Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 131-140
Received: 04-03-2017
Accepted: 05-04-2017

KK Chahal
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

K Dhaiwal
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

A Kumar
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

D Kataria
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

N Singla
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Correspondence
Alfonso Alexander-Aguilera
Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab, India

Chemical composition of *Trachyspermum ammi* L. and its biological properties: A review

KK Chahal, K Dhaiwal, A Kumar, D Kataria and N Singla

Abstract
Trachyspermum ammi L. (Apiaceae) commonly known as ajwain is an important medicinal, aromatic and spice plant. It was originated in Egypt and widely distributed throughout the World. Ajwain seeds yield 2-5% brownish essential oil, with thymol as the major constituent along with *p*-cymene, γ-terpinene, α-pinene, β-pinene and α-terpinene. Due to presence of various chemical constituents in ajwain, various biological and pharmacological properties have been reported. The present study is an effort to collect all the information regarding chemical composition and biological activities of ajwain.

Keywords: *Trachyspermum ammi* (L.), Essential oil, Chemical composition, Biological properties

1. Introduction
Natural products, such as essential oils are produced by the secondary metabolism in plants. Their constituents are used in human consumption as functional food, food additives, medicines, nutritional supplements and for the manufacture of cosmetics (Burt and Reinders 2003) [15]. The volatile components of essential oils mainly consist of monoterpenes, sesquiterpenes and their oxygenated derivatives such as alcohols, aldehyde, ketones, acids and esters (Suntar *et al.* 2014) [76]. Terpenes having a diverse variety of structures with specific functions constituted the largest amount (71.10%) of essential oils (Zule *et al.* 2003) [84]. There are several methods to extract essential oil from herbs and spices like steam distillation, hydrodistillation, carbon-dioxide and solvent extraction. Essential oils possess various biological activities such as antioxidant, antibacterial, anti-mutagenic and antimicrobial (Dadalioglu and Evrendilek 2004) [20]. *Trachyspermum ammi* L. is a medicinal plant that belongs to family Apiaceae comprising 270 genera and species. It is commonly known as Ajwain in Hindi, Bishop's weed in English, Yamini in Sanskrit, Lodhar Bengali in Punjabi, Ajma in Gujrati, Kath in Kashmiri and Omam in Tamil (Chauhan *et al.* 2012) [18]. The name ajwain derived from the Sanskrit words yavanaka or ajomoda. Ajwain is a very old and well known Ayurvedic spice. It is an annual, aromatic, erect herb bearing white flowers and small brownish fruit. Ajwain seeds are small, grey with bitter taste and quite peppery when raw, but milder when cooked. The most commonly used part of this plant is the seeds or fruit. It looks like cumin or caraway seeds. It has many branches of leafy stems, small feather like leaves, 4-12 rays of flower heads, each bearing 6 to 16 flowers (Bairwa *et al.* 2012) [5]. The dark green leaves of ajwain contain many valuable nutrients, especially the antioxidant carotenoids, lutein and zeaxanthin. These leaves are used as green vegetable for salad (Sies 1997) [72].

2. Taxonomy, Distribution and Morphology
Kingdom : Plantae
Division : Magnoliophyta
Order : Apiales
Class : Magnoliopsida
Family : Apiaceae
Genus : *Trachyspermum*
Species : *ammi*
Source: (Zargari 1996)

Ajwain is originated in the Eastern Mediterranean region, probably in Egypt, and came to India with the Greeks (Chatterjee *et al.* 2012) [16]. This plant grows in arid and semiarid fields in different regions of Central Europe, Asia, India, Iran, Iraq, Afghanistan and Pakistan. In India, ajwain is grown on a large scale in the states of Rajasthan, Gujarat and Andhra Pradesh and grows on a smaller scale in Uttar Pradesh, Punjab, Bihar, Madhya Pradesh, Tamil Nadu,

West Bengal and Karnataka (Zahin *et al.* 2010; Zarshenas *et al.* 2013) [81,83]. Ajwain grows well on all kind of soil but does well on loam soil with a pH 6.5-8.2 at temperature of 15-25 ºC and relative humidity between 65-70%. Harvesting is done during later parts of winter or earlier in spring. The umbels of the plant mature and produce the seeds (Hassanshahian *et al.* 2014) [35]

3. Phytochemical investigation

The ajwain essential oil contained thymol (87.75%) and carvacrol (11.17%) as major constituents and major non-phenolic components quantified were p-cymene (60.78%) and γ-terpinene (22.26%) (Nagalakshmi *et al.* 2000; Pruthi 1992) [60, 64].

3.1 Essential oil content: The essential oil of ajwain seeds can be obtained by hydrodistillation using a Clevenger-type apparatus (Masoudi *et al.* 2002) [53]. The principal constituents which are responsible for typical flavour of ajwain seed essential oil are thymol and carvacrol. Ajwain seeds generally contain 2.5-5 % essential oil and 26% fatty oils (Raghavan 2007) [66]. Ajwain from Pakistan yielded 3.5 and 5.2 % essential oil from large and small size fruits, respectively (Ashraf and Bhatty 1975) [4]. Thymol main component in ajwain seeds, typically constitutes about 50% of the total essential oil. It acts as a strong germicide, anti-spasmodic and fungicide and used in toothpaste and perfumery (Anonymous 1959 and Joshi 2000) [3,42]. Essential oil obtained from freshly harvested ajwain fruit collected from Jodhpur, Rajasthan contained high level of moisture content (1.90%), saponification value (235.98 mg/KOH/g), ester value (231.04) congealing point (15.7 ºC) and peroxide value (7.35 mEq/kg) as compared to essential oil obtained from New Delhi which had moisture content (1.25%), saponification value (121.39 mg/KOH/g), ester value (116.39), congealing point (15.4 ºC) and peroxide value (5.5 mEq/kg). Other physicochemical parameters such as refractive index (1.345), optical rotation (-32.10 – 44.35) and specific gravity (0.7521) of essential oil collected from Jodhpur were found to be comparable with the essential oil obtained from New Delhi having refractive index (1.315), optical rotation (-32.25 – 44.25) and specific gravity (0.7445). Both the oils were soluble in alcohol, chloroform, carbon tetrachloride and hexane (Saini *et al.* 2014) [67].

3.2 Phytochemical characteristics

The chemical composition of ajwain seed essential oil is influenced by various factors. Different parameters such as pressure, temperature, and modifier volume and extraction time have significant effect on the percentage yield and composition of ajwain oil. Extraction yield based on the supercritical fluid extraction varied in the range of 1.0-5.8 % (w/w) under different conditions. Supercritical fluid (CO_2) extraction (SFE) of ajwain oil, under pressure of 30.4 mpa, temperature 35 °C and dynamic extraction time of 30 min, only 3 components including *γ*-terpinene (14.2%), p-cymene (23.1%) and thymol (62.0%) constituted more than 99 % of the oil. It was considered to be the most selective method for the extraction of thymol. However in hydrodistilled oil, there were 8 components including thymol (49.0%), *γ*-terpinene (30.8 %), p-cymene (15.7%), β-pinene (2.1%), myrcene (0.8%) and limonene (0.7%) were identified. The extraction yield, based on hydrodistillation was 2.8 % (w/w) (Minija and Thoppil 2002) [58]. Nutrition analysis of ajwain seeds indicated higher energy value (31.55%), carbohydrates (47.54%), protein (20.23%), fat (4.83%), moisture (11.6%), fibre (4.3%) and ash content (11.5%) in ajwain seeds (Javed *et al.* 2012) [41]. Gas Chromatography-Mass Spectrometry (GC–MS) analysis of ajwain seed essential oil revealed the presence of 27 compounds, of which thymol (40%) was present in the largest amounts, with p-cymene (15.6%) and γ-terpinene (11.9%) whereas β-pinene (4%), limonene (4%), carvacrol (5 %), camphene and myrcene present in trace amounts. However oil obtained from aerial parts and fruits of ajwain was found to contain thymol (42.7-46.2%), γ-terpinene (38.5-38.9%) and p-cymene (14.1-13.9%) as the main components (Krishnamoorthy and Madalageri 1999; Abdolali *et al.* 2007; Bhattacharya *et al.* 1998) [47,1,9]. The phenolic components of the ajwain essential oil contained thymol (87.75%) and carvacrol (11.17%) as major constituents and major non-phenolic components quantified were p-cymene (60.78%) and γ-terpinene (22.26%) (Nagalakshmi *et al.* 2000; Pruthi 1992) [60,64]. Variations in aroma compounds have been observed in seeds collected from different geographical regions and plant parts (flowers, seeds, leaves) of ajwain. In Algeria, isothymol (50%) was found to be the dominant constituent. Other components present were p-cymene, thymol, five new monoterpenoid glucosides, a new monoterpenoid, limonene and γ-terpinene. Essential oil of ajwain seeds collected from South India was found to be rich in thymol (98%). However oil extracted from ajwain leaves was found to be composed of monoterpenoids and sesquiterpenoids: Cadinene (43%), longifolene (11%), thymol (5%), and camphor (3%) (Farooq *et al.*1953) [25]. Water-soluble portion of methanol extract of ajwain seeds contained 25 compounds, including two new aromatic glucosides and two new glucides (Ishikawa *et al.* 2003) [38]. On analysis of the fixed oil from the seeds of ajwain in Indian origin, reported petroselinic acid as the major component (Dwivedi *et al.* 2012) [22]. Major components present in acetone extract of ajwain seeds were found to be thymol (39.1%) followed by oleic acid (10.4%), linoleic acid (9.6%), γ-terpinene (2.6 %), *p*-cymene (1.6%), palmitic acid (1.6%) and xylene (0.1%). Thymol easily crystallizes from the oil on cooling and commonly known as ajwain ka phool or Sat of ajwain (Farooq *et al.* 1953) [25]. A yellow, crystalline flavone and a steroid-like substance were also isolated from ajwain seeds which contained 6-O-β-glucopyranosyloxythymol, a glucoside and a yield of 25% oleoresin containing 12% volatile oil (thymol, γ-terpinene, *p*-cymene and α-pinene and β-pinene) (Garg 1998) [27]. Dichloromethane extract of cell suspension culture of *Carum copticum* revealed the presence of 41 compounds. Major constituents were found to be elemol (11.5%), cadinol (10.6%), cadinene (7.8%), caryophyllene (6.2%), muurolol (4.9%), eudesmol (3.1%), elemene (3.9%), muurolene (2.6 %), limonene (2.4 %) and humulene (2.0%) while other compounds were present in trace amount (Lockwood *et al.* 2002) [49].The sowing dates had significant effect on the percentage and composition of essential oil (Table 1). Essential oil percentage was significantly influenced by planting time so that highest yield obtained in October was 3.3%. By delayed sowing date, essential oil yield decreased gradually. Lowest amount of essential oil was obtained (2.14% or 10.42 kg/ha) in March which was significantly different in comparison with other dates (at 5% of probability level). Ajwain seeds from all treatments contained normal to high amount of thymol. The other main constituents in the essential oil were γ-terpinene, p-cymene and β-pinene that are ranging from 12.52-27.35, 4.28-11.79 and 1.93-39.17%, respectively. The concentration of thymol and p-cymene were greater in seed oil from the first seedling date, whereas

concentration of γ-terpinene was greater in January and a greater content of β-pinene was found in February. The highest amount (49.62%) of minor components was observed in March. October was suitable time to convert precursor's p-cymene and γ-terpinene to thymol. It seems that higher temperature in October and long-time flowering had suitable time for synthesis essential oils. Effects of seeding date on ajwain essential oil yield and composition showed that for ajwain seed production, sowing in March was not preferable. Significant changes were attributed to long duration of crop which provided long photoperiod to synthesize more quantity of essential oil or in other words might be due to availability of higher temperature from flowering. The environment during seed development was a major determinate of seed quality. For successful seed production at this site, ajwain must be sown in early sowing date as delaying sowing until March reduced yield and composition (Syed *et al.* 2015) [77].
Non-polar fraction of ajwain oil contained *p*-cymene, γ-terpinene, α-pinene, β-pinene, α-terpinene, styrene, δ-3-carene, β-phyllanderene, terpinene-4-ol and carvacrol. Also oleic, linoleic, palmitic, petroselinic acid, resin acids were isolated from fruits of ajwain (Qureshi *et al.* 2010). New glycosyl constituents such as 6-hydroxycarvacrol 2-O-β-D-Glucopyranoside and 3, 5-Dihydroxytoluene 3-O-β-D-Galactopyranoside were recently reported from fruits of ajwain (Gang *et al.* 1980) [26].

Table 1: Effect of planting dates on main components of *Trachyspermum ammi* L. essential oil.

Sowing time	(%) Composition					Essential oil (kg/ha)
	Major components				Minor components	
	Thymol	γ-terpinene	p-cymene	β-pinene		
October	61.85	20.16	11.79	1.93	4.27	16.01
November	54.70	23.50	8.87	6.35	6.58	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

Source: Sayed *et al.* 2015

Phytochemical studies revealed that ajwain seed oil contained fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron and nicotinic acid (Zarshenas *et al.* 2013; Bairwa *et al.* 2012) [83, 5].

3.3 Effect of Geographical Climatic Conditions on Yield, Carbon Isotope Composition and Chemical Composition

Different geographical regions effect the chemical composition of ajwain oil. Various scienists reported the chemical composition of ajwain oil (Table 2). The results of these findings revealed the presence of thymol, γ-terpinene, γ-terpinolene, *p*-cymene, o-cymene and β-pinene as the major constituents. However, major component of ajwain oil collected from Pakistan was found to be p-cymene-3-ol. Other minor constituents present in ajwain oil were ethylene methacrylate, carvacrol, β-myrcene and α-pinene (Mahboubi and Kazempour 2011; Shojaaddini *et al.* 2014; Singh *et al.* 2004; Soni *et al.* 2016; Hassan *et al.* 2016; Balbaa *et al.* 1973, Lucchesi *et al.* 2004) [51, 71, 73, 74, 34, 7, 50]. The chemical composition of ajwain seed oil collected from Sabzevar (Iran) was found to be different as compared to other studies. Forty four compounds consisting 91.6 % of the total oil were identified. The ajwain seed oil was rich in non-terpenoids (56.0 %) and the main components of the oil were hexadecanoic acid (27.5 %), ethyl linoleate (8.5 %), 6-methyl-α-ionone (8.0 %), isobutyl phthalate (5.8 %), α-cadinol (4.7 %), germacrene D (4.3 %) and δ-cadinene (3.5 %) (Hashem *et al.* 2014) [33].
Ajwain seed essential oil consist of monoterpenes such as *p*-cymene, γ-terpinene, β-pinene, β-phellandrene, myrcene, α-pinene, α-terpinene, α-thujene, β-selinene and their phenol derivatives such as thymol, terpinene-4-ol, carvacrol, terpinolene, trans-sabinenhydrate, linalool and α-terpineol, reported in earlier studies (Kazemi *et al.* 2011; Chialva *et al.*1993) [44, 19].

Table 2: Chemical composition of ajwain seed oil in different geographical regions

S.No.	Components	Concentrations (%)							
		Iran		India		Pakistan		Egypt	Turkey
		Kehsan	Tehran	Delhi	Gorukhpur	Jaipur	Peshawar	Cairo	Sanliurta
1	α-Thujene	0.4	0.17	-	0.2	-	-	-	-
2	α-Pinene	0.3	0.06	2.29	0.2	2.91	0.20	-	-
3	β-Pinene	1.9	0.39	8.12	1.7	8.95	1.42	-	-
4	p-Cymene	-	16.16	12.30	30.8	13.50	-	24.00	33.1
5	β-Myrcene	0.7	0.33	1.67	0.4	1.11	0.60	-	-
6	o-Cymene	19.0	-		-	-	37.44	-	-
7	α-Terpinene	-	-	1.32	0.2	2.62	0.36	-	-
8	α-Phellendrene	-	-	-	-	-	0.52	-	-
9	β-Phellendrene	0.4	-	0.97	0.6	0.91	-	-	-
10	Limonene	0.2	-	0.44	-	0.57	-	-	-
11	γ-Terpinene	20.6	17.52	55.75	23.2	-	21.07	24.00	28.6
12	γ-terpinolene	-	-	-	0.2	55.63	-	-	-
13	4-Terpineol	0.1	-	0.65	0.8	-	-	-	-
14	Sabinene	-	0.02	0.29	-	0.44	-	-	-
15	cis-Limonene oxide	0.7	-	-	-	-	-	-	-
16	cis-β-terpineol	-	-	0.42	-	0.39	-	-	-

17	p-Cymene-3-ol	-	-	-	-	-	38.00	-	-
18	Dodecane	0.2	-	-	-	-	-	-	-
19	β-Fenchyl alcohol	0.1	-	-	-	-	-	-	-
20	Thymol	45.9	64.51	15.56	39.1	16.77	-	42.00	24.1
21	Ethylene methacrylate	6.9	-	-	-	-	-	-	-
22	p-Menth-1-en-1-ol	-	-	-	-	0.39	0.12	-	-
23	Tetra decane	0.2	-	-	-	-	-	-	
24	Carvacrol	-	-	-	0.3	-	-	4.70	-
25	Hexadecane	1.1	-	-	-	-	-	-	-

Source: Mahboubi and Kazempour, 2011; Shojaaddini *et al*., 2014; Chauhan *et al*., 2012; Singh *et al*., 2016; Soni *et al*., 2016; Hassan, 2016; Balbaa *et al*., 1973; Lucchesi *et al.*, 2004

The structures of compounds are given in Figure 1.

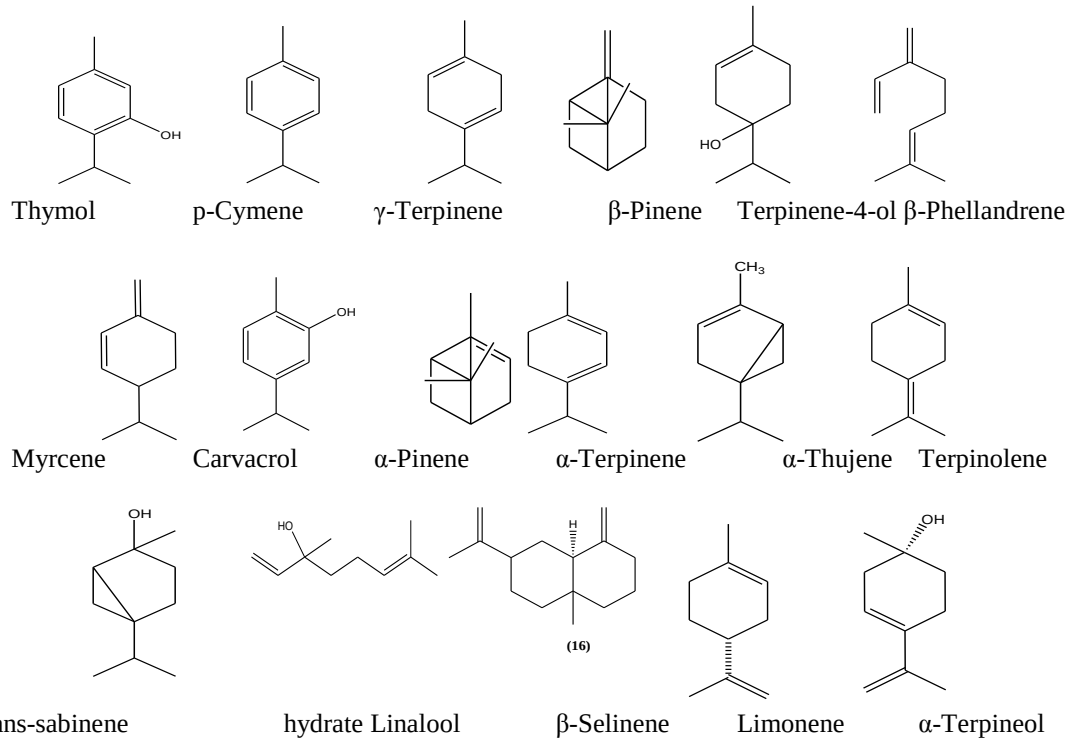

Fig 1: Structure of *Trachyspermum ammi* constituents

4. Biological properties

4.1 Insecticidal

Plant secondary metabolites play an important role in the plant-insect interactions. Some compounds extracted from plants have insecticidal activity. Essential oil extracted from the seeds of ajwain exhibited insecticidal activity against *Callosobruchus chinensis* in the ovi-position step as well as egg hatching and developmental inhibitory activities (Chaubey 2008; Kostyukovsky *et al.* 2002) [17, 46]. Alcoholic seed extract of *T. ammi* was found to be effective against different developmental stages of *Aedes aegypti* i.e. larva and pupa and considered as eco-friendly remedy. Mortality rate of larva was three times faster than the pupa stage (Sharanappa *et al.* 2015) [70]. Ajwain oil was found to showed fumigant activity against adults of *Oryzaephilus surinamensis*, *Rhyzopertha Dominica* and *Tribolium confusum* at different concentrations and exposure times. LC_{50} values obtained were 1.69, 19.01 and 58.70 µl/L air after 24 hrs of exposure, 0.80, 15.12 and 51.96 µl/L air after 48 hrs of exposure and 0.43, 12.83 and 47.05 µl/L air after 72 hrs of exposure for *O. surinamensis*, *R. dominica and T. confusum*, respectively. These values demonstrated that *O. surinamensis* was more susceptible than *R. Dominica* and *T. confusum*. Therefore, *T. confusum* was found to be more resistant than other two pests. Results showed that the increasing of exposure time was caused to decreasing LC_{50} value in all of three tested insects (Habashi *et al.* 2011) [31].

4.2 Antibacterial

Ethanol and acetone extract of ajwain seeds possessed an antibacterial activity against two Gram negative food spoilage bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. The *in vitro* antibacterial activity was performed by disc diffusion method. Ethanol extract of ajwain seeds possessed highest activity against *P. aeruginosa* whereas acetone extract exhibited highest activity against *E. coli* (Masih *et al.* 2012) [52]. Methanol extract of ajwain seeds showed significant antibacterial activity against various strains of bacteria like *P. aeruginosa*, *Bacillus pumilus*, *Staphylococcus aureus*, *S. epidermidis*, *E. coli*, *Klebsiella pneumonia* and *Bordetella bronchiseptica* (Shahidi 2004) [69]. However, acetone and aqueous extract of ajwain seeds possessed antibacterial efficacy against *Enterococcus faecalis*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *Salmonella typhi*, *S. typhimurium*, *Shigella flexneri* and *S. aureus* using agar diffusion assay (Kaur and Arora 2008) [43].

Antibacterial effect of ajwain was studied by applying cream containing 5% essential oil of ajwain for healing wound in rabbits and compared its effect with iodine solution. Wound contraction on the 15th day in ajwain group was 99.68%, compared with the healing effect of iodine solution group and non-treatment group which was found to be 100 and 96.57%, respectively which indicated a wound healing effect of ajwain (Gilani *et al.* 2013) [28]. Ajwain seed essential oil also showed antibacterial activity against Gram-negative bacteria *Erwinia*

carotovora with 43.00 and 46.67 mm diameter of growth inhabitation at a concentration of 300 and 400 ppm. Antibacterial properties of ajwain might be due to presence of phenolic compounds such as thymol and Carvacrol (Jafarpour *et al.* 2013) [40].

Essential oil of ajwain exhibited antibacterial activity against three Gram negative bacterial strains (*E. coli* -MTCC 443, *Proteus vulgaris* -MTCC 1771 and *K. pneumonia* -MTCC 7028) and three Gram positive bacterial strains (*S. aureus* - MTCC 3381, *B. subtilis* -MTCC 10619 and *B. megaterium* - MTCC 2412). The zones of inhibition for ajwain were found to be 18 mm, against *S. aureus, P. vulgaris, B. subtilis* and *B. megaterium* whereas 16 mm for *K. pneumonia* and 14 mm for *E. coli* (Hassan *et al.* 2016) [34].

4.3 Antifungal

Ethanol extract of ajwain seeds showed antifungal activity against selected fungi (*Aspergillus flavus, A. ochraceus, A. niger, A. orzyae, Fusarium moniliforme, Penicillium sp*.) using agar well diffusion assay (Odhav *et al.* 2002). Ajwain seed essential oil also exhibited a broad spectrum of fungitoxic behavior against *A. niger*, *A. flavus*, *A. oryzae*, *A. ochraceus*, *F. monoliforme*, *F. graminearum*, *P. citrium*, *P. viridicatum*, *P. madriti* and *Cheilomenes lunata* and absolute mycelial zone inhibition was obtained at a 6 µl dose of the oil (Thangam and Dhananjayan, 2003) [79].

Antifungal action of volatile constituents of ajwain seeds on ten fungi (*Acrophialophora fusispora, C. lunata, F. chlamydosporum, F. poae, Myrothecium roridum, Papulaspora sp., A. grisea, Alternaria tenuissima, D. tetramera and Rhizoctonia solani*) was evaluated. Ajwain seeds inhibited the growth of all test fungi by 72-90%. Fungicidal effect of ajwain seed essential oil on *A. Niger* and *Cochlostyla ovoidea* was also evaluated and minimum inhibitory concentration was observed at 5000µg/ml. (Dwivedi and Singh 1998) [22].

4.4 Antioxidant

Ajwain is rich in vitamins and minerals; it is also concentrated in health-promoting phytonutrients such as carotenoids (β-carotene and lutein) and flavonoids which provides powerful antioxidant protection. Antioxidant potential of blend of ajwain seed extract was evaluated by two different *in vitro* assays; the hydrogen peroxide (H_2O_2) scavenging assay and nitric oxide radical scavenging assay. Results revealed that ajwain extract exhibited significant effect in inhibiting hydrogen peroxide reaching up to 70.04% at concentration of 200µg/ml. In nitric oxide radical scavenging assay it showed maximum inhibition of 72.80% at concentration of 200µg/ml, whereas ascorbic acid at the same concentration exhibited 94.96% inhibition (Bajpai and Agrawal 2015) [6]. Ethanol extract of ajwain oil possessed a strong antioxidant capacity determined by various scavenging models including DPPH, nitric oxide, superoxide and hydroxyl radical scavenging assay as well as its lipid peroxidation ability in bovine brain extract. Ethanol extract of ajwain oil quenched 73.41% of DPPH radicals. It had potent inhibitory effect on scavenging nitric oxide, superoxide and hydroxyl radicals by 67.33, 63.22 and 62.48% respectively. Moreover, ethanol extract displayed concentration-dependent reducing power ability and remarkable ferric ion-induced lipid peroxidation inhibitory effect (69.22%) in bovine brain extract. All these properties confirmed the pharmacological efficacy of ajwain as a potential source of natural antioxidant (Mazumder *et al.* 2014) [57]. Antioxidant activity in the extract of fresh and frozen ajwain leaves was subjected to *in vitro* DPPH free radical scavenging assay. Results showed that frozen ajwain leaves exhibited significantly higher antioxidant activity than fresh one (Mazahir *et al.* 2015) [56]. Free radical scavenging potential and oxidative DNA damage preventive activity of potential of aqueous, methanol and acetone extracts of ajwain seeds was determined using different *in vitro* methods, namely, DPPH radical scavenging assay and ferric reducing antioxidant power assay along with the protection against oxidative DNA damage. The results revealed that acetone extract of ajwain seeds possessed comparatively high amount of total phenols whereas methanol seed extract was found to have highest amount of total flavonoids. At 1mg/ml acetone extract was recorded with highest FRAP value (2270.27 ± 0.005 μmol/l) as compared to aqueous and methanol extract of ajwain seeds. All the seed extracts showed mitigation of damage induced by Fenton reaction on calf thymus DNA. Therefore, the study suggested that ajwain seed extracts could contribute a highly significant bio-resource of antioxidants to be used in our day-to-day life and in food and pharmaceutical (Goswami and Chatterjee 2014) [30].

4.5 Antimicrobial

Ajwain seed oil showed antimicrobial activity on fifty-five bacterial strains with minimum inhibitory concentration (MIC) < 2% (v/v) except against *P. aeruginosa* (Mayaud *et al.* 2008) [55]. Diethyl ether fraction of ajwain exhibited good antibacterial and antifungal activity against multi drug resistant (MDR) strains of *C. albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, *E coli* and reference strains of *Streptococcus mutans* and *S. bovis* (Khan *et al.* 2010) [45]. Ajwain can lead to reduction in pathogenic microorganisms such as *C. albicans*, *Clostridium spp*. and *B. fragilis* and therefore could be effective in dysbiosis treatment (Myers *et al.* 2009) [59]. Ajwain seed essential oil also exhibited considerable *in vitro* antimicrobial activity against bacterial strains such as *P. destructum*, *A. niger* and *A. flavus* and results were comparable with the standard bactericide (Abdolali *et al.* 2007) [1]. The active compounds, responsible for the antimicrobial activity of ajwain were carvacol and thymol (Saxena and Vyas 1986) [68]. Ajwain seed essential oil showed a broad range of antimicrobial activity against thirty six isolates of twelve *K. pneumoniae*, twelve *E. coli* and twelve *S. aureus* isolated from urine culture of hospitalized patients were evaluated. Results showed that *E. coli* isolates were resistance to four of the antibiotics including ceftazidime (50%) cefixime (41.6%), tetracyclin (75%) and erythromycin (58.3%). However *K. pneumonia* isolates were resistant to three of the agent including ceftazidime (33.3%), cefixime (58.3%) and erythromycin (75%) while *S. aureus* isolates were resistant to six agents including cefixime (33.3%), trimethoprim-sulfamethoxazol (41.66%), penicillin (50%), oxacillin (3.3%), ceftazidime (66.6%) and vancomycin (8.3%). Minimum inhibitory concentration (MIC) was determined to characterize the antimicrobial activity of ajwain seed essential oil against all the tested bacteria. The highest MIC values of ajwain seed essential oil against *E. coli* and *K. pneumoniae* were observed at a concentration of 100 and 250 µg/ml respectively. It was concluded that ajwain essential oil inhibited the growth of all of the tested bacteria (Hassanshahian *et al.* 2014) [35]. Antimicrobial activity of the ajwain seed essential oil was determined using the micro-broth dilution technique in 24 multi-well plates, in triplicates. The microorganisms obtained from Persian Type Culture Collection (PTCC) Iran, included three Gram-positive

microorganisms: *S. aureus* (PTCC 1337), *B. subtilis* (PTCC 1023) and *C. albicans* (PTCC 5027) and three Gram-negative bacteria: *E. coli* (PTCC 1330), *K. pneumoniae* (PTCC 1053) and *P. aeruginosa* (PTCC 1047). Standard antibiotic (Gentamycin) was used as control for the sensitivity of the tested bacteria and ketoconazole was used as control for the tested fungi. Results revealed that ajwain seed essential oil exhibited antimicrobial activity against all the tested bacteria in dilution 0.05% (v/v) except for *P. aeruginosa*. It showed strong antimicrobial activity against *S. aureus* and *B. subtilis* (MIC, 0.00025% v/v) as compared to *E. coli* (MIC, 0.0005% v/v) (Eloff 1998) [24]. The antimicrobial activity of essential oil and different solvent extracts (petroleum ether, benzene, chloroform & methanol) of ajwain seeds were analysed against four bacterial species (*S. typhi*, *E. coli*, *Lactobacillus* and *B. lichenifomis*) by agar disc diffusion method. Results showed that the essential oil showed better antimicrobial activity in comparison to extracts with zone of inhibition 40.45, 37.12 and 44.54 mm against *S. typhi*, *E. coli* and *Lactobacillus* respectively. However no inhibition was observed against *B. lichenifomis*. Among the different solvent extracts, chloroform extract showed more antimicrobial activity in comparison to the other extracts (Aggarwal and Goyal 2012) [2].

4.6 Anti-inflammatory

Anti-inflammatory potential of alcoholic and aqueous extract of ajwain seeds was studied using acute rat model and a sub-acute rat model. Aspirin and phenylbutazone were used as standard positive controls. Total alcoholic and aqueous extract of ajwain seeds in 100 mg/kg doses exhibited significant ($P<0.001$) anti-inflammatory activity in both the animal models. In carrageenan induced rat paw oedema, aspirin and phenylbutazone inhibited 45.23 and 43.83% respectively, while total alcoholic and aqueous extracts showed an inhibition of 38.32 and 41.11% respectively. In cotton pellet induced granuloma studies, total alcoholic and aqueous extract produced 38.05 and 43.87% inhibition of the pellets weight, whereas aspirin and phenylbutazone produced 44.69 and 42.04% inhibition. The weight of the adrenal glands were found to be significantly increased in total alcoholic and aqueous extract treated animals (25.53 and 32.2%) whereas aspirin and phenylbutazone showed an increase of 18.86 and 10.00% respectively. It showed that both the extracts from the seeds of ajwain, exhibited significant anti-inflammatory potential (Thangam and Dhananjayan 2003 and Boskabady *et al.* 2005) [79, 12].

4.7 Anti-filarial

In vitro activity of a methanol extract of ajwain fruit against *Setaria digitata* worms was investigated. The crude extract and the active fraction showed significant activity against the adult *S. digitata* by both a worm mortality and MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide] reduction assays. Ajwain crude extract also exhibited macrofilaricidal activity. The *in vivo* effect of thymol was evaluated against the *B. malayi* parasite in a Mastomys coucha model. It also showed macrofilaricidal activity and female worm sterility *in vivo* against *B. malayi.* The IC_{50} values for thymol at two incubation periods 24 and 48 hrs were 0.024 and 0.002 mg/ml, respectively. The mean percentage mortality of adults (58.93%) in the group, treated with 50 mg/kg was significantly ($P < 0.0001$) higher than that obtained in the control group (19.05%) (Mathew *et al.* 2008) [54].

4.8 Nematicidal

Ajwain seed essential oil exhibited nematicidal activity against *Bursaphelenchus xylophilus* with LC_{50} value of 0.431 mg/ml (Kwon *et al.* 2007) [48]. Ajwain oil constituents (camphene, pinene, myrcene, limonene, terpinene, terpinen-4-ol, thymol and carvacrol) showed nematicidal activity against pinewood nematode (Pelczar *et al.* 1998; Wright 1981) [62, 80].

4.9 Anthelmintic

Anthelmintic activity in ajwain was exerted by interference with the energy metabolism of parasites through potentiation of ATPase activity and thus loss of energy occured. Anthelmintic activity of ajwain, showed its effect against specific helminths, e.g. *Ascaris lumbricoides* in humans and *Haemonchus contortus* in sheep. The plant was also reported to possess cholinergic activity with peristaltic movements of the gut, thus helped in expulsion of intestinal parasites which might also be a contributory factor to its anthelmintic activity (Tamurab and Iwamoto, 2004; Jabbar *et al.* 2006) [78,39].

4.10 Hypotensive

Ajwain seed extract in 70% methanol was found to cause a dose-dependent hypotensive effect ranging from about 6 % reduction in mean arterial blood pressure (BP) at a dose of 3.0 mg/kg to about 42% reduction in mean arterial blood pressure at a dose of 100.0 mg/kg. Acetylcholine also caused a hypotensive effect at 1 µg/kg comparable in magnitude to the effect of ajwain extract at 30.0 mg/kg (Gilani *et al.* 2005) [28].

4.11 Analgesic and anti-nociceptive

In order to evaluate the analgesic and anti-nociceptive activity of ajwain, an *in vivo* investigation was carried out using a Tail-flick Analgesiometer Device (Dashti-Rahmatabadi *et al.* 2007) [21]. The study revealed that the ethanol extract significantly increased in Tail-Flick Latency within 2 hrs post drug administration. An experimental trial study was carried out to compare the anti-nociceptive effect of the hydro-alcoholic extract of ajwain with morphine sulfate using formalin test. Findings revealed that ajwain extract exhibited anti-nociceptive effect on both early and late phases (Hejazian *et al.* 2008) [36]. Similar study was carried out on the ajwain total essential oil which showed significant effect on the late phase of formalin test and it may be due to the presence of thymol in essential oil (Hejazian 2006) [37]. In addition, under a randomized controlled placebo control clinical trial, the herb essential oil was assayed for the analgesic effect in neuropathic feet burn. Results revealed that ajwain essential oil significantly reduced the feet burn compared to placebo (Petramfar *et al.* 2013) [63].

4.12 Antitussive and bronchodilatory

Antitussive effect of ajwain was reported in traditional medical manuscripts. In this regard, the clinical effect of aerosols related to two different concentrations of aqueous and macerated extracts of ajwain seeds as well as carvacrol, codeine and saline were evaluated by counting the number of coughs produced. According to the results, both concentrations of ajwain seeds revealed significant reduction of cough number which may be due to its potent antitussive effect (Boskabady *et al.* 2005) [12]. Relative studies showed the inhibitory effect of both ajwain extract and essential oil on histamine (H1) receptors of isolated guinea-pig tracheal chains (Boskabady and Shaikhi 2000) [10]. The respiratory, bronchodilatory effects of different fractions of ajwain seed essential oil were examined. Results showed that the relaxant

and bronchodilatory effect of essential oil fractions may be due to the amount of Carvacrol (Boskabady *et al.* 2003) [13]. The bronchodilatory effect of decocted extract of ajwain on the asthmatic patients' airways was examined in a subsequent trial study. According to the results, the extract showed relatively bronchodilatory effect on asthmatic airways compared to the effect of theophylline at concentrations used (Boskabady *et al.* 2007) [11].

4.13 Hepatoprotective

This study describes the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the aqueous-methanol extract of ajwain seeds to rationalize some of its traditional uses. Aqueous-methanol extract of ajwain seeds (3-100 mg/kg) caused a dose-dependent fall in arterial blood pressure in anaesthetized rats. In isolated rabbit aorta and jejunum preparations, aqueous-methanol extract of ajwain (0.1-3.0 mg/ml) caused an inhibitory effect on the K^+-induced contractions. In isolated guinea-pig tracheal preparations, it inhibited carbachol and K^+-induced bronchoconstriction at 0.1-1.0 mg/ml as well as shifted the dose-response curves (DRCs) of carbachol and histamine to the right with suppression of maximum response suggestive of non-specific bronchodilator effect mediated possibly through CCB. Pretreatment of rats with aqueous-methanol extract of ajwain seeds (500 mg/kg orally for 2 days at 12 hrs intervals) prevented paracetamol (640 mg/kg) and carbon tetrachloride (CCl_4) (150 mg/kg) induced rise in serum alkaline phosphatase (ALP) and aminotransferases. The same dose of these extracts prevented the CCl_4-induced prolongation in pentobarbital-induced sleeping time in mice confirmed its hepatoprotectivity. These results indicated the presence of calcium antagonist (s) in ajwain seeds and thus provide sound mechanistic basis for some of their folkloric uses (Srivastava *et al.* 1999) [75].

4.15 Medical uses

In Indian system of medicine, ajwain is administered for stomach disorders, a paste of crushed fruits is applied externally for relieving colic pains; a hot and dry fermentation of the fruits was lapped on the chest to cure asthma. Aqueous extract of ajwain which was also known as Ajwan-ka-arak used for the treatment of diarrhoea (Krishnamoorthy and Madalageri 1999; Soni *et al.* 2016) [47, 74]. Ajwain leaves can be crushed and used for skin infections. People in Middle East countries, tied ajwain powder in a thin cloth and smelt it frequently to decreases the pain, in the acute phase of common cold or migraine (Nagalakshmi *et al.* 2000) [60]. The seeds are small but taste hot, penchant, bitter, appetizer and was used as effective remedy in managing ailments like vomiting, disease of heart and mouth etc. It was considered as carminative and diuretic in nature and effective remedy of disease like paralysis, weakness of limbs. Ajwain seeds consisted of, chest pain, liver disease, hiccups, kidney and spleen problem etc. The ajwain seeds intake with honey moisture, protein, fat, minerals, fibre, carbohydrates, calcium, phosphorus, iron, carotene, thiamine, riboflavin and niacin. People in India, Pakistan and in the South East of Iran used ajwain seeds in vegetable curries, steamed cabbage, carrots, potato and pumpkin. In Indian cuisine, this seed was usually crushed in a mortar and then fried in butter to make an aromatic butter sauce, used to flavour vegetables and bean dishes. It is well-known for its medicinal effects, one of the chief being its ability to reduce flatulence. It can also be brewed into tea, to treat colic and diarrhoea. It has also important germicidal and fungicidal properties. Ajwain roots are diuretic in nature. Its seeds contain about 50% thymol, a well-known and antibacterial essential oil, and along with thyme that can be used to enhance the immune system to ward off colds and flu and other viral infections. Ajwain seeds with the kernel of tamarind seeds act as an effective aphrodisiac, prepared by frying an equal quantity of both in pure ghee, dried and then powdered and preserved in airtight container. This is taken at bedtime as a promoter for virility. For an earache, a half a teaspoon of the ajwain seeds heated in 30 ml of milk till the aroma of the seeds permeate the milk, which is then filtered and used as an ear drop. To overcome ear ache, 3 g of ajwain seeds, with garlic boiled together in 40 g of sesame oil were used. The ajwain seed oil was strained while cooling to body temperature and used as ear drops. A habit of chewing ajwain seeds prevents bad breath. For bronchial asthma, ajwain seeds are tied in the cotton cloth, heated in a frying pan and applied on the chest and neck when still warm. It helps curing conditions like ringworm and itching and menstrual and postnatal disorders (Dwivedi *et al.* 2012; Brul and Coote 1999) [22, 14]. It is the most known spice in kitchen can be easily take in daily diet as a cure or prevent many more diseases of life (Bhargava and Hakasa 1959) [8].

5. Conclusions

Ajwain has been well known as an Ayurvedic spice since ancient times. It has traditionally been used as a medicinal plant for the treatment of indigestion and dyspepsia and many other gastric disorders. Ajwain is also rich in moisture, protein, carbohydrates, fat, minerals, fiber, calcium, phosphorus, iron, carotene, thiamine, riboflavin and niacin. Chemical composition of essential oil and presence of variety of diverse constituents in it are responsible for a wide range of biological properties.

6. References

1. Abdolali M, Pouya F, Younes G. *Carum copticum* essential oil chemotypes. Food Chemistry. 2007; 100(3):1217-1219.
2. Aggarwal S, Goyal S. *In vitro* antimicrobial studies of *Trychyspermum ammi*. International Journal of Pharma and Bio Sciences. 2012; 3:64-68.
3. Anonymous. The Wealth of India. Publication and Information Directorate (CSIR), New Delhi. 1959, 266-268.
4. Ashraf M, Bhatty MK. Studies on essential oils of the Pakistan species of *Trachyspermum ammi*, ajwain seed oil. Pakistan Journal of Scientific and Industrial Research. 1975; 18:232-235.
5. Bairwa R, Sodha RS, Rajawat BS. *Trachyspermum ammi*. Pharmacognosy Reviews. 2012; 6(11):56-60.
6. Bajpai KV, Agrawal P. Studies on phytochemicals, antioxidant, free radical scavenging and lipid peroxidation inhibitory effects of *Trachyspermum ammi* seeds. Pharmaceutical Research. 2015; 49(1):58-65.
7. Balbaa SI, Hilal SH, Haggag MY. The volatile oil from the herb and fruits of *Carum copticum* at different stages of growth. Planta Medica. 1973; 23(4):312-320.
8. Bhargava PP, Hakasa, CN. Examination of essential oil from ajwain seeds. Journal of Essential Oil Research. 1959; 50:204-206.
9. Bhattacharya AK, Kaul PN, Rajeshwara RBR. Essential oil composition of ajwain seed production in Andhra Pradesh. Indian Perfumer. 1998; 42:65-67.
10. Boskabady MH, Shaikhi J. Inhibitory effect of *Carum*

copticum on histamine (H_1) receptors of isolated guinea-pig tracheal chains. Journal of Ethno pharmacology. 2000; 69(3):217-227.
11. Boskabady MH, Alizadeh M, Jahanbin B. Bronchodilatory effect of *Carum copticum* in airways of asthmatic patients. Therapie. 2007; 62(1):23-29.
12. Boskabady MH, Jandaghi P, Kiani S, Hasanzadeh L. Antitussive effect of *Carum copticum* in guinea pigs. Journal of Ethno pharmacology. 2005; 97(1):79-82.
13. Boskabady MH, Ramazani M, Tabei T. Relaxant effects of different fractions of essential oil from *Carum copticum* on guinea pig tracheal chains. Phytotherapy Research. 2003; 17(10):1145-1149.
14. Brul S, Coote P. Preservative agents in foods: Mode of action and microbial resistance mechanisms. International Journal of Food Microbiology. 1999; 50(1):1-17.
15. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against *E coli*. Letters in Applied Microbiology. 2003; 36(3):162-167.
16. Chatterjee S, Goswam N, Bhatnagar P. Estimation of phenolic components and *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) and ajwain (*Trachyspermum ammi*) seeds. Journal of Life Sciences. 2012; 3(2):109-118.
17. Chaubey MK. Fumigant toxicity of essential oils from some common spices against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Journal of Oleo Science. 2008; 57(3):171-179.
18. Chauhan B, Kumar G, Ali M. Composition of volatile oil of *Carum copticum* benth and hook fruits. International Research Journal of Pharmacy. 2012; 3(7):131-132.
19. Chialva F, Monguzzi F, Manitto P, Akgül A. Essential oil constituents of *Trachyspermum copticum* (L.) link fruits. Journal of Essential Oil Research. 1993; 5:105-106.
20. Dadalioglu I, Evrendilek GA. Chemical compositions and antibacterial effects of essential oils of turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), spanish lavender (*Lavandula stoechas* L.) and fennel (*Foeniculum vulgare*) on common foodborne pathogens. Journal of Agricultural and Food Chemistry. 2004; 52(26):8255-8260.
21. Dashti-Rahmatabadi MH, Hejazian SH, Morshedi A, Rafati A. The analgesic effect of *Carum copticum* extract and morphine on phasic pain in mice. Journal of Ethnopharmacology. 2007; 109(2):226-228.
22. Dwivedi SK, Singh KP. Fungitoxicity of some higher plant products against *Macrophomina phaseolina* (Tassi) goid. Flavour and Fragrance Journal. 1998; 13(6):397-399.
23. Dwivedi SN, Mishra RP, Alava S. Phytochemistry, pharmacological studies and traditional benefits of *Trachyspermum ammi* L. Sprague. International Journal of Pharmaceutical and Life Sciences. 2012; 3(5):1705-1709.
24. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica. 1998; 64(8):711-713.
25. Farooq MO, Osman SM, Ahman MS. The fixed oil from the seeds of *Carum copticum* Benth. Journal of the Science of Food and Agriculture. 1953; 4:132-134.
26. Gang SK, Sharma ND, Gupta SR. A phenolic glucoside from the seeds of *Carum copticum*. Phytochemistry. 1980; 9(10):2215-2216.
27. Garg S. A new glucoside from *Trachyspermum ammi*. Fitoterapia. 1998; 69(6):511-512.
28. Gilani AH, Jabeen Q, Ghayur MN, Janbaz KH, Akhtar, MS. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. Journal of Ethno pharmacology. 2005; 98(12):127-135.
29. Gilani SR, Mahmood Z, Hussain M. Preliminary evaluation of antimicrobial activity of cream formulated with essential oil of *Trachyspermum ammi*. Pakistan Journal of Pharmaceutical Science. 2013; 26(5):893-896.
30. Goswami N, Chatterjee S. Assessment of free radical scavenging potential and oxidative DNA damage preventive activity of *Trachyspermum ammi* L. (Carom) and *Foeniculum vulgare Mill.* (Fennel) seed extracts. International Journal of Biological and Medical Research. 2014; 3:1-8.
31. Habashi AS, Safaralizadeh MH, Safavi SA. Fumigant toxicity of *Carum copticum* L. oil against *Tribolium confusum* du val, *Rhyzopertha dominica* F. and *Oryzaephilus surinamensis*. Munis Entomology and Zoology Journal. 2011; 6(1):282-289.
32. Hajare SS, Hajare SN, Sharma A. Aflatoxin inactivation using aqueous extract of ajowan (*Trachyspermum ammi*) seeds. Journal of Food Science. 2005; 70(1):29-34.
33. Hashem A, Bhnam M, Hasan R. Characterization of chemical composition and antioxidant properties of *Trachyspermum ammi* seeds as a potential medicinal plant. Journal of Chemical Health Risks. 2014; 4(4):9-16.
34. Hassan W, Gul S, Rehman S, Noreen H, Shah Z, Mohammadzai I., Zaman B. Chemical composition, essential oil characterization and antimicrobial activity of *Carum copticum*. Vitamin Minerals 2016; 5: 1-5.
35. Hassanshahian M, Bayat Z, Saeidi S, Shiri Y. Antimicrobial activity of *Trachyspermum ammi* essential oil against human bacterial. International Journal of Advanced Biological and Biomedical Research. 2014; 2(1):18-24.
36. Hejazian SH, Mosaddegh MH, Dashti Rahmatabadi HM. Anti-nociceptive effects of *Carum copticum* extract in mice using formalin test. World Applied Sciences Journal. 2008; 3(2):215-219.
37. Hejazian S. Analgesic effect of essential oil (EO) from *Carum copticum* in mice. World Applied Sciences Journal. 2006; 1(2):95-99.
38. Ishikawa T, Sega Y, Kitajima J. Water-soluble constituents of ajwain. Chemical and Pharmaceutical Bulletin. 2001; 49(7):840-844.
39. Jabbar A, Iqbal Z, Khan MN. *In vitro* anthelmintic activity of *Trachyspermum ammi* seeds. Pharmacogonosy Magazine. 2006; 2:126-129.
40. Jafarpour M, Golparvar AR, Lotfi A. Antibacterial activity of essential oils from *Thymus vulgaris*, *Trachyspermum ammi* and *Mentha aquatic* against *Erwinia carotovora in vitro*. Journal of Herbal Drugs. 2013; 4(3):115-118.
41. Javed S, Shahid AA, Haider MS, Umeeram A, Ahmad R, Mushtaq S. Nutritional, phytochemical potential and pharmacological evaluation of *Nigella sativa* (kalonji) and *Trachyspermum ammi* (ajwain). Journal of Medicinal Plants Research. 2012; 6(5):768-775.
42. Joshi SG. Medicinal Plant, Oxford and IBH Publisher, New Delhi (India). 2000, 51-52.
43. Kaur GJ, Arora DS. *In vitro* antibacterial activity of three plants belonging to the family Umbelliferae. International Journal of Antimicrobial Agents. 2008; 31(4):380-399.

44. Kazemi OR, Behravan J, Ramezani M. Chemical composition, antimicrobial activity and antiviral activity of essential oil of *Carum copticum* from Iran. Avicenna Journal of Phytomedicine. 2011; 1(2):83-90.
45. Khan R, Zakir M, Afaq SH, Latif A, Khan AU. Activity of solvent extracts of *Prosopis spicigera*, *Zingiber officinale* and *Trachyspermum ammi* against multi drug resistant bacterial and fungal strains. Journal of Infection in Developing Countries. 2010; 4(5):292-300.
46. Kostyukovsky M, Rafaeli A, Gileadi C, Demchenko N, Shaaya E. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: Possible mode of action against insect pests. Pest Management Science. 2002; 58(11):1101-1106.
47. Krishnamoorthy V, Madalageri MB. Bishop weeds (*Trachyspermum ammi*): An essential crop for North Karnataka. Journal of Medicinal and Aromatic Plant Sciences. 1999; 21(4):996-998.
48. Kwon PIL, Junheon K, Sang-Gil L, Sang-Chul S. Nematicidal activity of plant essential oils and components from ajowan (*Trachyspermum ammi*), allspice (*Pimenta dioica*) and litsea (*Litsea cubeba*) essential oils against pine wood nematode (*Bursaphelenchus Xylophilus*). Journal of Nematology. 2007; 39(3):275-279.
49. Lockwood GB, Asghari G, Hakimi B. Production of essential oil constituents by cultured cells of *Carum copticum.* Flavour and Fragrance Journal. 2002; 17: 456-458.
50. Lucchesi ME, Chemat F, Smadja J. An original solvent free microwave extraction of essential oil from spices. Flavour and Fragrance Journal. 2004; 19:134-138.
51. Mahboubi M, Kazempour N. Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trachyspermum copticum* essential oil. Iranian Journal of Microbiology. 2011; 3(4):194-200.
52. Masih U, Shrimali R, Naqvi SMA. Antibacterial activity of acetone and ethanol extracts of cinnamon (*Cinnamomum zeylanicum*) and ajwain (*Trachyspermum ammi*) on four food spoilage bacteria. International Research Journal of Biological Sciences. 2012; 1(4):7-11.
53. Masoudi S, Rustaiyan A, Ameri N, Monfared A, Komeilizadeh H, Kamalinejed M, Janu roodi J. Volatile oils of *Carum copticum.* Journal of Essential Oil Research. 2002; 14:288-289.
54. Mathew N, Bhattacharya SM, Perumal V, Muthuswamy K. Antifilarial lead molecules isolated from *Trachyspermum ammi*. Molecules. 2008; 13:2156-2168.
55. Mayaud L, Carricajo A, Zhiri A, Aubert G. Comparison of bacteriostatic and bactericidal activity of thirteen essential oils against strains with varying sensitivity to antibiotics. Letters in Applied Microbiology. 2008; 47(3):167-173.
56. Mazahir R, Anshul KS, Tatheer F, Shaista A. Comparative study of antioxidant activity of polyphenols isolated from frozen and fresh leaves of *Trachyspermum ammi* (ajwain). Journal of Pharmacognosy and Phytochemistry. 2015; 6:122-124.
57. Mazumder J, Kumria R, Pathak D. Evaluation of synergistic antimicrobial activity and antioxidant activity of blend of essential oil contains fennel, coriander, ajowan and caraway. International Journal of Pharmacy and Biological Sciences. 2014; 9(1):87-94.
58. Minija J, Thoppil, JE. Essential oil composition of *Trachyspermum ammi* L. sprague from South India. Indian Journal of Pharmaceutical Sciences. 2002; 64(3): 250-251.
59. Myers SR, Hawrelak JA, Cattley T. Essential oils in the treatment of intestinal dysbiosis: A preliminary *in vitro* study. Alternative Medicine Review. 2009; 14(4):380-384.
60. Nagalakshmi G, Shankar Acharya NB, Puranaik J. Studies on chemical and technological aspects of ajwain (*Trachyspermum ammi*) syn (*Carum copticum* Hiren) seeds. Journal of Food Science and Technology. 2000; 37(3):277-281.
61. Odhav B, Juglal S, Govinden R. Spices oils for the control of co-occurring mycotoxins producing fungi. European Food Research and Technology. 2002; 65:683-687.
62. Pelczar MJ, Chan ECS, Krieg NR. Microbiology. *Tata Mcgraw Hill Publisher,* New York (USA). 1998, 80-100.
63. Petramfar P, Moein M, Semani S, Zarshenas MM. Ajwain ten percent cream versus placebo in feet burning; a randomized, double-blind, placebo-controlled trial. Neurological Sciences. 2013; 37:1449-1455.
64. Pruthi JS. Spices and Condiments. National Book Trust Publisher, New Delhi (India). 1992, 1-195.
65. Qureshi AA, Kumar KE. Phytochemical constituents and pharmacological activities of *Trachyspermum ammi*. Plant Archives. 2010; 10(2):955-959.
66. Raghavan S. Handbook of Spices, Seasoning and Flavourings, 2nd ed, CRC Press, Taylor & Francis group, Boca Raton, FL, New York. 2007, 107-119.
67. Saini N, Singh GK, Nagori BP. Physicochemical characterization and spasmolytic activity of essential oil of ajwain (*Trachyspermum ammi* L.) from Rajasthan. International Journal of Pharmacological Screening Methods. 2014; 4(1):49-55.
68. Saxena AP, Vyas KM. Antimicrobial activity of seeds of some ethnomedicinal plants. Journal of Economic and Taxonomic Botany. 1986; 8:291-300.
69. Shahidi B. Evaluation of antibacterial properties of some medicinal plants used in Iran. Journal of Ethno pharmacology. 2004; 94(2):301-305.
70. Sharanappa K, Kadabinakatti Patil S, Kulkarni GV, Kumar R, Arya MC. Potential of *Trachyspermum ammi* (Linn.) seed extract against larva and pupa of Aedes species. *Natural Sciences*. 2015; 13(12):127-130.
71. Shojaaddini M, Moharramipour S, Saha ZB. Fumigant toxicity of essential oil from *Carum copticum* against Indian meal moth, *Plodia interpunctella*. Journal of Plant Protection Research. 2014; 48:411-419.
72. Sies H. Oxidative stress: Oxidants and antioxidants. Experimental Physiology. 1997; 82(2):291-295.
73. Singh G, Maurya S, Catalan C, Lampasona DMP. Chemical constituents, antifungal and antioxidative effects of ajwain essential oil and its acetone extract. Journal of Agricultural and Food Chemistry. 2004; 52(11):3292-3296.
74. Soni R, Sharma G, Asuja DN. Essential oil yield pattern and antibacterial and insecticidal activities of *Trachyspermum ammi* and *Myristica fragrans*. Scientifica. 2016; 2:1-7.
75. Srivastava M, Baby P, Saxena A. GC-MS investigation and antimicrobial activity of the essential oil of *Carum copticum* benth and hook. Acta Alimentaria. 1999; 28(3):291-295.
76. Suntar I, Akkol EK, Tosun A, Keles H. Comparative pharmacological and phytochemical investigation on the

wound-healing effects of the frequently used essential oils. J Essent Oil Res. 2014; 26(1):41-49.
77. Syed S, Muhammad IA, Saddiqui HM, Aslam Z. Growth and yield response of ajwain (*Carum copticum* L) to sowing date and row spacing. Pakistan Journal of Life and Social Sciences. 2015; 13:157-161.
78. Tamurab T, Iwamoto Thymol H. A classical small molecule compound that has a dual effect (potentiating and inhibitory) on myosin. Biochemical and Biophysical Research Communication. 2004; 18: 786-789.
79. Thangam C, Dhananjayan R. Anti-inflammatory potential of the seeds of *Carum copticum* Linn. Indian Journal of Pharmacology. 2003; 35:388-391.
80. Wright Nematicides DJ. Mode of action and new approaches to chemical control. Zukerman and Rhode Publisher, New York (USA). 1981, 421-429.
81. Zahin MI, Ahmad Aqil F. Antioxidant and antimutagenic activity of *Carum copticum* fruit extracts. *Toxicology in Vitro*. 2010; 24:1243-1249.
82. Zargari A. Medicinal plants. Tehran University Press, Tehran, (Iran). 1996.
83. Zarshenas MM, Moein M, Samani SM, Petramfar P. An overview on ajwain (*Trachyspermum ammi*) pharmacological effects; Modern and traditional. Journal of Natural Remedies. 2013; 14:98-105.
84. Zule J, Tisler V, Zurej A, Torelli N. Isolation and characterization of essential oils from the cones of Norway Spruce (*Picea abies* Karst.), European Larch (*Larix decidua* Mill.) and Scots Pine (*Pinus sylvestris*). Zborniku Gozdarstva in Lesar. 2003; 71:159-172.