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Antibacterial Activity of Ajowan (*Trachyspermum copticum*) Seed Extract

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ABSTRACT

The antibacterial activity of ajowan (*Trachyspermum copticum*) seed extract (ASE) against four Gram-negative and one Gram-positive bacteria were evaluated with the agar disc diffusion and MIC methods. *Pseudomonas aeruginosa* and *Acinetobacter lwoffii* were resistant to cephalosporin and hexane (0.01 mg/ml) as control drugs but sensitive to ASE. ASE also exhibited antibacterial activity against *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Staphylococcus aureus*. The thymol in ajowan seeds extracts maybe the cause of this antibacterial effect.

Keywords: ajwain, antibiotic, antimicrobial activity, disc diffusion method

INTRODUCTION

Many Gram-positive and Gram-negative bacteria cause different diseases in humans. Ajowan or ajwain (*Trachyspermum copticum* Link syn *Trachyspermum ammi* (L.) Sprague syn *Carum copticum* Hiren) is a beautiful Umbelliferous plant with white flowers and compact bushy-like growth habit, ranging from the north of India and Nepal to Afghanistan.

Ajowan seeds include phenols (30-50% thymol, 1-7% carvacrol) and monoterpenes (20-35% γ-terpinene and 20-25% paracymene, α-pinene and limonene). Thymol, the major phenolic compound present in ajowan, has been reported to be a germicide, antispasmodic, and antifungal agent (Nagalakshmi *et al*. 2000). Ajowan is used in the treatment of intestinal dysbiosis (Nagalakshmi *et al*. 2000). Its benefit derives from being able to inhibit the growth of undesired pathogens while not adversely affecting the beneficial flora (Murthy *et al*. 2009; Siripornvisal 2010). The present study deals with the investigation of the antimicrobial activity of Iranian ajowan seed extract (ASE) against some resistance bacteria to control drugs.

MATERIALS AND METHODS

Preparation of ajowan seeds

Ajowan seed were obtained from the Iranian Agricultural Organization of Zabol City. The seeds were powdered and then dissolved in 200 ml ethanol 96% using a shaker water bath for 24 h at room temperature. After filtration with Whatman No. 1 filter paper, the resulting solution was concentrated by a rotary evaporator at 50°C for 30 min to remove solvent from the extract. Solid extract was dissolved in 50 ml of distilled water. This working solution was used for the minimal inhibition concentration (MIC) test.

Microorganisms

Four Gram-negative (*Staphylococcus aureus*) and one Gram-positive (*Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Acinetobacter lwoffii*) bacteria were obtained from the microbiological laboratory of the central hospital in Shiraz, Iran. The bacteria included. The microorganisms were inoculated onto nutrient agar slants at 37°C and maintained at -80°C. These bacteria were isolated from MRI patients that provided written permission to do so.

Antimicrobial activity

Antimicrobial activity was based on the disc diffusion method (Thitilertdecha *et al*. 2008) using a cell suspension of microorganisms. The concentration of the cell suspension was equilibrated to a 0.5 McFarland standard and 50 µl of each microorganism's suspension was spread on a Mueller-Hinton agar plate. In addition, 50 µl of diluted seed extract was pipetted onto sterile paper discs (6 mm in diameter), which were allowed to dry in an open sterile Petri dish in a biological laminar flow bench. Discs were placed on the surface of inoculated plates and incubated at 37°C for 24 h. Diameters (mm) of the zones of bacterial inhibition minus the discs diameter were recorded (Aureli *et al*. 1992; Ilçim *et al*. 1998). Discs with hexane and cephalosporin (Padtan Teb Co., Iran) were used as positive controls. Cephalosporin is an antibiotic with antibacterial activity that inhibits cell wall synthesis although some bacteria like *Klebsiella* spp., *Proteus* spp and *Pseudomonas* sp. are resistance to it (Pfeifer *et al*. 2010; Wright 2010). The experiment was performed in duplicate and the results were expressed as average values.

Determination of MIC

The MIC values were determined for the bacterial strains to the ASE. The inoculum (100 µl), initially adjusted to the density cited above, was spread onto 20 ml Mueller–Hinton agar supplemented with the seed at concentrations ranging from 2–10 µl/ml in Petri dishes, with each one of its equivalent in DMSO. These serially diluted cultures were then incubated at 37 ± 1°C for 24 h. The MIC is defined as the lowest concentration at which the microorganism does not demonstrate visible growth. As control, DMSO was used (Khadidja *et al*. 2010).

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Research Note

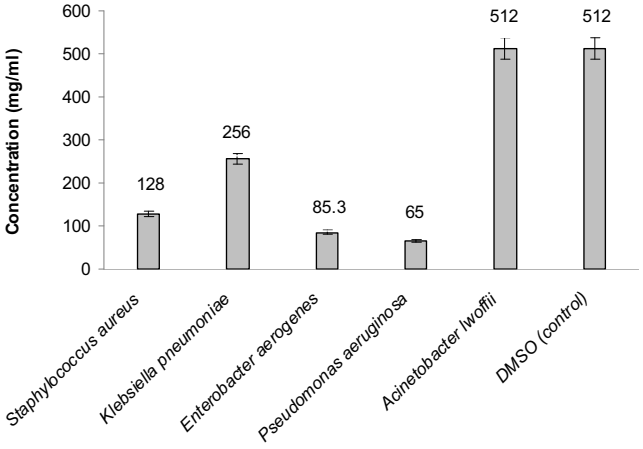

Fig. 1 MIC results of ajowan seed extract against five tested bacteria.

Table 1 The mean values diameter of inhibition zone with ajowan seed extract and two positive controls.

Microorganism	Inhibition zone diameter (mm)		
	Extract (512 mg/ml)	Hexane	Cephalosporin
Staphylococcus aureus	12	16	16
Pseudomonas aeruginosa	10	0	0
Enterobacter aerogenes	10	22	22
Klebsiella pneumoniae	14	20	28
Acinetobacter lwoffii	18	0	0

RESULTS AND DISCUSSION

The ASE inhibited the growth of all tested bacteria (**Fig. 1**). *P. aeruginosa* and *A. lwoffii* were resistant to cephalosporin and hexane (0.01mg/ml) as control drugs but sensitive to ASE (**Table 1**). ASE also exhibited antibacterial activity against *K. pneumoniae*, *E. aerogenes* and *S. aureus*. The thymol in ajowan seeds extracts maybe the cause of this antibacterial effect. Ajowan seeds yield 2 to 4% brownish essential oil, with thymol as the major constituent at about 35 to 60% (Dwivedi *et al*. 2012). The thymol exhibited potent antimicrobial activity with minimum inhibitory concentrations ranging from 0.625 to 10.0 mg/mL (Botelho *et al*. 2007). The *in vitro*-obtained results suggested that thymol may be successfully used as a alternative preservative to increase the lag time as well as to decrease the maximum cell load reached in the stationary phase of growth cycle of bacteria (Falcone *et al*. 2007). The structure of thymol may potentially be used as a basic structure for development of drugs aimed against the bacterial virulence factors (Qiu *et al*. 2010).

P. aeruginosa is intrinsically resistant to all penicillins and some other antibiotics (Chopra 2007; Pfeifer *et al*. 2010; Wright 2010). This Gram-negative bacterium has become an important cause of infection, especially in patients with compromised host defense mechanisms (Tepe *et al*. 2004; Mujeeb *et al*. 2008). It is the most common pathogen isolated from patients who have been hospitalized for longer than one week (Syed *et al*. 1991). It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections, and bacteremia. Pseudomonal infections are complicated and can be life threatening. *Acinetobacter* are a key source of infection in debilitated patients in the hospital (Visca *et al*. 2011).

K. pneumoniae can cause destructive changes to human lungs inflammation and hemorrhage with cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum (Podschun and Ullman 1998). *E. aerogenes* is a nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections (Rhondina *et al*. 2006). Gram-positive bacteria such as *S. aureus* are primarily responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mylotte *et al*. 1987). Our results suggest the possibility of using ajowan seed, which possesses strong antibacterial activity, in the treatment of diseases caused by the microorganisms tested.

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