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ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACTS OF *SAMBUCUS EBULUS* AND *URTICA DIOICA* AGAINST CLINICAL ISOLATES OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Abstract

Background: Increase in the emergence of drug - resistant pathogens led to the development of natural antimicrobials. In this study the antimicrobial effect of methanolic extracts of *Sambucus ebulus* and *Urtica dioica* on 16 skin and wound infections isolates of methicillin resistant *S. aureus* have been studied.
Material and Methods: Solvent extraction procedure was done using soxhlet apparatus for extracting antimicrobial agents from freeze dried plants. Antibacterial activity was measured using agar well diffusion method.
Results: The MIC of *Sambucus ebulus* and *Urtica dioica* extracts against the standard strain of *S. aureus* ATCC 6538 were determined using the micro dilution method at 15 mg and 20 mg respectively. All the test bacteria were found sensitive to the *Sambucus ebulus* extract and only one isolate was resistant to *Urtica dioica* extract.
Conclusion: Extracts of *Sambucus ebulus* and *Urtica dioica* possess antibacterial potency against MRSA isolates and may be used as a natural antiseptics and antimicrobial agents in medicine.

Key words: Antimicrobial activity, *Sambucus ebulus*, *Urtica dioica*, *S. aureus*, (MIC) minimum inhibitory concentration, MRSA (Methicillin Resistant *S. aureus*).

Introduction

Staphylococcus aureus is a major human pathogen associated with invasive disease such as deep abscess formation, endocarditis, osteomyelitis, and sepsis (Lowy, 1998). Because of the great genetic variability of *S. aureus* and the ability to develop changes in sensitivity to antimicrobials, most clinical isolates of *S. aureus* are resistant to a number of antibiotics (Sibanda et al., 2010). The emergence of methicillin-resistant *S. aureus* (MRSA) worldwide is a major concern as this dramatically reduces the choice of effective antibiotics for prevention and treatment of a very common infection in both hospitals and communities (Gould, 2005). Again, there is the need for new antimicrobial agents to control the spread of MRSA which is recognized globally as a clinically significant pathogen, associated with skin and soft tissue infections**.** An alternative in searching-out new effective drugs are natural products, especially those of plant origin (Hemaiswarya et al., 2008). Medicinal plants have a great potential for providing novel drug leads with proven mechanism of action (Singh et al., 2012). *Sambucus ebulus* L. and *Urtica dioica* L. grown extensively within the northern regions of Iran and are frequently used as medical plants. In traditional medicine *Sambucus ebulus* L. is used for treating some inflammatory cases such as arthritis, anti-hemorrhoid, treating burns and infectious wounds (Ebrahimzadeh et al., 2009) and *Urtica dioica* L. is used to treat allergies, kidney stones, burns, anemia, rashes, internal bleeding, diabetes, etc. (Eloff, 1998). It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Ojala et al., 2000). So the present study was conducted to investigate *in vitro* antimicrobial activity of methanolic extracts of *Sambucus ebulus* and *Urtica dioica* against clinical isolates of methicillin resistant *S. aureus.*

Material and Methods

Isolation and identification of bacteria

Swab samples obtained from skin and wound infections were enriched in Tryptone Soya Broth containing 6.5% NaCl at 37°C for 24 hrs prior to culturing (Safdar et al., 2003). Enriched growths were cultured onto blood agar and incubated aerobically at 37°C for 24 hrs. and suspect colonies from each sample were further identified by Gram-staining, catalase, DNase and coagulase tests and culturing on mannitol salt agar . All *S. aueus* isolates were screened for methicillin resistance by disc diffusion (6 µg/ml oxacillin) on Mueller Hinton agar with 2% NaCl. The susceptibility pattern of the MRSA isolates to the selected antimicrobial agents including vancomycine (30µg), Amoxicillin (25µg), Cefazolin (30µg) and Cefalexin (30µg) provided from Padtan Teb company (Iran), was determined by Kirby – Bauer disk diffusion method. Standard strain of *S.aureus* ATCC 6538 was used as control.

Preparation of the extracts

Sambucus ebulus and *Urtica dioica* were collected from Rasht city in north of Iran.. The voucher number IBRC PH100487 and IBRC PH100362 has been deposited in Iranian biological resource centre. The aerial parts of these plants were freeze-dried and then grounded to fine powder using grinder. Powdered plant materials were extracted with methanol using soxhlet apparatus. The extracts were filtered using Wathman no.1 filter paper and concentrated on a Rotary evaporator at 45°C to give 1 g/ml concentration of extracts.

Antimicrobial activity of the extracts

Sixteen MRSA isolated from skin and wound infections were used. Antimicrobial activity of extracts was evaluated by using agar well diffusion method. Muller – Hinton agar plates were inoculated with 100 µl of standardized inoculum (10^8 CFU/ m1) of each bacterium and spread with sterile swabs. Wells of 6 mm in diameter were made with sterile borer into each agar plate containing the bacterial inoculum. A little

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molten agar medium was used to seal the bottom of the wells. All the wells filled with 25µl (25 mg per well) of each plant extract. One well in each plate was set up as control by adding 25 µl of freshly prepared sterile distilled water. The plates were left at room temperature for 15 minutes to allow the diffusion of plant extract in to the agar (Riose J.L. et al., 1988). After incubation at 37°C for 24 hrs; the inhibition zone around each well was measured in millimeter. If the diameter of the inhibition zone, less than 9 mm was considered as inactive, then 9 – 12 mm as partially active, 13 – 18 mm as active and more than 18 mm as very active (Junior and Zanil, 2000).

The MIC of two plants extracts against the standard strain of *S. aureus* ATCC 6538 was determined using the micro dilution method (Eloff, 1998). Fourteen different concentrations of extracts ranging from 10 mg to 100 mg were prepared in Muller – Hinton Broth medium. Then 100 µl of standardized *S. aureus* ATCC 6538 suspension (10^8 CFU/ml) was inoculated into each tube. Tubes containing growth medium and different concentration of extract without inoculums were used as controls. All tubes were incubated at 37°C for 24 hrs. Then the tube with lowest concentration without visible growth when compared with control was considered as the MIC.

Results

Antimicrobial susceptibility test in *S. aureus* isolates

The results of antimicrobial susceptibility of methicilline resistant *S.aureus* isolates and antimicrobial activity of *Sambucus ebulus* and *Urtica dioica* extracts against test bacteria are shown in table 1. The zone of inhibition of the growth of the isolates is a function of antimicrobial activity of the extracts.

Table 1: Antimicrobial sensitivity testing of methicillin resistant *S. aureus* isolates (Diameter of zone of inhibition in millimeter)

Test bacteria	V	Amx	CZ	CN	*S. ebulus*	*U. dioica*
MRSA - 1	16	13	0	16	20	18
MRSA - 2	15	15	10	21	19	18
MRSA - 3	16	13	11	23	19	12
MRSA - 4	17	16	16	20	21	16
MRSA- 5	13	17	0	16	15	0
MRSA - 6	17	24	19	28	18	16
MRSA - 7	14	9	10	0	14	10
MRSA - 8	16	16	15	21	17	15
MRSA - 9	17	19	14	14	20	21
MRSA - 10	10	0	9	8	18	10
MRSA - 11	10	14	18	16	19	17
MDR - 12	17	18	21	20	14	18
MRSA - 13	11	14	11	8	14	18
MRSA - 14	15	16	16	16	19	18
MDR - 15	16	14	21	24	20	15
MRSA - 16	15	8	13	17	21	19

KEY*; MRSA= Methicillin resistant S.aureus , V = vancomycin, Amx =Amoxicillin, CZ = Cefazolin, CN = Cefalexin*. Minimum inhibitory concentration of the *Sambucus ebulus* and *Urtica dioica* against the standard strain of *S. aureus* ATCC 6538 were 15 mg and 20 mg respectively.

Discussion

The practice of traditional medicine is widespread and natural products derived medicines are widely used for effective infectious disease eradication. In The present study, the effects of methanolic extracts of *Sambucus ebulus* and *Urtica dioica* on the growth of methicillin resistant *S .aureus* isolates were investigated *in vitro*. The results revealed the antimicrobial potential of these extracts. All the test organisms were susceptible to extracts of *Sambucus ebulus* with inhibition zone diameter between 14-22 mm. One MRSA isolate was resistant to *Urtica dioica* extract and the diameter of inhibition zone around the rest ranged from 10-21 mm.

In agreement with the results obtained from the present study, previous studies found that *Urtica dioica* have noticeable antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Ilhami et al., 2004; Nuriye et al., 2009). According to Zoran et al the ethanolic extract of nettle (*Urtica dioica*) leaves diluted with methanol showed antibacterial activity with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract ranging from 9.05 to more than 149.93 mg/ ml respectively (Zoran et al., 2012). In a study conducted by Suntar et al, the methanolic extract of *Sambucus ebulus* leaves displayed remarkable wound healing activity (Suntar et al., 2010). Yesilada et al reported that aqueous and methanol extracts and n-Butanol fraction of herbaceous parts of *S. ebulus* showed no inhibitory activity against the microorganism (Yesilada et al., 1999).

Ghesmati has studied the antibacterial activity of *Sambucus ebulus* extracts against *Staphylococcus aureus* ATCC 1341 and *Pseudomonas aeruginosa* ATCC 2785 (Ghesmati, 2008). The results indicated that three extracts of leaf ،flower and fruits of *S. ebulus* showed inhibition zones against *S. aureus* ATCC 1341 about 10-12mm ،11-14mm and 11-13mm, respectively and no inhibition zones were observed against *P. aeruginosa* ATCC 2785. Hearst et al, reported that Elder (*Sambucus nigra* L.) flower and elder berry in particular and their concomitant, exhibited strong antimicrobial effects on various nosocomial pathogens notably upon methicillin-resistant *Staphylococcus aureus* (Hearst et al. 2010). Also, the results obtained from the present research showed antimicrobial potential of *Sambucus ebulus* and *Urtica dioica* extracts against skin and wound infections isolates of methicillin resistant *S.aureus.* So these plants extracts can be used as antiseptics and antimicrobial agents in medicine. The antibacterial activity in Urtica *dioica* may be due to presence of fatty acids and phenolic compounds in their composition (Yiiksel et al., 2009) and ursolic acid as active compound of leave extract of *Sambucus ebulus* L. Also flavonoids of Sambucus extract have several therapeutic effects such as antioxidant and anti-inflammatory (Okuda, 2005).

Conclusion

The research showed extracts of Sambucus *ebulus* and *Urtica dioica* possess antibacterial potency against MRSA isolates and may be used as a natural antiseptics and antimicrobial agents in medicine.

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