

ORIGINAL RESEARCH ARTICLE

Antimicrobial Activity of Essential Oils of *Trachyspermum ammi*

Shailendra Wadhwa*, Manish Bairagi, Govinda Bhatt, Mahesh Panday, Ankit Porwal

Department of Pharmaceutics, Mandsaur Institute of Pharmacy, Mandsaur, M. P. India

Received 13 April 2010; Revised 22 May 2010; Accepted 5 May 2010

ABSTRACT

Overuse of antibiotics has selected new strains of bacterial pathogens that are resistant to the very antibiotics used to combat them. Many plants have been used for centuries by traditional healers and may have antibacterial properties. Ajowan (*Trachyspermum ammi*) is one such plant, having been prescribed for digestive, respiratory, renal, dental, and many other maladies in Asian traditional medicine. Our research aim was to determine whether essential oils obtained from the plant's fruit could inhibit growth of gram-positive bacteria and gram-negative bacteria. The essential oils were obtained using the hydrodistillation method. Our methods included a well-diffusion assay.

Key Words: Antimicrobial activity, Ajowan (*Trachyspermum ammi*), Antibacterial activity.

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way [1].

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. For example, vincristine (an antitumor drug), digitalis (a heart regulator) and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [2]. Thousands of secondary plant products have been identified and it is estimated that thousands of these compounds still exist. Since secondary metabolites from natural resources have been elaborated within living systems, they are often perceived as showing more "drug – likeness and biological friendliness than totally synthetic molecules" making them

good candidates for further drug development [3-5].

Ajowan is one of the aromatic seed spices, which is generally used for medicinal purposes as a digestive stimulant or to treat liver disorders. Thymol, the major phenolic compound present in Ajowan, has been reported to be a germicide, antispasmodic, and antifungal agent [6]. Ajowan, is reported to have platelet aggregation inhibitory action [7], antifungal potency [8] and blood pressure lowering action [9]. Recently, antihyperlipidaemic effect of *T. ammi* seed has been evaluated in albino rabbits.

MATERIALS AND METHODS**Plant Materials**

Plant materials were collected at the local market, Mandsaur. Plant authenticated from K.N.K. College of horticulture by Dr. Gyanendra Tiwari and Mandsaur. The voucher specimen (MIP/C/VSNTS-18) was submitted in Department of Pharmacognosy at Mandsaur Institute of Pharmacy, Mandsaur.

Isolation of Essential oils

The seeds, roots and leaves for oil were dried and ground to semi-powdered state. The air-dried aerial parts (50 g) were hydro distilled in a clevenger apparatus (sigma chemical company) for 5 h. in accordance with the British pharmacopoeia. The yield was 0.62% dry

weight. The aqueous phase was extracted with dichloromethane (Qualigens) (3 x 50 mL). The organic phase was dried with sodium sulphate (Bio-RAD), filtered and the solvent evaporated until dryness by air-dry. The fractions obtained were combined into calibrated flasks, evaporated to dryness and weighted in order to determine the extraction's efficiency. The oils were solubilized in DMSO (Bio-RAD) to a final concentration 5 mg/mL^[10]. The oils were stored in a sealed glass vial (bijoux bottle) in a refrigerator at 4 °C until required. These all oils of above plants were screened for their antimicrobial activity.

Test Microorganism

In the study *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas arginosa*, *Staphylococcus aureus* were used.

SCREENING OF ANTIBACTERIAL ACTIVITY

Media

Mueller-Hinton agar (MHA) (Merck) was used as base medium for screening of antibacterial activity and Mueller-Hinton broth (MHB) (Merck) for preparation of inoculum.

Preparation of McFarland Nephelometer standard

McFarland tube number 0.5 was prepared by mixing 9.95 ml 1% sulphuric acid in MHB and 0.05 ml 1% barium chloride in distilled water in order to estimate bacterial density^[11]. The tube was sealed and used for comparison of bacterial suspension with standard whenever required.

Preparation and standardization of inoculum

Four to five colonies from pure growth of each test organism were transferred to 5 ml of MHB. The broth was incubated at 35-37°C for 18-24 hours. The turbidity of the culture was compared to 0.5 McFarland Nephelometer standards to get 150x10⁶ CFU/ml, the standardized inoculum suspension was inoculated within 15-20 minutes.

Well diffusion technique

Screening of antibacterial activity was performed by well diffusion technique^[12], the MHA plates were seeded with 0.1 ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 100 µl of each infusion and decoction of *E. officinalis* and *c. sativum* was introduced in the well.

Incubation

The inoculated plates were incubated at 35-37°C for 24 hours and zone of inhibition was measured to the nearest millimeter (mm).

RESULTS AND DISCUSSION

Different strain of gram negative bacteria and positive bacteria isolated from urine specimens, viz., *Escherichia coli* (433), *Pseudomonas aeruginosa* (741), were used in the present study. The results of *in vitro* antibacterial activity of *Trachyspermum ammi* are presented in **Table 1**. The diameter of inhibitory zones recorded includes the size of filter paper discs (6mm in diameter).

The *in vitro* anti microbial activity of essential oil of *Trachyspermum ammi* on gram-positive and gram-negative bacteria collected from local market, Mandasaur were studied. This result are shown in Table 1 the maximum activity was on *B. subtilis*(16-17 mm) and minimum activity was on *S. aureus*(11-12mm), among the gram positive bacteria. The inhibition zone, especially on Ofloxacin resistant was 26-27mm respectively.

On the other hand the maximum activity was observed on *E. coli* (14mm) among the gram negative bacteria. The anti bacterial activity of the plant on antibiotic resistant strains was especially notable.

The yield of essential oil obtained from air dried plant material was 8.13% (v/w) these plant material showed strong antibacterial activity on *B. Subtilis* and *P. Srginosa*. It is important that the essential oil of *Trachyspermum ammi* have anti bacterial activity on *E-coli*, which are multiple antibiotic bacteria, because *E-coli* is a biogenic amine procedure in food^[13]. Also *E-coli* has become an important agent of nosocomial infection^[14].

The essential oil also inhibited the growth of multiple antibiotic resistant *Staphylococcus* stain, tested. The effect of essential oil on *S. aureus* and *P. aurious* were high. *S. aureus* is a one of the most common causes of both hospital and community-acquired infection worldwide^[15]. *S. aureus* is a major cause of cutaneous infections, furunculosis, impetigo and arthritis, and toxinoses, such as food poisoning, septic shock, scalded skin syndrome and toxic shock syndrome^[16]. The presence of antibiotic resistant staphylococci is of concern due to the possible spread of resistance determinants among the staphylococcus species. This could lead to the survival, growth and spread

of enterotoxigenic staphylococci and staphylococci of clinical significance.^[17]

The inhibition zone of the essential oil of these materials collected from different location,

on bacteria, were similar [Table-1]. The study demonstrates that *Trachyspermum ammi* had antimicrobial activity on Gram-positive and Gram-negative bacteria.

Table 1 Antimicrobial Activity of compounds

S.No.	Microorganisms	Zone of inhibition(in mm)	Activity index	% activity
1.	E.C.	14	0.53	53%
2.	P.A.	12	0.46	46%
3.	B.S.	16	0.61	61%
4.	S.A.	11	0.42	42%

Where as:-E.C. = *Eschericia coli* (Gram negative) P.A.=*Pseudomonos Arginosa* (Gram negative)
B.S. = *Bacillus subtilis* (Gram positive); S.A. = *Styphylococcus Aureus* (Gram positive)

REFERENCES

- Parekh, J. and S.Chanda, *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* kurz. flower (lythraceae).Brazilian J. Microbiol.,2007; 38: 204-207,
- Iwu, M.W., A.R. Duncan and C.O. Okunji, New antimicrobials of plant origin In: Janick, J. (Ed.), Perspectives on New crops and New Uses. ASHS Press, Alexandr, VA,1999; pp: 457-462.
- Koehn, F.E. and G.T. Carter, The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov; 2005; 4: 206-220.
- Balunas, M.J. and A.D. Kinghorn. Drug discoveryfrom medicinal plants. Life Sci, 2005; 78: 431-441.
- Drahl, C., B.F. Cravatt and E.J. Sorensen. Protein-reactive natural products. Angew Chem. Int. Ed. Engl, 2005; 44: 5788-5809.
- Nagalakshmi G, Shankaracharya NB, Puranaik.J, et al. Studies on chemical and technological aspects of ajowan (*Trachyspermum ammi*) syn (*Cerum copticum* Hiren) seeds. J of Food Sci Techno, 2000; 37: 277- 281.
- Srivastava, K. C., Extract of *Trachyspermum ammi* shows antiaggregatory effects and alters arachidonic acid metabolism in human platelets. Prostaglandins Leukot. Essent. Fatty Acids, 1988; 33 (1): 1-6.
- Dwivedi, S. K. and N. K. Dubey. Potential use of the essential oil of *Trachyspermum ammi* against seed-born fungi of guar. Mycopathologia, 1993; 121(2): 101-104.
- Aftab, K., A. Rahman and K. U. Ghani. Blood pressure lowering action of active principle from *Trachyspermum ammi* (L) Sprague. Phyto-medicine, 1995; 2(1): 35-40.
- Glowniak, K., T. Wolski and T. Dragan, Sposób wyodrebniania Kumaryn Z Surowców roelinych zwlaszcza Z owoców arcydziegla olesnika. Pat pol, 1991; 15: 1-5.
- Saeed S and Tariq P. Effects of some seasonal vegetables and fruits on the growth of bacteria. Pakistan Journal of Biological Sciences, 9(8), 2006; 1547- 1551.
- Saeed S and Tariq P. Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. Pakistan Journal of Botany, 37(4), 2005; 997- 1001.
- Ben-Gigirey B,Vieites-Baptista de Sousa JM, Villa TG,Baros-Velazquez J. Characterization of biogenic amine producing *Stenotrophomonas maltophilia* strains isolated from white muscle of fresh and frozen albacore tuna. Int J food microbial, 2000; 57:19-31.
- Denton M, Kerr KG. microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*, clin microbial rev ,1998;11:57-80
- CDC. Staphylococcu Aureus with reduced susceptibility to vancomycin- United states 1997. MMVR morb Mortal Wkly Rep, 1997; 46; 765-6.
- Projan SJ, Novick RP. The molecular Basis of Pathogenicity. In:Archer G, Crossley K, editors. The Staphylococci in Human Disease. New York: Churchill Livingstone, 1997; p. 55-81.
- Heir E, undhein G, Holck AL. identification and characterization of quaternary ammonium compound. Int J food microbial, 1999; 48: 211-9.