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Research Article

Antibacterial Effects of 4 Methanol Plant Extracts (Lamiaceae) against 10 Methicillin Resistant *Staphylococcus aureus* Isolates

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ABSTRACT

Antibiotic resistance has paved the way for replacing conventional medications with herbal therapies that supposedly have less side-effect. This research is an experimental study and a fundamental-functional one. The present study aimed to investigate the antibacterial effect of 4 plant extracts from Lamiaceae on 10 methicillin resistant *Staphylococcus aureus* isolates. Methanol extracts were prepared by maceration method for 10 days in room temperature and then filtered with whatman paper No.1 and concentrated by rotary evaporator system. Different concentration of each extract were prepared in dimethyl sulfoxide: methanol (1:1 v/v) and bioassayed on 10 MRSA isolates by agar well diffusion method in Muller-Hinton agar medium. Plates were incubated in 37˚C for 24 hours. After incubation period, zone of inhibition was measured in millimeter and the antibacterial effect of extracts were evaluated. According to antibiogram test, some of *Staphylococcus aureus* isolates were sensitive to the used extracts with different MIC. MIC values about all *Staphylococcus aureus* isolates about *Zataria multiflora, Mentha longifolia*, *Ziziphor clinopodioidae* and *Satureja hortensis* were 15, 30, 30 and 15 mg/ml respectively. Because of regarding the fact that antibiotic resistance is growing, according to the acquired results we expect to be able to use plant extracts against *Staphylococcus aureus* resistant to meticillin in controlling the infections or as preservatives in food sciences and in the next step separating of effective substances were suggested.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that is resistant to many antibiotics (Chambers, 2001). In 1959 methicillin was licensed in England and introduced as the first beta-lactamase-resistant penicillin to treat penicillin-resistant *S. aureus* infections (Benner, 1968). In 1961 the first known MRSA isolates were reported in a British study and between 1961-1967 there were infrequent hospital outbreaks in Western Europe and Australia (Johnson, 2011). Nosocomial MRSA strains are now distributed worldwide and responsible for numerous difficult-to-treat infections in humans (Daum, 2007). Humans are a natural reservoir for *S. aureus* (Reinoso, 2006). Skin and the nostrils are the typical sites of MRSA colonization, which may persist for years (Clement, 2005, Yan et al., 2013) and open wounds, intravenous catheters and the urinary tract are also potential sites for infection (Evellard et al., 2004). In the community, most MRSA infections are skin infections. In medical facilities, MRSA causes life-threatening bloodstream infections, pneumonia, wounds and surgical site infections (Wagenaar, 2011). MRSA infections can also occur in healthy people who have not recently been in the hospital. Most of these MRSA infections are on the skin or less commonly lung infections (Bratu et al, 2005). People who may be at risk are athletes and other people who may share items such as towels or razors, children in daycare, members of the military and people who have gotten tattoos (Jones, 2006). Traditional medicinal plants are valuable natural sources effective against various infectious agents. Recently, there has been an increasing
Materials and Methods

Staphylococcal isolates were screened from nasal and skin of Afzalipoor hospital personnel in Kerman. The sample swabs were inoculated to transport medium and transferred to microbiology laboratory and cultured on blood agar. After 24 hours incubation in 37°C, beta hemolytic, gram positive cocci and catalase and coagulase test positive colonies were isolated and cultured on mannitol salt agar for detection of mannitol fermentation (Tacconelli, 2009). After diagnosis and confirming of Staphylococcus aureus isolates, the antibiotic sensitivity of the all isolates were determined according to the method of Bauer-Kirby (Winn, Jret al. 2006) using methicillin disc placed on the surface of Muller-Hinton medium (Merck Company) seeded with screened MRSA isolates. After incubation period in 37°C for 24 hours, antibiotic susceptibility was determined by measuring zone of inhibition in mm and the isolates were defined as MRSA based on their resistance to methicillin.

Plant Materials and Extraction

Test plants including Zataria multiflora, Mentha longifolia, Ziziphor clinopodiode and Satureja hortensis were collected from Shahrebabak, Kerman, Iran, and were identified by Dr. P. Rajaei, department of biology, Islamic Azad University, Kerman Branch. The plant samples were washed with water and air dried in room temperature and ground into a fine powder (Shakibaa et al., 2011). For preparation of methanol extract by maceration method, 50 gram of each powdered plant were soaked separately in 500ml of methanol (Merck Company) for 7 days at room temperature in shaking conditions (Salehi et al., 2014). Obtained extracts were filtered by Whatman paper no.1 and then concentrated using rotary evaporator system (Heidolph, Germany) at 42°C (Shahidi-Bonjar and Kariminik, 2004).

Antimicrobial Assay

The well diffusion method was used to determine the antibacterial activity (Shahidi Bonjar, 2004). The bacterial suspension equal 1.5×10⁸ CFU/ml in sterile normal saline (adjusted to 0.5 McFarland standards) was prepared as described by Nalubega et al., The Muller-Hinton agar (Merck Company) medium with depth of 4 mm was poured into petri dishes to give a solid plate and inoculated with 100 μl of suspension containing 1.5×10⁸ CFU/ml of MRSA isolates by sterile cotton swabs (Nalubega, Kabasa, Oliia.D, and Kateregga, 2011). Wells in 6 mm diameter were punctured in the media by sterile cork borers and filled with 20 μl of the extracts. The first concentration used was 80 mg/ml (Shahidi-Bonjar and Kariminik, 2004). The plates were incubated at 37°C for 24 hours. Following incubation, antibacterial activity was determined by measuring the inhibition zones around each of the wells in mm (Shakibaa et al., 2011). All tests were done in triplicate. DMSO: Methanol (1:1 v/v) solvent was considered as a negative control.

Determination of Minimum Inhibitory Concentration (MIC)

To determine Minimum Inhibitory Concentration (MIC), Two fold dilution series (80, 40, 20, 10, 5, 2.5 and 1.25 mg/ml) of each crude extract in the solvent of DMSO: Methanol (1:1 V/V) was prepared and bioassayed using well diffusion agar assay as mentioned above (Shahidi-Bonjar et al., 2003). The plates were then incubated at 37°C for 24 hrs.

Results

10 Methicillin-resistant Staphylococcus aureus (MRSA) were isolated based on diagnostic tests: Gram positive cocci, catalase and coagulase positive, β hemolysis on blood agar media, mannitol fermenter and resistant to methicillin. According to antibiogram test by agar well diffusion assay, Some of MRSA isolates were sensitive to the used extracts.60 mg/ml concentration of Zataria multiflora methanol extract was effective on 9 of 10 MRSA isolates with zone of inhibition 13-21 mm. MIC value of Zataria multiflora methanol extract was 15mg/ml. 60 mg/ml concentration of Mentha longifolia methanol extract was effective on 3 of 10 MRSA isolates with zone of inhibition 13-15 mm. MIC value of Mentha longifolia methanol extract was 30mg/ml. 60 mg/ml concentration of Satureja hortensis methanol extract was effective on 8 of 10 MRSA isolates with zone of inhibition 14-21 mm. MIC value of Satureja hortensis methanol extract was 15mg/ml. 60 mg/ml concentration of Ziziphor clinopodiode methanol extract was effective on 3 of 10 MRSA isolates with zone of inhibition 13-14 mm. MIC value of Ziziphor clinopodiode methanol extract was 30mg/ml. Based on obtained results, the most effective plant extract was Zataria multiflora and Satureja
hortensia had low antibacterial activity against 10 studied MRSA isolates.

DISCUSSION

MRSA is methicillin-resistant Staphylococcus aureus, a type of gram positive cocci bacteria that is resistant to many antibiotics. In a healthcare setting, such as a hospital or nursing home, MRSA can cause severe problems such as bloodstream infections, pneumonia and surgical site infections. Of the people with S. aureus present, about 1 percent has MRSA, according to the centers for disease control and prevention (CDC) reports (Wendakoon et al, 2012). Hence, a proper and rapid detection of methicillin resistance in Staphylococci is very important, not only for choosing the appropriate antibiotic therapy, but also for the control of the endemicity of the MRSA. This situation has placed limits on our options to treat infections by this organism. Antibiotics like vancomycin and teicoplanin, are now considered to be agents of last resort for the treatment of MRSA infections. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant S. aureus (VRSA) strains exhibiting two different resistance mechanisms. Therefore, before therapy with vancomycin and teicoplanin fails completely, it is necessary to find some alternative antibacterial agents against MRSA infections (Sucilathangam, 2012). Our investigation presented antibacterial activity of 4 plant extracts related to Labiata family against 10 MRSA isolates. The effective plant extracts were Zataria multiflora and Satureja hortensis with MIC value 15mg/ml (Abu-Shanab et al, 2006). Our results agree with the mentioned antibacterial studies. In the present investigation, we used the well diffusion assay. It is undoubtedly the most convenient way of evaluating the antibacterial potential of plant extracts because the extracts can diffuse more easily into the media. Results of Valgas and co-workers indicated that agar well diffusion method proved to be more sensitive than disc diffusion method (Valgas et al, 2007).

CONCLUSION

In conclusion, our results showed that two plant extracts of Labiata family (Satureja hortensis and Zataria multiflora) possesses potential antibacterial activity against MRSA. We believe that these findings will be helpful to many researchers in the field of the evolution of antibacterial activities plant extracts or essential oil. It must be considered that the type and level of biological activity exhibited by any plant material depends on many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions, and post-harvest processing. We suggest antimicrobial activity of traditional plants in every geographic region are investigated.

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