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Research Article

### ANTI-BACTERIAL ACTIVITY OF EXTRACTS OF *TACHYSPERMUM* *AMMI* FRUITS

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#### ABSTRACT

This study was carried out with an objective to investigate the antibacterial activity of *Tachyspermum ammi* fruits extracts. In the present study, the anti-bacterial activity of aqueous and ethanolic extracts of *Tachyspermum ammi* fruits was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined using agar disc diffusion method. The antibacterial and antifungal activities of extracts were tested against Gram-positive—*Staphylococcus aureus* and Gram-negative—*Escherichia coli* human pathogenic bacteria. Zone of inhibition of extracts were compared with that of different standard drugs. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The antibacterial activity of the *Tachyspermum ammi* fruits was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

**Keypoints:** Anti Bacterial Activity; *Tachyspermum ammi* Fruits

#### INTRODUCTION

Medicinal plants are still major part of traditional medicinal system in developing countries many infection disease are known to be treated with herbal remedies throughout the history of mankind even today plant material continue to play a major role in primary health care as therapeutic remedies in many developing countries.

The alkaloids are one of the most diverse groups of secondary metabolites found in plants and have an array of structure type, biosynthetic pathway, and pharmacological activities. Plants with their wide variety of chemical constituents offer a promising source as well as specific activity.<sup>1</sup>

There is a need to develop alternative antimicrobial drugs for the treatment of infectious

diseases from medicinal plants. Several screening studies have been carried out in different part of the antimicrobial activity of different herbal extract in different regions of the worlds.

In an effort to expand the spectrum of antibacterial agents from natural resources *Tachyspermum ammi* was selected. It consists of dried fruits of "*Tachyspermum ammi*" belonging to the family Apiaceae.

*Tachyspermum ammi* is a native of Egypt and is cultivated in Iraq, Iran, Afghanistan, Pakistan, and India. In India, it is cultivated in Madhyapardesh, Uttarpardesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal.<sup>2</sup>

The oil exhibits fungicidal, antimicrobial and Anti-aggregatory effects on humans. Ajwain is a traditional potential herb and is widely used for curing various diseases in humans and animals. The fruit possesses stimulant, antispasmodic and carminative properties. It is an important remedial agent for flatulence, atonic dyspepsia and diarrhea. The seed of Ajwain is bitter, pungent and it acts as anti-helminthic, carminative, laxative, and stomachic. It also cures abdominal tumors, abdominal pains and piles.

Seeds contain an essential oil containing about 50% thymol which is a strong germicide, anti-spasmodic and fungicide. Thymol is also used in toothpaste and perfumery.<sup>3</sup>

## MATERIALS

### Plant sample collection

The materials used in this study consisted of dried fruits of *Tachyspermum ammi* which are grown in Rajasthan India. These dry fruits were collected from grocery shop at Begumbazar, Hyderabad, Telangana, India.

### Chemicals

These chemicals media and the solvent were purchased from Unique traders Abids Hyderabad.

### Antibiotics

Pencillin, Tetracyclines, Ampicillin, Cefotaxime. These were collected from Med Plus drug store Begumbazar Hyderabad.

## METHOD

### Preparation of aqueous extract (maceration)

Aqueous extract (Maceration method)<sup>4</sup> 500 gm of seeds of *Tachyspermum ammi* was kept for maceration with 1000 ml of distilled water for 7 days. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper.

### Preparation of the Ethanolic extract (soxhlation)

Over 100g of the processed Ajwain powder was extracted with 200ml of ethanol using Soxhlet apparatus for a period of 6-8h. The extract was further concentrated at 40°C on a water bath to obtain a semisolid mass. This mass was re-suspended in ethanol to get the required concentration of the extract for carrying out further analysis. This concentrated extract was prepared in large volume and preserved at 4°C in sealed vials until further use. This procedure avoids batch to batch variations.<sup>5</sup>

### Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of Saponins, Tannins, Alkaloids, Flavonoids, Triterpenoids, Steroids, Glycosides, Anthraquinones, Coumarin, Saponins, Gum, Mucilage, Carbohydrates, Reducing sugars, Starch, Protein, and Amino acids, as described in literatures.<sup>6-8</sup>

### Preparation of Plant Extracts and Standard Drug Concentrations

One gram of each drug aqueous extract and alcohol pre-prepared (each separately) was taken and the aqueous extract was dissolved in 5 grams sterile distilled water, while alcoholic extracts were dissolved in 5 ml of Di-Methyl Sulphoxide (DMSO). Thus 200 mg / ml of stock was obtained as a standard concentration of aqueous and alcoholic extracts. Aqueous extracts were sterilized using 0.22 µm membrane filters and alcoholic extracts were pasteurization for 15 minutes at temperature 62 °C<sup>9</sup>.

## Bacterial Cultures

Pathogenic strains of *Staphylococcus aureus* and *Lactobacillus* were obtained from microbiology department from K.P.Labs Hyderabad, and were maintained on agar medium at 4 °C for further experiments. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar medium.

## Spread plate method

By spread plate method the effectiveness of test and a range of antibiotics was determined against *Staphylococcus aureus* (Gram +ve) and *E. coli* (Gram –ve). About 1.0.ml of inoculated bacteria will be transferred to a sterile Petri dish containing Nutrient medium. The glass spreader (Hockey Stick) is used to evenly distribute<sup>10</sup>. Upon Incubation isolated pure colonies of the Bacteria will be formed .In an individual petriplate single sample was taken and the activity was compared by

the minimum inhibitory concentration and zone of inhibition studies.

## EVALUATION OF ACTIVITY

### Minimum inhibitory concentration

In microbiology, the minimum inhibitory concentration (MIC)<sup>11</sup> is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

### Zone of Inhibition (Kirby-Bauer test)

In this, the size of the zone of inhibition indicates the degree of sensitivity of bacteria to a drug. In general, a bigger area of bacteria-free media surrounding an antibiotic disk means the bacteria are more sensitive to the drug the disk contains.

## RESULTS

Sample	Concentration	Inhibition zone(mm)
Aqueous extract	300µg/ml	14
Ethanollic extract	300µg/ml	18
Pencillin	300µg/ml	0
Ampicillin	300µg/ml	4
Cefotaxime	300µg/ml	15

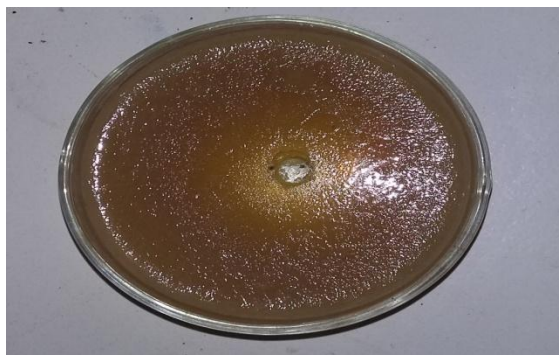
### Evaluation of Activity against E.coli



Penicillin on E.coli colonies



Fig.4.5 Activity of Cefotaxime on E.coli colonies



Aqueous extract of Ajwain on E.coli colonies



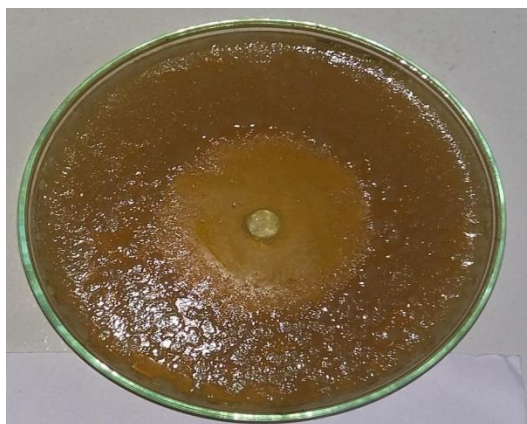
Fig.4.6 Activity of Ethanolic Extract of Ajwain on E.coli colonies

#### Evaluation of Activity against *Staphylococcus aureus*

Sample	Concentration	Inhibition zone(mm)
Aqueous extract	300 $\mu$ g/ml	21
Ethanolic extract	300 $\mu$ g/ml	28
Penicillin	300 $\mu$ g/ml	10
Ampicillin	300 $\mu$ g/ml	15
Tetracycline	300 $\mu$ g/ml	23



**Aqueous Extract on Ajwain *Staphylococcus aureus* colonies**



**Tetracycline on *Staphylococcus aureus* colonies**



**Ethanolic extract of Ajwain on *Staphylococcus aureus* colonies**

## DISCUSSIONS

The ethanolic and aqueous extracts of *Tachypernum ammi* are more efficient than the standard drugs like Pencillin, Ampicillin, Tetracycline and Cefotaxime. With these

encouraging results, the extracts can be further explored for detailed microbiological investigations to arrive at possibly newer potent antibacterial agent with various other activities.

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