

Research Article

Open Access

Antibacterial *In-Vitro* Activities of Selected Medicinal Plants against Methicillin Resistant *Staphylococcus Aureus* from Libyan Environment

Yousef M Abouzeed^{1,2*}, Abdurrezagh Elfaheem³, Fraje Zgheel³ and Mohamed O Ahmed¹

¹Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

²National Authority of Scientific Research P.O. Box 80045, Tripoli, Libya

³Biotechnology Research Centre, P.O. Box 30313, Tripoli, Libya

Abstract

Medicinal plants are valuable natural sources effective against various infectious agents. Extracts from Libyan traditional medicinal plants were investigated for antibacterial activity. In this study, the potential antibacterial activity of extracts from eight Libyan traditional medicinal plants against methicillin-resistant *Staphylococcus aureus* (MRSA) was investigated *in-vitro*. Susceptibility assays using disc diffusion and broth microdilution test for the determination of minimum inhibitory concentration (MIC) were used to assess the antibacterial activity of methanolic extracts from medicinal plants. Extracts from all eight plants showed anti-MRSA activity with MIC values ranged between 25-50 mg/ml. *Cistus salvifolius*, *Salvia officinalis*, *Pistacia atlantica*, *Arbutus pavarii*, and *Myrtus communis* exhibited the most potent anti-MRSA activity, whereas extracts from *Teucrium polium*, *Thymus capitellatus*, and *Euphorbia dendroides* showed weak anti-MRSA activity. Medicinal plants may serve as useful bactericidal agents and warrant further investigation to better evaluate their particular therapeutic potentials and optimize their application.

Keywords: Medicinal plants; *In-vitro* antibacterial activity; Methicillin-resistant *Staphylococcus aureus* (MRSA); Libya

Introduction

Medicinal plants can be valuable therapeutic resources [1-3]. The treatment of infections with plant-derived compounds is an age-old practice that is employed throughout the world, especially in developing countries where traditional medicines are used to treat a variety of diseases [1]. Interest in plants with antimicrobial properties has been revived as a result of current resistance profiles associated with over- and inappropriate use of antibiotics [3,4].

Staphylococcus aureus is a gram-positive bacterium responsible for morbidity and mortality [5]. It is one of the leading causes of human infections of the skin, soft tissues, bones and joints, abscesses, and normal heart valves. *Staphylococcus aureus* flourishes in the hospital setting and is associated with bloodstream and surgical wound infections [6] and has become important nosocomial organism. Methicillin Resistant *Staphylococcus aureus* (MRSA) first recognized in the 60s [7] is a multiresistant strain that has been documented worldwide showing risen resistance to different classes of antimicrobials [8].

Over the past decades, MRSA has spread throughout the world and has become highly endemic in many geographic areas [9]. MRSA infections are difficult to treat because of their resistance to many of the commonly used antibiotics (e.g., macrolides, tetracyclines, aminoglycosides). Some of these MRSA strains are resistant to even the most powerful antibiotics, including vancomycin [8,10]. The WHO has acknowledged the need to identify new antibiotics and/or new approaches to overcome the growing problems associated with such infectious agents.

In Libya, MRSA and other epidemic diseases have emerged as a serious emerging problem [11,12]. Limited studies in Libya have assessed the antibacterial activity of medicinal plants against different types of bacteria [13]. In the current study, only eight Libyan traditional medicinal plants commonly used for the treatment of several clinical conditions were tested for *in-vitro* antimicrobial activity against the pathogenic virulent bacteria methicillin resistant *Staphylococcus aureus* (MRSA).

Materials and Methods

Plants

Eight Libyan traditional medicinal plants were collected in the period of spring 2010 from north-east region (Al Jabal al Akhdar "Green Mountains") of Libya (Table 1). Fresh plants material (Table 1) were collected and processed within hours after collection. The collected plants were identified and classified according to the herbarium at the biotechnology research centre Tripoli, Libya.

Preparation of extracts

Plants parts (Table 1) were first cleaned, shade dried and grounded to a fine powder and extraction process was next carried out using a conventional solvent extraction procedure. The extraction method was adapted from Hassawi and Karma [14] and in accordance with Motamedi et al. [3] as following: 10grams of each plant materials were extracted with 100 ml of alcohol (i.e. methanol with concentration of alcohol:water = 8:2, v/v), filtered through Whatman No. 1 filter paper, and the residual material was re-extracted with methanol. The two resulting filtrates were combined and dried using a rotary evaporator.

6-Bacterial strain

The MRSA strain used in the current study was in-house confirmed clinical isolate from Libyan hospital environment provided by MO

***Corresponding author:** Yousef M Abouzeed, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli P.O. Box 13662, Tripoli, Libya, Tel: +218925400914; Fax: +218214628421; E-mail: yabouzeed@yahoo.com

Received August 23, 2013; **Accepted** September 27, 2013; **Published** September 30, 2013

Citation: Abouzeed YM, Elfaheem A, Zgheel F, Ahmed MO (2013) Antibacterial *In-Vitro* Activities of Selected Medicinal Plants against Methicillin Resistant *Staphylococcus Aureus* from Libyan Environment. J Environ Anal Toxicol 3: 194. doi: 10.4172/2161-0525.1000194

Copyright: © 2013 Abouzeed YM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Ahmed from the department of Microbiology and parasitology, faculty of veterinary medicine at University of Tripoli, Libya.

Antimicrobial Susceptibility Assays

Disk diffusion assay

The anti-MRSA activity of the plant extracts was initially determined using the disc diffusion assay [15]. Each dried plant extract was dissolved and prepared as described by Gangoué-Piéboji [16]. MRSA were over night cultured (24 h) at 37°C on nutrient agar for the preparation of cell suspensions. MRSA cell suspensions adjusted to 0.5 McFarland standards (10⁷ CFU/ml) were prepared and 0.1 ml of the suspension was poured on Muller Hinton agar (MHA) (Oxoid, UK) and dispersed using a cotton swab to ensure robust cell growth. Plates were then allowed to dry for 5 min prior incubation.

The sterile filter paper discs (Whatman No. 3, diameter=6 mm) were soaked in 30 µl of each plant extract for 30 min. The extract-soaked filter paper discs were then placed on the inoculated MHA plates, allowed prior incubation to stand for 30 min at room temperature to permit proper diffusion of the extract. All plates were incubated at 37°C for 24 h, and the resulting inhibition zones were measured in mm for each of the medicinal plant extracts. This experiment was done in triplicate and the antimicrobial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts. As positive controls, discs (oxoid) containing oxacillin 1 µg were used.

Minimum inhibitory concentration (MIC)

The determination of the lowest concentration of the extracts capable of inhibiting the growth of (MRSA) (i.e. minimum inhibitory concentration (MIC)) for each of the eight medicinal plant extracts was performed using broth microdilution test [17] as following: A stock solution of 200 mg/ml was prepared for each extract by dissolving 200 mg of each extract in 1 ml dimethyl sulfoxide solvent (DMSO:Water, 2:4 v/v). Two hundred microliters of each of the eight extract stock solutions were pipetted into the first row of a 96-well plate, and 100 µl of nutrient broth (Oxoid) were pipetted into each of the remaining

wells. Serial dilutions of the stocks were performed such that each of the following wells contained 100 µl of the stock solution in serially descending concentrations (1:2) until a final concentration of 6.25 mg/ml was achieved. One hundred microliters of MRSA culture (10⁷ cells/ml) were added to each well. All of the plates were incubated at 37°C for 24 h. The least concentration of the extract showing no visible growth was initially taken as the MIC. The MIC was established by determining the lowest extract concentration corresponding to a lack of turbidity in the wells, and further confirmed by culturing at 37°C for 24 h on nutrient agar. MIC values were evaluated based on positive interpretation for both no visible turbidity and negative culturing.

Results

The antimicrobial activities for each of the eight extracts according to a zone of inhibition ranged from 6-18 mm (Table 2). Only five out of the eight plants (*Cistus salvifolius*, *Salvia officinalis*, *Arbutus pavarii*, *Pistacia atlantica*, and *Myrtus communis*) exhibited broad spectrum activity against MRSA with corresponding inhibition zones of 18 mm, 14 mm, 9 mm, 11 mm, and 9 mm respectively. The remaining crude methanolic extracts from *Teucrium polium*, *Thymus capitellatus*, and *Euphorbia dendroides* showed selective activity (inhibition zone =6mm) against MRSA (Table 2).

The MIC values obtained using methanolic extracts derived from the eight plants exhibited anti-MRSA activity ranged between 25-50 mg/ml (Table 2). MIC values for the *Cistus salvifolius* extract was the most active against MRSA with an MIC value of 25 mg/ml. In contrast, *Salvia officinalis*, *Arbutus pavarii*, *Pistacia atlantica*, and *Myrtus communis* exhibited an MIC value of 50 mg/ml. *Teucrium polium*, *Thymus capitellatus*, and *Euphorbia dendroides* had no effect on MRSA.

Discussion

Medicinal plants are commonly available resources, have less if no side effects, economic and have antimicrobial properties [14]. The majority of these medicinal plants used in this study are applied in traditional medicine in many Libyan regions to cure different disorders. Medicinal plants are considered one of the most valuable resources for antibiotic development. Pathogenic strains of antibiotic-resistant bacteria have emerged due to the misuse of antibiotics. As a result, bacteria become resistant to antibiotics, which are in turn less effective after extended periods of use. Pharmaceutical companies whose efforts are focused on the production and manufacture of antibiotics strive to manufacture new generations of antibiotics capable of treating such antibiotic-resistant bacterial strains. In recent years, publications from several countries have reported the use of active compounds extracted from medicinal plants, which may benefit antibiotic development [4,18]. Many studies have extracted active compounds from medicinal plants that exhibit a synergistic effect against some bacterial species [4,18].

Plant Botanical Name	Family	Local name	Plant part
<i>Cistus salvifolius</i>	Cistaceae	Parbish	Leaves
<i>Salvia officinalis</i>	Labiatae	Tea apple	Leaves
<i>Arbutus pavarii</i>	Ericaceae	Shmary	Leaves
<i>Pistacia atlantica</i>	Anacardiaceae	Battom	Leaves
<i>Myrtus communis</i>	Myrtaceae	Marseen	Leaves
<i>Teucrium polium</i>	Labiatae	Jeheda	Leaves and flowers
<i>Thymus capitellatus</i>	Labiatae	Zahter	Leaves and flowers
<i>Euphorbia dendroides</i>	Euphorbiaceae	Halablib	Leaves and flowers

Table 1: Plants names and plant parts used in the anti-MRSA activity assays.

Plant	Disk diffusion(mm)	Minimum inhibitory concentration (MIC) (mg/ml)					
		50	37.5	25	12.5	6.25	3.125
<i>Cistus salvifolius</i>	18	No growth	No growth	No growth	Growth	Growth	Growth
<i>Salvia officinalis</i>	14	No growth	No growth	Growth	Growth	Growth	Growth
<i>Arbutus pavarii</i>	9	No growth	No growth	Growth	Growth	Growth	Growth
<i>Pistacia atlantica</i>	11	No growth	No growth	Growth	Growth	Growth	Growth
<i>Myrtus communis</i>	9	No growth	No growth	Growth	Growth	Growth	Growth
<i>Teucrium polium</i>	6	Growth	Growth	Growth	Growth	Growth	Growth
<i>Thymus capitellatus</i>	6	Growth	Growth	Growth	Growth	Growth	Growth
<i>Euphorbia dendroides</i>	6	Growth	Growth	Growth	Growth	Growth	Growth

Table 2: Results of disk diffusion and minimum inhibitory concentration (MIC) assays to determine the anti-MRSA activity of methanolic extracts from eight Libyan traditional medicine plants.

MRSA is one of the leading causes of skin, soft tissue, bone, joint, abscess, and normal heart valve infections. Herein, we examined the anti-MRSA activity of extracts from eight different Libyan traditional medicinal plants. Taken together, the results of disk diffusion and MIC assays show that five out of the eight plant extracts (i.e., *Cistus salvifolius*, *Salvia officinalis*, *Arbutus pavarii*, *Pistacia atlantica*, and *Myrtus communis*) exhibited bactericidal activity against MRSA. Previously observation indicated that the relationship between inhibition zone diameters and the MIC values was not correlated [18] however this was not the case in our study. It is generally held that for evaluating bactericidal activity of medicinal plants, both MIC and MBC values are often near or equivalent [19]. Previously it has been acknowledge that in the determination of the antibacterial activity of plant extracts, methanolic extract had a better efficacy than its ethanolic extract [3].

The results obtained from this study provide evidence that methanolic extracts of the Libyan traditional medicinal plants *Cistus salvifolius*, *Salvia officinalis*, *Arbutus pavarii*, *Pistacia atlantica*, and *Myrtus communis* exhibit useful bactericidal activities against locally isolated MRSA strain, suggesting that they may be clinically useful. Further search in respect of these findings are needed and promising.

However, while *in vitro* results are encouraging and merit further study, the *in-vivo* efficacy remains to be confirmed. In general, the results revealed significant antibacterial activity of the studied medicinal plants, which could be a potential source of new antibacterial agents. To our knowledge, there was no previous report on the antimicrobial activities on selected resistant bacteria in Libya.

Acknowledgment

This project has been funded by the National authority For Scientific Research. Also we acknowledge the contribution of both the Biotechnology research centre and faculty of veterinary medicine at Tripoli University, Libya.

References

- De Albuquerque UP (2006) Re-examining hypotheses concerning the use and knowledge of medicinal plants: a study in the Caatinga vegetation of NE Brazil. J Ethnobiol Ethnomed 2: 30.
- Yineger H, Yewhalaw D (2007) Traditional medicinal plant knowledge and use by local healers in Sekoru District, Jimma Zone, Southwestern Ethiopia. J Ethnobiol Ethnomed 3: 24.
- Motamedi H, Darabpour E, Gholipour M, Seyyed Nejad SM (2010) In vitro assay for the anti-brucella activity of medicinal plants against tetracycline-resistant *Brucella melitensis*. J Zhejiang Univ Sci B 11: 506-511.
- Chattopadhyay RR, Bhattacharyya SK, Medda C, Chanda S, Bag A (2009) A comparative evaluation of antibacterial potential of some plants used in indian traditional medicine for the treatment of microbial infections. Braz Arch Biol Technol 52: 1123-1128.
- Woodford N, Livermore DM (2009) Infections caused by Gram-positive bacteria: a review of the global challenge. J Infect 59: S4-S16.
- Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, et al. (2003) Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. Int J Antimicrob Agents 22: 406-419.
- Barber M (1961) Methicillin-resistant staphylococci. J Clin Pathol 14: 385-393.
- Ness T (2010) Multiresistant bacteria in ophthalmology. Ophthalmologie 107: 318-322.
- Ayliffe GA (1997) The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 1: S74-S79.
- Centre for Disease Control and Prevention (CDC) (2008) *Staphylococcus aureus* resistant to vancomycin. JAMA 288: 825.
- Ahmed MO, Elmeshri SE, Abuzweda AR, Blauo M, Abouzeed YM, et al. (2010) Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya December 2006-January 2008. Euro Surveill 15.
- Ahmed MO, Alghazali MH, Amri SG, Abuzweda AR (2010) Detection of Inducible Clindamycin Resistance (MLSBi) among Methicillin-resistant *Staphylococcus aureus* (MRSA) from Libya. Libyan J Med 5.
- Abdelsalam H, Bogdadi A (2010) Antimicrobial activities of ten medicinal plants used in the folk medicine of Libya. Acta Hort 853: 419-422.
- Hassawi D, Kharm A (2006) Antimicrobial activity of some medicinal plants against *Candida albicans*. J Biological Sci 6:109-114.
- Anon (1997) Performance standards for antimicrobial disk susceptibility tests. 6th ed. Approved standard M2 A6 (M100-S7). Wayne, PA: National Committee for Clinical Laboratory Standards.
- Gangoue-Pieboji J, Eze N, Ngongang DA, Ngameni B, Tsabang N, et al. (2009) The in-vitro antimicrobial activity of some medicinal plants against beta-lactam-resistant bacteria. J Infect Dev Ctries 3: 671-680.
- Barry AL, Jones RN, Gavan TL and Washington JA (1991) In manual of clinical microbiology. American Society for microbiology, Washington, DC 972-977.
- Motamedi H, Darabpour E, Gholipour M, Seyyed Nejad SM (2010) Antibacterial effect of ethanolic and methanolic extracts of *Plantago ovata* and *Olivaria decumbens* endemic in Iran against some pathogenic bacteria. Int J Pharmacol 6: 117-122.
- Reuben KD, Abdulrahman FI, Akan JC, Sodipo OA (2008) Phytochemical screening and in vitro antimicrobial investigation of the methanolic extract of *Croton zambesicus* Muell ARG stem bark. Eur J Sci Res 23: 134-140.