

Anti Bacterial and Phytochemical Screening of Trachyspermum Ammi – An In Vitro Approach

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Abstract—Treatment with medicinal plants is considered very safe as there is no or minimal side effects. *Trachyspermum ammi* is a folklore medicinal plant. The roots, seeds and leaves are used in traditional & folklore Medicine. The study was carried out to ascertain the anti bacterial properties present in ethanol extract of dried scale leaves of *Trachyspermum ammi*. The Anti bacterial testing of leaves extract *Trachyspermum ammi* was evaluated by Cup Plate Method and Minimum Inhibitory Concentration (MIC) by Broth Dilution Method using gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae*. The potent activity was observed at 300µg/ml and other concentrations also shown the as well. The extract was more active against Gram negative bacteria such as *Klebsiella* and *E. coli* and did not show the marked activity on Gram positive bacteria such as *Staphylococcus*. It is considered as a valuable source of natural products for development of medicines against various diseases.

Keywords— *Trachyspermum ammi*, ajwain, broth dilution, cup plate method, phytochemicals, flavonoids, anti- bacterial activity.

I. INTRODUCTION

As our lifestyle is now getting techno-savvy, we are moving away from nature, while we cannot escape from nature because we are part of nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally there are lots of herbs used for the ailments related to different seasons. There is a need to promote them to save the human lives. These herbal products are today the symbol of safety in contrast to the synthetic drugs that are regarded as unsafe to human being and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. It's time to promote them globally¹.

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extracts have been developed and proposed for use as antibacterial substances. Many of the plant materials used in the traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. Thus it is important to characterize different types of medicinal plants for their antioxidant and antibacterial potential. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs. Antibacterial activities of many plants have been reported by the researchers². Medicinal plants represent a rich source of antibacterial agents. The antibacterial activities of medicinal plants can be attributed to be the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants. A wide range of medicinal

plants parts is used for extracts as raw drugs and they possess varied medicinal properties. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extract have been developed and proposed for use as antibacterial substances. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. Medicinal herbs practiced in traditional folk medicine in India were screened for the presence of antibacterial activity³.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age group and the gender⁴.

Plant Profile

Trachyspermum ammi (Ajwain) is an annual herb in the family Apiaceae. It originated in the eastern Mediterranean, possibly Egypt and spread up to India from the near east. Both fruit pods and leaves of the plant are consumed by humans. The plant is also called as Bishop's weed, but this is a common name it shares with some other different plants. As medicinal herb it is used for curing a wide variety of ills from tumors to flatulence. The plants are highly branched and about two feet tall by sixteen inches wide. The finely cut leaves are pinnately compound and small wide flowers are reduced in mid-summer in compound umbels of sixteen umbles each with sixteen flowers. The tear-shaped, light brown seeds have Aphrodisiac properties and contain the thymol used in cough syrups. They are also valued as a spice and are added to vegetable and carbohydrate recipes where they provide pungent peppery thyme like flavour. The seeds contain 2-4.4% brown coloured oil known as ajwain oil. The main component of oil is thymol, which is used as Gastro Intestinal Aliments, lack of appetite and bronchial problems. The oil exhibits

fungicidal, anti-bacterial and anti aggregatory effects on humans. Leaves of Ajwain, *Trachyspermum ammi* is used as green vegetable for salad has high nutrition values. The dark green leaves contain many valuable nutrients, especially the anti-oxidant carotenoids, lutein and zeaxanthin. To get availability of ajwain leaves throughout the year supply of frozen ajwain has become popular now days^{6,7}.

Common names: Ajwain, Ajowan

Taxonomical classification⁸

Kingdom: Plantae

Subkingdom: *Tracheobionta*, Vascular plants

Division: Magnoliophyta-Flowering plant

Class: Magolopsida-Dicotyledon

Order: Apiales

Family: Apiaceae

Genus: *Trachyspermum*

Species: *T. ammi*



Fig. 1. *Trachyspermum ammi* plant.



Fig. 2. *Trachyspermum ammi* leaves.

Objective of the Study

The main objective of this research is to screen the anti-bacterial activity of ethanolic extract of *Trachyspermum ammi* leaves.

II. MATERIALS AND METHODS

Collection and Authentication of Plant

This plant was collected from Hyderabad and authenticated by Dr. Ram Mohan, Department of

Pharmacognosy, School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar. The plant specimen was deposited in the college herbarium at School of Pharmacy, Anurag Group of Institution, Venkatapur, Ghatkesar.

Preparation of Plant Extract

The collected plant material was air dried pulverised passed through sieve no.40 and used for further studies.

Soxhlet Extraction

This method is convenient and widely used for extraction because of its continuous process, less time and solvent-consumption than maceration and percolation. The powdered plant material was placed in a Soxhlet apparatus, which is on top of a collecting flask beneath a reflux condenser. A suitable solvent (ethanol) was added to flask and the setup is heated under reflux. The steam of the solvent, which contacts with the material will dissolve metabolites and bring it back to flask. The powder was successively extracted with ethanol by soxhlet extraction method for 48 hrs. Solvent recovery was done by using simple distillation method and extract was collected and stored in refrigerator for future use.



Fig. 3. Soxhlet extraction.

Preliminary Phytochemical Screening

The different chemical tests were performed for establishing profile of the leaves extract and separated phytoconstituents to explore the possible primary and secondary active constituents present in the extract.

Qualitative Phytochemical Screening for Primary Metabolites

The different chemical tests were performed on plant extract to explore Carbohydrates, Proteins, Amino acids, Fats and Fixed oils present in it.

Qualitative Phytochemical Screening for Secondary Metabolites

The different chemical tests were performed on plant extract to explore alkaloids, glycosides, flavonoids, tannins, steroids and terpenoids.

Assessment of Anti-bacterial Activity

The term microbiological assay is a biological assay performed with microorganisms like bacteria, yeast, moulds etc. This involves the measurement of the relative potency or activity of compounds by determining the amount of test material required for producing stipulated effect on suitable organism under standard conditions. The procedures employed in bacterial assays were,

- Cylinder Plate Method or Cup Plate Method
- Broth Dilution Method.

Test organisms

Gram-positive organisms

Staphylococcus aureus, *Bacillus subtilis*

Gram-negative organisms

Escherichia coli, *Klebsiella pneumoniae*

Standardization of Micro-organisms

One loop-full of micro-organisms were inoculated into 100 ml of sterile medium and incubated for 24 hrs at 37 °C for bacterial culture. After 24/48 hours of incubation, 1 ml of broth containing the micro-organisms was added to 9 ml of peptone water. 10 fold serial dilutions were made in the range of 10⁻¹ to 10⁻¹⁰. 100 µl of the dilutions ranging from 10⁻⁵ to 10⁻⁸ were spread over the sterile nutrient agar (SNA) plates and kept at 37 °C and 27 °C for 24/48 hours respectively. The number of colony forming units (CFU) was counted and number of micro-organisms per 1 ml of stock culture was calculated.

Preparation of Test and Standard Solutions

The stock solution of test compound was prepared by dissolving the extract at a concentration of 1mg/ml in water respectively. The stock solutions of reference standards (*Streptomycin*) were prepared at a concentration of 1mg/ml in sterile water. Anti-bacterial activity was screened by adding stock solution to each cup by micropipette. The following media were used for the present antibacterial studies.

Nutrient Broth for Bacteria

Beef extract - 0.35%, Sodium chloride - 0.5%, Peptone - 0.5%. The above ingredients were weighed and 37 g were dissolved in distilled water (1000 ml). pH was adjusted to 7.2 - 7.4 and sterilized by autoclaving at 15 lbs/inch² for 20 min. The above constituents were dissolved in distilled water and pH was adjusted to 5.6 ± 0.2 and then sterilized by autoclaving at 15 lbs/inch² for 20 min.

Sterilization

Sterilization of the media, water etc. was carried out by autoclaving at 15 lbs/inch² for 20 minutes. The glassware like syringes, petri dishes, pipettes, empty test tubes were sterilized by dry heat in an oven at a temperature of 160 °C for one hour.

Nutrient Agar for Bacteria for Cup Plate Method

Beef extract - 0.3%, Sodium chloride - 0.5%, Peptone - 0.5%, Agar - 2.0%, pH - 7.2-7.4. The sterilized medium was cooled to 40 °C and poured into the petri dishes to contain 6 mm thickness. The media was allowed to solidify at room temperature.

Broth Solution for MIC

Nutrient broth was prepared which is devoid of solidifying agent. The prepared liquid broth was poured into test tubes and kept for sterilization.

Evaluation of Anti-Bacterial Activity

Determination of zone of inhibition by cup plate method (Indian Pharmacopoeia 1996)

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of bacterial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculums. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 °C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 °C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated anti-bacterial activity.

Determination of Minimum Inhibitory Concentration (MIC) by Broth Dilution Method (Tube dilution)

The MIC of the extract was determined by using Broth dilution technique. The MIC helps to measure more exactly the concentration of an antibiotic necessary to inhibit the growth of standardised inoculums under defined conditions. Serial dilutions of the extract in liquid medium were prepared. These were then tested with small inoculums of an overnight broth culture of the test organisms. The culture was then incubated at 37 °C for 48 hours. The smallest concentration that inhibits growth was taken as the MIC. The determination of the value of MBC follows the determination of MIC by broth dilution technique. The MBC is the lowest concentration of the anti-bacterial agent that kills 99.9% of the test organisms. To determine this value about 0.5ml of the sample was removed from the test tubes used in the determination of MIC in which there was no desirable growth was spread over the surface of the dried nutrient agar plates. The lowest concentration of the agent that prevent the growth of less than 0.1% of the test organism on the recovery plate was taken to be the MBC value for the extract.

III. RESULTS AND DISCUSSION

Pharmacognostic Evaluation

In this present research, macroscopic characteristics of aerial parts of *Trachyspermum ammi* were studied. The observations of the investigations were shown below:

Organoleptic Characters

The aerial parts powder of the plant was studied for their organoleptic characters like colour, odour and taste. The results of this study were shown below:

Colour - Greenish

Taste - Bitter

Odour – Characteristic

TABLE 1. Percentage yield and physical appearance of leaf ethanol extract of *Trachyspermum ammi*.

Extract	% Dry weight	Colour	Consistency
Ethanol	7.3%	Dark green	Semi solid

Qualitative phytochemical screening

TABLE 2. Preliminary phytochemical screening of *Trachyspermum ammi* leaves.

Test name	Ethanol extract
Test for carbohydrates: Mollisch test :-	+
Test for proteins: Biuret test:-	+
Test for amino acids: Ninhydrin test:-	-
Test for steroids and terpenoids: Salkowaskii test:-	+
Test for steroids and terpenoids: Liebermann-burchards test:-	-
Test for alkaloids: Mayers test:-	-
Dragondroff's test	-
Test for glycosides: Keller killiani test	+
Tannis	-
Flavonoids	+

‘+’ Present, ‘-’ Absent

Cup Plate Method

After successive solvent extraction the obtained extract was tested for anti-bacterial activity. With the method of cup plate we came to know that the ethanol extract of plant *Trachyspermum ammi* shows positive in case of anti-bacterial activity. The anti bacterial activity of the ethanolic extract shows high effect at all concentration. *Trachyspermum ammi* ethanolic extract at concentration 300 µg/ml was found to be more effective than the other concentrations.



Fig. 4. Standard Streptomycin showing anti-bacterial activity.

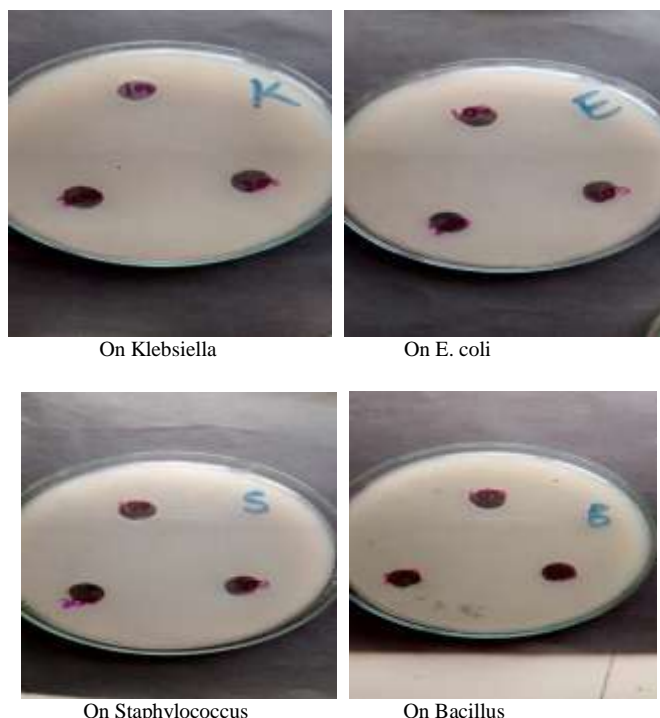


Fig. 5. Ethanol extract of plant leaf showing anti-bacterial activity on different test organisms.

TABLE 3. Inhibitory zone.

Inhibitory zone(diameter/cm)							
Kl.pneumoniae		E.coli		St.aureus		B.subtulis	
Stand ar d	Leaf extract	Standa rd	Leaf extract	Standa rd	Leaf exteact	Stand ar d	Leaf extrct
2.5	1	2.0	1	2.1	1.1	2.3	-
2.4	1.1	2.3	1	2.2	1.1	2.3	-
2.8	1.1	2.5	1.1	2.4	1.1	2.6	-

TABLE 4. Minimum Inhibitory Concentration (MIC).

Test organism	Concentration (MIC)
<i>Klebsiella</i>	10 ⁻⁶
<i>E. coli</i>	10 ⁻⁷
<i>Staphylococci</i>	10 ⁻⁶
<i>Bacillus</i>	-

IV. DISCUSSION

The presence of anti-bacterial substances in the higher plants is well established. Plants have provided a source of inspiration of novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurveda system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of medicine. Successive isolation of botanical compounds from the plant material is largely dependent upon the type of solvent used in the extraction procedure. The selected plant *Trachyspermum ammi* leaves are primarily subjected to extraction by Soxhlet method of extraction using ethanol. The obtained extract is tested for phytochemicals such as primary metabolites and secondary metabolites. The plant extract consists of glycosides, thymol which is present in the plant and responsible for the anti-bacterial activity. The plant extract is evaluated for the anti-bacterial activity by using Cup Plate Method and Minimum Inhibitory Concentration (MIC) by Broth Dilution Method. In cup-plate method the small wells are made using cork borer and different concentrations of 100µg/ml, 200µg/ml and 300µg/ml are added in to the wells using micropipette and incubated for 24-48 hours. The activity is indicated by Zone of Inhibition. It is measured in mm. The potent activity was observed at 300µg/ml and other concentrations also shown the as well. The extract was more active against Gram negative bacteria such as *Klebsiella* and *E. coli* and did not show the marked activity on Gram positive bacteria such as *Staphylococcus*. The extract did not show activity against *Bacillus* species. In broth dilution method 1mg/ml of extract was used to determine the MIC by serial dilution method and compared with positive control. Significance activity is marked on the activity on different concentrations. No activity is observed in the *Bacillus* species. From the studies it is noted that the ethanol leaf extract is active against Gram negative than Gram positive micro-organisms.

V. CONCLUSION

The results of preliminary phytochemical screening suggest extracts of *Trachyspermum ammi* are good sources of beneficial phytochemicals. The anti-bacterial activities of the prepared extract indicated that ethanol extracts at higher concentration of 300µg/mL was more potent and no activity on *Bacillus* species was observed. The statistical analyses performed with regard to anti-bacterial activities of different extract at various concentrations were collaborated with the present findings. Thus, further research is warranted to determine the efficacy of these extracts against various other pathogenic bacteria. The anti-bacterial activity was due to presence of flavonoid and phenolic compounds. Also, there is

call for isolation and identification of active principles of the plant extracts responsible for anti-bacterial activity in order to develop future pharmaceuticals. From the above results, we can conclude that *Trachyspermum ammi* extract exhibited significant anti-bacterial activity; therefore further study must be carried to know the active chemical constituents responsible for anti-bacterial activity.

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Conflict of interest

There is no conflict of interest.

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