

Study the anti-microbial effects of *Artemisia santonica* extract on some pathogenic bacteria

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ABSTRACT

Background and aims: It is essential to find new compounds with antimicrobial activity, for rapid growing drug resistance among pathogenic microorganisms. Medicinal herbs application in traditional treatment for thousand years makes this natural compound to the best choice for antimicrobial studies. The aim of this study was to determine the antimicrobial activity of *Artemisia* against some important pathogenic bacteria.

Methods: In the present study, antimicrobial activity of ethanolic and methanolic extracts of *A. santonica* against 4 pathogenic bacteria was evaluated. *A. santonica* dry powder was extracted with ethanol and methanol solvents in maceration method. Assessment of antimicrobial efficacies of extracts and MIC were conducted by disk diffusion manual and macro broth dilution method, respectively.

Results: Both ethanolic and methanolic extracts could inhibit microorganism in this test. *Staphylococcus aureus* with MIC=1.56 mg/ml indicates more sensitive to the *Artemisia*. In addition, *Bacillus cereus* showed medium sensitivity. *E.coli* and *Pseudomonas Aeruginosa* became involved in less sensitivity to the *A. santonica* extracts.

Conclusion: The result of this study confirmed that *A. santonica* extracts were the best inhibitory effect on *S. aureus*. Then it can be concluded that by using herbal plant against antibiotics some antibiotic resistant problem can be resolved.

Keywords: *Artemisia santonica*, Pathogenic bacteria, Antimicrobial activity, Drug resistance.

Original article

INTRODUCTION

Nowadays, increase in resistance to anti-bacterial factors among the pathogenic micro-organisms is considered as one of the most important barriers for control of septic diseases. Therefore, several efforts were carried out to find new compounds with anti-bacterial properties in order to substitute or improve antibiotics and antiseptics. Medicinal Plants and their natural derivatives had some evidences about curing diseases because herbal compounds had been one of the first choices of human being for disease

curing from thousands years ago and amount of their qualities has been identified during the years. Additionally, public acceptability of using the medicinal plants, easy access, little production cost, and low side effects of these compounds to common anti-bacteria factors make herbal derivatives a suitable choice for controlling the pathogenic micro-organisms.¹

A. santonica with scientific name of (*Artemisia*) belongs to succory family consisting of diverse species. This plant consists of shrubs with aromatic leaves having

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deep cuts. In traditional medicine, *Artemisia* is used for excreting of intestinal worms, headache treatment, tremor, epilepsy and rheumatism.² It is proved that the most chemical compounds are from flavonoids and terpenoids. The anti-bacterial property of this group of compounds is approved in many plants.³ These anti-bacterial effects of many *Artemisia* species on the pathogenic micro-organisms have been determined in literature. *Artemisia sieberi* is one of the existent *Artemisia* species in Iran that has shown a suitable controlling effect on several important pathogens particularly *Yersinia enterocolitica*.⁴ Hakimiet et al. investigated anti-bacterial effect of *Artemisia persica* on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Enterobacter*, *Enterococcus* and *Candida albicans*.⁴

This study was conducted in microbiology laboratory of Science School of Bahonar University of Kerman. Anti-bacterial effect of *A. santonica* on positive hot bacterium of *S. aureus* and *B. cereus* and also on negative hot bacterium of *E. coli* and *P. aeruginosa* was studied in this research. Mentioned microorganisms are main human pathogens that have made different clinical troubles by resistance to various antibiotics during recent years.

METHODS

The micro-organisms of *S. aureus*, *P. aeruginosa*, and *E. coli* strains were isolated levels of patients and were provided from microbiology laboratory of medical Science University of Kerman. Also, *B. cereus* was the isolated level of livestock supplied from Veterinary School of Livestock.

Collection, identification and preparing the herbal extracts *A. santonica* was done from environs of Kerman and after scientific recognition was completely blasted on shade. Then, dried pieces were ground as much as possible and this powder was used for

producing the herbal extracts. This extraction was done in the manner of Maceration with ethanol and methanol solvents. Two gram *A. santonica* powder was added to both Erlenmeyer containing 100 ml ethanol 80% and 100 ml methanol 96% in order to prepare an extract with 100 mg/ml concentration. Foresaid Erlenmeyer reposed in darkness with room temperature for 24h. Then, for removing the plant pieces, the solution was passed Whatman filter paper No.1 and was held in dark containers in the refrigerator until utilization time.⁵

The antibacterial property of *A. santonica* extract studied bacterium was analyzed in the manner of disk diffusion. For preparing disks containing the extract, 10 ml ethanol and methanol extract of *A. santonica* was sterilized by passing Millipore filter and then sterilized blank disks was added to each one for one hour. After this period, the disks were bought out of extract solution by forceps under specific conditions and transferred to dry heat 40°C.

In order to evaporate additional solvent in the sterilized plate, antibiotic chloramphenicol utilized as positive control and disks impregnated with ethanol and methanol was also used as negative. 24 hours cultivation of tested bacterium in nutrient broth has been implemented to prepare the disk diffusion test. At first, microbial suspensions were diluted until reaching a turbidity equivalent to 0.5 Mac forland and then a sample was taken from each of them by sterile swab and table culture was done in Muller Hinton broth. The disks containing extract, solvent and antibiotic were put on the medium at regular intervals by sterile forceps and plate was incubated at temperature of 37°C for 18 to 24h. Inhibitory zones diameter of mentioned disks were measured after incubation by millimeter ruler. This phase was repeated three times.⁶

Minimum inhibitory concentration (MIC) and Minimal Bactericidal Concentrations (MBC) of the extracts of *A. santonica* were

determined by macro broth dilution method according to CLSINC protocols.

Stored extract solution has been used to prime 10 dilution 0.02 to 50 mg/ml by the method of successive dilution and determining minimum inhibitory concentration. In this test, 10 sterile test tubes were selected and 1 ml. Nutrient broth brought in tubes from 2 to 10.

Then, we transferred 1ml. from first tube containing 2ml. extract 100 mg/ml. to second tube and mixed well unit final concentration reached 50 mg/ml. Similarly next dilutions of extract were prepared in tubes from 3 to 10. Then, 1 ml. 24 hours bacteria suspension that had grown in nutrient broth and was reached to 0.5 Mac for land turbidity was added to each dilution. In this way, final concentration of extract was hold in tubes. For example, final concentration of extract was 50 mg/ml in tube No.1. In this test, positive control tubes contained microbial suspension and antibiotic ciprofloxacin 0.2%, negative control tubes

contained microbial suspension and distilled water and tubes contained intended dilution of extract and nutrient broth. From the experiments, all mentioned steps performed in sterile condition and tubes were incubated at 35°C to 37°C for 16 to 18 hours. After this time, positive and negative control were compared and minimum concentrations in bacteria growing were inhibited, and were selected as MIC.⁷ The results reported from the tests were analyzed using ANOVA test at significant level less than 0.05.

RESULTS

The zone of inhibition (ZOI) for methanolic and ethanolic extracts of *A. santonica* and the MIC and MBC values of these extracts were illustrated in Table 1. The extracts of *A. santonica* have the best inhibitory effect on *S. aureus* in disc diffusion analysis,

Table 1: Inhibitory zones diameter of ethanol and methanol extract of *Artemisia* by the method of disk diffusion (mm) and calculated MIC (mg/ml) and MBC (mg/ml)

Microorganism	Methanol extract (mm)	Ethanol extract (mm)	Chloramphénicol (mm)	Solvent control	MIC mg/ml	MBC mg/ml
<i>Staphylococcus aureus</i>	15±0.6	13±0.5	30±0.8	0	1.56	3.12
<i>Bacillus cereus</i>	13±0.2	11±0.3	20±0.6	0	3.12	4.12
<i>E.coli</i>	12±0.3	9±0.7	28±0.5	0	6.25	7.56
<i>Pseudomonas aeruginosa</i>	12±0.8	9±0.5	25±0.4	0	6.25	7.78

Accordingly to Table 1 methanolic extract of *A. santonicat* have more antibacterial property than ethanolic extract. Also the results of the test determining minimum inhibitory concentration is related to *Staphylococcus aureus* (MIC=1.56 mg/ml) and *Bacillus cereus* have medium sensitivity (MIC=3.12 mg.ml) and *P.aeruginosa* shows minimum sensitivity (MIC=6.25 mg/ml). In this test, calculated MIC for methanol and ethanol extracts were equal (Table 1).

DISCUSSION

Despite increasing advances in medical science and the development of treatment techniques, infectious diseases are still considered as major cause of worldwide deaths. Pathogenic microorganisms have different ways to deal with antimicrobial agents such as antibiotics and indiscriminate uses of these compounds have led to the development of drug resistance. Drug-resistant bacteria, is easily crossed antibiotic treatment

and create many clinical problems. Even drug-sensitive bacteria that are able to form biofilms, when placed in this structure will respond differently to antibiotics.

In this research, antimicrobial effect of *Artemisia* against selected bacterial pathogen was evaluated. These results show that the antibacterial effect of *A. santonica* extracts increased after concentrating of extracts by maceration method. The results of this research and other similar researches confirm the presence compounds with antibacterial properties in *A. santonica*. Erel and et al. studied similar species of *Artemisia*.⁸ They reported the inhibitory zones diameters for standard levels of *S. aureus*, *P. aeruginosa* and *E. coli*, as 24, 14 and 10 mm, respectively.

Although difference between findings obtained by the current study and Erel research is marginal, this issue is related to the medicinal resistance of clinical bacterial levels toward standard levels applied in Erel's investigations and different extraction methods.⁸ Considering the antibacterial properties that are found in many *Artemisia* species in different studies, the most of researches are recommended to purify active compounds of this plant. Then, these compounds are used for providing new antibacterial factors and are applied in organisms, if it is possible.⁹

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest.

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