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Full Length Research Paper

Evaluating antibacterial activity from essential oil of *Artemisia fragrans* Willd. in North-Western of Iran

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The antibacterial effects of *Artemisia fragrans* essential oil were studied in this research. The composition of essential oil from aerial parts was analyzed by GC/MS and its antibacterial effects were determined by disk diffusion method. In this study the antibacterial effects of essential oil of *A. fragrans* on two types of Gram positive bacteria *Staphylococcus aureus*, *Strep. Enterococcus fecalis* and Gram negative bacteria; *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumonia* by disk diffusion method on Mueller Hinton agar medium, were evaluated. The oil in concentrations 0, 10, 25, 50, 75, 100 percent prepared in DMSO and then 50 mg ml^{-1} *A. fragrans* essential oil was each was added to the specified disks. The present study describes the phytochemical profile and antibacterial activity of essential oil of *A. fragrans.* The antibacterial test results showed that the oil had a potential antibacterial activity against Gram positive bacterial strains that were mentioned. Essential oil showed maximum zone of inhibition concentration against *Escherichia coli* and *Klebsiella pneumonia* and minimal inhibition concentration against other Gram-negative bacteria that were studied.

Key words: *Artemisia fragrans*, antibacterial activity, essential oil, Gram negative bacteria, Gram positive bacteria, disk diffusion.

INTRODUCTION

It has long been recognized that naturally occurring substances in higher plants have antibacterial activity (Cha et al., 2005). The genus *Artemisia* is one of the largest in the *Asteraceae* family, consisting of more than 800 species widely disturbed throughout the world, especially, in south-west of Asia and Central Europe (Mirjalili et al., 2007). There are approximately 34 native *Artemisia* spp. in Iran (Judzentiene and Buzelyte, 2006).

The Iranian species has been investigated chemically and presence of monoterpenes, sesquiterpenes, especially sesquiter-pene lactones and essential oils reported. In fact, the Iranian *Artemisia* spp. has yielded a considerable amount of new, interesting terpenoids. We have recently reported from the aerial parts of *Artemisia kulbadica* new sesquiterpene lactones and penta methoxylated flavones (Rustaiyan and Ezzatzadeh, 2011). In continuation of our studies on the chemical composition of the essential oils (Morteza-Semnani et al., 2002; Morteza-Semnani and Saeedi, 2003), we decided to investigate *Artemisia fragrans* Willd., a species which grows in Armenia, Iran, Russia and neighboring domains. *A. fragrans* Willd. is a perennial herb, which grows wild in Azerbaijan, Mazandaran, Qazvin and Tehran provinces of Iran (Rechinger et al., 1986). According to our survey of the available literature, previous reports including the isolation of germacranolides and a monoterpene cyclic peroxide from *A. fragrans* extract are available (Marco et al., 1998). Many members of the genus *Artemisia* (*Asteraceae*) are important medicinal plants. Thus, for example, *Artemisia vulgaris* (*mugwort*), native to Britain and Europe, has been used as a tonic, febrifuge, anthelmintic, female troubles, nervous disorders, complaints of the gastrointestinal tract (e.g. stomach ulcers and indigestion (Gruenwald, 2000). The value and

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importance of sagebrush in medical applications is so great that it is renowned in traditional medicine due to its longstanding use and gained many different names in different languages. For example, in Persian it is referred to as Dermaneh, Yooshan, Tarakh, Afsantin, Ghiosum, and Baranjasef (Mozaffarian, 2004).

Biological activities

Artemisia species, widespread in nature, are frequently utilized for the treatment of disease such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria and viruses. Many species have been used since ancient times as folk remedies for some treatment purposes (reducing phlegm, relieving cough, invigorating blood circulation, stopping pain, inducing sweat, diuresis, anti-hypertension, anthelminthic, antitoxic and antiallergy). According to the literature, over 260 *Artemisia* species have been investigated to reveal that they contain many classes of secondary metabolites including terpenoids, flavonoids, coumarins, glycosides, sterols and polyacetylenes (Rustaiyan and Masoudi, 2011).

Previous reports suggest that this group of medical and fragrant plants contain compounds like flavones, terpenes, and coumarins. This plant contains 2.1% of essential oil as regard with the dry weight of the plant. The analysis on the essential oil of the plant indicates that it contains 39/8% limonene and camphor, 15% 1, 8-cineol, 6% camphene, and 5% alpha pinene. Camphor is one the main components of the essential oil which has disinfectant properties (Zargari, 1997).

These plants often grow in temperate climate of northern and southern hemispheres and but usually grow in arid and semiarid areas. Due to having volatile oil (essential oil) and antimalarial and anti-worm compounds, sagebrush plants are very famous and therefore many studies have been conducted on chemical compounds and effect of environmental factors on quantity and quality of the essential oil of these plants. Results from these ecological studies suggest that as temperature, rainfall, and evaporation reduce the amount of monoterpenes will be decrease as well and the amount of sesquiterpene will increase (Ghasemi et al., 2009).

Pharmacological effects of the drug

Sagebrush essential oil has antispasmodic effects due to blocking calcium channels that will help to dilate the bronchial. After entering respiratory system, sagebrush essential oil transforms to lactones by the existing oxygen and will be disposed through the respiratory and urinary excretion. Also, sagebrush essential oil significantly influences dermatophytic fungus such as epidermophyton floccosum, trichophyton rubrum, trichophyton mentagrophytes, microsporum canis and helps prevent growth of fungi. Also prevents growth of bacteria causing unpleasant smell of sweat (Ayinechi, 2000; Amin, 2005; Mir Haider, 2001).

Health benefits

In different regions this plant is used for eliminating worms. In addition, it has carminative, cough and headache relief, anti-worm, anti-infection, and insecticide properties. The essential oil of this plant has a slight anti-worm effect. Various experiments have not suggested existence of santonin even in small amounts. According to the research of Ghasemi et al. (2009) the amount of sagebrush essential oil on candida albicans is considered as average (Morteza-Semnani and Saeedi, 2003).

This research aims to describe the phytochemical profile and test the antibacterial activity of essential oil of *A. fragrans* grown in various regions of East Azerbaijan in north-western of Iran on some of the standard Gram positive and negative bacteria and some strains isolated from clinical samples.

MATERIALS AND METHODS

Preparation of plant samples and essential oil production

The aerial parts in blooming of *A. fragrance* were collected in late August of 2011 from Tabriz, province of east Azerbaijan In north-western of Iran.

For preparing required essential oil, some aerobic organs of the *A. fragrance* was cut by a clipper and kept in a dark place at a temperature of 25℃ and controlled moisture and light conditions; then the organs was dried and chopped before grinding. Their botanical name identified in the plant systematic laboratory, collage of science, PNU University of Benis Branch, Iran where voucher specimens were deposited.

For extracting the essence, 100 g of the dried plant powder were separately subjected to hydro distillation for 3 h, in full glass apparatus. The oil was isolated using a Clevenger type apparatus and stored frozen in dark glass bottles until they were used (Orave et al., 2006).

Evaluation of antibacterial property of essential oil

In this research antimicrobial activity of sagebrush essential oil was studied by digging wells in the gel by diffusion using a Mueller Hinton agar medium. After preparing mediums, 6 wells with a diameter of 6 mm were created in each plate.

Some of the microbial strains being studied were isolated from clinical samples and some of the standard coded strains were obtained from Center of Scientific and industrial Research of Iran (PTCC). Microbial strains were prepared and used according to NCCL protocols. 15 min after three directional lawn cultures, 50 µl of each dilution were inoculated in a certain well and kept in refrigerator at 4℃ and then incubated at a temperature of 37℃ for 24 h. To increase the accuracy of the results obtained from each sample, the tests were replicated three times. After 24 h of incubation, the diameter of growth inhibition zone in all plates was

Table 1. Sensitivity pattern of gram positive and negative to essential oil of *Artemisia* spp. Cup diffusion method.

Microorganisms	PTCC/ATCC	Zone size of inhibition (mm)						
Concentration percentage		100%	75%	50%	25%	10%	DMSO	Tet 30 µl
Escherichia coli	*PTCC 1270*	0	0	0	0	0	0	14.73
Escherichia coli	*ATCC 25922*	19.06	18.65	17.15	18.75	15.49	0	16.23
Escherichia coli	*O157H7*	0	0	0	0	0	0	25
Klebsiella pneumonia	*PTCC 1298*	18.43	17.50	17.15	16.75	15.49	0	R
Klebsiella oxytoca	Pathological sample	10.94	10.14	10.13	9.99	9.02	0	R
Salmonella typhi	Pathological sample	0	0	0	0	0	0	34.20
Pseudomonas euriginosa	*ATCC27853*	0	0	0	0	0	0	R
Staphylococcus aurous	*ATCC 25923*	21.40	20.50	17.14	15.04	11.40	0	23.86
Streptococcus agalactia	Pathological sample	Max	Max	Max	Max	29.20	0	24.92
Enetrococcus fecalis	*ATCC 29212*	28.45	25.04	23.73	19.28	16.90	0	24.72

measured by a caliper and an antibiogram ruler and the results were recorded in Table1.

In the first method, before inoculating the essence in the created wells in the medium, serial concentrations of essential oil was collected in sterile DMSO solvents with 0, 10, 25, 50, 75, 100 v/v percent proportions and 50 µl from each dilution was inoculated in certain wells. In the second method before adding the essential oil with a serial dosage, 5, 10, 25, 50, 75, 100 µl raw disks were placed in the medium and then various amounts of essence were added to them. Each method was replicated three times to ensure the accuracy.

Tested organisms

In this studies evaluation of antibacterial effects of this essence in laboratory of PNU University (Benis Branch), its effect on two categories of pathogenic gram positive bacteria, such as *Staphylococcus aureus*, *Streptococcus agalactiae* and *Enterococcus faecalis*, and pathogenic Gram negative bacteria such as *Escherichia Coli (PTCC 1270, ATCC 25922, O157H7)*, *Salmonella Typhi (Pathological sample)*, *Klebsiella pneumonia (PTCC 1298), Klebsiella oxytoca (Pathological sample)* and *Pseudomonas aeruginosa (ATCC27853)*, and the sample was tested using disk diffusion method in Mueller Hinton agar medium.

RESULTS

Investigation of obtained results showed that *A. fragrans* has antibacterial activity. The results of the study are presented in Table 1. The essential oil of this plant, in concentrations of 10 to 100%, was effective on under study gram positive bacteria such as *S. agalactiae*, *E. faecalis* as well as *S. aureus* and the average diameter of growth inhibition zone in a 50% concentration of the oil was estimated to be approximately 23 mm which indicates the high sensitivity of these bacteria against the essential oil of the plant. On the other hand, the results showed that the essential oil of this plant has no effect on gram negative bacteria except *E. coli* and *K. pneumonia* though it has an insignificant effect on *K. oxytoca* (10 mm).

DISCUSSION

In this study antibacterial activity of *Artemisia fragrans* on 10 Gram positive and gram negative bacteria was investigated using disk diffusion method.

The results indicated that by using the suitable extraction conditions, SFE is more selective than the conventional hydro-distillation method in the extraction of the essential oil and preservation of this quality. The composition of the oil obtained by hydro-distillation from *A. fragrans* collected from Tabriz, North West of Iran was analyzed by GC/MS. Twenty-eight compounds were identified, among which 1,8-cineole (52.1%) and a-thujone (34.8%) were the major constituents that this result exactly confirm by Barazandeh (2003) (Ayinechi, 2000). Another sample for the same species (*A. fragrans*) collected from the suburb of Behshahr, Province of Mazandaran Iran, the composition of the essential oil obtained from the dried flowering aerial parts was analyzed by GC and GC/MS. Twenty-seven components were identified, whose major constituents were camphor (46.0%), 1,8-cineole (23.7%) and camphene (7.9%) (Amin, 2005).

Compounds that are the most important of them were 1, 8-Cineole (52.1%) and α-thujone (34/8%) and according to the research by Morteza Semnani et al. (2005) this essential oil is composed of 27 compounds among which camphor compounds (46%), 1,8-Cineole (23.7%) and camphene (7.9%) were the main ones (Ghasemi et al., 2009). In reports by Ramezani et al. (2004) who studied most species of *Artemisia*, the main compounds of them contained saponin and tannin (Ghasemi et al., 2009). According to the reports by Babakhanloo et al. (1998), the main compounds of *Artemisia* were cyber α-thujone (16%) beta-bisabolol (6.9%) (Mozaffarian, 2004; Rustaiyan and Masoudi, 2011; Ghasemi et al., 2009). Studying the results of this research revealed that the gram positive bacteria being studied had a full sensitivity in concentrations higher than

50% with much greater growth inhibition zone but this oil had no significant effect on gram negative bacteria except *E. coli*. Juteau et al. (2002) suggested that this oil is not effective on *E. coli* and *S. aureus* but influences *S. aureus* (Zargari, 1997). Guangrong et al. (2002) suggested that methanol extract of *Artemisia anomala* is relatively effective on Gram negative bacteria such as *E. coli*, *S. typhi* and *P. vulgaris* but it influences Gram positive bacteria such as *S. aureus* and *Bacillus cereus* more effectively (Rechinger et al., 1986). Gupts Parakasha et al. (2010) believed that methanol extract of *Artemisia anomala* is completely effective on Gram positive bacteria such as *S. aureus*, bacilli, and micrococcus but does not affect Gram negative bacteria but, however, chloroform extract of the plant can affect Gram negative bacteria such as *E. coli*, *S. typhi* and *Pseudomonas* but has no effect on gram positive bacteria (Gruenwald, 2000). Naili et al. (2010) found that methanol extract of *Artemisia campestris* highly affects Gram positive bacteria rather than Gram negative bacteria (Amin, 2005). According to Mighri et al. (2010) and type IV extract analysis and matching some of its compounds with used *A. fragrans* oil in the present study it is found that the extract is effective on Gram negative bacteria (Ayinechi, 2000).

Ramezani et al. (2004) showed that all extracts were effective against two Gram positive bacteria, *Bacillus subtilis* and *S. aureus*. None of the extracts showed anti-bacterial activity against *E. coli* while *P. aeruginosa* was inhibited by *Artemisia oliveriana* and *Artemisia turanica* extracts. Antifungal activity was observed only for *Artemisia scoparia* extract. These results may partly justify the traditional use of *Artemisia* species.

SUGGESTIONS

We suggest that for medical applications of this extract and for using different plants collected from various regions, exactly evaluate the oil with Minimum Inhibitory Concentration Determination method and then determine the minimum concentration of fatality. And also considering the previous literature, this plant can be effective on some fungi but more investigation should be conducted to prove this claim.

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