Anti microbial potential of Ajwain (*Trachyspermum copticum*): An Immense Medical Spice

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**ABSTRACT**

Natural products and naturally derived components from plants have applications in controlling pathogens in food. The challenge is to isolate, purify, stabilize and incorporate natural oxidants and antimicrobials into foods without adversely affecting sensory, nutritional and safety characteristics. In this study leaves, seeds and oil of *Trachyspermum copticum* has been taken to analyze its antimicrobial effect. The different extracts of leaves and seed were prepared by using ethanol, petroleum ether, carbon tetrachloride, methanol, acetone, ethanol 70% and aqueous (water), whereas the oil was directly used for study. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *E. coli*, *Aspergillus niger* were selected for antimicrobial screening. The results of the study showed significant antibacterial effect of ethanol, ethanol 70%, acetone, carbon tetrachloride plus methanol extracts of seed and oil whereas petroleum ether and aqueous did not show any activity against bacteria and fungi. A major part of the endeavor is in the historically productive area of natural product screening, to make the best use of natural product resources for the discovery of novel antibiotics. The results conclude that the further study of *Trachyspermum copticum* extracts may lead to new drug discovery.

**Key words:** *Trachyspermum copticum*, Antimicrobial activity

**INTRODUCTION:**

Many plants have been used for different purposes such as foods and drugs. The use of spices has been highly valued from prehistorical times not solely for their food preparing power but also for their flavor and antimicrobial activity. Spices have the ability to kill microbes or prevent the growth of microorganisms and this inhibitory activity is known as antimicrobial activity¹. Antibiotics are among the most commonly used drugs. However, the inevitable consequence of wide spread and injudicious use of antibiotics has been the emergence of antibiotic resistant pathogens, resulting in a serious threat to global public health². This problem of resistance requires renewed efforts to be made to discover antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals³. A diverse range of bioactive molecules produced by plants, make them a rich source of different types of medicines. Most of these medicines are obtained from natural sources or semi synthetic derivatives of natural products⁴. Thus it is valuable to screen traditional natural products⁵. Approximately 20 percent of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural products or semisynthetic resources⁶. It has been revealed that between the year 1984 and 1994⁷, the screening of antimicrobial plant extracts has been done to find new compounds with the potential to act against multi resistant bacteria⁸. This multidrug resistance of microorganisms requires the search for natural antimicrobial compounds⁹. The resistance of multiple drugs or multidrug is referred to a condition in which a disease causing organism is enabled to resist many drugs or chemicals of a wide variety¹⁰ of structure and function aiming at eradicating the organism. Organisms that display multidrug resistance can be pathologic cells, including bacterial and neoplastic (tumor) cells¹¹. The aim of this conventional rediscovery process aims to identify a single pure active constituent from the extraction of *Trachyspermum copticum* [ajwain] (leaves, oil, and seeds) in the drug discovery process mainly against the bacteria and fungi as *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Aspergillus niger* respectively. Bacterial infections, over 80% while for anti-cancer agents over 60% of all medicines in clinical uses are natural products or their derivatives¹²,¹³. The rich biodiversity of India has remained untouched as far as discovery of new chemical entities is concerned¹⁴. Certain essential oils which have been discovered newly¹⁵,¹⁶ have shown good fighting potential against drug resistant pathogen¹⁷. Essential oils are aromatically oily liquids, which are medicines obtained from various plant parts such as Ajwain (*Trachyspermum copticum*) oils obtained from its steam distillation. Scientifically ajwain oil have been proved highly potent antimicrobial agent in comparisons to antibiotics. Ajwain oil show antibacterial activity and antioxidant properties. The oil of ajwain has thymol which acts as a germicide and antiseptic. Ajwain is also an Indian spice; it is widely used in curries due to its aromatic smell and pungent taste. It is basically used in small quantities for flavouring numerous foods, as preservatives and household remedy for several medical problems.

**MATERIALS AND METHOD:**

**Collection of plant material:**

**Leaves:** The Fresh leaves of *Trachyspermum copticum* were first collected from the plants. These leaves were washed thoroughly 10-15 minutes with running tap water and then with sterile water. They were dried in shade, powdered and used for extraction.

**Seed:** Dried ajwain seeds were grounded and powdered with the help of mortar and pestle.
Oil: Commercially prepared Oil was used.

Preparation of solvent extractions:
20gm of the *copicum* seeds and leaves powder was dissolved in 100 ml of distilled water, carbon teta chloride and methanol, ethanol, ethanol 70%, petroleum ether and acetone to prepare the aqueous extract, carbon teta chloride and methanol extract, ethanol extract, petroleum ether extract and acetone extract respectively.

**TEST MICROORGANISMS:**

Microorganisms used for the antimicrobial activity as follows:

- *Staphylococcus aureus* MTCC-3160
- *Pseudomonas aeruginosa* MTCC-4673
- *Bacillus subtilis* MTCC-441
- *E.coli* MTCC-443
- *Aspergillus niger* MTCC-1344

These microorganisms were collected from Microbial Type culture collection (MTCC) Chandigarh India.

**Bacterial culture:**
Bacterial cultures were maintained on Nutrient agar slants and broth.

**Fungal culture:**
Fungi was cultured on potato dextrose agar (PDA) as the growth medium to maintain the culture. For the inoculum of overnight culture of each bacteria was dispensed into 20 ml of sterile nutrient broth and incubated for 3-5 hrs at 37°C to standardize the culture to 6-10^5 cfu/ml. Screening of antibacterial activity was done by Agar disc diffusion/well diffusion method.

**Antimicrobial activity assay:**

**Anti-bacterial screening:** In vitro antibacterial activity of ajwain seeds, leaves and oil extracts were tested against *E.coli, P.aeruginosa, S.aureus, B.subtilis*. Above bacterial culture were maintained on the agar slants. 0.2 ml of overnight culture of each bacteria was dispensed into 20 ml of sterile nutrient broth and incubated for 3-5 hrs at 37°C to standardize the culture to 6-10^5 cfu/ml. Screening of antibacterial activity was done by Agar disc diffusion/well diffusion method.

**Anti-fungal activity:** In vitro anti fungal activity of Ajwain leaves, seed and oil were determined against the *Aspergillus niger*. Fungal culture was maintained on the potato dextrose agar as the growth medium. For the inoculum preparation the tested fungi was grown on PDA (Potato dextrose agar) slant and incubated at 25°C for 5 days. Anti fungal activity was determined by the Disc diffusion/Well diffusion method.

**RESULTS:**
Total 6 samples were prepared in extracts of two forms namely aqueous and organic solvent and were tested against 5 microbial species (four bacterial strains and one was fungal).

**Antimicrobial activity of Trachyspermum coticum (ajwain) seeds**
Ethanol, acetone, petroleum ether, carbon teta chloride (organic solvents), ethanol 70% and Distill water (for aqueous extraction) were used for extraction of seeds of *Trachyspermum coticum* (ajwain). Out of the all extracts, acetone, ethanol and 70% ethanol extracts of ajwain seeds showed significant antibacterial activity against *Staphylococcus aureus* (fig.4.1,4.2 & 4.3), *Pseudomonas aeruginosa* 4673 (fig: 4.5, 4.6 and 4.7) and *Bacillus subtilis* 441 (fig:4.8,4.9 & 4.10) (Table 1). Carbon teta chloride + methanol extract of ajwain seeds also showed the antibacterial activity against *Staphylococcus aureus* (fig:4.4, table 1). Moreover it was found that ethanol 70% extract showed activity against *E.coli* (fig:4.11, table 1). Petroleum ether and aqueous extract of ajwain seeds did not show antibacterial and antifungal activities against the test microorganisms.

**Antimicrobial activity of Trachyspermum coticum (ajwain) oil**
The oil of Trachyspermum coticum showed good antimicrobial activity against *Staphylococcus aureus* (fig: no:4.12), *Bacillus subtilis* (fig:4.13 & Table 2) and *E.coli* (fig no:4.14 & Table 2). Oil of ajwain did not show any antimicrobial activity against *Aspergillus niger*.

**Antimicrobial activity of Trachyspermum coticum (ajwain) leaves**
All the extracts of leaves of ajwain did not show the antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, E.coli and Aspergillus niger*. (table no.2.2)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>EXTRACT</th>
<th>TEST MICROORGANISM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td><em>E.coli</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MTCC-443</td>
</tr>
<tr>
<td>1</td>
<td>Acetone</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>CCl₄+ Methanol</td>
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</tr>
<tr>
<td>4.</td>
<td>70% Ethanol</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Petroleum Ether</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Aqueous</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Anti microbial activity of *T.coticum* seed (Inhibition Zone)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Source</th>
<th>TEST MICROORGANISM</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td><em>E.coli</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MTCC-443</td>
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<tr>
<td>1</td>
<td><em>Trachyspermum Coticum</em> oil</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Figure 4.1: Zone of inhibition of Acetone extract of *T.coticum* seeds against *S.aureus*
Figure 4.2: Zone of inhibition of Ethanol extract of *T.coticum* seeds against *S.aureus*
DISCUSSION:

Trachyspremum copticum (Ajwain) is an annual herbaceous plant. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, and anti-inflammatory properties of plants\cite{18-29}. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

In this study some of the seed extracts and Oil have showed anti-bacterial activities against the E.coli, S. aureus, P. aureuginosa & B.subtilis. Whereas any of the extracts and oil did not show anti fungal activity. This data is in contrast with the results of Bazzaz et al\cite{28} The result of the study is in accordance to Mahboubi M et al 2011 & Goudarzi et al 2011\cite{30-31}. The study suggests that T.copticum may be an important source of potentially useful structure for the development of new chemotherapeutic agents.

REFERENCES:

5. Nakanishi, K. An historical perspective of natural products chem-