Assessment of *in vitro* Antibacterial activity of *Trachyspermum ammi*

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ABSTRACT: PURPOSE: Antimicrobial Resistance of bacterial infections to drugs presently used are major problem for curing the infections. The aim of this study is to check the potential of *Trachyspermum ammi* seeds against four different bacterial pathogens. METHODS: Evaluation of antibacterial activity of ethyl acetate extract prepared from the powdered seeds of *Trachyspermum ammi* was determined by agar well diffusion method against bacterial pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa, Hafnia alvei* and *Micrococcus luteus*. RESULTS: Various concentrations of extracts such as 25µg/ml, 50µg/ml, and 100µg/ml was tested with chloramphenicol (100µg/ml) as positive control. Zone of inhibition was measured and calculated. CONCLUSIONS: *Trachyspermum ammi* extract showed highest antibacterial effect against *hafnia alvei* while it is least effective against *micrococcus luteus*.

KEYWORDS: Antibacterial activity, *Trachyspermum ammi*, bacterial pathogens, *hafnia alvei*, *micrococcus luteus*.

1. INTRODUCTION:

Microbes are becoming resistant to the present day antibiotics due to its overuse and there is need for development of drugs to combat this. Many plants are known to have antimicrobial potential since many effective drugs have been developed from plants. [1] Also plants form an integral part of traditional medicinal system all over the world. Natural products from plants are safe and also effective against many microbes. [2] Usage of herbal medicine in the form of liquids, powders or mixtures for treatment has been carried out in India since ancient times. [3]

The health effects of spices for seasoning in cooking have been claimed for prevention of food spoilage due to having antimicrobial action against microbial pathogens. Antimicrobial activity of different medicinal plants are studied throughout the world extensively. [4],[5],[6],[7] Plant based antimicrobials are packed with therapeutic agents have lesser side effects compared to synthetic antibiotics. [8]

Trachyspermum ammi (L.) Sprague is an annual herb belonging to family Apiaceae and grows in many parts of the world. [9] It is also known as Bishops weed, yavaani, ajwain, ajowan, ajma, omam in different parts of India. [10] Ajwain is traditionally used and act as a carminative, stimulant, flatulence, diarrhea, atonic dyspepsia, piles, abdominal tumours, abdominal pains and lack of appetite, bronchial problems, galactogogue, asthma and amenorrhea. Therapeutically, it has been proven to possess various pharmacological activities like antioxidant, antimicrobial, antifungal, Hypolipidaemic, Antihypertensive, antinociceptive, cytotoxic activity, broncho-dilating actions, antispasmodic, Antilithiasis, Abortifacient, diuretic, Antitussive, Anthelmintic, Nematicidal, Antifilarial Activity [11] and Antidiabetic. [12] Owing to the much renowned use of *T. ammi* for longer times, the study to investigate the antibacterial activity of *T. ammi* and add to its scientific evidence for its usage was done. In the present study extract was prepared with ethyl acetate as a solvent from *T. ammi* seeds. Then we investigated the antibacterial activity of this extract against four different bacterial pathogens.

2. MATERIALS AND METHODS

2.1 Sample preparation:

The Ajwain seeds were purchased from local markets in Chennai, Tamil Nadu, India. Taxonomical identification and authentication was done at Department of Medicinal Botany, National Institute of Siddha, Tambaram, Tamil Nadu, India. The voucher specimen obtained was NISMB2202016. The seeds were cleaned physically to remove foreign particles. Then the seeds were grounded in a mechanical grinder. 50 g dried and powdered seeds were mixed separately with 250 ml of ethyl acetate and continuous extraction was done for 5 to 6 h in mechanical shaker. [13] Then the mixture was filtered using Whatmann no.1 filter paper. The extract thus obtained was concentrated by using rotary vacuum evaporator under reduced pressure and stored at 4 ºC in labelled sterile screw-capped bottles for further analysis.

2.2 ANTIBACTERIAL ACTIVITY

2.2.1 Preparation of inoculum:

The pathogenic cultures of *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424), *Hafnia alvei* (MTCC 1426) and *Micrococcus luteus* (MTCC 1809) were obtained from the King Institute of Preventive Medicine, Guindy, Chennai, Tamil Nadu, India, and were periodically subcultured and maintained at 4°C on nutrient agar slant. Active cultures for experimentations were prepared by transferring a loop full of bacterial culture from the stock cultures to test tubes of Luria Bertani (LB) broth for bacteria and were incubated for 24 hours at 37°C. The assay was performed by agar well diffusion method.

2.2.2 Agar Well Diffusion Method:

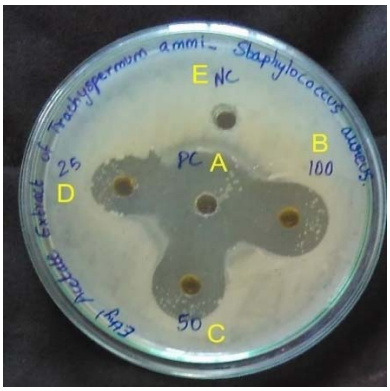
Antibacterial activity of ethyl acetate extract was determined by agar well diffusion method. [14] The Nutrient agar plates were swabbed with 0.5 ml of the respective 24 hours broth culture of organisms and kept for 15 minutes in laminar chamber for absorption of cultures. Wells were made in agar plates using a sterile cork borer of 5 mm. The ethyl acetate extract of various concentrations such as 25 µg/ml, 50 µg/ml, 100 µg/ml were prepared and 20 µl from each concentration was added to each well. Distilled water was used as negative control and chloramphenicol (100µg/ml) used as positive control. The plates were incubated at 37°C for 24 hours. The diameters of the zone of inhibition were measured in millimeter by using antibiotic zone measuring scale.

2.3 STATISTICAL ANALYSIS:

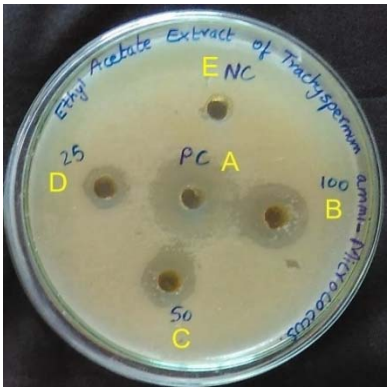
Studies were performed in triplicates. Data were expressed as mean ± SEM.

3. RESULTS AND DISCUSSION

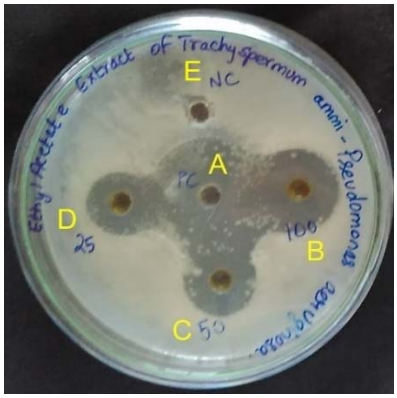
The antibacterial activity of ethyl acetate extract of *T. ammi* (Figure 1) was studied against two gram positive bacterial pathogens *S. aureus* and *M. luteus* and two gram negative bacterial pathogens *P. aeruginosa* and *H. alvei*. Highest activity was observed against *H. alvei* with largest zone of inhibition whereas ethyl acetate extract showed less inhibition against *M. luteus*. Measurement of maximum zone of inhibition (Table 1) for each bacteria were 18.6±0.4 mm, 11.1±0.3 mm, 17.8±0.5 mm and 22.4±0.6 mm against *S. aureus*, *M. luteus*, *P. aeruginosa* and *H. alvei*, respectively. It can be seen that there was increase in zone of inhibition with increased concentration of ethyl acetate extract of *T. ammi*. The result of this study indicates that many antimicrobial substances are present in *T. ammi* seeds.



Staphylococcus aureus



Micrococcus luteus



Pseudomonas aeruginosa

Hafnia alvei

Fig. 1. Antibacterial activity of Ethyl Acetate extract using agar well diffusion method: A - Positive control, Chloramphenicol 100 µg/mL; B - 100 µg/ml of Ethyl Acetate extract; C - 50 µg/ml of Ethyl Acetate extract; D - 25 µg/ml of Ethyl Acetate extract; E - Negative Control.

Table 1. Antibacterial activity of Ethyl Acetate extract using agar well diffusion method.

Bacterial strain	Zone of inhibition in mm				
	Concentration of Ethyl acetate extract			Negative Control	Standard Chloramphenicol 100 µg/mL
	25 µg/ml	50 µg/ml	100 µg/ml		
Staphylococcus aureus	11.2 ± 0.3	13.3 ± 0.2	18.6 ± 0.4	-	22.1 ± 0.3
Micrococcus luteus	4.6 ± 0.2	8.8 ± 0.1	11.1 ± 0.3	-	13.8 ± 0.4
Pseudomonas aeruginosa	11.6 ± 0.4	14.5 ± 0.3	17.8 ± 0.5	-	23.1 ± 0.5
Hafnia alvei	15.7 ± 0.5	19.3 ± 0.3	22.4 ± 0.6	-	22.5 ± 0.5

The data are expressed as mean ± SD values (n=3).

Our results are in parallel line with the recent study by Bashyal and Guha who also studied the antimicrobial activity of different extracts of *T. ammi* seeds against *E. coli*. [15] Khan *et al.* also studied and proved the antibacterial activity of different extracts of *T. ammi* against 4 different bacteria. [16] Furthermore the GC-MS analysis also confirmed the presence of high contents being phenols and flavonoids. Sharma *et al.* have also performed antibacterial activity of *T. ammi* extracts with two different solvents against 4 different bacteria. [17] Hassan *et al.* have demonstrated the acetone extract of *T. ammi* against 4 gram positive bacteria and 6 gram negative bacteria. [18] Oueslati *et al.* did investigation on phenolic content and antibacterial activities of *T. ammi* seeds against 6 bacterial pathogens. [19] Usha masih have also positively tested the antibacterial activity of various extracts of *T. ammi* seeds against four different strains of *E. coli*. [20] Vazirzadeh *et al.* also checked antibacterial activity of ajwain seed extract against 5 different bacteria and obtained positive results. [21] Malik *et al.* also tested the antibacterial activity of *T. ammi* against three different bacteria. [22] Reji and Maheshwari also have demonstrated antibacterial assay of acetone and ethanol extract of *T. ammi* against three bacteria and had good inhibitory results. [23] Faiza Ahad *et al.* also proved the synergistic antidiabetic activity of methanolic and ethanolic extracts of ajwain with metal salts against seven isolated bacterial species. [24] *T. ammi* seeds extracts prepared in different solvents demonstrated variable antibacterial activity against *E. coli*, *P. aeruginosa, S. typhi* and *S. aureus*, which suggests their century's old usage for treating gastrointestinal disorders. [25],[26] Kaur and Arora also reported the antibacterial effect of aqueous and organic extracts of ajwain seeds.[3] Our results also have exhibited the antibacterial activity which are in good agreement with previous studies as mentioned. The present study of antibacterial activity of ethyl acetate extract of *T. ammi* against pathogens *H. alvei* and *M. luteus* are reported to be first time to the best of our knowledge.

During extraction procedure the compounds extracted are mainly dependent on the type of the solvent used. In traditional medicine, water was the primary solvent used, but in the present study, compounds extracted in organic solvent (ethyl acetate) may have exhibited more significant antibacterial activity. [27] Phytochemical analysis of ajwain seeds has shown to contain carbohydrates, fat, protein, fibre, moisture, saponins, tannins, glycosides, flavone and mineral matter containing iron, calcium, phosphorous and nicotinic acid. [28] *T. ammi* seeds contain 2.5 - 5% essential oil and the principal constituents of essential oil are phenols- thymol (35 - 60%), carvacrol (11%). The rest of the oil is called thymene which contains p-cymene (50 - 55%), limonene with gamma, betaterpinenes (30 - 35%) and beta-pinene (4 - 5%). [29] Based on the past studies and their phytochemical investigation it can be suggested that this antibacterial effect of ethyl acetate extract of *T. ammi* in our study could be due to the major phenols being thymol and carvacrol present in the seeds. Thymol can kill bacteria resistant to even prevalent third-generation antibiotics and multidrug-resistance microbial pathogens. [30]

4. CONCLUSION

The results indicate the presence of different natural antimicrobial substances in ethyl acetate extract of *T. ammi*. Maximum antibacterial activity was found against *Hafnia alvei*. The extract has great potent to inhibit the growth of common pathogenic bacteria. This extract shall be subjected to further purification and isolation of bioactive compounds and their characterization to identify them. *T. ammi* might serve as useful sources for new antibacterial agents.

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CONFLICT OF INTEREST

No conflict of interest was declared by the author.

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