Antibacterial activity of methanolic extract and essence of sagebrush (*Artemisia vulgaris*) against pathogenic bacteria

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Abstract: Antibiotic resistance is one of the common issues in the modern medicine so finding new agents with low side effects is necessary. Considering the biological active components exist in the sagebrush and its usage in the traditional medicine, it seems that, it may have significant antibacterial activity. The objective of present study was to evaluate antibacterial activity of methanolic extract and essence of sagebrush against four strain including Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa. In this study, the effect of methanolic extract of sagebrush was tested against above mentioned strains after achieving by Soxhlet method. The concentrations of 20, 30, 50 and 400 mg/ml of plant were prepared using dimethyl sulfoxide then its antimicrobial activity was evaluated using well distribution and tube dilution. Data were analysed by ANOVA and chi-square at the level $P<0.01$. Results showed that methanolic extract of sagebrush prevents of growth of S.aureus, B.cereus and E.coli that was dose-dependently. Also, concentration of 1000 µg/ml of plant's leaves has shown preventive activity against S.aureus, B.cereus and E.coli. The results showed antibacterial activity of sagebrush.
[Changhiz Ahmadizadeh, Alireza Monadi, Ali Rezaie, Mahdi Ghiami Rad, Behbood Jafari. **Antibacterial activity of methanolic extract and essence of sagebrush (*Artemisia vulgaris*) against pathogenic bacteria.** *Life Sci J* 2018;15(5):69-73]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 12. doi:10.7537/marslsj150518.12.

Keywords: sagebrush, antibacterial, herbal extract.

Introduction:

Plants still are known as source of pharmaceutical compounds. Medicinal plants are used for treatment of several diseases like infectious one such as diarrhea, fever, the flu, also are used for controlling the breeding and hygiene of oral cavity (Mitscher et al., 1981). By increasing the strains resistant to antibacterial compounds, much effort has been made to use of medicinal plants. On the other hands, emerging the resistant strains among gram positive and negative genera like pseudomonas, kelebsiella, entrobacter, staphylococcus and enterococcus have made problems in the treatment of infections due to them (Oussalah et al., 2007).

Artemisia vulgaris (mugwort or common wormwood) is one of several species in the genus Artemisia commonly known as mugwort, although Artemisia vulgaris is the species most often called mugwort. This species is also occasionally known as felon herb, chrysanthemum weed, wild wormwood, old Uncle Henry, sailor's tobacco, naughty man, old man or St. John's plant (not to be confused with St John's wort). Mugworts are used medicinally and as culinary herbs. It is native to temperate Europe, Asia, northern Africa and Alaska and is naturalized in North America, where some consider it an invasive weed. It is a very common plant growing on nitrogenous soils, like weedy and uncultivated areas, such as waste places and roadsides. It is a tall herbaceous perennial plant growing 1–2 m (rarely 2.5 m) tall, with a woody root. The leaves are 5–20 cm long, dark green, pinnate, with dense white tomentose hairs on the underside. The erect stem often has a red-purplish tinge. The rather small flowers (5 mm long) are radially symmetrical with many yellow or dark red petals. The narrow and numerous capitula (flower heads) spread out in racemose panicles. It flowers from July to September.

The genus Artemisia L. is among the largest and most widely distributed genera of the family Asteraceae, consisting of 522 small herb and shrub species native to the northern hemisphere, South America, southern Africa, and the Pacific Islands (Wright, 2002; Oberprieler, 2005).

These herbs have been used worldwide in folk medicine since ancient times (Furlenmeir, 1983; Hose, 2002). They have been used as tonics, antimalarials, antihelmintics, and antidiabetics, and in treating wounds, bronchitis, ulcers, and tuberculosis in traditional Anatolian medicine (Baytop, 1999; Uzun et al., 2004; Tümen and Sekendiz, 1989; Akalın, 1993). There are also several reports concerning the

antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial, and antifungal activities of different Artemisia species (Wright, 2002; ESCOP, 1997; Korkmaz and Gürdal, 2002; Kalemba et al., 2002; Tan et al., 1998). The chemical studies on Artemisia species indicate that all classes of compounds are present in the genus with particular reference to terpenoids and flavonoids. The rich accumulation of essential oils and other terpenoids in the genus is responsible for the use of various members for flavoring foods or liqueurs (Wright, 2002). The objective of present study was to evaluate antibacterial activity of methanolic extract and essence of sagebrush against four strain including Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa.

Materials and methods:

Fresh leaves of plants was prepared from Shiraz and approved by botanical Department of Islamic Azad University, Ahar branch. Clevenger apparatus was used for obtaining the essence. For this mean, 300g of dried plant powder was heated with 1 liter distilled water by Clevenger apparatus. Obtained essence was dehumidified using the sodium sulfate and was kept in dark and closed places at 20℃. for extracting, 1000g of dried plant was poured into the mixture of chloroform and methanol at the ratio 70:30 for 24 hour. Then the mixture was underwent rotary evaporator (Heidolph wb2001, Germany) in order to obtain the net extract.

The resulting crude extract dissolved in a minimum of hot methanol then was putted into the refrigerator at -15℃ then were filtered in order to obtaining the fat-free extract. Then extract was dehydrated using the rotary evaporator and sodium sulfate. The different concentrations of extract 20, 30, 50 and 400mg/ml which were obtained by using DMSO were used in MIC and disc diffusion exams.

The used genera in this experiment were Bacillus cereus (ATCC:1247), Staphylococcus aureus (ATCC:25923), Pseudomonas aeruginosa (ATCC:27853), and Escherichia coli (ATCC:25922) which were in lyophilize form. Bacterial concentration was prepared according to the McFarland standards. For assessment of anti-microbial activity of extract, 4 concentration of methanolic extract (20, 30, 50 and 400 mg/ml) in DMSO 5% were prepared.

In this study, both of Agar well diffusion and dilution test were used. In former, 500 ml of bacterial suspension was transferred onto the Mueller-Hinton agar medium and was cultured using the swab in 3 different directions. Then wells with 6mm in diameter and distance 2.5cm from each other were made on medium and extract was poured into these wells. DMSO 5% and chloramphenicol were used as negative and positive controls, respectively.

Mediums were incubated at 37℃ for 24 hours then were assayed in point of existence or absence of shadow.

For determination the MIC, serial dilutions of extract (6.25, 12.5, 25, 50, 100 and 200 mg/ml) in Mueller-Hinton broth medium were prepared. Then 1ml of bacterial suspension was added for each tube. Beside of this, positive (medium with bacterium without extract) and negative (medium without bacterium) controls was also prepared. Then, tubes were incubated at 37℃ for 24 hours. At the end, tubes were assayed in point of opacity. The latest dilution without opacity considered as MIC. Then, from tubes without bacterial growing, cultures were made in order to determination the minimum bactericidal concentration (MBC). Then, plates were incubated at 37℃ for 24 hours and plate with minimum extract content without bacterial growth considered as MBC. The same method used for determination of antibacterial effects of plant essence.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P<0.001$ was considered statistically significant.

Results:

The results of well diffusion method are given in table 1. Data showed that Staphylococcus aureus and Bacillus cereus have the most susceptibility against extract dose dependently as showed as increase in halo diameter.

Also, data showed that the anti-bacterial effect of extract on gram negative bacteria was at minimum level so that there was no growth preventive activity on pseudomonas. The dilution 400 mg/ml showed a little preventive activity against E.coli. The values of MIC and MBC are given in table 2. Data showed that the dilution 25mg/ml has bactericidal activity on S.aureus. The concentration was obtained 12.5 mg/ml. Thus, there was statistically significant among tested bacteria ($P<0.001$). The maximum and minimum susceptibility to extract was due to B.cereus and P.aeruginosa, respectively. Data related to antibacterial effect of essence showed that the concentration of 1000mg/ml has preventive activity against B.cereus, S.aureus, and E.coli and don't has activity against P.aeruginosa.

Table 1: The diameter of halos in term of mm obtained from bacterial strains against different concentrations of extract

Bacterial Strain \ Concentration (mg/ml)	400	50	30	20	Negative Control	Positive Control
Staphylococcus aureus	18	10	9	7	-	20
Bacillus cereus	18	8	8	-	-	19
Escherichia coli	12	-	-	-	-	26
Pseudomonas aeruginosa	-	-	-	-	-	22

Table 2: MIC and MBC of extract against tested bacteria

Bacterial Strain \ Concentration (mg/ml)	MBC	MIC
Staphylococcus aureus	25	12.5
Bacillus cereus	12.5	6.25
Escherichia coli	200	100
Pseudomonas aeruginosa	-	-

Discussion:

Commonly known as mugwort, Artemisia vulgaris is a perennial herb that can reach 60-160 cm high, with many thin lateral roots. The branched, purplish brown stems are parallel grooved, with ascending twigs covered with short hairs. Leaves are papery, pubescent, dark green on the upper surface, and have various shapes depending on their position on the plant. The leaves near the base are elliptic and oblong, bipinnate (divided two times) deeply even nearly to midrib. The leaves in the middle are elliptic to ovate, 3-10 cm long and 1.5-6 cm wide, pinnate or bipinnate, with four to five lobes that are elliptic lanceolate or linear lanceolate, 3-5 cm long and 1-1.5 cm wide, with more than one tooth on the tip. The leaves near the top are small, also pinnate and lanceolate-lobed, not significantly toothed or even entire. Head inflorescences are oblong, 2.5-3 mm in diameter, borne densely in a spike on the branched twigs as well as spreading panicles on the stems. There are seven to ten purple female flowers, with narrow tube-shaped corollas. Bisexual flowers number 8-20, with tube or goblet shaped corollas, and long densely ciliate hairs at the top of the style. Fruits, appearing from August to October together with flowers, are obovate or ovate achenes.

Akrout et al. (2010) reported the antimicrobial and antiradical activities of the essential oil of A.campestris originating from Tunisia. Their low observed antiradical effect is similar to that of our sample, but their observed antimicrobial activities were quite different. Tunisian essential oil showed a strong effect on E. coli, while S. aureus was the most sensitive bacteria in our samples. This difference might be due to the different essential oil compositions. Methanolic extracts of A.campestris were also evaluated for antibacterial properties by Naili et al., 2010. The extract was reported to have a strong effect on S. aureus and Bacillus subtilis strains.

The essential oil of A.scoparia was also reported to have an antimicrobial effect on 15 oral bacteria when tested using the minimum inhibitory concentration method by Cha et al., 2005. Limited reported effects on S. epidermidis and E. coli are similar to our results, but we report a higher effect on S. aureus. Ramezani et al. (2004) also reported that Iranian A.scoparia extract showed an inhibition zone (13.6 mm) against S. aureus but not against P. aeruginosa.

In one study by Satyal et al., 2012 it has been revealed that the essential oil of Nepalese A. vulgaris was rich in alpha-thujone (30.5%), 1,8-cineole (12.4%), and camphor (10.3%). The essential oils were screened for phytotoxic activity against Lactuca sativa (lettuce) and Lolium perenne (perennial ryegrass) using both seed germination and seedling growth, and all three Artemisia oils exhibited notable allelopathic activity. A. dubia oil showed in-vitro cytotoxic activity on MCF-7 cells (100% kill at 100 microg/mL) and was also marginally antifungal against Aspergillus niger (MIC= 313 microg/mL). DFT calculations (B3LYP/6-31G*) revealed thermal decomposition of ascaridole to be energetically accessible at hydrodistillation and GC conditions, but these are spin-forbidden processes. If decomposition does occur, it likely proceeds by way of homolytic peroxide bond cleavage rather than retro-Diels-Alder elimination of molecular oxygen.

Masoudi et al., 2012 showed that Menthyl acetate (26.5%, 22.0%, 20.5% and 20.5%) and (Z)-nerolidol (20.8%, 26.3%, 14.7% and 18.1%) were the main constituents in the aerial parts, stem, leaf and flower oils, respectively. The other main component in the aerial parts, leaf and flower oils was 1, 8-cineole

(13.9%, 11.7% and 12.8%, respectively). Yomogi alcohol (10.4%) and artemisyl acetate (10.4%) were the main components of the leaf and flower oil of the plant, respectively. their data showed no significant difference between compositions of the aerial parts, stem, leaf and flower oils. They identified Twenty-four compounds representing 90.5% of the oil of the aerial parts of A. turcomanica, of which 1,8-cineole (15.5%), spathulenol (15.2%), camphor (14.8%), santolina alcohol (14.6%) and trans-beta-terpineol (11.6%) were the major ones.

According to the results obtained by Raeisi et al., 2012, minimum inhibitory concentrations (MIC) for E. coli and S. aureus were 2500 and 1250 µg/mL, respectively. Also, minimum bactericidal concentration (MBC) for the mentioned microorganisms was 5000 and 2500 µg/mL, respectively. All the EO concentrations for each bacteria result in reducing bacterial count of cheese samples compared to control ($P<0.05$). Also, with increasing concentration of EO in cheese samples, the bacterial count was reduced further ($P<0.05$).

Habibi et al., 2013 also mentioned that the essential oil of Artemisia was highly active against Escherichia coli and Enterococcus faecalis.

By comparison of our results with those of previous studies shows that the locality of the plant material and the extraction procedure cause differences in the antimicrobial activity of the plants. The strong effects of the essential oils of A.vulgaris are probably due to the high α-thujone content.

Acknowledgment:

This study was Adapted from a research plan which was supported financially by Islamic Azad University, Ahar branch. So, author declare own thankful from grant staff of research deputy of Islamic Azad University, Ahar branch.

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5/21/2018