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Antimicrobial Activity of *Prosopisfarcta* L. and *Datura stramonium* L. Extracts Against *Staphylococcus aureus*

Saeide Saeidi[1], Nafiseh Mahdi Nezhad[2], Majid Sharifi-Rad[3], Fereshteh Javadian[1*], Elham Javadian[1]

[1]*Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, Iran;*
Email:** ***Fereshteh.Javadian@yahoo.com

[2]*Department of plant Breeding and biotechnology, University of Zabol, Zabol, Iran;*

[3]*Department of Range and Watershed Management, Faculty of Water and Soil, University of Zabol, Zabol, Iran;*

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ABSTRACT

Background & Aim: The aim of this study was to investigate the antimicrobial activity of two rangeland-medical plants extracts, *Prosopis farcta* L. and *Datura stramonium* L., against *Staphylococcus aureus* isolated from sheep in Zabol city.
Experimental: The *P. farcta* and *D. stramonium* were collected from the rangelands of Zabol city. *Staphylococcus aureus* strains were isolated from the nose of sheep in Zabol city. Antibiotic resistance pattern was determined by Kirby Bauer method. Finally, the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) were determined by microdilution method.
Results: The results of this study showed that the *P.farcta* extract had a MIC of 25 ppm, and 10 strains of the bacterium were inhibited in this concentration. The lowest inhibitory concentration of *D.stramonium* extract was 6.25 ppm, and one strain was inhibited in this concentration. On the other hand, the highest inhibitory concentration was 50 ppm and the highest bactericide concentration was 100 ppm.
Recommended applications/ industries: The results of this study showed good antimicrobial effects of *D. stramonium* and *P. farcta* extracts that can be used to treat *Staphylococcus aureus* infections.

1.Introduction

Staphylococci are one of the most resistant bacteria that are scattered and expanded. These bacteria are among the first known human pathogens that can be colonized on the skin and mucous membranes (Japooni *et al.*, 2004). Among the various species of this genus, *Staphylococcus aureus* is the most important pathogen that has become one of the major public health concerns due to its inherent ability to gain resistance to antimicrobial agents (Rahimi *et al.*, 2008).

Medicinal plants are rich sources of natural antibacterial. These plants use in traditional medicine for the control and treatment of many diseases. Without any academic confirmation, the *Prosopis farcta* is used to treat diabetes by the tribal people in Jordan (Al-Aboudi and Afifi, 2011). Leaves and beans of *P. farcta* have been used as traditional medicine (Ali-Shtayeh *et al.*, 2008). *P. farcta*, the Syrian mesquite, is a species of the genus Prosopis, growing in and around the Middle East. *P.farcta* is a below-ground tree. Above ground, it looks like a shrub with a height of 20–100

cm (in rare cases up to 4 m height). In addition, below ground the mesquite has a root system which is really a trunk with branches going as deep as 20 m or more underground. So, it is really a tree, and only the treetop protrudes above ground level. The "treetop" consists of a collection of shrubs which can extend over 1000 square meters or more, all of them connected to the same trunk. Medicinal properties of this plant include the treatment of gastric ulcer, abortion, bloody diarrhea, rheumatism, laryngeal inflammation, heart disease and shortness of breath (Al-Qura, 2008). In other studies, it has shown anti-diabetic properties (Jarald *et al.*, 2008) and the antispasmodic, anti-inflammatory and anti-inflammatory properties of the plant have also been reported (Fraz, 2009).

Datura stramonium is a widespread annual plant from the Solanaceae family. It is one of the widely well-known folklore medicinal herbs. It is a wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom ($N\text{-}CH_3$) and include the anti-cholinergic drugs atropine. *D. stromonium* is probably originated in Caspian Sea territories and spreaded to Europe in the first century. At present, it grows in waste places in Europe, Asia, America and South Africa. *D. stromonium* is cultivated in Germany, France, Hungary, South America and throughout the world (Jarald and Edwin, 2007).

The aim of this study was to investigate the antimicrobial activity of *P.farcta* and *D. stromonium* extracts on *Staphylococcus aureus* isolated from sheep nose in Zabol city.

2. Materials and Methods

2.1. Plant materials

The plant material used in this study consisted of fruits of *P. farcta*and leaves of *D. stromonium*, collected from the varied rangeland areas in Zabol, Iran.

2.2. Preparation of ethanol extract

The samples of each plant were separately dried, powdered and dissolved in 200 ml ethanol 85% using a shaker water bath for 24 h at room temperature. After filtration with Whatman No. 1 filter paper, the extract was concentrated using a rotary evaporator (Heidolph-Germany) at 40°C for 40 min. The semisolid extract produced was kept in at -80°C overnight and then subjected to freeze dried for 24 h, at -70°C in 200 ml vacuum. For further use, the extract was stored in airtight container at 4°C in refrigerator (Miri *et al.*, 2013).

2.3. Isolation of bacteria

Isolation of bacteria was carried out on the anterior part of the nose of 60 sheep in Zabol city by sterile cotton swab. Samples were cultured on a medium of Blag Agar. Suspected colonies were confirmed by biochemical and enzymatic tests and were tested by catalase, coagulase, mannitol fermentation and hot dyeing.

2.4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Concentration Bactericide (MBC)

Sensitivity of the bacteria samples with multiple resistances to the *P.farcta* and *D.stramonium* was analyzed by dilution method in broths. To this end, seven broths of microtitre plates were injected 100 ml of MHB. One hundred milliliter of the diluted extract was added to the first broth. Then, 100ml of the first broth was transferred to the second one and the same was done to the last broth. One hundred milliliter of the last broth was removed and 100ml of the microbial suspension with 10^7 units per ml was added to all broths. The mixture was kept 24 hours at temperature of 37 °C. The first broth that inhibits the growth of bacteria after being positioned in the incubator was considered as MIC and for more precision, 10 ml of the light broths was transferred to Moller environment. After 24 h, the lowest concentration that kills 99.9% of the bacteria was regarded as MBC.

3. Results and discussion

The results of this study showed that *P. farcta* extract had MIC of 25 ppm, and 10 strains of the bacteria were inhibited at this concentration, while the maximum MBC was 50 ppm. On the other hand, the lowest inhibitory concentration of *D. stromonium* extract was 6.25 ppm, and one strain was inhibited at this concentration while the highest inhibitory concentration was 50 ppm and the highest bactericide concentration was 100 ppm (Table 1).

Normano *et al.* (2007) tested 1634 specimens, including 641 and 993 samples of milk and meat products respectively. They reported that 109 samples (17%) of milk products and 100 samples (10%) of meat

products and in total 209 samples (12.8%) were contaminated with *Staphylococcus aureus*. Out of the 1634 meat and milk products, 6 samples (3.75) contaminated with *Staphylococcus aureus* were resistant to methicillin (Normano *et al.*, 2007).

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Prosopis farcta* extract.

Bacteria code	MIC(ppm)	MBC(ppm)
1	25	50
2	25	50
3	25	50
4	25	50
5	25	50
6	25	50
7	25	50
8	25	50
9	25	50
10	25	50

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Datura stramonium* extract.

Bacteria code	MIC(ppm)	MBC(ppm)
1	12.5	25
2	6.25	12.5
3	25	50
4	25	50
5	25	50
6	25	50
7	25	50
8	25	50
9	25	50
10	50	100

In previous studies the resistance of bacteria isolated from mastitis against penicillin has been reported (Moroni *et al.*, 2006; Chanda, 1989; Gentilini *et al.*, 2002; Nazer and Tavakoli, 1994). Guerinfaublee and Tardy (2002) reported resistance to tetracycline. Nunes *et al.* (2007) reported high sensitivity of *Staphylococcus epidermidis* and *Staphylococcus aureus* against endofloxacin.

The antimicrobial activity of *D. stromonium*, *Terminalia arjuna* and *Withania somnifera* extracts was determined by microdilution method. The extracts have good antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus* and *Candida albicans* as compared to the antibiotic ciprofloxacin (Sharma and Sharma, 2010).

Eftekhar *et al*. (2005) examined the antimicrobial activity of *D. innoxia* and *D. stramonium* extracts. The results showed that the extracts inhibited Gram-positive bacteria at different doses, although they exhibited very little antimicrobial activity against *E. coli* and *Pseudomonas aeruginosa* (Eftekhar et al., 2005).

In another study, the results showed that the minimum inhibitory concentration of Jimsonweed against *Pseudomonas aeruginosa*, *Klebsiell apneumoniae* and *Escherichia coli* was 15-25 and 20 (mg/ml) (Baso and Adeyemo, 2006).

The study of Priyatama *et al.* (2016) revealed that *D. stramonium* chloroform seed extract produced maximum zone of inhibition (26 mm) against *Klebsiella pneumonia*, 12 mm against *Bacillus subtilis* and 13 mm against *Escherichia coli. D. Stramonium* methanol seed extract produced maximum zone of inhibition 27 mm against *Pseudomonas aeruginosa*, 15 mm against *Bacillus subtilis*, 14 mm against *Staphylococcus aureus* and 19 mm against *Escherichia coli*. *D. stramonium* petroleum ether seed extract produced 16 mm zone of inhibition against *Escherichia coli*. *D. stramonium* aqueous seed extract exhibits 24 mm zone of inhibition against *Bacillus subtilis* (Priyatama *et al*., 2016).

The study of Sharma et al. (2013) indicated that methanol leaf extract exhibited antimicrobial activity against *S. aureus* (IZ=18.2mm), *E. coli* (IZ=19.8mm), *P. aeruginosa* (IZ=22.2mm), *R. stolonifer* (IZ=21.5mm), and callus exhibited antimicrobial activity against *A. niger* (IZ=12.1mm), *F. culmorum* (IZ=18.9mm) and *A.flavus* (IZ=12.8mm). (Sharma *et al*., 2013).

4. Conclusion

Considering the bacterial resistance to chemical antibiotics and the subsequent present results, it is suggested that, with further studies on the main and effective compounds of this plant, antibacterial compounds of this plant should be used to treatment of bacterial infection.

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