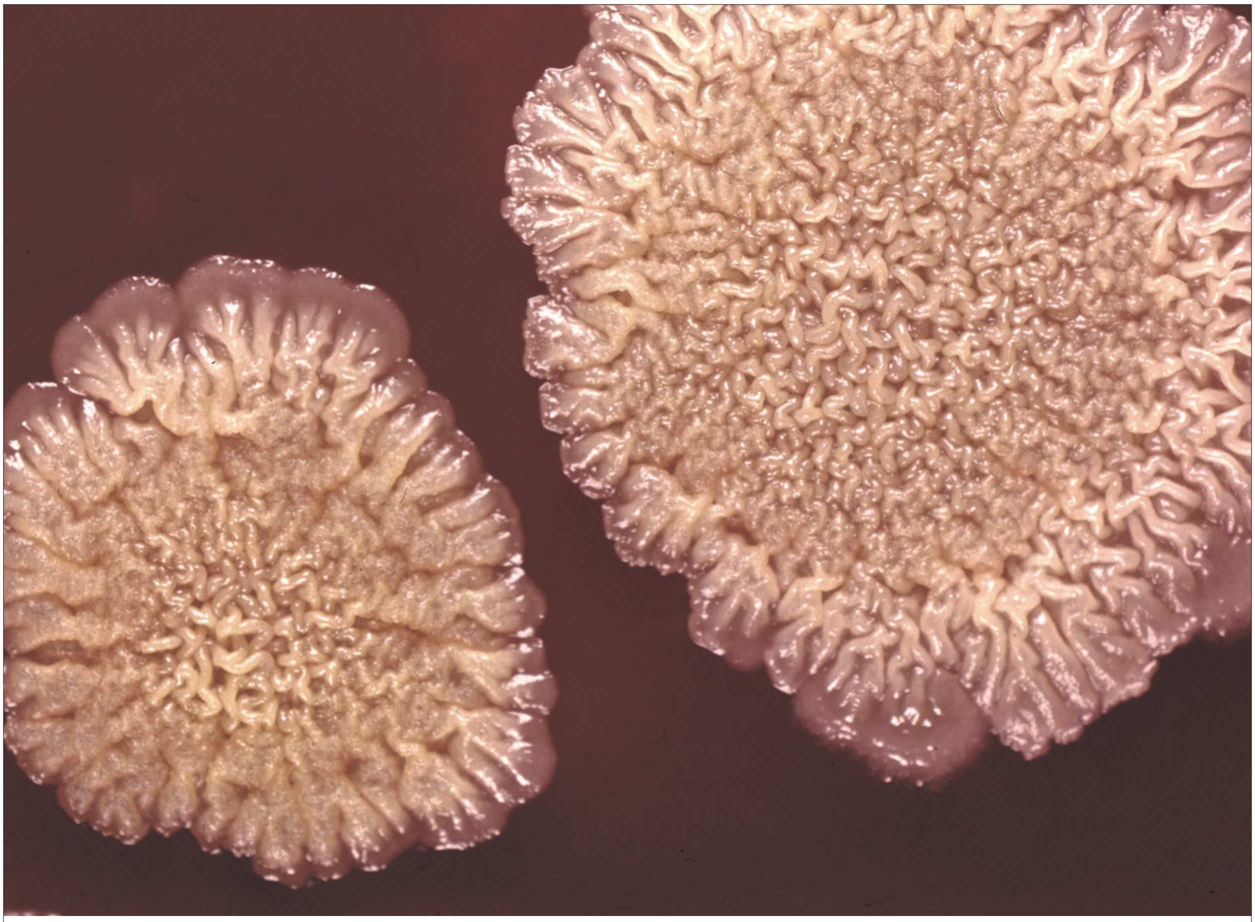

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Antibacterial Effects of *Artemisia dracunculus* Essential Oil on Multi-drug Resistant Isolates of *Acinetobacter baumannii*

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ABSTRACT

Artemisia dracunculus is an appetizer plant, its anti fungal and anti tumor properties has been demonstrated previously, but there are a few studies on its antibacterial effects. The aim of the present study was the evaluation of the antibacterial activity of *Artemisia dracunculus* essential oil on burn isolates of *Acinetobacter baumannii*. Forty eight isolates of *Acinetobacter baumannii* were collected from the burn wound infections. Muller-Hinton Broth was supplemented with 0.002% (v/v) tween 80. Serial doubling dilutions of *Artemisia dracunculus* essential oil over the range 0.03-25% were prepared. 1.5×10^6 inoculums of the isolates were added to each concentration. Antibacterial activity was measured by determining Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of isolates to *Artemisia dracunculus* essential oil. The susceptibilities of isolates to different antibiotics were tested using agar disc diffusion method. The rates of resistance were determined to antibiotics as follows: gentamicin 70.8%, ticarcillin 93.7%, ceftizoxime 75%, co-trimoxazole 79.1%, amikacin 52%, carbenicillin 93.7%, cefalotin 60.4%, cefazolin 100%, piperacillin 88.9%, imipenem 14.6%, kanamycin 95.8% and ofloxacin 95.8%. 14.5% of isolates were sensitive to all tested concentrations of *Artemisia dracunculus* essential oil. For the 10.4, 20.8 and 54.1% of remaining isolates Minimum Inhibitory Concentration and Minimum Bactericidal Concentration values were 1.56×10^{-2}, 7.8×10^{-2} and -3.9×10^{-3}, respectively. These results suggest the potential use of the *Artemisia dracunculus* essential oil for the control of Multi-drug resistant *Acinetobacter baumannii* infections.

Key words: Herbal medicine, antibiotics, resistant bacteria, antimicrobials, hospital acquired infections

INTRODUCTION

Medicinal plants contain biologically active compounds, many of which have been shown to have antibacterial properties (Mohsenzadeh, 2007). *Artemisia dracunculus* (tarragon) is a shrubby perennial herb belonging to Asteraceae family. Tarragon leaves, are commonly used on salad and soup in Iran (Shahriyary and Yazdanparast, 2007). Tarragon is an appetizer plant, its anti fungal and anti tumor properties has been demonstrated previously (Firestone and Sundar, 2009; Li and Wu, 1998; Kordali *et al*., 2005). The extract of *Artemisia dracunculus* leaves appears potentially useful for decreasing the incidence of coronary diseases in human and used for treatment of headaches, dizziness and epilepsy (Shahriyary and Yazdanparast, 2007). But there are a few studies on its antibacterial effects.

Acinetobacter baumannii is a gram-negative, nonmotile, nonfermentative and oxidase-negative bacillus, whose natural reservoir has not been clearly determined. It is found in many hospital environments and can be colonize in human body in the hospital environments. The combination of its environmental colonization and its very high resistance to antimicrobials renders it as a successful nosocomial pathogen (Nordmann, 2004). There are many reports of Multi Drug Resistant (MDR) *A. baumannii* from hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan and Korea and many other areas (Barbolla *et al.*, 2003; Houang *et al.*, 2001; Lee *et al.*, 2004; Liu *et al.*, 2006; Naas *et al.*, 2006; Nishio *et al.*, 2004; Quale *et al.*, 2003; Van Looveren and Goossens, 2004; Yu *et al.*, 2004). These MDR strains often spread to cause outbreaks throughout hospital wards. *Acinetobacter* sp. is usually considered to be opportunistic pathogens. They cause a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection and meningitis, especially in immunocompromised patients. MDR *A. baumannii* infections tend to occur in immunosuppressed patients, in patients admitted in intensive care and burn units and in those subjected to invasive procedures and treated with antibiotics. In respect of its very high resistance to antimicrobials, introducing of the new antimicrobial agents against this bacterium is one of the most important goals in treatment of such infections (Perez *et al.*, 2007).

In this study, we evaluated the antibacterial activity of *Artemisia dracunculus* on 48 hospital isolates of MDR *A. baumannii*.

MATERIALS AND METHODS

Essential oil: *Artemisia dracunculus* essential oil from Barije Essence Pharmaceutical Company, Iran (commercial producer of plant essential oils and aromatic substances) were bought on 2007, used in this study. The oil was selected based on literature survey and its use in traditional medicine. Quality of the oil ascertained to be more than 65% pure. The main effective components of the *Artemisia dracunculus* essential oil were anethole (81.0%), beta-ocimene (6.5%), beta-ocimene (3.1%), limonene (3.1%) and methyleugenol (1.8%) (Kordali *et al.*, 2005).

Bacterial strains and culture media: A total of 48 isolates were collected from clinical specimens from burn wards of hospitals in Tehran, Iran during a 6 months period between April and September 2006. The isolates were further processed by the standard methods to identify as the *A. baumannii* (Baron and Finegold, 1990). Isolated bacteria were maintained for long storage on skimmed milk medium (BBL) by adding 10% glycerol in -60°C, cultures were maintained for daily use on Nutrient agar (BBL) slants on 4°C. The Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) medium (Pronadisa) were used for detection of antibiotic resistance of isolates. Acinetobacter *calcuaceticus* PTCC 1318 has been used as reference strain.

Determination of antimicrobial activity of *Artemisia dracunculus* essential oil: The susceptibility of *Acinetobacter* isolates to *Artemisia dracunculus* essential oil was determined using a broth microdilution method based on CLSI guidelines. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Artemisia dracunculus* essential oil for isolates were determined. Muller-Hinton Broth (MHB; Oxoid) was supplemented with 0.002% (v/v) tween

80 (Sigma) (MHB-T) to enhance dispersion of the *Artemisia dracunculus* oil (Jazani *et al.*, 2009b; Papadopoulos *et al.*, 2006). The initial concentration of *Artemisia dracunculus* essential oil in the first tube contains MHB-T was 1/2. This was used to prepare serial doubling dilutions over the range 0.03-25% (v/v). 1.5×10^6 inoculums of the isolates were added to each concentration in MHB-T. A tube containing growth medium without essential oil and an un-inoculated tube were used as a positive and negative growth control respectively. Antibacterial activity was measured by determining MICs and MBCs. The MIC was the lowest concentration of essential oil that resulted in a clear tube. Ten microlitres from each tube was spot-inoculated onto Nutrient Agar (NA) and incubated overnight at 37°C to determine the MBC. The highest dilution that inhibits bacterial growth on nutrient agar after overnight incubation was taken as MBC (Baron and Finegold, 1990; Papadopoulos *et al.*, 2006). Experiments were performed at least three times and the modal value selected.

Determination of the isolates sensitivity to antibiotics: The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. To represents the different classes of antimicrobial agents commonly used for the treatment of *Acinetobacter* sp. Infections, we used piperacillin, Gentamicin, Ofloxacin, Cephalotin, Ticarcillin, Kanamycin, Imipenem, Amikacin, Co-Trimoxazole, Ceftizoxime, cefazolin, Carbenicillin (Hi-media, Mombay, India).

RESULTS AND DISCUSSION

The rates of resistance to different antibiotics for 48 isolates of Acinetobacter baumannii have been showed in Table 1. Cefazolin (100%), Ofloxacin (95.8%) and Kanamycin (95.8%) showed the highest rate of resistance and Amikacin (52%) and Imipenem (14.6%) demonstrated the lowest. 45.8% of isolates showed resistance to the 11 tested antimicrobials (Table 1).

Results showed that *Artemisia dracunculus* essential oil possessed antibacterial effect against all isolates of *Acinetobacter* sp., 7 isolate were sensitive to all tested dilutions and other 41 remained isolates showed MIC and MBC values in the range of 3.9×10^{-3} to 1.56×10^{-2} mm^3 mm^{-3} (Table 2). Also MIC and MBC values of *Artemisia dracunculus* essential oil for *Acinetobacter baumannii* PTCC1318 was of 3.9×10^{-3} mm^3 mm^{-3}.

Antibiotic resistance is a major problem in treating infection with *A. baumannii,* which has become resistant to almost all available antibacterial drugs play an important role in colonization

Table 1: The rates of resistance to different antibiotics for 48 burn wound isolates of *Acinetobacter baumannii*

Antibiotics	Resistance (%)
Cefazolin (Cz)	100.0
Ofloxacin (Of)	95.8
Kanamycin (K)	95.8
Carbenicillin (Cb)	93.7
Ticarcillin (Ti)	93.7
Piperacillin (Pc)	88.9
Cotrimoxazole (Co)	79.1
Ceftizoxime (Ce)	75.0
Gentamicin (G)	70.8
Cefalotin (Ch)	60.4
Amikacin (Ak)	52.0
Imipenem (I)	14.6

Table 2: Antibacterial activity of *Artemisia dracunculus* essential oil against 48 burn isolates of *Acinetobacter baumannii*

MBCs for each isolate ($mm^3\,mm^{-3}$)	No. of isolates
Sensitive to all tested dilutions	7
1.56×10^{-2}	5
7.8×10^{-2}	10
3.9×10^{-3}	26
Total	48

MIC and MBC amounts of *Artemisia dracunculus* essential for *Acinetobacter baumannii* PTCC1318 was 3.9×10^{-3}

and infection of patients admitted to hospitals. They have been implicated in a variety of hospital acquired infections, including bacteremia, urinary tract infection, meningitis and pneumonia. Treatment of such infections are very difficult because of high resistance of these bacteria to the several antibiotics, also, *Acinetobacter* have a high capacity for long-term survival in the hospital environment with enhanced opportunities for transmission between patients (Perez *et al*., 2007; Jazani *et al*., 2009a; Jazani *et al*., 2007) so it seems reasonable to explore new sources of natural compounds with antibacterial activity against *Acinetonacter* sp. In our study burn wound isolates of *Acinetobacter* baumannii showed high resistance to tested antibiotics (Table 1).

Artemisia is a large, diverse genus of plants with between 200 to 400 species belonging to the daisy family Asteraceae. Common names used for several species include wormwood, mugwort, sagebrush and sagewort, while a few species have unique names, notably Tarragon (*A. dracunculus*) and Southernwood (*A. abrotanum*). The antioxidant, antifungal and antibacterial activities of *Artemisia absinthium, A. dracunculus, Artemisia santonicum* and *Artemisia spicigera* essential oils has been shown previously (Kordali *et al*., 2005).

Also the Antibacterial activity of some *Artemisia* species extract has been demonstrated against some of Gram-positive Gram-negative bacteria and fungal strains (Poiata *et al*., 2009).

Nagy and Tengerdy (1967) showed that some of the *Artemisia* species (*Artemisia tridentate* and *A. nova*) has antibacterial effects on different species of bacteria. Among the four species examined, *Escherichia coli* was the most resistant to the essential oils, followed by *Neisseria sicca, Bacillus subtilis* and *Staphylococcus aureus*. Also they showed the essential oils of *Artemisia* had a marked antibacterial effect on the rumen bacteria (Nagy and Tengerdy, 1968). The antimicrobial activity of the essential oil from *Artemisia feddei* was tested against 15 different genera of oral bacteria. The essential oil from *A. feddei* had a considerable inhibitory effect on all the obligate anaerobic bacteria tested (MICs, 0.025 to 0.05 mg mL^{-1}; MBCs, 0.025 to 0.1 mg mL^{-1}) (Cha *et al*., 2007). Ahameethunisa and Hopper (2010) showed antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. The agar disk diffusion method was used to study the antibacterial activity of *A. nilagirica* extracts against 15 bacterial strains. The extracts showed inhibitory activity for gram-positive and gram-negative bacteria except for *Klebsiella pneumoniae, Enterococcus faecalis* and *Staphylococcus aureus*. Valdés *et al*. (2008) investigated in vitro anti-microbial activity of the Cuban medicinal plants, namely *Simarouba glauca, Melaleuca leucadendron* and *Artemisia absinthium*. Antiprotozoal and antitrypanosomal activity of *A. absinthium* has been demonstrated against *Naegleria fowleri, Giardia lamblia* and all tested protozoa, including *P. falciparum*, but the lack of antimicrobial activity of *Artemisia absinthium* demonstrated by these researchers. Overall findings indicate that Bacterial response to different essential oils varies significantly according to species of the *Artemisia* and tested bacteria (Nagy and Tengerdy, 1968).

There are little studies in the literature on the antibacterial effects of *Artemisia dracunculus*, on the other hand in some studies tarragon essential oil were less effective against tested microorganisms, like *Escherichia coli* and *Staphylococcus aureus* (Mohsenzadeh, 2007). However in our study *Artemisia dracunculus* essential oil showed antibacterial effect against all isolates of *Acinetobacter* sp. (Table 2), since the medicinal plants studied appear to have a broad antimicrobial activity spectrum, they could be useful in antiseptic and disinfectant formulations as well as in chemotherapy. The essential oil shows antioxidant, antibacterial, antifungal and some other therapeutic activities. However, there are often large differences in the reported antimicrobial activity of oils from the same plant.

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