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, aldehydes, ketones, acids and esters (Suntar et al. 2014) \[16\]. Terpenes having a diverse variety of structures with specific functions constituted the largest amount (71.10%) of essential oils (Zule et al. 2003) \[17\]. 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) \[18\]. *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 Punjab, Ajma in Gujarati, Kath in Kashmiri and Omam in Tamil (Chauhan et al. 2012) \[19\]. 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) \[3\]. 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) \[20\].

2. Taxonomy, Distribution and Morphology

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Order</td>
<td>Apiales</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Family</td>
<td>Apiceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Trachyspermum</td>
</tr>
<tr>
<td>Species</td>
<td>ammi</td>
</tr>
</tbody>
</table>

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) \[10\]. This plant grows in arid and semi-arid 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, ~ 131 ~
West Bengal and Karnatak (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 °C 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 (Hassanahabian 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) [31]. 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) [60]. 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, 44]. 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), conealing point (15.7 °C) 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), conealing point (15.4 °C) 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 (CO2) 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) (Minjia and Thoppil 2002) [59]. 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) [27, 19]. 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 monoterprenoid 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 monoterprenoids and sesquiterpenoids: Cadinene (43%), longiroleone (11%), thymol (5%), and camphor (3%) (Faruq et al. 1953) [29]. 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) [34]. On analysis of the fixed oil from the seeds of ajwain in Indian origin, reported petrocellinic acid as the major component (Dwiwedi et al. 2012) [24]. 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-terpine (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 (Faruq et al. 1953) [29]. A yellow, crystalline flavone and a steroid-like substance were also isolated from ajwain seeds which contained 6-O-β-glucopyranoslythymol, a glucoside and a yield of 25% oleoresin containing 12% volatile oil (thymol, γ-terpine, 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%), carophyllene (6.2%), muurosol (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) [40]. 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-terpine, 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 sowing date, whereas

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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 (Sayed et al. 2015). Non-polar fraction of ajwain oil contained p-cymene, γ-terpinene, α-pinene, β-pinene, α-terpinene, styrene, δ-3-carenene, β-phellandrene, 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).  

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

<table>
<thead>
<tr>
<th>Sowing time</th>
<th>(% Composition)</th>
<th>Essential oil (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Major components</td>
<td>Minor components</td>
</tr>
<tr>
<td></td>
<td>Thymol</td>
<td>γ-terpinene</td>
</tr>
<tr>
<td>October</td>
<td>61.85</td>
<td>20.16</td>
</tr>
<tr>
<td>November</td>
<td>54.70</td>
<td>23.50</td>
</tr>
<tr>
<td>January</td>
<td>42.78</td>
<td>27.35</td>
</tr>
<tr>
<td>February</td>
<td>24.56</td>
<td>15.01</td>
</tr>
<tr>
<td>March</td>
<td>30.69</td>
<td>12.52</td>
</tr>
</tbody>
</table>

**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).  

**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 scientists 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, α-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; Shoaiaaddini et al. 2014; Singh et al. 2004; Soni et al. 2016; Hassan et al. 2016; Balbaa et al. 1973, Lucchesi et al. 2004). 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 (8.0 %), isobutyl phthalate (5.8 %), α-cadinol (4.7 %), germacrene D (4.3 %) and δ-cadinene (3.5 %) (Hashem et al. 2014).  

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).  

**Table 1:** Composition of ajwain seed oil in different geographical regions

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Components</th>
<th>Concentrations (%)</th>
<th>Iran</th>
<th>India</th>
<th>Pakistan</th>
<th>Egypt</th>
<th>Turkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kishan</td>
<td>Tehran</td>
<td>Delhi</td>
<td>Gorukhpur</td>
<td>Jaipur</td>
</tr>
<tr>
<td>1</td>
<td>α-Thujene</td>
<td>0.4</td>
<td>0.17</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>α-Pinene</td>
<td>0.3</td>
<td>0.06</td>
<td>2.29</td>
<td>0.2</td>
<td>2.91</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>β-Pinene</td>
<td>1.9</td>
<td>0.39</td>
<td>8.12</td>
<td>1.7</td>
<td>8.95</td>
<td>1.42</td>
</tr>
<tr>
<td>4</td>
<td>p-Cymene</td>
<td>-</td>
<td>16.16</td>
<td>12.30</td>
<td>30.8</td>
<td>13.50</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>β-Myrcene</td>
<td>0.7</td>
<td>0.33</td>
<td>1.67</td>
<td>0.4</td>
<td>1.11</td>
<td>0.60</td>
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<tr>
<td>6</td>
<td>α-Cymene</td>
<td>19.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>37.44</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>α-Terpine</td>
<td>-</td>
<td>-</td>
<td>1.32</td>
<td>0.2</td>
<td>2.62</td>
<td>0.36</td>
</tr>
<tr>
<td>8</td>
<td>β-Phellandrene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.52</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>α-Phellandrene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.97</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>Limonene</td>
<td>0.2</td>
<td>0.44</td>
<td>-</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>γ-Terpine</td>
<td>20.6</td>
<td>17.52</td>
<td>55.75</td>
<td>23.2</td>
<td>55.63</td>
<td>21.07</td>
</tr>
<tr>
<td>12</td>
<td>γ-Terpinolene</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>0.43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>δ-Terpine</td>
<td>0.1</td>
<td>0.65</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Sabine</td>
<td>-</td>
<td>0.02</td>
<td>0.29</td>
<td>0.44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>cis-Limonene oxide</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>cis-β-Terpineol</td>
<td>-</td>
<td>0.42</td>
<td>-</td>
<td>0.39</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
The structures of compounds are given in Figure 1.

![Chemical structures](image)

**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 show fumigant activity against adults of *Oryzaephilus surinamensis, Rhyzopertha Dominica* and *Tribolium confusum* at different concentrations and exposure times. LC<sub>50</sub> 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<sub>50</sub> value in all of three tested insects (Habashi *et al.* 2011) [51].

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* (Shahid 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 15<sup>th</sup> 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. oryzae, 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. moniliforme, F. graminearum, P. citrinum, P. viridicatu, P. madriti and Chelomenes lunata and absolute mycelial zone inhibition was obtained at a 6 µl dose of the oil (Thangam and Dhananjayan, 2003) [39]. Antifungal action of volatile constituents of ajwain seeds on ten fungi (Acrophialophora fassispora, C. lunata, F. chlamydosporum, F. paeae, Myrothecium roridum, Papulaspora sp., A. grisea, Alternaria tenuissima, D. tetramera and Rhizoctonia solani) was evaluated. Ajwain seeds inhibited the growth of all tested 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. (Dwiwedi 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 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. (Dwiwedi and Singh 1998) [22].

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, K. 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 dysbosis treatment (Myers et al. 2009) [59]. Ajwain seed essential oil also exhibited considerable in vitro antimicrobial activity against bacterial strains such as P. dextructum, 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) [60]. 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 (41.6%), penicillin (50%), oxacillin (3.3%), cefazidime (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) [5].

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 43.05 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 cholnergic 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) [78,30].

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) [30]. 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 (CCl₄) (150 mg/kg) induced rise in serum alkaline phosphatase (ALP) and aminotransferases. The same dose of these extracts prevented the CCl₄-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, 71]. 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, phantax, 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 effects, one of the spices. Ajwain seeds intake with honey results in a thin cloth and smelt it 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) [9].

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


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