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Antibacterial and Antifungal activity of ajwain (*Trachyspermum ammi*) in different solvent

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Abstract
Ajwain with its characteristic aromatic smell and pungent taste is widely used as a spice in curries. Several studies have been carried out in the past validating its antimicrobial, anti-mycobacterial and antiviral properties. Present study was conducted to validating anti-bacterial and anti-fungal properties of methanolic and hexane seed extract of two ajwain genotypes commonly grown in India. The findings indicated significant anti-microbial activity in seed extract. The study showed genotypic variation in anti-microbial effect of both the extracts. Ajwain has potential to be used as a good source of traditional medicine and it provides a noteworthy basis in pharmaceutical biology for the development/formulation of new drugs and future clinical uses.

Keywords: ajwain seed extract, antimicrobial activity, genetic variation, gram positive bacteria, gram negative bacteria

Introduction

Ajwain (*Trachyspermum ammi*)

Ajwain is a small, erect, annual, herbaceous plant with branched leafy stems, feather like leaves (2.5 cm long), and 4-12 ray flower heads bearing 6 - 16 flowers. The fruits are minute, greyish-brown coloured and egg shaped. Cultivation of this plant originated in Egypt. It grows widely around Mediterranean sea and in South-West Asia extending from Iraq to India, in India it is growing in - Madhya Pradesh, Gujarat, Maharashtra, Uttar Pradesh, Punjab, Haryana, Rajasthan, Bihar and West Bengal. Ajwain seeds are used in small quantities for flavouring numerous foods, as preservatives, in medicine and for the manufacture of essential oil for ultimate use in perfumery (Pruthi, 1992) [7]. In Indian system of medicine, ajwain is administered as a household remedy for stomach disorders, a paste of crushed fruits is applied externally for relieving colic pains and a hot and dry fermentation of the fruits applied on chest is used as a common remedy for asthma (Anonymous, 1995) [2]. Ajwain has been shown to possess anti-aggregatory, anthelmintic, antihyperlipidaemic, antifilarial, insecticidal, kidney stone inhibitory, molluscicidal, mosquito repellent and nematicidal activities. Present investigation has been carried out to evaluate methanol and hexane seed extract of two popular ajwain genotypes for its anti-microbial effects.

Material and Methods

Seed collection

Mature seeds of ajwain varieties Ajmer Ajwain-2 (AA-2) and Ajmer Ajwain-93 (AA-93) were taken from gene bank of ICAR-NRCSS, Ajmer, India from fresh harvest. Pure and healthy seeds of each variety were used for preparation of crude seed extract in different solvents.

Anti-bacterial and anti-fungal activity

Bacterial strains Gram positive *Staphylococcus aureus, Streptococcus Pyogenes*, Gram negative, *Escherichia Coli, Pseudomonas aeruginosa and* Fungal strains *Candida albicans,* and *Aspergillus clavatus* were chosen based on their clinical and pharmacological importance. The bacterial micro organisms were cultured on nutrient agar/YEPD by using spread plate technique and fungal stock cultures were incubated for 24 hours at 37°C on potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Determination of zone of inhibition method

In vitro anti-bacterial and antifungal activities of methanolic and hexane extracts of ajwain were examined against two Gram positive and two Gram negative pathogenic bacteria and two fungi by the agar disk diffusion method. Each sample was dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, Gram-positive, Gram-negative and fungal strains were taken with a standard antibiotic and fungicide for comparison of the results. The dilutions (25 µg/ml) of sample and standard drugs (25 µg/ml) were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using a cefixime and griseofulvin as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivity of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active.

Results and Discussion

Anti-bacterial potency of ajwain genotypes in different solvent extracts

In Gram positive bacterial group *Staphylococcus aureus* had minimum zone of inhibition in AA-2 (16 mm) whereas AA-93 showed maximum zone of inhibition (18 mm). This was highest in terms of percentage zone inhibition (56%) within methanol extracts of both genotypes in both the bacterial groups (Table 1), Next bacteria under this group *Streptococcus pyogenes* had minimum zone of inhibition in the methanol extracts of genotype AA-2 (11 mm) and AA-93 (19 mm) respectively. In case of seed extract in hexane solvent Gram positive bacterial group *Staphylococcus aureus* had minimum zone of inhibition in AA-2 (18mm) whereas AA-93 showed maximum zone of inhibition (23 mm), Another bacteria under this group *Streptococcus pyogenes* had minimum zone of inhibition in the hexane extracts of genotype AA-2 (18 mm) and maximum zone of inhibition in AA-93 (20 mm). Seed extract of genotype AA-93 was most effective in terms of percentage (72%) in both the bacterial groups (Table 1).

In Gram Negative bacterial group *E. coli* showed maximum zone of inhibition in AA-93 (20 mm) followed by AA-2 (16 mm). Next bacteria under this group *P. aeruginosa had* minimum zone of inhibition in the methanol extracts of genotype AA-2 (20 mm) highest zone of inhibition was observed in AA-93 (21 mm) (Table 1). In case of seed extract in hexane solvent *gram* negative *E. coli* showed the maximum zone of inhibition in AA-93 (19 mm) and minimum zone of inhibition in AA-2 (17 mm). Next bacteria under this group *P. aeruginosa* had minimum zone of inhibition in genotype AA-2 (17 mm) Highest zone of inhibition was observed in AA-2 (28 mm) (Table 1). When compared both the extract, hexane seed extract showed more effective against the gram positive bacteria's where methanol seed extract showed more effectiveness against the gram negative bacteria's.

Table 1: Antibacterial activity of Ajwain in different solvent extract (zone of inhibition in mm)

	Zone of inhibition in mm							
Seed extract (25 µg/ml)	***Staphylococcus aureus***		***S. pyogenes***		***E. coli***		***P. Aeruginosa***	
	Methanol	**Hexane**	**Methanol**	**Hexane**	**Methanol**	**Hexane**	**Methanol**	**Hexane**
Cefixime	32	32	35	35	37	37	37	37
AA-2	*16*(50)	*18*(56)	*11*(31)	*18*(51)	*16*(43)	*17*(46)	*20*(54)	*17*(46)
AA-93	*18*(56)	*23*(72)	*13*(37)	*20*(57)	*20*(54)	*19*(51)	*21*(57)	*18*(49)

Value in bracket indicate percent effectiveness

Anti-fungal potency of ajwain genotypes in different solvent extracts

Antifungal activity of methanol and hexane extract of fennel was analyzed using Griseofulvin as standard drug against the fungal infection. Griseofulvin 25 µg/ml was used and the zone of inhibition in mm was consider as the control (100%), which was 46 mm in *Candida albicans,* 45 mm *Aspergillus clavatus.* Similar concentration of fennel genotypes was used to detect the antifungal activity.

When methanol seed extract of each genotype was used as drug, ajwain strain *Candida albicans* had zone of inhibition in AA-2 and AA-93 (21 mm) (Table 2). *Aspergillus clavatus* had minimum zone of inhibition in the methanol extracts of genotype AA-2 (11 mm) and maximum zone of inhibition in genotype AA-93 (19 mm).

In place of methanol, hexane seed extract was used to compare both extract. Ajwain strain *Candida albicans* had minimum zone of inhibition in AA-2 (18 mm) whereas AA-93 showed maximum zone of inhibition (23 mm) (Table 2). Another strain *Aspergillus clavatus* had minimum zone of inhibition in genotype AA-2 (16 mm) and maximum in genotype AA-93 (21 mm). AA-93 was show maximum effectiveness in terms of percentage (50%) in both genotypes in both fungal strains (Table 2). Anti-fungal activity was more in methanol seed extract as compared to hexane.

Table 2: Antifungal activity of Ajwain in different solvent (zone of inhibition in mm)

	Zone of inhibition in mm			
Seed extract (25 µg/ml)	***Candida albicans***		***Aspergillus clavatus***	
	Methanol	**Hexane**	**Methanol**	**Hexane**
Griseofulvin	46	46	45	45
AA-2	*21*(46)	*18*(39)	*11*(24)	*16*(36)
AA-93	*21*(46)	*23*(50)	*19*(42)	*21*(47)

Value in bracket indicate percent effectiveness

There are many reports showing various medicinal potential of ajwain including antimicrobial activity. Essential oil of ajwain also exhibited fungal toxicity against *Epidermophyton floccsum*, *Microsporum canis* and *Trichophyton*

mentagrophytes at 900 ppm concentration. Fungitoxicity of the oil was thermo-stable up to 150°C and thymol was identified as the fungitoxic chemical in essential oil (Singh *et al*., 1986) [9]. Ajwain seed extract at 1:20 dilution was reported to possess fungicidal action against *Rhizoctonia solani*, a causative agent of sheath blight of rice (Ansari, 1995) [3]. Ajwain oil exhibited a remarkable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibrio cholera* (Syed *et al*., 1986; Anonymous, 1995) [10, 2]. The essential oils extracted from the seeds of ajwain showed antibacterial activity (Mayaud *et al*., 2008; Singh *et al*., 2002) [5, 8]. Extracts prepared in different solvents exhibited variable activity against *E. coli*, *P. aeruginosa*, *S. typhi* and *S. aureus* (Ahmad *et al*., 1998; Patel *et al*., 2008) [1, 6], suggesting their centuries old usage in the treatment of gastrointestinal disorders. This historical use of ajwain seeds to cure various gastrointestinal disorders has also been scientifically proved in another study carried out by Kaur and Arora (2009) [4] wherein aqueous and organic extracts of Ajwain seeds have also shown their antibacterial effect.

Present study further validates the earlier reports as we report significant antimicrobial properties of methanol and hexane seed extracts. Further, genetic variability may be explored to identify specific genotypes possess more medicinal potential.

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