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RESEARCH ARTICLE

# Comparative study of antibacterial efficacy of Ajwain seed oil and hexane extract against *Escherichia coli*

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## ABSTRACT

Ajwain is an annual tropical aromatic herb widely known as *Trachyspermum ammi* (Apiaceae) also it is an annual plant which is widely cultivated in India, Iran and Persia. The fruits of *T. ammi* (Ajwain) traditionally were used as diuretic, carminative, and anthelmintic. Ajwain has been found to have numerous biological effects, including antibacterial, antiviral, antifungal, and antioxidant properties. Evaluating the antibacterial activity of hexane extract of seeds and essential oil against Escherichia coli bacterial strains was the purpose of this present research. After the preparation of the extracts and essential oil, the growth inhibitory zone assay was used to evaluate the antibacterial activity utilizing the disc diffusion, weight variation, and colony counting methods. Minimum inhibitory concentration (MIC) was measured by comparing results of growth inhibitory zone assay. The Amoxicillin stock solution (1 mg/ml) for zone of inhibition served as the benchmark for the comparative evaluation. The different outcome data was examined and results were compared among each other. After conducting a thorough analysis, we have determined that ajwain essential oil exhibits greater antibacterial activity as compared to ajwain seed extract.

**Keywords:** Ajwain, Antibacterial, *Escherichia coli,* Inhibitory zone, Minimum inhibitory concentration.

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## INTRODUCTION

Plants have been traditionally proved for supporting the human health and enhances the quality of human life for many thousands of years. Based to a WHO estimation, 80% of the world's population receive their primary medical attention from traditional medicine.[1] Throughout ancient times, mankind have used medicinal herbs both for their medicinal qualities to treat and also to flavor their food. Presently, there's an interest in utilizing dry powdered samples and crude extracts from medicinal and aromatic vegetation and their species for the production of alternative traditional medicines and food additives.[2-5]. Previous investigations provide information on the chemical aspects that compose this plant species' essential oil and its ability to kill bacteria The essential oil shown productive in the production of a range of commercial products, essentially as an anti-bacterial; studies also proved it also worked as an antioxidant.[6,7] Various researchers have executed and documented significant clinical studies on several plant species for anti-bacterial, anti-oxidant, anti-fungal, insecticidal, and anti-platelet characteristics.[8,9] Many herbs are reported to have antimicrobial effects against various infections, gynecological disorders, and act smooth muscle contractile effects.[10] In broad terms, variations in chemical composition may cause the effect of crude extracts taken from medicinal plants to differ. Also, variations in the activities might be caused by the location, climate, and harvest time of the harvested plant material and by the activity's place of origin.[1,5,6] It has been proven that the chemical components found in plants or its crude extracts are physiologically active substances. Many chemical components are considered as secondary metabolites have shown many properties such as anti-oxidant, anti-bacterial, anti-fungal, and anti-cancer are directly associated with them. Through various processes, all of these secondary metabolites displayed anti-oxidant and anti-bacterial abilities. These components of secondary metabolites are typically extracted from polar plant extracts. The most effective sources of chemical components, anti-oxidants, and anti-microbial agents for treating different medical conditions are medicinal plants.[1,5,10]

Ajwain (*Trachyspermum ammi*) is an Indian herb its seeds are traditionally used as an ingredient of Indian cuisine. Ajwain leaves are used traditionally to treat a wide range of disorders such as acidity, colds, asthma, diabetes, kidney stones, flatulence and gas. It also help in weight loss. Strong antibacterial and anti-fungal qualities can be found in carom seeds. It helps with indigestion, blood pressure reduction, peptic ulcer treatment, cholesterol reduction, cough prevention, and enhanced breathing.[10,11]

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**Figure 1:** Ajwain seeds

India, Afghanistan, Iran, Egypt and Iraq are the major countries that grow *Trachyspermum ammi*. In India, it is widely cultivated in Uttar Pradesh, Madhya Pradesh, Maharashtra, Gujarat, Rajasthan, Bihar and West Bengal. *Trachyspermum ammi* L., is a highly valued and essential medicinal spice, is a member of the Apiaceae family. The roots have a diuretic effect in addition to the remarkable aphrodisiac qualities of the seeds.

Ajwain contains an essential oil (2-3.5%), protein (17.1%) and fat (21.8%). Ajwain oil is a colourless or brownish yellow liquid possessing a characteristic odour of thymol and a sharp taste. The principal constituents of the oil are phenol, mainly thymol (35-60%), carvacrol, p-cymene, γ -terpinene, α- and β -pinenes, and dipentene. The fatty oil is composed of palmitic, petroselinic, oleic, linoleic and 5.6-octa-decanoic acids.

The objective of this work to find out comparative antibacterial effects of Ajwain seeds oil and hexane extract of seeds against *E. coli*.

## MATERIALS AND METHODS

### Material

The seeds of Ajwain was purchased from a nearby supermarket in a packed manner while being observed for the necessary quality marks.

### Extraction of Volatile Oil

Ajwain seeds measuring 100 gm were reduced to coarsely powdered and placed in a Clevenger apparatus for 5 to 6 hours. Yellow colored oil was obtained and anhydrous sodium sulfate was added to remove traces of moisture. The oil was then stored in refrigerator.

### Preparation of the Hexane Extract

100 gm seeds were shade dried and extracted with hexane as solvent for 5-6 hours using Soxhlet apparatus (continuous hot maceration). The solution was cooled and filtered. The solvent was concentrated until a viscous residue was obtained and stored.

### Preparation of Nutrient Broth Media for Culture

Nutrient broth was prepared and sterilized using autoclave. The pH was maintained at 7.2 by using 0.1N HCl & 0.1N NaOH. Distilled water was added to increase the volume to the desired amount.

### Revival and Isolation of Pure Culture

*(Escherichia coli)* -The revival of bacteria was done by serial dilution method and the pure culture was isolated through streak plate method and kept as bacterial suspension.

### Evaluation of Anti-bacterial Activity of Volatile Oil and Hexane Extract

The antibacterial activity was determined by weight variation, colony counting method and zone of inhibition. The nutrient broth was poured in nutrient agar media and incubated for 24 and 48 hrs. The different concentration of volatile oil and hexane extract were prepared and tested against bacterial colony. The zone of inhibition was calculated with compared to standard drug Amoxicillin.

## RESULTS

### Sensory Evaluation and Percentage Yield of Volatile Oil from Ajwain Seeds

The ajwain oil from seeds was successfully isolated using hydro-distillation method and the percentage yield was obtained as 1.8%. The oil appears as yellowish green, clear transparent with aromatic and strong characteristic odor. The taste was found to be pungent mentioned in Table 1.

### Sensory Evaluation and Percentage Yield of Hexane Extract from Ajwain Seeds

The hexane extract from seeds of ajwain was prepared by continuous reflux process in soxhlet apparatus using hexane as solvent. The percentage yield of extract was found out to be 14.91 % w/w appears as viscous semisolid mass dark green in color shown in Table 2.

### Comparative Study of Anti-bacterial Activity

*Weight variation method*

The weight variation between different concentrations of volatile oil and hexane extract was determined after

**Table 1:** Sensory evaluation and percentage yield of Ajwain oil

| | |
|---|---|
| Color | Yellowish green |
| Odor | Aromatic, Characteristic |
| Taste | Pungent |
| Appearance | Clear transparent |
| % yield | 1.8 % |

**Table 2:** Sensory evaluation and percentage yield of hexane extract

| | |
|---|---|
| Color | Dark green |
| Nature | Viscous semisolid mass |
| Weight | 14.91 gm |
| % yield | 14.91 % (w/w) |

**Table 3:** Weight variation in bacterial culture (in mg) after 24 hrs

| *Different Concentration of Volatile oil and hexane extract (µl)* | *Weight variation in bacterial culture (in mg) after 24 hrs* | |
|---|---|---|
| | *Volatile oil* | *Hexane extract* |
| 25 | +2 | +2 |
| 50 | No growth | +1 |
| 75 | No growth | No growth |
| 100 | No growth | No growth |

**Table 4:** Weight variation in bacterial culture (in mg) after 48 hrs

| *Different Concentration of Volatile oil and hexane extract (µl)* | *Weight variation in bacterial culture (in mg) after 48 hrs* | |
|---|---|---|
| | *Volatile oil* | *Hexane extract* |
| 25 | +2 | +3 |
| 50 | No growth | +1 |
| 75 | No growth | No growth |
| 100 | No growth | No growth |

24 and 48 hrs respectively (shown in table 3 and 4). It was found that Ajwain oil have more effects in growth inhibition as compared to hexane extract as mentioned in Figures 2 and 3.

### *Colony counting method*

The colony counting method was performed on different concentrations of Ajwain oil and hexane extract using colony counter. The data was expressed in colony forming unit (cfu) and it was found that ajwain oil produces no growth at concentrations of 50, 75 and 100 µl compared

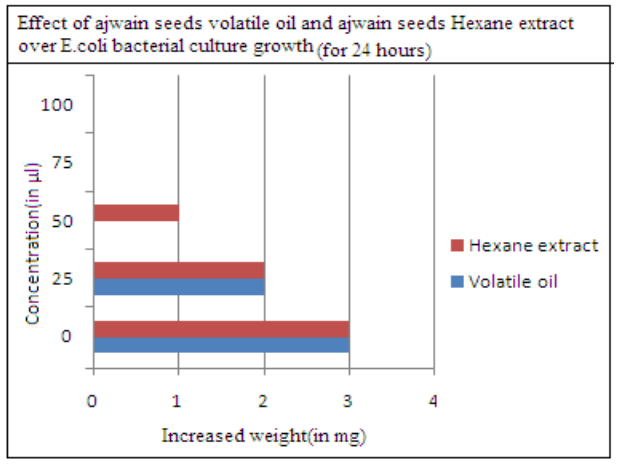


**Figure 2:** Effect of Ajwain seeds volatile oil and hexane extract over *E. coli* bacterial culture growth (for 24 hrs)

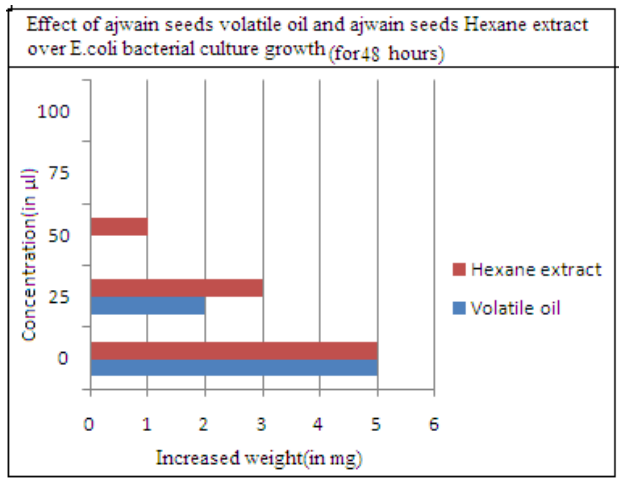


**Figure 3:** Effect of Ajwain seeds volatile oil and hexane extract over *E. coli* bacterial culture growth (for 48 hrs)

with hexane extract at 75 and 100 µl. The results are mentioned in Tables 5 and 6.

### *Zone of inhibition*

The zone of inhibition of different concentrations of Ajwain oil and hexane extract was compared with standard drug amoxicillin (Figure 4). It was found ajwain

**Table 5:** Volatile oil

| *Volatile oil concentration (µl)* | *Zone 1* | *Zone 2* | *Zone 3* | *Zone 4* | *Zone 5* |
|---|---|---|---|---|---|
| 25 | > 300 | 184 | 279 | > 300 | 253 |
| 50 | No growth | No growth | No growth | No growth | No growth |
| 75 | No growth | No growth | No growth | No growth | No growth |
| 100 | No growth | No growth | No growth | No growth | No growth |

**Table 6:** Hexane extract

| *Hexane extract concentration (µl)* | *Zone 1* | *Zone 2* | *Zone 3* | *Zone 4* | *Zone 5* |
|---|---|---|---|---|---|
| 25 | 285 | >300 | 290 | 230 | >300 |
| 50 | 20 | 16 | 19 | 13 | 15 |
| 75 | No growth | No growth | No growth | No growth | No growth |
| 100 | No growth | No growth | No growth | No growth | No growth |

**Table 7:** Different concentrations of amoxicillin in stock solution of 1 mg/mL

| Concentration (µl) | Zone | Diameter (cm) | | | Average |
|---|---|---|---|---|---|
| | | I | II | III | |
| 25 | A | 2.2 | 2 | 2.1 | 2.1 |
| 50 | B | 2.3 | 2.1 | 2.1 | 2.2 |
| 75 | C | 2.1 | 2.3 | 2.2 | 2.2 |
| 100 | D | 2.3 | 2.4 | 2.3 | 2.35 |

**Table 8:** Volatile oil at different concentrations

| Concentration (µl) | Zone | Diameter (cm) | | | Average |
|---|---|---|---|---|---|
| | | I | II | III | |
| 25 | A | 3.4 | 3.7 | 3.8 | 3.6 |
| 50 | B | 3.7 | 3.6 | 3.7 | 3.67 |
| 75 | C | 3.7 | 3.8 | 3.8 | 3.76 |
| 100 | D | 3.8 | 3.8 | 3.9 | 3.87 |

**Table 9:** Hexane extract at different concentrations

| Concentration (µl) | Zone | Diameter (cm) | | | Average |
|---|---|---|---|---|---|
| | | I | II | III | |
| 25 | A | 2.3 | 2.7 | 2.6 | 2.6 |
| 50 | B | 1.8 | 3.2 | 2.8 | 2.6 |
| 75 | C | 2.7 | 2.9 | 3.0 | 2.9 |
| 100 | D | 3.1 | 3.0 | 3.1 | 3.1 |

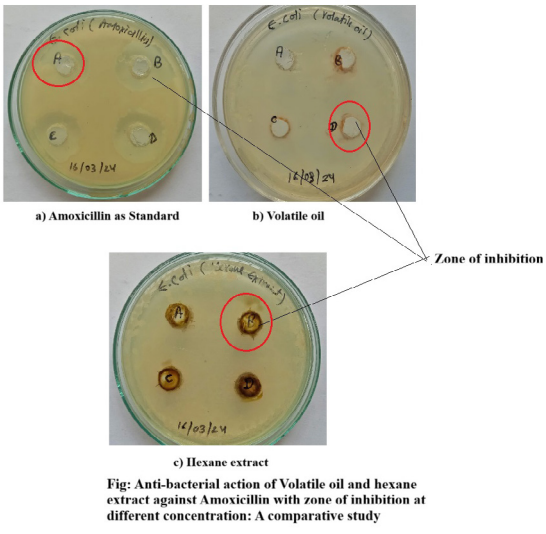


**Figure 4:** Antibacterial activity of volatile oil and hexane extract

oil have more potential antibacterial effects compared to standard and hexane extract. The results are shown in Tables 7, 8 and 9.

## DISCUSSION

The present study investigates the comparative effects of volatile oil and hexane extract of Ajwain seeds against inhibition and anti-bacterial effects on *E. coli*. It was found that the volatile oils exhibits greater effects against *E. coli* as compared to the hexane extract. However, both have valuable role and significance in controlling the growth of bacteria as compared to the standard Amoxicillin. The weight variation observed to have better result in volatile oil in comparison to hexane extract. The colony counting method signifies low colony formation in volatile oil at low concentrations. The volatile oil's zone of inhibition is broader than that of the standard used and hexane extract.

Essential oils are highly complex mixtures that might include hundreds of different aromatic components which are very well reported against various microbes. The role of antibacterial action of volatile oil from Ajwain seeds is due the presence of terpenes such as thymol, carvacrol, p-cymene, γ -terpinene, α- and β -pinenes, dipentene etc. The oils contains phenolic group in their structure which may increases the bacteriostatic and bactericidal action towards *E. coli*. However, hexane extract also controlled the growth of bacteria due to variety of phytoconstituents exhibiting free radical scavenging activity.

## CONCLUSION

Before executing the experiment, we decided the topic of the subject and study design. The sample was obtained. Chemical & reagents were examined before experimentation. We acquired the essential oil and hexane extract of Ajwain for the study by treating the sample seeds for hydro-distillation and administering them in hexane for solvent extraction. The essential oil & hexane extract were tried against a standard and among each other for microbial inhibitory concentration against *E. coli* bacteria. The results of the experiment showed that, out of all the samples, ajwain essential oil possesses the best microbial inhibitory concentration against *E coli*.

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