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Antibacterial Activity of Four Plant Extracts Used in Palestine in Folkloric Medicine against Methicillin-Resistant *Staphylococcus aureus*

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Abstract: Ethanolic and hot water extracts from 4 different plant species used in Palestine in popular medicine for the treatment of several ailments of microbial and non-microbial origin were evaluated for potential antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Both water and ethanol extracts of *Mentha longifolia, Melissa officinalis* and *Rosa damascena* were effective on MRSA. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the ethanolic extract of *M. longifolia* and *M. officinalis* were in the range of 3.125 to 12.50 mg/ml and 12.50 to 25.00 mg/ml, respectively. The ethanolic extract with the greatest antimicrobial activity was that of *R. damascena* (MIC 0.395 to 0.780 mg/ml and MBC 1.563 to 3.125 mg/ml). The combination of ethanolic extracts of the plants studied showed synergistic antibacterial activity against MRSA strains.

Key Words: Antibacterial activities, plant extracts, methicillin-resistant *Staphylococcus aureus*

Introduction

The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases (1,2). Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (3,4).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is now common in many areas of the world. The frequencies of infections and outbreaks due to MRSA have continued to increase. MRSA is often multidrug resistant and therapeutic options are limited (5-8).

In Palestine, antimicrobial resistance has clearly emerged as a serious problem with MRSA (8). In this study, 4 medicinal plants in popular use in Palestine for the treatment of several ailments of microbial and non-microbial origins were tested for in vitro MRSA activity.

Materials and Methods

Plant material

Four plant samples purchased from Palestinian markets were studied. All the plant materials were further identified in the Department of Biological Sciences, An-Najah National University, Palestine. Table 1 shows the botanical name, local name and plant part used of the plants under study.

Table 1. List of medicinal plants used in the antimicrobial assay.

Botanical Name	Family	Local name	Plant part used
Althaea officinalis	Malvaceae	Marsh mallow	Aerial part
Mentha longifolia	Lamiaceae	Wild mint	Aerial part
Melissa officinalis	Lamiaceae	Lemon balm	Aerial part
Rosa damascena	Rosaceae	Damask rose	Flower

Preparation of extracts

Dried and milled plant materials were extracted sequentially with hot water and 80% ethanol. Each solvent was replaced 3 times with fresh solvent and was allowed to remain in contact with the plant material for 48 h. After filtration of total extracts, the extracts were evaporated to dryness in vacuo and weighed.

Microorganisms used

The MRSA strains used in this study were clinical isolates from patients presenting with symptoms of *S. aureus*-associated diseases. The isolates were identified as *S. aureus* according to colonial and microscopic morphology, positive catalase, and coagulase production. All *S. aureus* isolates were tested for methicillin resistance. The disk diffusion method outlined by the National Committee for Clinical Laboratory Standards (NCCLS) (9) was used with a 1 µg oxacillin disk (Oxoid). Zone sizes were read after incubation at 35 °C for 24 h. Isolates with zone sizes ≤ 10 mm were considered methicillin resistant.

Antibacterial activity

The antibacterial activity was determined by the well diffusion method according to NCCLS (9). Three to five identical colonies from each agar plate were lifted with a sterile wire loop and transferred into a tube containing 5 ml of tryptic soy broth (TSB). The turbidity of each bacterial suspension was adjusted to reach an optical comparison to that of a 0.5 McFarland standard, resulting in a suspension containing approximately 1 to 2 x 10^8 CFU/ml. Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum. As a final step, the rim of the agar was also swabbed. After allowing the inoculum to dry at room temperature, 6-mm-diameter wells were bored in the agar. Each extract was checked for antibacterial activity by introducing 50 µl of a 100 mg/ml concentration into duplicate wells. The plates were allowed to stand at room temperature for 1 h for extract to diffuse into the agar and then they were incubated at 37 °C for 18 h. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter (IZD) measured to the nearest millimeter.

The MIC was determined by micro-broth dilution (10) methods. The reconstituted extract was serially diluted 2-fold in Mueller-Hinton broth (Oxoid) medium. Duplicate tubes of each dilution (0.391, 0.780, 1.563, 3.125, 6.25, 12.5, 25.0 and 50.0 mg/ml) were inoculated with 5 x 10^5 cells (cfu) of the test bacterial strain and cultures incubated at 37 °C for 18 h. MIC was taken as the highest dilution (least concentration) of extract or drug showing no detectable growth.

MBC was determined by subculturing the test dilution on to a fresh drug-free solid medium and incubating further for 18-24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

Results

Among the total crude extracts tested by the disk diffusion method, only 3 of the 4 plants (*M. longifolia, M. officinalis* and *R. damascena*) presented antibacterial activity against the MRSA used. The crude extracts (water and ethanol) of *Althaea officinalis* did not show activity against any of the test MRSA strains (Table 2).

The antimicrobial activity of the extracts and their potency were quantitatively assessed by determining the MIC and MBC, respectively, as given in Table 3. Only the alcoholic extract was tested, as alcohol was found to be a better solvent for extraction of antimicrobially active substances compared to water (11,12).

The MIC and MBC values obtained for the ethanolic extracts against the MRSA varied from one plant extract to the other. For instance, MIC and MBC values of *M.*

Table 2. Antibacterial activity of the crude plant extracts on MRSA.

Plants used	MRSA (n = 20)	
	Water extract	Ethanol extract
A. officinalis	-	-
M. longifolia	8	14
M. officinalis	12	15
R. damascena	18	34
Oxacillin (1 µg)	-	-

Figures indicate average zone of inhibition (in mm), (-) = no inhibition, n = number of MRSA tested.

Table 3. Antibacterial activity (MIC and MBC in mg/ml) of the ethanol extracts and their combinations on MRSA.

Plant tested	Ethanol extract	
	MIC	MBC
A. officinalis	na	na
M. longifolia	3.125 to 12.50	12.50 to 25.00
M. officinalis	3.125 to 12.50	12.50 to 25.00
R. damascena	0.3951 to 0.780	0.780 to 1.563
A. officinalis / *M. longifolia* / *M. officinalis* and *R. damascena*	12.50 to 25.00	25.00 to 50.00

a: MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration;
b: Not active at concentration range from 0.195 to 50.00 mg/ml

longifolia and *M. officinalis* were in the range of 3.125 to 12.50 mg/ml and 12.50 to 25.00 mg/ml, respectively. *R. damascena* extract showed the most potent inhibition for the test MRSA strains (MIC 0.395 to 0.780 mg/ml and MBC 1.563 to 3.125 mg/ml).

The results of screening are discouraging as the combination of ethanol extracts of *A. officinalis, M. longifolia, M. officinalis* and *R. damascena* not only led to a loss of their original spectrum antibacterial activity against MRSA, but their antagonistic effects also caused poorer activity up to a range of MIC of 12.50 to 25.00 mg/ml and of MBC from 25.00 to 50.00 mg/ml.

Discussion

The increasing occurrence, particularly in hospitals, of *S. aureus* resistant not only to methicillin but to a wide range of antimicrobial agents, including all kinds of β-lactams, has made therapy more difficult (5-8). Although strategies have been proposed in an attempt to control the spread (13), the search for new ways to treat MRSA infections stimulates the investigation of natural compounds as an alternative treatment of these infections.

In the present study, the analysis of the growth inhibition activity by the disk diffusion method showed that 3 out of 4 medicinal plants (*M. longifolia, M. officinalis* and *R. damascena*) commonly used by traditional medical practitioners in Palestine were active against hospital strains of MRSA under test conditions with crude extract concentrations as high as 5 mg/ml. Our results agree with the previous antibacterial studies related to these 3 botanical species (3,14-16). The extract with the greatest antimicrobial activity was that of *R. damascena* (inhibition zone 8-13 mm, MIC 0.395 to 0.780 mg/ml and MBC 1.563 to 3.125 mg/ml). It is known that Rosaceae is rich in corilagin and tellimagrandin (3) and this class of compounds has remarkable antimicrobial activity.

The MIC and MBC values obtained for the extracts against the MRSA also support the findings of the diffusion method. The area of concern is that MIC values of the active plant extracts obtained in this study were lower than the MBC values, suggesting that the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration.

The ethanol extract of the 3 plants exerted greater antibacterial activity than corresponding water extract (Table 3) at the same concentrations. These results confirmed the evidence in previous studies reported that ethanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as water (11,12).

The most important conclusion drawn from our study is the antibacterial diminution (antagonistic effect) of the combination of ethanol extracts of *A. officinalis, M. longifolia, M. officinalis* and *R. damascena* as detected in this study. This finding may disagree with the traditional therapeutic indications claimed on the use of these combinations of traditional plant medicines in Palestine against a number of infections for generations.

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