IJNLS
INTERNATIONAL JOURNAL OF NATURE AND LIFE SCIENCES
Research article

International Journal of Nature and Life Sciences (IJNLS)
https://www.dergipark.gov.tr/ijnls
e-ISSN: 2602-2397
Vol. 4(1), June 2020, pp. 40-46

Antibacterial Effect of Methanolic Extract of *Rosa damascena* on Standard Bacteria *Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa* in Vitro

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***Received:** 14 March 2020, **Revised:** 17 April 2020, **Published Online:** 01 June 2020*

Abstract

Staphylococcus aureus, Bacillus cereus, Escherichia coli and *Pseudomonas aeruginosa* are the most important bacteria causing nosocomial infections that have multiple antibiotic resistance. These bacteria can also cause food poisoning and gastrointestinal diseases. Difficulties in treating infections caused by these bacteria have made it a reason to consider alternative medicines, including medicinal plants. In this descriptive-in vitro study, after collecting and drying under appropriate conditions, the extract was prepared by Soxhole method at concentrations of 20 mg/ml to 400 mg/ml. *S. aureus, B. cereus, E. coli* and *P. aeruginosa* were cultured separately in Mueller Hinton Agar medium. MIC and MBC of *Rosa damascena* methanolic extract were evaluated by Agar Well Diffusion and Dilution Test. The results showed that the antibacterial effects of methanolic extract of *R. damascena* against Gram-positive bacteria were more than Gram-negative bacteria, with the highest diameter of growth zone in *S. aureus* (26 mm) and *B. cereus* (24 mm) observed. MIC and MBC of methanolic extract of *R. damascena* on *S. aureus*, *B. cereus* and *E. coli* and *P. aeruginosa* were 6.25 -12.5, 12.5 -25, 50-100, 100-200 mg/ml, respectively. Considering the antimicrobial effect of methanolic extract of *R. damascena* on *S. aureus*, *B. cereus* and *E. coli* and *P. aeruginosa* in vitro, by evaluating its effects in vivo, it can be used as an alternative to conventional chemical drugs in the treatment of infections

Key words: Antibacterial effects, extract, in vitro, *Rosa damascena*

1. Introduction

The medicinal plant is herbs with organs contain substances that affect living things. The use of medicinal plants has been one of the oldest human achievements to treat most diseases. So that in the development of all human civilizations there has always been a close relationship between man and plant (Jafari-Sales et al., 2019). Although most plant species are known to date, there is still much time left to discover new and valuable plant resources (Jafari-Sales and Hossein-Nezhad, 2020). Which are only partially identified so far. These chemicals can be used as a drug but also as a unique starting point for the manufacture of pharmaceutical analogs, as well as an interesting tool to better understanding biological phenomena (Jafari-sales and Shadi-Dizaji, 2019; Skaltsa et al., 1999). *Rosa damascena* as an ornamental plant commonly known in Iran as "Mohammadi flower". This plant has an important place in Iranian traditional medicine and is economically valuable (Mahboubi, 2016). Because of the side effects of antibiotics and also because of the increased resistance of microorganisms, the use of medicinal plants is more popular against bacterial infections (Gholami et al., 2019). This plant has antidepressant effects, sedative, anti-itch and dry skin effects, reduces sympathetic system and strengthens parasympathetic system. Other properties of this herb include treatment for inflammation of the liver, asthma and cough (Shahinfar et al., 2017). Citronellol and geraniol are the main constituents of the essential oils of *R. damascena* essential oils, making the plant antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-diabetic and antidepressant. *R. damascena* Mill is a hybrid between *R. gallica* and *R. phenicia* and is a member of the Rosaceae family with over 200 species and 18,000 varieties worldwide (Mahboubi, 2016). It is a shrub 2 m high and its leaves 2 to 6 cm high. Its flowers are pink to red, as well as the oval-shaped fruit, and red when it arrives (Masoumi et al., 2014). Extraction of essential oils from its flowers begins from the seventh month (Mahboubi, 2016). The *R. damascena* extract extracted from sepals and petals of the plant contains a number of effective substances, of which 300 are different types, 50 of which are known. The most effective ingredients of this plant are: 20-25% Stearapten, 40-60% Citronellol, 30-40% Geraniol and 20-30% Linalool (Zarghami et al., 2001). Compounds including glycosides, anthocyanins, flavonoids and terpenes have also been isolated from *R. damascena*. It has also been reported to have vitamin C, quercetin, carboxylic acid, camphor and myrcene (Katayoun and Mehdi, 2015; Jager et al., 2007). The aim of this study was to evaluate the antimicrobial properties of methanolic extract of *R. damascena* on some pathogenic bacteria in vitro.

2. Materials and Methods

In this descriptive in vitro study, saffron petals were collected from a field around the town of Marand in East Azarbaijan province. The petals were placed at ambient temperature for drying in the dark and were subjected to several steps until complete drying. After the samples were completely dried, the petals were prepared for grinding. Soxhlet method was used to extract 60 grams of petal powder with 300 ml of methanol as solvent for 8 hours in Soxhlet extractor. This solvent was slowly evaporated at 40 °C using rotary apparatus. The concentrated extract was obtained from it. Extracts of solvent concentrated 5% DMSO (Dimethylsulfoxide) at concentrations of 20, 30, 50 and 400 mg/ml for use in Minimum Inhibitory Concentration (MIC), Minimum

Bactericidal Concentration (MBC) and Agar Well Diffusion experiments was prepared. The microorganisms studied in this study were: *S. aureus* ATCC 25923, *B. cereus* ATCC 1052, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 (Microbial collection from University of Tehran). A separate culture was performed on the Mueller Hinton Agar medium to allow emerging colonies to be prepared with a 0.5 McFarland turbidity solution (1.5 × 106 cfu/ml). For this purpose, for preparation of microbial suspension, 4-5 colonies of bacterial culture were transferred to Mueller Hinton Broth to adjust the microbial suspension turbidity according to standard 0.5 McFarland tube. To reach a concentration of 1.5 ×106 cfu/ml, the microbial suspension was diluted to 0.01. In order to evaluate the antibacterial effect of methanol extract 4 concentrations of 20, 30, 50 and 400 mg/ml of methanolic extract were prepared in 5% DMSO solvent. In this study, the antimicrobial effect of methanolic extract was investigated by agar well diffusion and dilution test. In the agar well diffusion method, 500 ml of microbial suspension was transferred onto Mueller Hinton Agar medium and cultured by sterile swab in 3 directions. Then wells were prepared at 6 mm in diameter and 2.5 cm apart on agar surface. Then 100 µl of 20, 30, 50 and 400 mg/ml concentrations of methanol extract were injected into each well. Negative control was obtained using a solution used to dissolve the extracts (5% DMSO) and chloramphenicol antibiotic was used as positive control. Plates were then incubated at 37 °C for 24 hours and microbial cultures were measured for the presence or absence of growth zone in millimeters. The MIC and MBC of methanol extract were determined by tube dilution method. In this method, to determine the MIC of methanolic extract prepared by dilution serial dilutions of 6.25, 12.5, 25, 50, 100 and 200 mg/ml in Mueller Hinton Broth. Then, 1 ml of 1.5 × 106 cfu/ml active bacterial suspension was added to each dilution. Positive control (culture medium containing no bacterial extract) and negative control (culture medium without bacterium) were added to the tubes. Finally, the tubes were incubated at 37 °C for 24 hours. After incubation, the tubes were examined for turbidity caused by inoculated bacterial growth and the last dilution in which no turbidity was observed (non-growth) was considered as MIC. Samples were then taken from all tubes in which bacterial growth was observed and MBC was determined by plate culture. Plates were then incubated for 24 hours at 37 °C. The tube containing the lowest concentration of the extract that had no visible bacterial growth on the plate was considered MBC of that material. Each experiment was repeated 5 times to reduce the error of the experiment. SPSS software version 18 was used for data analysis. Analysis of variance and chi-square test were used to investigate the significant differences between the two groups and the significance level was set at $p < 0.05$.

3. Results

Comparison of different concentrations of methanolic extract of *R. damascena* with Agar Well Diffusion and Dilution Test methods on four strains of *S. aureus, B. cereus, E. coli* and *P. aeruginosa* showed that the growth inhibitory effects of methanolic extract of this plant on Gram-positive bacteria were more than Gram-negative bacteria. The methanolic extract of this plant has the most effect on *S. aureus* and *B. cereus* (Table 1). The concentrations of the MIC and MBC of methanolic extract of *R. damascena* against the four tested bacteria showed that the lethal concentration of this extract against *S. aureus* and *B. cereus* was 6.25 -12.5, 12.5 -25, 50-

100, 100-200 mg/ml, respectively. These results indicated that there was a significant difference between the tested bacteria in the sensitivity of the extract of *R. damascena* ($P < 0.05$).

Table 1. Mean diameter of non-growth zone of methanolic extract of *R. damascena* against selected bacteria (mean ± standard deviation).

Concentration of extrac (mg/ml) / Bacterial strain	20	30	50	400	Negative control	Positive control
Staphylococcus aureus	9.71±.14	15±1.70	20.2±0.83	26.2±1.30	--	22
Bacillus cereus	8.2±1.34	14±1.22	18.4±1.14	24.6±0.54	--	20
Escherichia coli	0	10.2±0.54	14.8±0.83	18.4±1.09	--	25
Pseudomonas aeruginosa	0	0	7.2±1.30	10.6±1.14	--	22

Table 2. MIC and MBC values of methanol extract of *R. damascena* (mg/ml).

Concentration of extract / Bacterial strain	MIC	MBC
Staphylococcus aureus	6.25	12.5
Bacillus cereus	12.5	25
Escherichia coli	50	100
Pseudomonas aeruginosa	100	200

4. Discussion

Due to the increased resistance of bacteria to a variety of antibiotics, the use of antimicrobial compounds in plants as natural compounds that have lethal and inhibitory effects on pathogens has become more popular (Jafari-Sales et al., 2019; Izadi et al., 2014). From the past to the present day, herbs have been widely used in traditional medicine and modern medicine due to the medicinal properties of their compounds (Gholami et al., 2019). Given the predominant resistance of *S. aureus, B. cereus, E. coli*, and *P. aeruginosa* to a wide range of antibiotics, we decided to investigate the antibacterial effect of the extract of *R. damascena* as a suitable alternative to antibiotics. Mankar (2015) concluded in a study that screened the antibacterial activity of rose species against pathogenic bacteria that among the species of rose family plants, 19 species had moderate to strong antimicrobial effects and 3 species had weak antimicrobial effects. Iisin et al., (1999) in a study on the antibacterial effects of essential oils including *R. damascena* on *S. aureus, E. coli*, and yeast *C. albicans* by Agar Diffusion Test and Serial Dilution Test, concluded that all the tested oils had antimicrobial effects against *S. aureus, E. coli* and yeast *C. albicans*, but none of them were effective against *P. aeruginosa*. In a study on the biological properties of roses, Dehghan Kashani et al., (2010) investigated the antimicrobial effects of the plant by disc diffusion on *E. faecalis, E. coli, S. aureus, P. aeruginosa* and *K. pneumoniae*, concluded that both alcoholic and aqueous extracts of *R. damascena* only had antimicrobial activity against *S. aureus* and other microorganisms were resistant to this extract. Eman (2014), in a study entitled antimicrobial activity of *R. damascena* petal extract and chemical composition using gas chromatography-mass spectrometry analysis, concluded by agar diffusion method that the methanolic, ethanolic and aqueous extracts of *R. damascena* have antimicrobial activity against Gram-negative and positive bacteria and the highest antimicrobial activity is related

to the ethanolic extract that affects *E. coli* and *P. aeruginosa* bacteria. Shohayeb et al., (2014) in a study of the antimicrobial and antifungal effects of *R. damascena*, showed that the susceptibility of Gram-positive bacteria was higher than Gram-negative. Tsai et al., (2008) reported MIC of methanolic extract of *R. damascena* in *S. mutans, S. sobrinus, S. sanguis* more than 8 mg/ml. Ozkan et al., (2004) in a study on antioxidant and antibacterial activities of *R. damascena* extracts, investigating the effect of *R. damascena* on 15 bacterial species *B. cereus, M. smegmatis, E. coli, E. faecalis, P. vulgaris, E. aeroginosa, K. pneumoniae, P. aeruginosa, S. enteritidis, S. typhimurium, S. aureus, A. hydrophila, P. fluorescens* and *Y. enterocolitica* concluded that the extract of this plant inhibits other pathogenic bacteria except for *E. coli*. Some differences in the amount of antimicrobial effects observed in this study and similar investigations may be due to differences in plant growth, different extraction methods, and so on. Differences in antimicrobial effects indicate differences in the effective components of the plant.

5. Conclusion

In conclusion, it can be concluded that the extract of *R. damascena* had appropriate antibacterial activity against Gram-negative and Gram-positive bacteria tested in this study that Gram-positive bacteria were more sensitive to this extract. Further in vivo studies are needed to evaluate the efficacy of this extract on clinical isolates and their adverse effects (if any) so that the extract can be introduced to the world as a new antimicrobial drug after its completion. It is also suggested that the effect of different extraction methods on its antimicrobial properties be investigated in order to understand the potentials of the extract.

Conflicts of Interests

Authors declare that there is no conflict of interests

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