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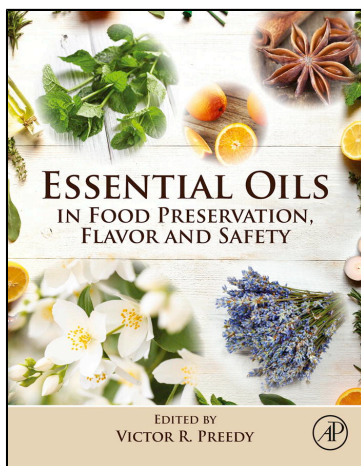
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Chapter 19

Ajwain (*Trachyspermum ammi* L.) OilsSirajudheen Anwar¹, Nafees Ahmed², Sofiane Habibatni^{3,4}, Yousef Abusamra⁵

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List of Abbreviations

ATP Adenosin triphosphate
ESBLs Extended-spectrum- β -lactamases
H₂O₂ Hydrogen peroxide
LAB Lactic acid bacteria
MBC Minimum bactericidal concentration
MIC Minimum inhibitory concentration
US-FDA United States-Food and Drug Administration

INTRODUCTION

Researchers are attempting to replace synthetic preservatives with natural botanicals and essential oils due to the established drawbacks of synthetic preservatives. Experimentation has revealed that natural methods of preservation are more beneficial than their unnatural counterparts. Essential oils have proven to be better than synthetics in preventing the growth of pathogens and delay of spoilage in food. Moreover, these essential oils do not have the harmful health risks that come with the use of synthetics. Moreover, essential oils are environmentally friendly, degradable, and cheaper than chemical preservatives. Thus, essential oils should be at the forefront of the food industry (Das et al., 2012).

Botanicals are environmentally sustainable because their manufacturing process is clean, inexpensive, and produces very little waste, even on an industrial scale. Steam extraction and hydrodistillation are two common high-output methods of essential oil extraction, both of which only produce two byproducts: biodegradable, compostable biomass and water vapor. Both of these methods require only simple machinery and a source of plant material, such as rosemary (whose essential oil is very effectively extracted by steam); they produce a potent product containing all the terpenes and phenols that make essential oils effective and economic (Boutekedjiret et al., 2003).

The seeds of *Trachyspermum ammi* (L.)—commonly known as ajwain or caraway—have been reported for wide range of medicinal applications. They show antifungal, antioxidant, antibacterial, cytotoxic, antilithiasis, nematocidal, anthelmintic, and antifilarial activities, which has generated interest in the use of ajwain as a food preservative. Among its other constituents, volatile oil (thymol, γ -terpinene, *para*-cymene, α - and β -pinene, α -thujen, myrcene, 1,8-cineole, and carvacrol) has effective antimicrobial and antifungal activity, especially thymol and carvacrol. Ajwain oil contains about 50% thymol, which is a strong germicide, antibacterial, antispasmodic, and fungicidal. Although antimicrobial studies have been carried out for ajwain oil against many bacterial and fungal species, food spoilage has been credited to broader range of microorganisms. Hence, it is confined to be used synergistically with other essential oils. Furthermore, ajwain oil possesses antioxidant properties; the high percentage of oxygenated monoterpenes, particularly thymol, and the high level of γ -terpinene and *p*-cymene of ajwain oil might be related to its high antiradical activity. In fact, thymol and carvacrol have been considered to be safe by the European Commission and US Food and Drug Administration (FDA) and are classified as flavoring agents; hence, they undergo regulatory requirements as additives for food preservation.

BOTANICAL ASPECTS

Trachyspermum ammi (L.) Sprague ex Turrill, commonly known as ajwain or caraway, is an erect, glabrous, or minutely pubescent, branched annual herb, 60–90 cm tall, belonging to the family Apiaceae. Other synonyms are *Ammi copticum* (L.), *Carum copticum* (L.), and *Trachyspermum copticum*. It is native to Egypt and widely grown all over Iraq, Iran, Afghanistan, Pakistan, and India, in arid to semi-arid regions where soils contain high levels of salts. *T. ammi* L. contains striated stems, with an inflorescence that is a compound umbel. Each umbel contains 16 umbellets, each with up to 16 actinomorphic, white, and small flowers with five petals, five stamens, and ovary inferior. Its leaves are rather distant and pinnately divided, with a terminal and seven pairs of lateral leaflets. Fruits are ovoid, muricate, aromatic cremocarps that are grayish-brown; the mericarps are compressed, with distinct ridges and tubercular surface and one-seeded (Joy et al., 2001).

Microscopically, transverse section of fruit exhibits two hexagonal structures linked by carpophores. Epicarp is made of a single layer of tangentially elongated tabular cells, while mesocarp is slightly thick-walled, with polygonal tangentially elongated cells having some vittae. Fruiting bodies and vascular bundles appear as groups of thick-walled elongated cells; the endosperm consists of thin-walled cells filled with embryos, small circular oil globules, and is composed of thin-walled polygonal cells. The powder exhibits oil-globules and groups of endosperm cells (Ayurvedic Pharmacopoeia of India, 1999–2011).

Seeds of *T. ammi* L. are widely used in India and eastern Asia, both in diet and in traditional medicine. They contain fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone, and mineral matter (7.1%) (Bairwa et al., 2012). Moazeni et al. identified 18 compounds in the essential oil, representing 99.54% of the total oil. The major constituents were thymol (50.07%), γ -terpinene (23.92%), and *p*-cymene (22.9%), along with α - and β -pinenes, α -thujen, myrcene, 1,8-cineole, and carvacrol, present in very low concentrations (Moazeni et al., 2012; Zachariah, 2008).

Trachyspermum ammi L. is known as a popular aromatic herb and an important commercial product for the food/flavoring industries. The fruits are edible and usually used as spice in India, Middle East Asia, and some parts of America. The seeds possess remarkable digestive and antiseptic properties and are used in traditional medicine, primarily to control bowel disorders such as indigestion, flatulence, colic, and diarrhea (Khan et al., 2010). They are also used as a stimulant, stomachic, carminative, aromatic, antispasmodic, antihypertensive, antiseptic, antiparasitic, antiscorbutic, antihistamine, vermicide, emmenagogue, sialagogue, and anti-inflammatory (Aftab et al., 1995; Kamal et al., 2012).

Ajwain oil contains around 50% thymol, which is a strong germicide, antibacterial, antispasmodic, and fungicide (Bairwa et al., 2012). The diuretic properties of *T. ammi* L. seeds have been widely reported and are used in some drug formulations for kidney stone treatments (Kaur et al., 2009). The seeds are also used in traditional ethno-veterinary for treating diarrhea, indigestion, and constipation in animals. It is given orally to animals in case of weakness and to increase milk-yielding capacity (Sharma et al., 2012).

USAGE AND APPLICATION IN FOOD SCIENCE

Ajwain Oil as an Antibacterial Agent

The bacteria that are involved in specific food spoilage and food products are summarized in Table 1. Mayaud et al. evaluated the antimicrobial activity of 13 essential oils, including ajwain oil, against antibiotic-resistant bacterial strains using two methods: the agar dilution method and the time-killing curve method. The ajwain oil was effective against *Escherichia coli*, *Enterobacter cloacae*, *Salmonella*, *Citrobacter*, *Hafnia alvei*, *Vibrio cholerae*, *Klebsiella*, *Pasteurella multocida*, *Bordetella bronchisepta*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococci*, *Streptococci*, *Enterococci*, *Listeria monocytogenes*, and *Corynebacterium* with varying degrees of sensitivity (Table 2). They classified the bacteriostatic efficacy of tested essential oils, including ajwain oil, based on chemical composition: phenols, aldehydes, and monoterpenols were more potent than the essential oil containing oxides and hydrocarbons. This classification is in general agreement with previously reported results (Mayaud et al., 2008).

Kaur et al. studied the antibacterial activity of aqueous and organic seed extracts of *T. ammi* L. using agar diffusion assay, minimum inhibitory concentration, and viable cell count studies; their antibacterial effect was compared with some standard antibiotics. The phytochemical constituents of the extracts were alkaloids (4.23%), flavonoids (8.58%), tannins (22.77%), and saponins (0.71%). Although they did not study the presence of monoterpenoids, this has been identified and reported elsewhere. The aqueous extracts have shown variable zones of inhibition (11–25 mm) for all the tested bacteria except *Klebsiella pneumoniae* 1 and 2 and *Pseudomonas aeruginosa* 1, which were completely resistant (Table 2). Organic

Food and Food Products	Different Types of Bacterial Species Involved in Spoilage
Fish	Shewanella, Pseudomonas, Photobacterium, Shewanella
Smoked fish	Lactic acid bacteria (LAB), Enterobacteriaceae, Photobacterium
Marinated fish	LAB
Meat	Pseudomonas, <i>Lactobacillus curvatus</i> (LAC), Enterobacteriaceae, Brocothrix, Clostridia
Meat products	<i>L. curvatus</i> (LAC), Enterobacteriaceae, Brochotrix
Milk	Pseudomonas, Bacillus
Raw vegetables	Erwinia, Pseudomonas
Eggs	Pseudomonas, Enterobacteriaceae
Fruits	LAB
Mayonnaise salads	LAB
Beer	LAB
Wine	LAB
Cereals	LAB
Nuts	LAB

Species of bacteria involved in food spoilage.

extracts showed similar results as observed in the case of aqueous extracts with some variations. The extracts prepared in hexane and acetone gave relatively better inhibitory zones, ranging from 9 to 30 mm. Indeed, the strains that exhibited considerably good sensitivity to *T. ammi* L. extracts were selected further to determine minimum inhibitory concentration (MIC) of aqueous and acetone extract of seeds. The higher value of MIC was observed for the aqueous extract (MIC: 60–80 mg/mL), whereas lower value of MIC was shown for the solvent extract (MIC: 5–15 mg/mL) against Gram-positive bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus* and Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, and *Shigella flexneri* (Table 2). The viable cell count for hot water extracts (200 mg/mL) of the *T. ammi* L. seeds against strains depicted complete killing of bacteria, mainly within a 10-h time span. Their bactericidal nature was further confirmed when no regrowth occurred, even after 24 h of incubation (Kaur et al., 2009). While comparing the antibacterial potential between aqueous and acetone extracts of *T. ammi* (L.) seeds with standard antibiotics, acetone extract implicated statistically significant activity in comparison to its aqueous extract ($p < 0.05$), while an insignificant difference was observed for *Anethum graveolens* and *Foeniculum vulgare* extracts.

Khan et al. (2010) investigated different solvent extracts of *T. ammi* (L.) seeds to determine their efficacy against multidrug-resistant (MDR) strains of five extended-spectrum- β -lactamase (ESBL)-producing strains of *E. coli* (nosocomial infection) confirmed by polymerase chain reaction and reference strains of *Streptococcus mutans* ATCC-700610 and *Streptococcus bovis* ATCC-9809. Alkaloids, amino acids, proteins, sterol terpenes, and glycosides were identified in the solvent extract. The MIC and minimum bactericidal concentration (MBC) were tested by using a broth microdilution method. Various solvent extracts of *T. ammi* seeds were found to be bactericidal against five ESBL-producing strains of *E. coli*, *S. mutans*, and *S. bovis* with MIC (1250–5000 $\mu\text{g/mL}$) and MBC (312.5– \sim 5000 $\mu\text{g/mL}$) shown in Table 2 (Khan R et al., 2010). In this study, the authors qualitatively identified terpinene as one of the components; these findings are in agreement with other studies reporting that thymol and carvacrol, a monoterpenoid phenol, are the major components of ajwain oil responsible for antimicrobial action.

Terpenoids are terpenes that undergo biochemical modifications via enzymes that add oxygen molecules and move or remove methyl groups. Naturally occurring compounds such as thymol and carvacrol can reduce bacterial resistance to common drugs, such as penicillin (Palaniappan and Richard, 2010). Thymol and carvacrol were found to be more effective in killing bacteria (Bairwa et al., 2012). Another study showed that thymol and carvacrol were the most effective

TABLE 2 Antibacterial Activity of Ajwain (*Trachyspermum ammi* L.) Oil and Seeds Extract

Parts/Extracts of <i>T. ammi</i> Seeds	Susceptible Bacterial Species			References
		MBC (mm)	MIC (mg/mL)	
Hot water extract				Kaur et al. (2009)
	<i>Enterococcus faecalis</i>	22	60	
	<i>Staphylococcus aureus</i>	23	70	
	<i>Escherichia coli</i>	17	80	
	<i>Klebsiella pneumoniae 1</i>	–	–	
	<i>K. pneumoniae 2</i>	–	–	
	<i>Pseudomonas aeruginosa 1</i>	–	–	
	<i>P. aeruginosa 2</i>	24	80	
	<i>Salmonella typhi</i>	–	80	
	<i>Salmonella typhimurium 1</i>	–	–	
	<i>S. typhimurium 2</i>	12	80	
	<i>Shigella flexneri</i>	15	80	
Solvent extract (acetone)	<i>E. faecalis</i>	28	05	Kaur et al. (2009)
	<i>S. aureus</i>	30	05	
	<i>E. coli</i>	23	10	
	<i>Klebsiella pneumoniae 1</i>	13	–	
	<i>K. pneumoniae 2</i>	12	–	
	<i>P. aeruginosa 1</i>	15	–	
	<i>P. aeruginosa 2</i>	25	05	
	<i>S. typhi</i>	18	10	
	<i>S. typhimurium 1</i>	14	–	
	<i>S. typhimurium 2</i>	24	05	
	<i>S. flexneri</i>	24	05	
	Solvent extract (acetone)		MBC (µg/mL)	
<i>E. coli 1</i> ESBL		5000	2500	
<i>E. coli 2</i> ESBL		>5000	2500	
<i>E. coli 3</i> ESBL		5000	2500	
<i>E. coli 4</i> ESBL		2500	5000	
<i>E. coli 5</i> ESBL		2500	2500	
<i>Streptococcus mutans</i>		1250	1250	
<i>Streptococcus bovis</i>		2500	2500	
Ajwain (<i>T. ammi</i> L.) oil			MIC % v/v	
	<i>E. coli</i>	0.37 ± 0.15		
	<i>Enterobacter cloacae</i>	0.2 ± 0.07		
	<i>Salmonella</i>	0.31 ± 0		
	<i>Citrobacter</i>	0.26 ± 0.07		
	<i>Hafnia alvei</i>	0.24 ± 0.08		
	<i>Vibrio cholerae</i>	0.16 ± 0		

TABLE 2 Antibacterial Activity of Ajwain (*Trachyspermum ammi* L.) Oil and Seeds Extract—cont'd

Parts/Extracts of <i>T. ammi</i> Seeds	Susceptible Bacterial Species		References	
	<i>Klebsiella</i>	0.31 ± 0		
	<i>Pasteurella multocida</i>	0.27 ± 0.07		
	<i>Bordetella bronchisepta</i>	0.47 ± 0.16		
	<i>Acinetobacter baumannii</i>	0.27 ± 0.15		
	<i>Stenotrophomonas maltophilia</i>	0.16 ± 0		
	<i>Aeromonas hydrophila</i>	0.14 ± 0.04		
	<i>Branhamella catarrhalis</i>	ND		
	<i>P. aeruginosa</i>	7.4 ± 2.5		
	<i>S. aureus</i>	0.24 ± 0.08		
	<i>Coagulase-staphylococci</i>	0.25 ± 0.07		
	<i>Streptococci</i>	0.31 ± 0.14		
	<i>Enterococci</i>	0.47 ± 0.15		
	<i>Listeria monocytogenes</i>	0.47 ± 0.15		
	<i>Corynebacterium</i>	0.27 ± 0.06		
Ajwain (<i>T. ammi</i> L.) oil		MBC (mm)	MIC (ppm)	Gandomi et al. (2013)
	<i>Bacillus cereus</i>	35	500	
	<i>S. aureus</i>	33.5	500	
	<i>L. monocytogenes</i>	36	500	
	<i>S. typhimurium</i>	34	500	
	<i>E. coli</i>	39	500	
Ajwain (<i>T. ammi</i> L.) oil			MIC (µg/mL)	Paul S et al. (2011)
	<i>S. aureus</i> ATCC 6538		162.5	
	<i>S. aureus</i> KCTC 1916		175	
	<i>E. coli</i> O157:H7ATCC 43888		450	
	<i>Bacillus subtilis</i> ATCC 6633		12.5	
	<i>P. aeruginosa</i> KCTC 2004		12.5	
	<i>S. typhimurium</i> KCTC 2515		225	
	<i>E. coli</i> ATCC 8739		462.5	
	<i>Enterobacter aerogenes</i> KCTC 2190		12.5	
	<i>Salmonella enteritidis</i> KCYC 12021		250	
The above values shows minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum bactericidal zone (mm) of Ajwain (<i>Trachyspermum ammi</i> L.) oil against bacterial strains.				

components against *Aeromonas hydrophila*, followed by *Brochothrix thermosphacta* and *E. coli*. (Klein et al., 2013). The antibacterial activity of ajwain oil and seed extracts are shown in Table 2.

Mode of Action

Thymol is a phenolic monoterpenoid and one of the major constituents of ajwain oil. Thymol is structurally very similar to carvacrol, having the hydroxyl group at a different position on the phenolic ring. The antimicrobial action of phenolic compounds, such as thymol and carvacrol, are anticipated to cause structural and functional damage to the cytoplasmic

membrane. Studies have shown that thymol interacts with cell membranes. This interaction affects membrane permeability, leading to a loss of membrane potential and leakage of potassium ions and adenosine triphosphate. Thymol integrates at the polar head-group region of a lipid bilayer, causing alterations to the cell membrane. Also, it interacts with membrane proteins and intracellular targets. It forms a complex with membrane-bound or periplasmic proteins by means of hydrogen bonds and hydrophobic interactions. It also impairs the citrate metabolic pathway and affects many enzymes directly or indirectly involved in the synthesis of ATP (Hyldgaard et al., 2012).

The antimicrobial action of carvacrol, a phenolic monoterpenoid, is similar to that of thymol. It causes structural and functional damage to the cell membrane. It has been demonstrated to affect the outer membrane of Gram-negative bacteria. Disintegration of the outer membrane caused release of lipopolysaccharides from Gram-negative bacteria. Although carvacrol affects the outer membrane, its site of action is thought to be the cytoplasmic membrane, resulting in passive transport of ions across the membrane. Besides interaction with membranes, carvacrol has been proposed to interact with membrane proteins and periplasmic enzymes, but the evidence for this is limited (Sikkema et al., 1995; Hyldgaard et al., 2012).

Ajwain Oil as an Antifungal

Molds not only contaminate our air but also contaminate our food. As the molds grow on food, they produce enzymes that break down the food, resulting to spoilage. Details of food spoilage and the mold and fungal species involved are listed in Table 3 (Hocking, 2006; Filtenborg et al., 1996). Gilani et al. (2013) formulated 5% ajwain oil into dermal cream and tested against molds (*Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum*, *Mucor*) and yeast (*Candida utilis*) in rabbits, comparing the antifungal activity with an iodine tincture. The gas chromatographic analysis of ajwain oil revealed the presence of monoterpenes, hydrocarbons, and oxygenated fraction. The identified components of the essential oil were α -thujene (0.2983%), α -pinene (0.1509%), *p*-cymene (0.185%), myrcene (1.7638), limonene (20.5294%), α -terpinene (18.9218%), Δ careen (0.1561%), carveol acetate (0.1462%), carvone (0.441%), thymol (55.308%), α -terpineol (0.2404%), carveol (0.2813%), and bergapten (0.3705%). They found that the antifungal and wound-healing activity of ajwain oil was similar to iodine tincture (Table 4). These findings generate speculation that ajwain oil can protect cereals, nuts, fruits, vegetables, meat, eggs, fish, and vegetables from spoilage exhibited by *Aspergillus* and *Penicillium* species (Table 3).

Uniyal and co-workers assessed the antifungal action of ajwain oil against *A. niger*, *Aspergillus fumigatus*, and their spores using an agar well diffusion assay and gaseous contact method. They found antifungal activity against *A. niger* and *A. fumigatus* with inhibition zones of 20 ± 1.5 and 27 ± 1.0 mm, respectively. The gaseous contact assay revealed complete spore inhibition of both species (Uniyal et al., 2012). Similarly, Bansod and Rai studied ajwain oil as a preservative for antimycotic activity against *A. fumigatus* and *A. niger*; MICs of oils (%v/v) were determined by an agar dilution method, with MIC and MCC data (%v/v) obtained by the broth microdilution method (Bansod and Rai, 2008). The MIC and MCC values are given in Table 4. These authors classified ajwain as having low antimycotic activity when compared with other potent essential oils from *Cymbopogon martini*, *Eucalyptus globulus*, and *Cinnamomum zylenticum*. The different observations obtained by Uniyal et al. and Bansod are due to the assay techniques used. Agar diffusion technique assesses qualitative data only, as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium. Agar and broth dilution methods are also commonly used. The results obtained by each of these methods may differ as many factors vary between assays—differences in microbial growth, exposure of microorganisms to plant oil, the solubility of oil or oil components, and the use and quantity of an emulsifier.

Antifungal activities of various essential oils have been reviewed (Tabassum and Vidyasagar, 2013; Kamal et al., 2012). Tabassum et al. classified and described antifungal essential oils on the basis of the family of plant of origin. *Trachyspermum ammi* (L.) belongs to Apiaceae (or Umbelliferae), commonly known as the carrot or parsley family, which is a family of mostly aromatic plants. Ajwain oil is composed of 26 identified components, which account for 96.3% of the total amount. Thymol was found to be a major component along with *p*-cymene, γ -terpinene, β -pinene, and terpinen-4-ol. Ajwain oil exhibited a broad spectrum of fungitoxic activity against fungi, such as *A. niger*, *Fusarium moniliforme*, and *Curvularia lunata*; absolute mycelial zone inhibition was found to at a 6- μ l dose of the oil (Tabassum and Vidyasagar, 2013). Kamal et al. listed the medicinal uses of *T. ammi* (L.). The volatile constituents of *T. ammi* seeds inhibited 72–90% growth of 10 fungi (*Acrophialophora fusispora*, *Curvularia lunata*, *Fusarium chlamydosporum*, *Fusarium poae*, *Myrothecium roridum*, *Papulaspora* sp., *Alternaria grisea*, *Alternaria tenuissima*, *Drechslera tetramera*, and *Rhizoctonia solani*; Table 4). These findings reinforce that phenolic components, such as thymol and carvacrol in ajwain oil, exhibit antifungal activity depending on the concentration used (Kamal et al., 2012).

TABLE 3 Types of Food and Fungi Involved in Spoilage

Type of Food	Molds and Fungi
Molds commonly found on cereals, nuts, and their products	<i>Aspergillus candidus</i>
	<i>Aspergillus flavus</i>
	<i>Aspergillus glaucus</i>
	<i>Aspergillus niger</i>
	<i>Aspergillus ochraceus</i>
	<i>Aspergillus parasiticus</i>
	<i>Chrysonilia sitophila</i>
	<i>Fusarium</i> spp., e.g., <i>Fusarium graminearum</i>
	<i>Penicillium citreoviride</i>
	<i>Penicillium citrinum</i>
	<i>Penicillium expansum</i>
	<i>Penicillium islandicum</i>
	<i>Penicillium stoloniferum</i>
	<i>Penicillium verrucosum</i>
<i>Rhizopus stolonifer</i>	
Molds commonly found on high-sugar foods	<i>Aspergillus glaucus</i>
	<i>Penicillium corylophilum</i>
	<i>Wallemia sebi</i>
Molds commonly found on fruits and vegetables	<i>Alternaria</i> spp.
	<i>A. niger</i>
	<i>Botrytis cinerea</i>
	<i>Cladosporium</i> spp.
	<i>Fusarium</i> spp.
	<i>Gloeosporium</i> spp.
	<i>Penicillium digitatum</i>
	<i>Penicillium expansum</i>
	<i>Penicillium italicum</i>
	<i>Rhizopus stolonifer</i>
<i>Sclerotinia</i> spp.	
Molds commonly found on animal products such as meat, eggs, fish, and milk	<i>Aspergillus</i> spp., e.g., <i>Aspergillus versicolor</i>
	<i>Eurotium</i> spp., e.g., <i>Eurotium herbariorum</i>
	<i>Penicillium</i> spp. e.g., <i>Penicillium commune</i>
	<i>Scopulariopsis</i> spp.
Wilt disease on tomato and brinjal plants	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> and
	<i>Fusarium solani</i> f. sp. <i>Melonga</i> .
Species of fungi involved in food spoilage.	

TABLE 4 Antifungal Activity of Ajwain (*Trachyspermum ammi* L.) Oil and Seeds Extract

Source	Antifungal Activity			References
Ajwain (<i>T. ammi</i> L.) oil		MFC (mm)	Spore inhibition	Uniyal et al. (2012)
	<i>Aspergillus niger</i>	20+1.5	Yes	
	<i>Aspergillus fumigatus</i>	27+1.0	Yes	
Ajwain (<i>T. ammi</i> L.) oil		MFC (mm)	MIC (ppm)	Gandomi H et al. (2013)
	<i>Penicillium citrinum</i>	>80	2000	
	<i>Penicillium chrysogenum</i>	>80	2000	
	<i>Aspergillus flavus</i>	>80	2000	
	<i>A. niger</i>	>80	1000	
	<i>Aspergillus parasiticus</i>	>80	3000	
Ajwain (<i>T. ammi</i> L.) oil		MFC (µg/ml)	MIC (µg/ml)	Khan R et al. (2010)
	<i>Candida albicans 1</i>	2500	2500	
	<i>C. albicans 2</i>	1250	1250	
	<i>C. albicans 3</i>	2500	2500	
	<i>Candida glabrata</i>	5000	2500	
	<i>Candida krusei</i>	>5000	2500	
	<i>Candida tropicalis 1</i>	2500	1250	
	<i>C. tropicalis 2</i>	5000	1250	
Ajwain seeds ethanolic, benzene and petroleum ether extract	<i>A. niger</i>	Benzene and petroleum ether extract better fungitoxic activity		Rizki et al. (1997)
	<i>A. flavus</i>			
	<i>F. solani</i>			
	<i>Aspergillus alternata</i>			
	<i>Helminthosporium</i> sp.			
Ajwain (<i>T. ammi</i> L.) oil		Fungitoxicity		Singh et al. (1979); Kamal et al. (2012)
	<i>Acrophialophora fusispora</i>	72–90% inhibition		
	<i>Curvularia lunata</i>			
	<i>Fusarium chlamydosporum</i>			
	<i>Fusarium poae</i>			
	<i>Myrothecium roridum</i>			
	<i>Papulaspora</i> sp.			
	<i>Alternaria grisea</i>			
	<i>A. tenuissima</i>			
	<i>Drechslera tetramera</i>			
	<i>Rhizoctonia solani</i>			
Ajwain (<i>T. ammi</i> L.) oil		Fungitoxicity		Singh et al. (1986)
	<i>Epidermophyton floccosum</i>	900ppm (Ajwain)		
	<i>Microsporum canis</i>	<i>(T. ammi</i> L.) oil		
	<i>Trichophyton mentagrophytes</i>	1000ppm (Thymol)		

The above values show minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) of Ajwain (*Trachyspermum ammi* L.) oil against fungal strains.

Dwivedi and Enespa et al. (2012) studied aqueous extraction of *T. ammi* seeds for protection against wilt disease on tomato and brinjal plants, at three concentrations—25, 50, and 75% (v/v)—using an in vitro poisoned food technique against *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium solani* f.sp. *Melonga*. The antifungal activity was assessed in terms of the percentage of inhibition of mycelial growth of the test fungi. The magnitude of inhibition was greater for *F. oxysporum* f. sp. *lycopersici* than *F. solani* f.sp. *Melonga* at 75% (v/v) dose (Table 4).

Rizki et al. reported that the ethanolic extract of *T. ammi* was effective against *A. niger*, *A. flavus*, *F. solani*, *Aspergillus alternata*, and *Helminthosporium* sp. Benzene and petroleum ether extracts also exhibited some activity (Rizki et al., 1997).

Pandey and Pant et al. (1997) reported antifungal activity of ajwain oil against *Pythium aphanidermatum*, *Macrophomina phaseolina*, and *Rhizoctonia solani*. The essential oils of the seeds exhibited strong fungitoxicity and completely controlled mycelial growth of all the tested fungi, even at very low concentrations. Sadik Tuzun et al. (2000) patented their invention of a natural preservative containing multiple plant extracts as a safe fungicide, bacteriocide, nematocide, and insecticide for plant protection against household pests. Thymol and carvacrol were components of their natural preservative compound.

Fermented sausages are considered to be good substrates for the growth of yeasts. Encinas et al. (2000) identified the presence of *Trichosporon ovoides* during the stage of manufacturing of Spanish fermented sausages. Saxena et al. (2012) reported on the antifungal action of ajwain oil against *T. ovoides*, with no inhibition (0 mm), 50 μ L/mL (MIC), and 100 μ L/mL (MFC) determined by an agar diffusion assay and broth dilution method, respectively. These findings suggest that higher concentrations of ajwain oil can protect the fermented sausages against *T. ovoides*.

Antifungal Mode of Action

The antifungal activity of ajwain oil can be collectively attributed to thymol and carvacrol. The mode of action of thymol against yeast and fungi has been sparsely investigated, but studies point to interactions with the cell envelope and intracellular targets. Thymol disrupts vesicles and cell membranes and impaired ergosterol biosynthesis in *Candida* strains, which consequently affected cell membrane integrity (Ahmad et al., 2011; Hyldgaard et al., 2012). Thymol also induces cell lysis in *S. cerevisiae*, thereby altering the cell structure and thus preventing the proliferation of cells (Bennis et al., 2004). Carvacrol exhibits antifungal action similar to that of thymol, causing disruption of Ca^{2+} and H^+ homeostasis, upregulation and downregulation of gene transcription, disruption of membrane integrity, and impairment of ergosterol biosynthesis in *Candida* strains (Ahmad et al., 2011).

Ajwain Oil as an Antioxidant

Oxidation is one of the most important processes occurring in food systems. It affects many interactions among food constituents, leading to both desirable and undesirable products. Food lipids are very susceptible to oxidation; therefore, oxidation reactions are one of the major sources of deterioration that occurs during preparation, storage, and distribution of foods. Lipid oxidation products are ubiquitous in foods, although much variation exists in the kind and levels present. Although levels of these compounds are generally low, the problem of lipid oxidation severely compromises the quality and limits the shelf life of foods. All foods that contain lipids, even at a very low level (<1%), are susceptible to oxidation, leading to rancidity.

Changes in foods caused by lipid oxidation include loss of flavor, development of off-flavors, loss of color, loss of nutrient value, and the accumulation of compounds that may be detrimental to the health of consumers (W'sowicz et al., 2004). Oxidation affects the quality of food, but it also has an impact on the charge and conformation of the protein's three-dimensional structure, loss of enzyme activity, and changes in the nutritive value (Karel et al., 1975). Interactions between lipids and proteins have a significant effect on the progress of oxidative reactions in foods. Due to strong interactions, the oxidation reactions can easily transfer from lipids to proteins.

Gandomi et al. studied the antioxidant activity of ajwain oil by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and β -carotene-linoleic acid assay. Ajwain oil contributes as an antioxidant and the activity is confined to the concentration of the essential oil tested. The antioxidant potential of ajwain oil was less than the synthetic antioxidant butylated hydroxyl toluene. The activity of alcoholic extract oil is higher than that of aqueous extract. It seems that the high percentage of oxygenated monoterpenes, particularly thymol, and the high level of γ -terpinene and *p*-cymene of ajwain oil might be related to its high antiradical activity. The antioxidant properties of ajwain oil are attributed to oxygenated monoterpenes and monoterpene hydrocarbons such as thymol, carvacrol, γ -terpinene, and *p*-cymene (Gandomi et al., 2013).

Kamleshya et al. reported a comparative study of the antioxidative activity of ajwain oil; they found that the reducing power of the aqueous and methanolic extracts was 26% and 36%, respectively, as compared to ascorbic acid. Similarly, the DPPH assay revealed antioxidant activity of 39% and 55% for aqueous and methanolic extract, respectively, as

compared with ascorbic acid (Kamleshya et al., 2012). When compared with ascorbic acid, the H₂O₂ radical scavenging ability of the ajwain oil was higher than ascorbic acid at same concentration. The ability of the oil to quench hydroxyl radicals seems to be directly related to the process of preventing lipid peroxidation, thus indicating that they are good scavengers of active oxygen species (Chatterjee et al., 2013). Anti-inflammatory and antioxidant activity of *T. ammi* seeds were evaluated in collagen-induced arthritis in rats. It was concluded that seeds suppressed the induction of oxidative enzymes and markers in joint. It acts either by termination of cellular infiltration or reducing the generation of oxidants (Umar et al., 2012).

Ajwain Oil as an Antispoilage Agent

Almost all groups of microorganisms can contribute to the spoilage of foods under certain conditions. It is assumed that all microorganisms are initially present on a food product, but growth occurs based primarily on nutrient composition and on the chemical and physical parameters (Gram et al., 2002). Proteinaceous foods such as meat, poultry, fish, shellfish, milk, and some other dairy products undergo rapid spoilage. Because they are highly nutritious, with a neutral or slightly acid pH and have high moisture content, a wide range of microorganisms grow on them with similar pattern of microbial spoilage. Seafood spoilage organisms grow faster and produce the metabolites responsible for off-odors, off-flavors, and slime (Jos and Huis, 1996).

Ajwain oil has been demonstrated to have antibacterial, antifungal, antiparasitic, antigermicide, antifilarial, nematocidal, molluscicidal, fumigant, antitermitic, and anthelmintic activities. It has been used traditionally in India and other countries for this purpose. This exclusive property of ajwain oil accounts to be used in food preservation against spoilage. The antimicrobial activity of a given essential oil may depend on only one or two of the major constituents that make up the oil. However, increasing amounts of evidence indicate that the inherent activity of essential oils may not rely exclusively on the ratio in which the main active constituents are present, but also on interactions between these and minor constituents in the oils. Various synergistic antimicrobial activities have been reported for constituents or fractions of essential oils when tested in binary or ternary combinations. Henceforth, ajwain oil and its component can produce the best results as food preservative, when synergistic effects with other essential oils are taken into consideration.

The European Commission has registered thymol and carvacrol as flavoring agents, intended to be used in food products and considered to be safe for the consumer. The US-FDA has also classified these substances as generally recognized as safe. There are regulatory limitations on the accepted daily intake of essential oils or essential oil components; therefore, before they can be used in food products, a daily intake survey should be available for FDA evaluation.

SUMMARY POINTS

- *Trachyspermum ammi* L. (Ajwain) is widely grown in Egypt, Iraq, Iran, Afghanistan, Pakistan, and India, in arid to semi-arid regions where soils contain high levels of salts.
- Ajwain oil contains thymol as main active ingredient.
- Seeds are used traditionally in various bowel disorders, such as indigestion, flatulence, colic, and diarrhea.
- Ajwain oil exhibits a wide variety of pharmacological activities, including antibacterial, antifungal, and antioxidant activities.
- Ajwain oil is used by food industries to prevent the growth of molds and bacteria.
- Thymol and carvacrol have been considered safe by the European Commission and the US-FDA as a flavoring agent.

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