibiting E. coli by 25 mm. Compared with Ciprofloxacin, used as a control, Ajwain EO demonstrated superior antibacterial activity in most tested cultures, as evidenced by larger zones of inhibition. These results were attributed to key constituents of ajwain thymol and p-cymene. Thymol may disrupt bacterial membrane permeability, as mentioned earlier, leading to cell lysis or the

leakage of intracellular contents. In contrast, p-cymene could enhance this effect by causing membrane swelling, further compromising membrane integrity and function [27].
Furthermore, another study has shown that ajwain can enhance antibiotic activity, reduce side effects, and even decrease the resistance of Extended-Spectrum Lactamases (ESL)-producing bacteria, which are increasingly resistant to recent antibacterial drugs. Remarkably, 81% of the bacterial cultures were restimulated in their sensitivity towards Ciprofloxacin upon the addition of the methanolic extract of the seeds, thereby allowing the antibiotic to work to its full potential [28]. The growing concern about bacterial contamination in food and the potential health risks associated with synthetic preservatives have led researchers to explore safer, natural alternatives. One such study investigated whether ajwain EO could serve as a greener alternative to processed chemical food preservatives. The results suggest that even at higher temperatures of 70 °C, 80 °C, and 90 °C, ajwain EO can inhibit the growth of S. aureus and E. coli, with inhibition zones of 18.3 mm and 14.36 mm, respectively, suggesting that ajwain EO can potentially replace chemicals such as Sodium benzoate in the food industry [20].

Table 4: Compilation of zones of inhibition of bacteria upon addition of ajwain extract, collected from 5 research papers

Bacterial species	Zone of Inhibition (mm)	References
Staphylococcus aureus	28.3; 18.6; 25; 17.5; 18.3	20, 23, 26, 27, 29
Klebsiella pneumoniae	16; 20; 16	23, 27, 29
Proteus vulgaris	20.5	27
Salmonella paratyphi	18.5	27
Escherichia coli	15.6; 20; 29; 14.36	20, 23, 27, 29
Micrococcus luteus	11.1	26
Pseudomonas aeruginosa	17.8; 16; 15.5	26, 27, 29
Hafnia alvei	22.4	26
Bacillus cereus	11	29
Acinetobacter baumannii	7	29
Salmonella typhi	14.5	29
Erwinia carotovora	14.5	29
Agrobacterium tumefaciens	16	29
Bacillus subtilis	20.5	29
Bacillus atrophoeus	13.5	29

6.3 Anticancer Effect
Ajwain has been studied extensively for its anticancer properties, which include inducing apoptosis, reducing oxidative stress, and inhibiting cell proliferation in cancer cell lines. These effects are attributed to the chemical compounds present in its extracts and EOs.

6.3.1 Heavy metal poisoning
As demonstrated in the study, the ethanolic extract of ajwain inhibits Cd^{2+}-induced apoptosis through the intrinsic pathway, potentially by enhancing antioxidant defense mechanisms [30]. It was successfully demonstrated to significantly reduce Cd^{2+} induced cytotoxicity and apoptosis in PC12 cells by reducing cell death, increasing glutathione levels, decreasing DNA fragmentation, and lowering lactate dehydrogenase (LDH) activity. It also suppressed the Cd^{2+}-induced increase in Bax expression, while promoting the expression of anti-apoptotic proteins, including Bcl-2, Bcl-xL, and NF-κB.

6.3.2 Breast cancer
Another study investigated the anticancer properties of ethanolic ajwain extract against MCF7 breast cancer cells [31]. The extract caused important apoptotic features, including cell shrinkage, membrane blebbing, and DNA fragmentation. It also upregulated the tumor suppressor gene p53 while downregulating the anti-apoptotic gene Bcl-2, thereby inducing apoptosis in cancerous cells.

6.3.3 Gastric cancer
Ajwain has been further demonstrated to possess cytotoxic potential, with its seed methanolic extracts resulting in a significant reduction in the proliferation of AGS gastric cancer cells [32]. Its combination with *Foeniculum vulgare* seeds decreased tumour multiplicity not only in forestomach and skin cancer but also in the model. A combination of ajwain seed extract and n-hexane extract demonstrated notable anticancer effects against liver carcinoma cells, demonstrating broad-spectrum efficacy.

6.3.4 Colon cancer
The *In vitro* antiproliferative activity of ajwain oil has been investigated against various cell lines, including human colon carcinoma (HCT116), to identify the primary compound responsible for its cytotoxic effect. Thymol, as reported in this paper, was the main bioactive compound with activity against different cancer cell lines, including HL-60 (acute promyelocytic leukemia), glioblastoma, and H1299 (lung cancer). Additionally, γ-terpinene demonstrated cytotoxicity against lung carcinoma (A-549) and colon adenocarcinoma (DLD-1) cells, further enhancing the pharmacological value of ajwain [33]. Further validated the anticancer effects of ajwain by examining the impacts of ajwain EO on SW4 80 colorectal cancer cells. Notably, treatment with a combination of ajwain EO and irradiation further enhanced ROS production, leading to molecular damage, increased free radical levels, and higher cell death rates. The synergistic effect suggests that ajwain EO may enhance the effectiveness of radiotherapy.

6.4 Insecticidal
The use of ajwain as an insecticide has been demonstrated in several studies. It proved capable of either completely eradicating certain agricultural pests or, at the very least, controlling their population to the lowest degree. *Bemisia tabaci*, commonly referred to as the whitefly, is a pest that frequently causes leaf deterioration. Three EOs were tested against it in a study: Ajwain, Curry Tree, and Wood Apple. While all had effects as insecticides, insect repellents, and oviposition deterrents, ajwain exhibited remarkable results. It had the highest percentage of insecticidal activity in both the lab and the greenhouse. They found that as the EO concentration increased, extermination increased, with

optimal results at 10 mg/ml. It further prevents egg numbers from increasing by acting as an oviposition deterrent. Hence, it is evident that it is a successful bio-replacement for chemical insecticides with side effects [34]. In another study, encapsulating the EO in nanoparticles increases mortality rates of pests and enhances its efficacy as an insecticide. Results revealed a spike in the mortality rate from 62% to 74% in the case of *Rhyzopertha dominica* and from 44% to 57% for *Tribolium confusum* [35].

6.5 Antifungal effects

The antifungal activity of ajwain seeds' EO was reported against *Fusarium* and *Saprolegnia* due to the presence of thymol, *γ*-terpinene, and p-cymene. Ajwain is proposed as a natural bioactive molecule for the development of antifungal agents [36]. Furthermore, the mixture of ajwain EO and *Peganum harmala* extract encapsulated in chitosan nanoparticles was shown to induce inhibition of the growth of the pathogenic fungus *Alternaria alternata* in both *In vitro* and *in vivo* conditions [37]. Moreover, a study has reported that ajwain crude extract exhibits significant antifungal activity against *Candida albicans*, a pathogenic fungus commonly found in the human gut that causes candidiasis infections. The study also proposed that ajwain extract could serve as a valuable alternative medicine in the future, provided the bioactive compound is isolated from the crude extract. Thymol, a novel bioactive compound, may be isolated and further developed as an antifungal drug to combat infections caused by various species, especially *C. albicans*, in the future [38].

6.6 Muscle-relaxant

Although ajwain has been used in traditional medicine for muscle pain relief, there was no scientific documentation to explain this until recently. The EO was revealed to be a vasorelaxant upon an experiment on the aorta of Wistar rats. The aortas were isolated from the rats and contracted using either phenylephrine or potassium chloride (KCl). In both cases, the aortas were shown to dilate and relax with cumulative doses of Ajwain EO (20-100 µg/ml), by 93% and 104.4%, respectively, through inhibition of calcium channels [39]. Therefore, we can encourage the use of Ajwain EO within the mentioned concentrations as a muscle relaxant, although further research is needed to establish a stronger scientific foundation.

6.7 Anti-inflammatory effects

The anti-inflammatory activity of the aqueous extract of ajwain, when combined with ibuprofen, was reported in collagen-induced arthritis rats. The results indicated that treating with the aqueous extract of ajwain decreased COX2 mRNA levels, a gene expression marker of high inflammation. Additionally, the study suggests that the ajwain extract, either alone or in combination with ibuprofen, is a novel candidate for the manufacture of anti-inflammatory drugs [39]. Further, ajwain EO was evaluated for its potential as a component in root canal filling materials for primary teeth. The study suggests that they formulated new obturating materials by combining zinc oxide with ajwain EO, and the results showed decreased inflammation and enhanced vascularity, indicating that all the materials exhibited good tissue compatibility [14].

6.8 Gastroprotective

The most common household use of ajwain, as an aid in digestion and to relieve indigestion, highlights its gastroprotective effects. Compared with a standard gastric drug, Liv52, the methanolic seed extract of ajwain proved equally effective. Upon administration of 250mg/kg of Liv52 and ajwain seed extract on animals with induced gastric abnormalities using non-steroidal anti-inflammatory drugs, biochemical analysis revealed a healthy increase in SGOT and SGPT levels. The abnormality-induced animals had SGOT and SGPT concentrations of 52.85 IU/L and 57.11 IU/L, respectively, while Liv52 produced 71.12 IU/L and 74.23 IU/L, respectively. Ajwain extracts produced levels of 62.47 IU/L and 64.49 IU/L, demonstrating their adequacy as a gastroprotective agent [18].

6.9 Anti-viral effects

Japanese encephalitis is an encephalitic virus that is widespread throughout Asia, caused by the JEV virus, with yearly reported cases ranging from 35,000 to 50,000. JEV is a small RNA virus that belongs to the Flavivirus family and is generally transmitted by mosquitoes and ticks. The virus severely affects, particularly children below the age of 15 years and adults over the age of 65, who have compromised immunity. A study explored the antiviral activity of ajwain EO against the Japanese encephalitis virus (JEV) [40]. It was found that pre-exposure ajwain EO treatment outperformed post-exposure treatment in terms of viral inhibition. However, further research is needed to determine whether thymol, the active ingredient in ajwain EO, has potential as an antiviral medication. Additionally, the paper discusses how ajwain has demonstrated antiviral activity against other pathogenic viruses, namely hepatitis C virus (HCV), and has shown substantial inhibitory activity against the HCV protease.

6.10 Analgesic

Throughout history, ajwain has found its place in traditional medicines worldwide as a topical pain reliever when applied as a cream. This piqued scientists' interest in digging deeper to find logical, scientific reasoning behind this folklore. Leading to it being labelled as dopaminergic and cholinergic, a remedy to battle neuropathic pain [41]. One clinical study demonstrated an improvement in 92 patients with regular burning feet, who, after treatment with 10% ajwain cream, reported a pronounced reduction in neuropathic pain compared to the placebo [42]. Moreover, a hot plate assessment on rats exhibited antinociceptive activity a decrease in sensitivity to pain upon oral doses from 250 mg/kg, up to 2000 mg/kg, revealing an increase in reaction time by 0.58s (from 7.32s to 7.9s) and 1.1s (from 6.7s to 7.8s), correspondingly [43].

6.11 Anxiolytic effects

Researchers have investigated the anxiolytic activity of the methanolic extract of ajwain [44]. It was reported that the anxiolytic effects of ajwain are believed to be due to its high thymol content, which is considered to enhance the activity of *GABAA* receptors and promote the opening of chloride ion channels. The presence of a compound named alpha-pinene was also found to be a crucial contributor to the above-observed results.

6.12 Anti-diabetic

The global occurrence of diabetic patients is expected to rise to 830 million by 2022, with it prevailing in lower-income populations [45]. The treatment options do not get any cheaper as the years go by, and many are not able to afford it, or it

becomes a financial strain to keep the extensive therapies and medications. Additionally, such treatments have adverse side effects and may become ineffective over time. When tested against Acarbose, a standard Type 2 diabetes drug, ajwain essential oil was found to be a strong alternative. Amylase and glucosidase are key enzymes in regulating blood glucose levels. The ajwain EO was evaluated as an inhibitor of the two enzymes at a concentration of 4 µL/mL. Exhibiting an 88.55% inhibitory action on a-amylase compared to Acarbose's 90.96% and in the case of a-glucosidase, 89% and 91.67% respectively, showing how similar an anti-hyperglycaemic effect ajwain has to a standard drug. Moreover, the cytotoxicity tests showed that ajwain EO was required in a lower concentration than acarbose to achieve the same result an IC_{50} of 0.37 µL/mL for EO and 0.41 µL/mL for Acarbose [46]. Another study reported IC50 values of 160 µg/ml and 220 µg/ml for ajwain EO and its ethanolic extract, respectively [47]. The α-glucosidase inhibitory activity of ajwain was compared against cinnamon EO and acarbose, both of which produced relatively lower IC_{50} values-90 µg/ml and 75 µg/ml, respectively. The α-amylase inhibition was also demonstrated in another study, where an ethyl acetate extract was used; furthermore, it was noted that this enables the body to consume free blood glucose by the fat and muscles [48].

6.13 Antiulcer effects

It has been revealed that the ethanolic extract of ajwain fruit exhibits significant anti-ulcer activity, and the EO of ajwain fruit has been identified as a promising natural remedy for treating gastric ulcers. Additionally, thymol, present in ajwain, is a highly potent potential lead compound for developing a novel class of H+/K+-ATPase inhibitor drugs [49].

6.14 Anti-spasmodic

Drugs available in the market that are antispasmodic or anti-cholinergic are administered to patients who have periodic cramps in the stomach or the lower gastrointestinal tract; however, they also lead to unfavorable side effects. The visible effect of Ajwain seed essence as an antispasmodic agent was proven in a study on rat ileum, upon which spasms were induced using acetylcholine. When cumulative concentrations were applied, the contractions were reduced. A concentration of 0.002% resulted in a 47.34% reduction in spasms, while concentrations of 0.005% and 0.01% yielded reductions of 60.46% and 86%, respectively [50].

6.15 Hepatoprotective effects

At therapeutic dosages, Acetaminophen, a widely used analgesic, however, excessive use has been linked to liver damage in organisms where the compound can be converted into a reactive metabolite that leads to impaired function of the mitochondria. A study investigated the hepatoprotective effects of ethanolic extracts of ajwain, combined with *Elettaria cardamomum*, against acetaminophen-induced hepatotoxicity [51]. It was observed that ajwain and *E. cardamomum*, administered both individually and in combination, exhibited practical hepatoprotective effects, restoring liver structure and preventing degeneration in liver tissues by reducing oxidative stress, lipid peroxidation, and toxic molecular interactions.

6.16 Anti-biofilm

Microorganisms' communal defense mechanism, biofilm formation, which occurs when they coexist in a layer of slime, enables them to resist antibiotics and evade the host's immune system. This can be observed in implants or dentures, which can lead to infection and, in turn, encourage the manifestation of other diseases [52]. In the case of dentures, the tissue conditioner used can provide a medium for the growth of microorganisms, thereby facilitating the formation of biofilm. Bacteria that exist in biofilms have an exceptionally high resistance to antibiotics, but ajwain's antibacterial effect seems to penetrate their defenses, attributed to thymol.

A study conducted against five bacterial and two fungal species (*Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguinis, Streptococcus salivarius, S. aureus, Enterococcus faecalis, C. albicans, and Candida dubliniensis*) tested ajwain EO mixed with tissue conditioner.[53] It revealed the inhibition of biofilm growth, with the most significant effect observed on *S. salivarius*, where biofilm growth was reduced to 22.4%, and the least impact on *C. albicans*, at 71.42%. This was followed at an EO concentration of 64 µg/mL, and it was observed that antibiofilm activity was greater against bacteria than against fungi. A negative correlation between dosage and vitality was also observed in another study on the persistence of biofilm formations on mucosal linings, which can lead to infection. They tested concentrations up to 6.25 mg/mL of the methanolic extract and verified ajwain's role as a biofilm inhibitor and destroyer. Their results showed a maximum of 98% prevention against Acinetobacter baumannii and a minimum of 19% against K. pneumoniae among six tested bacteria (*B. cereus, S. aureus, P. aeruginosa, E. coli, A. baumannii, and K. pneumoniae*). The latter had an incredibly resistant biofilm structure; yet, the effect of ajwain was evident [29].

7. Toxicology

Ajwain is safe when used in moderation and offers a wide range of health-promoting benefits, but excessive intake of its seeds or EO may lead to undesirable effects. As with most medicinal plants, therapeutic efficacy depends on dose, and exceeding the recommended levels can result in toxicity. Most studies on ajwain suggest that, to achieve optimal pharmacological activity while minimizing risks, it is necessary to use controlled concentrations of ajwain or its EO. In recent years, some studies have revealed the adverse effects and highlighted the need for caution and further safety evaluation. In 1987, a survey was conducted among villages in northern India where women traditionally use ajwain as an abortifacient. Approximately two-thirds of the pregnant women who were interviewed in the study acknowledged past use of ajwain for this purpose. However, this practice was inconsistent and unsafe, and excessive consumption was sometimes associated with fetal malformations, suggesting a risk of fetotoxicity. Animal studies further support this concern, with reports of adverse developmental effects in rats [54]. Ethnobotanical surveys among Meccan women suggested that ajwain is most used as a spice, but its high intake was associated with constipation. The threshold dose leading to this effect was not clearly defined, indicating a need for dose-response studies [10]. Thymol, the major bioactive component of Ajwain EO, is considered safe at low concentrations but can cause toxic reactions at higher levels. A study reported fatal poisoning in cases of excessive Ajwain consumption. Experimental data suggest that Ajwain EO exhibits relatively low acute toxicity, with an estimated oral LD_{50} of 2294 mg/kg. Still, high doses of thymol have been linked to severe toxicities and potentially life-threatening outcomes [8]. Ajwain is generally considered safe when used in dietary and

therapeutic amounts; however, its overdosage may cause adverse effects, which include gastrointestinal discomfort, reproductive toxicity, and thymol-induced poisoning. More systematic studies, particularly clinical trials and toxicokinetic evaluations, are necessary to establish safe therapeutic windows for its use in integrative medicine.

8. Conclusion

Ajwain (*Trachyspermum ammi L.*) is a valuable seed whose traditional medicinal uses are now supported by scientific research. The primary phytochemicals present in it, including thymol, carvacrol, limonene, and p-cymene, contribute to a wide range of therapeutic effects, including antimicrobial and anti-inflammatory activities and anticancer potential. As per clinical and preclinical studies, ajwain, particularly in its EO form, exhibited efficacy comparable to or superior to conventional pharmaceuticals, often at lower doses and with minimal side effects.

In recent years, multiple studies have been conducted on ajwain, with promising outcomes; however, information on non-standardized extraction methods, clinical trials, pharmacokinetics, and bioavailability is limited. To overcome this gap, future research on ajwain should focus on rigorously designed clinical trials, standardized preparation protocols, and detailed mechanistic investigations. Furthermore, to enhance the stability and therapeutic efficacy of ajwain's bioactive compounds, advanced techniques such as nanoencapsulation and lipid-based formulations should be employed. By combining traditional knowledge with modern science, ajwain has the potential to become a safe and effective natural therapy, supporting preventive and integrative medicine and possibly reducing the need for certain chemical drugs.

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The authors used Grammarly to review the manuscript for grammar, punctuation, and clarity of language. All scientific content, analyses, and conclusions are solely the work of the authors.

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