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Full Length Research Paper

Anti-adhesive and anti-invasive activities of an oil based di-herbal extract against methicillin resistant *Staphylococcus aureus*

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Staphylococcus aureus **is a major human pathogen capable of causing a wide range of infections. Treatment of *S. aureus* infections is becoming more challenging with the continuous emergence of new strains of methicillin resistant *S. aureus* (MRSA) from hospital and community. The aim of the present study is to formulate an oil based combinatorial herbal extract which will not only have antibacterial effect for treatment, but also anti-adhesive and anti-invasive activities against MRSA for to be used as a prophylactic agent. Based on the Indian and Iranian traditional medicine, herbs such as barberry and turmeric which has medicinal properties are tested against MRSA. MRSA and *S. aureus* strains exposed to oil based di-herbal extract prepared from coconut oil, barberry and turmeric suppressed the partial expression of fibronectin binding protein (FNBP) encoded by fibronectin binding gene (*fnbA*), that establishes the attachment to and invasion of host cells. Results from the current study showed that the formulated extract has good anti-bacterial, anti-adhesive and anti-invasive activity against *S. aureus* and MRSA as it showed good zone of inhibition (15 mm) and inhibited the FNB protein synthesis. The formulated combinatorial herbal extract thus could be used for the treatment of *S. aureus* and MRSA infections as well as for prophylaxis; thus controls the spread of MDR *S. aureus* strains in hospital and community.**

Key words: Anti adhesive, Anti invasive, barberry, coconut oil, turmeric, MRSA.

INTRODUCTION

Staphylococcus aureus is an established nosocomial pathogen and capable of producing wide variety of infection diseases like soft tissue infection (wound infection, boil, eczema, blister and scalded skin syndrome), pneumonia and osteomyelitis (Diekema et al*.,* 2001). The ability of this *S. aureus* to cause infectious diseases depends on two important factors, antibiotic resistance and virulence factors. Methicillin resistant *S. aureus* (MRSA) have evolved from successful clonal lineage via acquisition of mobile genetic elements called staphylococcal chromosomal cassette *mec* (SCC*mec*) containing the *mecA* gene, which encodes penicillin-binding protein 2 (PBP2) with reduced affinity for -lactam antibiotics; hence they are resistant to treatment with methicillin, penicillin, amoxicillin, and cephalosporins (Robinson et al., 2003). In healthcare settings MRSA commonly causes serious and potentially life threatening infections, such as bloodstream and surgical site infections, or pneumonia (Cosgrove et al., 2003). Treatment failure with antibiotics in MRSA leads to the dissemination of multiple drug resistant (MDR) strains resulting in huge outbreaks in hospital and community (Pillar et al., 2003). Therefore the combination of enhanced virulence with resistance to the most commonly prescribed anti-staphylococcal antibiotics makes this organism an important public health threat. Hence for the effective treatment and control the emergence and dissemination of MRSA, it is high time to focus on an alternate medicine from natural source.

Malaysian hospitals, MRSA is a most important noso-

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comial pathogen where a gradual increase has been observed in its frequency in major hospitals rising from 10% in 1985 to 44.1% in 2007 (National Surveillance of Antibiotic Resistance (NSAR) System, Ministry of Health, Malaysia). In addition to healthcare associated infections, MRSA are frequently infecting people in the community with much mortality being reported (Mongkolrattanothai et al., 2003) . According to CDC, in 1974, MRSA infections accounted for two percent of the total number of staph infections; in 1995 it was 22%; in 2004 it was around 63% (http://www.cdc.gov/Features/MRSA/). One of the key steps in controlling nosocomial infectious by MRSA could be through preventing their colonization. Colonization can be prevented with antimicrobials with colonization inhibition properties. Due to the development of bacterial resistance to presently available antibiotics the search for new antibacterial agents is vital. Several products from natural sources have been shown to exert antimicrobial activities; however, very limited studies are available on anti-adhesive and anti-invasive agents. MRSA or *S. aureus* binds to the host tissue through adherence proteins such as fibronectin binding Proteins (FNBP) (Manzies, 2003), collagen-binding proteins (Cna) (Nashey et al., 2004). Among the mediators, FNBP plays the most vital part, hence targeting this mediator could efficient prevent colonization. Therefore the aim of this study is to investigate the antimicrobial, anti-adhesive and anti- invasive activities of previously established medicinal properties oriented natural products such as *Curcuma longa*, commonly known as 'turmeric (Kang-Ju Kim et al*.,* 2005), *Barberry vulgaris*, known as barberry (Fatehi et al., 2005) and coconut oil (Duke et al., 1991) against *S. aureus* and MRSA.

MATERIALS AND METHODS

Plant

The *B. vulgaris* is a deciduous shrub grows in central and southern Europe, northwest Africa and southwestern Asia. The dried *B. vulgaris* fruit was collected farms in the area of Mashhad, Iran. *C. longa* L. and coconut oil were obtained from India.

Preparation of *B. vulgaris* extract

Dried *B. vulgaris* fruits were shade dried at room temperature and powdered by electric blender. Hundred grams of the powdered material was added to 500 ml of distilled sterile water and agitated at room temperature for 24 h. The mixture was filtered (Nalgene filter) and stored at 4ºC (0.2 g/ml).

Preparation of oil based di-herbal extract

Oil based di-herbal extract was prepared by mixing 300 µl of coconut oil, turmeric 0.12 mg and 300 µl of *B. vulgaris* extract at room temperature.

Microorganisms tested

Methicillin resistant *S. aureus* (MRSA) ATCC 700698 and methicillin susceptible *S. aureus* (MSSA) ATCC 29247.

Studied activity

Antibacterial activity

The antibacterial activities of different extracts were tested using the disk-diffusion method standardized method. Bacterial strains were suspended in Luria Bertanii (LB) broth (Difco, USA) and incubated at 37°C for 24 h. Petri plates containing 15 ml of Mueller Hinton agar medium were seeded with 24 h old culture of a selec-ted bacterial strain. Sterile filter paper discs (6 mm in diameter) impregnated with plant extract, were placed on the surface of the medium and incubated overnight. Zone of inhibition was observed and measured after 18-24 h.

Anti adhesive and anti invasive activity

FnbA, gene coding for fibronectin binding protein (FNB) was amplified using published primers (forward 5 -GCGGATCCCATAT GGGCCAAAATAGCGGTAAC- 3 and Reverse: 5 -GCAAGCTTTAA GCTTACTTTTGGAAGTGT-3) with cycling conditions consisted of predenaturation at 98°C for 10 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 45 s from extract treated and non-treated MRSA and MSSA isolates (Zhou et al., 2006). Total cellular RNA was isolated using the Master Pure_ RNA Purification kits (Epicenter Technologies, USA). RNA quality was monitored by agarose gel electrophoresis and RNA quantity was measured by spectrophotometer.

Following amplification a reverse transcriptase (RT) reaction was performed using RNA extracted from treated and non-treated isolates by Monster Script RT-PCR kit (Epicenter, Technologies, USA) using specific primers. Amplified bands from PCR and RT-PCR from treated and untreated samples were confirmed by sequencing and compared for anti adhesive and anti invasive activity.

RESULTS

Antimicrobial activity

Antimicrobial activity of oil based aqueous di-herbal extract evaluated *in vitro* against MRSA and MSSA showed very good antimicrobial activity inhibiting the growth of MRSA and MSSA at concentration of 0.2 g/ml of *B. vulgaris*; 0.12 mg/ml of *C. longa* and 300 µl of oil. Antibacterial effect was highly significant with oil based aq -ueous di-herbal compared to individual extract (Table 1).

Anti adhesive and anti invasive activity

PCR amplification of *fnbA* gene before and after treatment showed good positive signal for both MRSA and MSSA (Figure 1). Sequencing of *fnbA* gene confirmed the identity. RT -PCR showed positive signal for non-treated samples while no amplification signal was seen for treated samples.

DISCUSSION

Secondary infections due to nosocomial multiple drug

Table 1. Antibacterial activity of oil based herbal extracts.

Extracts	Zone of inhibition[a]	
	MRSA	MSSA
Coconut oil	7	7
C. longa	8	7
B. vulgaris	11	10
cocktail	15	13
Oxa[b]	11	20
Van[c]	21	23

[a] Values expressed in mm, including diameter of filter paper (6 mm), are means of triplicates. [b] Oxacillin (1 μg) standard positive. [c] Vancomycin (30 μg) standard positive.

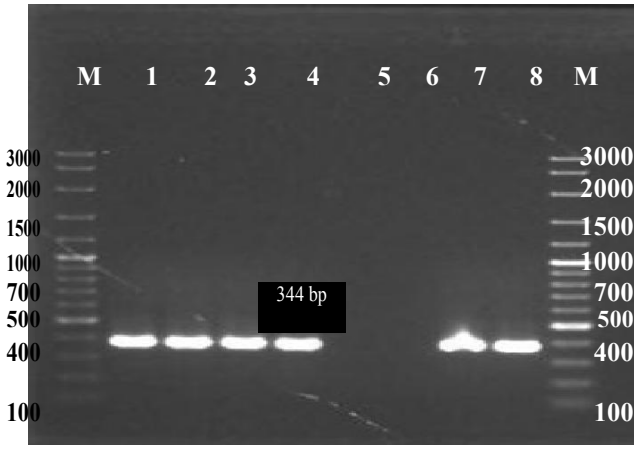

Figure 1. Lane M, 100 bp Plus DNA ladder; lane 1, DNA amplification of *fnbA* gene in MRSA before treatment; lane 2, DNA Amplification of *fnbA* gene in MSSA before treatment; lane 3, DNA amplification of *fnbA* gene in MRSA after treatment; lane 4, DNA amplification of *fnbA* gene in MSSA after treatment; lane 5, cDNA amplification of *fnbA* gene (RT-PCR) in MRSA after treatment; lane 6, cDNA amplification of *fnbA* gene (RT-PCR) in MSSA after treatment; lane 7, cDNA amplification of *fnbA* gene (RT-PCR) in MRSA before treatment; lane 8, cDNA amplification of *fnbA* gene (RT-PCR) in MSSA before treatment.

resistant bacterial strains are the most common complications reported in post surgical cases and cancer patients resulting in longer hospital stay, increase medical costs, unnecessary deterioration in quality of life and even mortality (Cosgrove et al., 2003). One of the predominant nosocomial pathogen is MRSA, frequently isolated from intensive care unit (ICU) and other critical wards. The ability of MRSA to colonize host tissues is mediated by adherence and invasive proteins encoded by *fnbp* genes. Any factor that interferes with the expre-ssion or function of this protein may prevent their attach-ment or invasiveness to host tissues, hence the esta-blishment of infection. Antibiotics are usually given as prophylaxis for prevention of colonization by bacterial pathogens in post surgical cases. Multiple drug resistant pathogens that do not respond to antibiotics may lead to further complications and also result in spread of resistant strains (Zinn et al., 2004). The oil based di-herbal extract formulated in this study shows a very good antimicrobial, anti-adhesive and anti-invasive activity against MRSA. The extracts when tested individually, except for *B. vulgaris* (10-11 mm), others showed small inhibition zone (7-8 mm). Combination of two herbs with coconut oil produced a significant inhibition zone (13-15 mm) against *S. aureus* and MRSA. Another interesting result observed in the present study was the growth of cultured organism on the inhibition zone of *B. vulgaris* extract treated plates after 18 h, while no growth was seen on the combination extract even after 48 h. This indicates that the combination extract gives enhanced protection.

Molecular analysis of *fnbA* (adhesin and invasive) genes and FNB proteins shows that the *fnbp* gene could be amplified in treated and non-treated extract, but absence of amplification signal in RT-PCR clearly indicates that the extract interferes with the protein synthesis and not the nucleic acid synthesis. The result is encouraging, as the extract not only has antibacterial activity which will be very useful in the treatment, but also has anti-adhesive and anti-invasive property that adds the value of the extract to be a colonization inhibitor, hence the extract could be used for treatment and prophylaxis.

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