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ORIGINAL ARTICLE

Activity of *Peganum harmala* extract against antibiotic resistant *Staphylococcus aureus*

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ABSTRACT

Many naturally occurring compounds found in plants herbal, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial against pathogens. The present study was carried out to determine the potential antibacterial effect of ethanol extracts of P. harmala against antibiotic resistant S. aureus. All 20 strains of S. aureus isolated from infected and the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this extract. The result show that the least MIC value of alcoholic extract of P.harmala was 0.62mg/ml and the highest MBC value of alcoholic extract of P.harmala was 10mg/ml.
Key word: Peganum harmala, Antibacterial activity, Staphylococcus aureus

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INTRODUCTION

The incidence of severe infections in humans caused by pathogenic microorganisms has increased globally and is a key cause of morbidity and mortality in developing countries (1). In recent years, resistance of human pathogenic microorganisms to drug has been frequently and extensively reported (2, 3, 4, 5). Medicinal plants were the first medicines and have been used since ancient times (6), and they continue to be used by various cultures around the world (7). Peganum *harmala* L. (Zygophyllaceae), that is also called Harmal, Suryin Rue, is a perennial, bushy, and wild-growing flowering plant with short creeping root which may grow to 30-100 cm high (8, 9, 10) is known as "Espand" in Iran and Harmal in North Africa and African Rue, Mexican Rue, Syrian Rue or Turkish Rue in United States (10). *Peganum harmala* is used as an analgesic and anti-inflammatory agent. *Peganum harmala* has antibacterial activity, including antibacterial activity against drug-resistant bacteria. The present study was carried out to determine the potential antibacterial effect of ethanol extracts of *P. harmala* against antibiotic resistant *S. aureus*.

Plant materials:

The seed *P. harmala* was collected in the region of Iran and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of extracts:

Plant was properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman No. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, dried extracts were obtained and then stored at 4ºC in air tight screw-captube.

***Staphylococcus aureus* isolation and culturing:**

Cross sectional study was performed and samples were collected from infected quarter just on time from each suffering man. An aliquot (10 µl) from each sample was spread over blood agar (Merck, Germany) (pH=6.5) plate and incubated at 37ºC for 24 h. Isolated Gram and catalase positive cocci were further tests for biochemical characterization viz. carbohydrates fermentation followed by urease, vogues-proskauer, arginine utilization, lysostaphin sensitivity, coagulase, clumping factor thermonuclease, haemolysin tests .

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts:

The broth microdilution method was used to determine MIC and MBC. Alltests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from0.3 mg/ml to 10.00 mg/mL. To each well, 10 µL of indicator solution (prepared by dissolving a 10 mg extract in 2 ml of DMSO) and 10 µL of Mueller Hinton Broth were added. Finally, 10 µL of bacterial suspension (10^6 CFU/mL) was added to each well to achieve the concentration of 10^4 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37ºC for 18- 24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested extracts. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

Statistical treatment of the results:

The mean values were analyzed with the MINITAB Release 13.20 program statistically by the general one-way analysis of variance (ANOVA) to find out the most effective plants and the most sensitive test organisms.

RESULTS AND DISCUSSION

Different inhibitory effects of alcoholic extract from *P. harmala* against most *S. aureus* isolates were demonstrated in table 1.The results in tables 1 showed that ethanol extract of *P.harmala* had inhibitory effect against most isolated plates. The least MIC value of alcoholic extract of *P.harmala* was 0.62mg/ml and the highest MBC value of alcoholic extract of *P.harmala* was 10mg/ml(Table1). The study of Darabpour, the result showed that MIC and MBC values for the seed and root extract of *P. harmala* against MRSA are equal (0.625 mg/ ml). Also, these values for seed extract against *E. coli* and *S. typhi* were the same (0.625 mg/ml) while for the root extract were different (11). An ethanolic *P. harmala* extract has been shown to have high antibacterial activity against *MRSA* (methicillinresistant *Staphylococcus aureus*) (12) and *CRSA* (cefixime resistant *S. aureus*) (13). The study of Fazal, *P. harmala* extracts in ethanol and hexane exhibited best activity against *K. pneumonia* (25.8 mm and 22.5 mm) respectively. The chloroform extract was best effective against *S. aureus* (24.5 mm) (14). Among the evaluated different parts of P. harmala, the seed and root extracts showed the best antibacterial activity against Gram positive bacterial species, including Bacillus anthracis, Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes and Streptococcus pyogenes and Gram negative bacterial species, including Pseudomonas aeruginosa, Brucella melitensis, Proteus mirabilis, Salmonella typhi, Escherichia coli and Klebsiella pneumonia (11). The observed antibacterial activity of *P. harmala* might also be attributed to the high quantity of polyphenols, which are known to possess efficient antibacterial activity (15).Thus on the basis of the results it is inferred that the ethanol extract of *P. harmala* whole plant had *in-vitro* antibacterial. Further phytochemical studies are needed to identify active constituents responsible for the observed activity.

Table1. Minimum inhibitory concentration of *P.harmala* extract against *S. aureus* (%)

***P.harmala* concentration(mg/ml)**	0.3	0.62	1.25	2.5	5	10
MIC	0	11.76	29.11	23.52	17.64	0
MBC	0	5.88	17.64	47.08	70.58	88.23

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