Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 2, 2012

Research Article

INHIBITION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* BY BOTANICAL EXTRACTS OF *WAGATEA SPICATA*

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Received: 23 Dec 2011, Revised and Accepted: 2 Mar 2012

ABSTRACT

Western Ghats of India is treasure house of medicinal plants, comprising of algae, herbs, shrubs, climbers and trees which are annuals to perennials. Some of these are used for traditional and folk medicinal practices. The present study is carried out to find the antibacterial efficacy of *Wagatea spicata* a shrubby medicinal plant of Western Ghats, whose roots, bark and leaves have been used for several ailments by local people against fever, cough, gastrointestinal disorders, skin ailments etc., Shade dried leaf, bark and flower powder of the plant has been extracted with water. Aqueous extracts of plant materials are tested against two Methicillin resistant (MRSA), two Methicillin sensitive (MSSA) Staphylococcus aureus and ten other species of pathogenic bacteria through agar diffusion method. *Wagatea spicata* having Phenolic and flavonoid rich plant materials significantly inhibited MRSA, MSSA and five other species of pathogenic bacteria at a very low concentration. The findings of this study showed antibacterial efficacy of *Wagatea spicata*.

Keywords: *Wagatea spicata, Staphylococcus aureus,* MRSA, MSSA, Phenolics, Flavonoid.

INTRODUCTION

Staphylococcus aureus is the main cause for skin infection, surgical wound infection, septicemia and toxic shock syndrome [1]. Methicillin resistant *Staphylococcus aureus* (MRSA), a multidrug resistant variation of common *S. aureus* has become a considerable public health issue during the past decade, due to a significant increase in the incidence of MRSA isolated from patients with complicated infections, including diabetic foot infections of varying severity [2]. Antibiotic resistance decreases the therapeutics effectiveness of antibiotics used to treat variety of bacterial infections [3]. This has necessitated the use of new antimicrobial substances from other sources including plants. Plants are rich in wide variety of secondary metabolites, such as poly phenols tannins, terpenoids, alkaloids, and flavonoids which have been found *in vitro* to have antimicrobial properties. These substances serve as plant defense mechanisms against microorganisms [4].

Wagatea spicata Wt. Syn. *Moullva spicata* (Dalz) (Family: Fabaceae) is commonly known as candy corn plant, a small prickly, woody ornamental plant with long flaming spike. This legume shrub is one of the most widespread medicinal plants in the Western Ghat forests of India. The whole plant possesses medicinal properties and useful in the treatment of various ailments such as fever, cough, gastrointestinal disorders, skin ailments etc., as reported by local people as they regularly use roots, leaves and bark of the plant. Bark is used as an application for skin diseases. Root extract of *Wagatea. spicata* is having high phagocytic co-efficient and found to be effective against skin infections [5]. Aqueous extracts and their solvent fractions were found to be potent scavengers of deleterious superoxide and hydroxyl radicals. Free radical scavenging action of *Wagatea spicata* is due to its rich phenolic and flavonoid contents [6].

In the present study, antibacterial efficacy of plant material was evaluated with respect to two Methicillin resistant (MRSA), two Methicillin sensitive (MSSA) *Staphylococcus aureus* strains and ten other species of pathogenic bacteria. Aqueous extract of leaf, bark and flower of *Wagatea spicata* were analyzed for phenolic and flavonoid contents. Phenolic rich plant extracts brought significant inhibition of MRSA, MSSA and five species of pathogenic bacteria *at* very low concentration.

MATERIAL AND METHODS

Plant Material

Wagatea spicata plant materials leaves bark and flowers collected soon after monsoon season from Western Ghats region of India.

Preparation of Aqueous extract

Leaves, Bark and flowers of *Wagatea spicata* were collected, dried in shade and powdered. The powder was used for extraction. 100 gms of plant material powder was refluxed with 750 ml of double distilled water for 1 h. at 75-80°C, cooled and filtered. This was repeated in 3 trials; extracts were pooled and evaporated using Lyophiliser[7]. The extract yield was 16% in leaf, 30% in bark 35% in flower.

Bacterial Culture

Two strains of MRSA and two strains of MSSA used in this study were clinical isolates from wound swab. *Klebsiella pneumoniae*, beta haemolytic *Streptococcus, Escherichia coli* and Proteus were also clinical isolates from wound swab. *Acinetobacter* collected from endotracheal tube, *Klebsiella* from Urinary tract infection, *Enterobacter* from sputum, *Shigella flexneri* from stool and *Salmonella paratyphi* from blood.

Antibacterial assay

The test isolates were freshly grown on nutrient agar plates, inoculated into nutrient broth and incubated for 4-6 h at 37^{0}C to obtain organisms in log phase of growth. Broth turbidity is adjusted to 0.5 Mc Farland units to get 10^8 organisms /ml. This standard inoculum was introduced to Muller Hinton agar plates/ blood agar plates with a sterile glass spreader, by distributing the inoculum equally. Wells were punched in the agar plate with help of sterile borer. 50 µl plant material extract was added to the well with the help of sterile pipette. Plant material in two concentrations 8 mg and 16 mg/50 µl were tried in duplicate. Then all the plates were incubated at 37^{0}C for maximum 48 hours. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well [8].

RESULTS

The antibacterial activity of aqueous extracts of leaf, bark and flower of *Wagatea spicata* is assessed by the presence of absence of inhibition zone and diameter of zone of inhibition is given in (Table 1). They showed strong antibacterial activity against four strains of *Staphylococcus aureus* and five species of pathogenic bacteria which are responsible for hospital borne wound infections. All plant materials showed zone of inhibition of more than 20 mm diameter with respect to all strains of *S. aureus, S. citreus and β Hemolytic Streptococcus*. The maximum size of zone of inhibition (≥26 mm) was

achieved against *β Hemolytic Streptococcus.* Bark and flower extracts showed zone of inhibition (≥20 mm) against two strains of MRSA. However antibacterial activity was absent in plant materials with respect *E. coli, Klebsiella, Enteterobacter,* and *Salmonella* species.

Flower, bark and leaf material of *W. spicata* are rich in phenolic content 227 mg, 127 mg and 47mg Gallic acid equivalent/g of dry weight of crude extract respectively. Flavonoid content is 4 mg, 9 mg and 13 mg Quercitin equivalent/g of dry weight of crude extract respectively[6]. Phenolics and flavonoids are useful antimicrobial phytochemicals[4].

Inhibitory zone for bark and flower are bigger than leaf material which can be attributed to rich phenolic contents of these materials.

Table 1: Antibacterial activity of aqueous extracts of leaf, bark and flower material of *Wagatea spicata*

Bacteria	Zone of Inhibition					
	8 mg/50µl			16 mg/50µl		
	Leaf	Bark	Flower	Leaf	Bark	Flower
1. MSSA	++	+++	++	+++	+++	+++
2. MSSA (Coagulase negative)	+++	+++	++	+++	+++	++
3. MRSA	++	+++	++	++	+++	+++
4. MRSA (Coagulase negative)	++	+++	++	++	+++	+++
5. Escherichia coli	--	--	--	--	--	--
6. Proteus	++	++	++	++	++	+++
7. β Hemolytic Streptococci	++	+++	+++	+++	+++	+++
8. Klebsiella pneumoniae	--	--	--	--	--	--
9. Klebsiella (Urinary tract infection)	--	--	--	--	--	--
10. Enterobacter	--	--	--	--	--	--
11. Shigella flexneri	++	++	++	++	++	+++
12. Acinetobacter	+	+	+	++	++	++
13. Staphylococcus citreus	+++	+++	+++	+++	+++	+++
14. Salmonella paratyphi	--	--	--	--	--	--

D = Diameter of zone of inhibition

+++ D> 20mm, ++ 20mm>16mm, + 16mm >12mm, -- No inhibition

DISCUSSION

Methicillin resistant Staphylococcus aureus is a major cause of nosocomial infection leading to a wide range of diseases including endocarditis, osteomyelitis, toxic shock syndrome, pneumonia, food poisoning and carbuncles. In Indian hospitals MRSA is responsible for 30-80% of hospital acquired infections [9]. The present study indicated that plant materials *W. spicata* have strong antibacterial effect on MRSA strains. They also inhibited other bacterial pathogens responsible for wound and other infections. Phytochemical screening revealed that plant materials of *W. spicata* are rich in phenolics. Hydroxyl groups on phenol groups are thought to be related to their toxicity on microorganisms. It was also reported that oxidized phenol have more inhibitory effect on microorganisms [4]. Flower and bark extracts were rich in phenolics where as leaf extracts are rich in flavonoids. Flavonoids are antimicrobial substances synthesized by plants against invading infective microbes. Flavonoids show in vitro antimicrobial activity for a wide range of microbes [10]. Polyphenols from tea, phenolics and flavonoids from other plants have showed antimicrobial activity and also found to be very effective against MRSA [11, 12, 13]. Hence antibacterial efficacy of *W. spicata* is due to its phenolics and flavonoid contents.

In summary, this study shows botanical extracts of *Wagatea spicata* leaf, bark and flower are rich in phenolics and flavonoid contents. These phytochemicals of plant material significantly showed antibacterial efficacy on two strains of MRSA, and MSSA at low concentrations. They also significantly inhibited the growth of five other species which cause wound infection such as *S. citreus*, *β Hemolytic Streptococcus*, *Shigella flexneri , Acinetobacter* and *Proteus* species at very low concentration. Antibiotic potentials of phytocompounds of *Wagatea spicata* can be exploited in combination therapy of infectious diseases caused by multi drug resistant organisms.

ACKNOWLEDGEMENT

This work is supported by University Grant Commission of India under UGC Minor Research Project. Prof. Sudhakar Nayak , Head of the Department of Biochemistry, KMC, MAHE, Manipal, Karnataka, India is thankfully acknowledged for supporting this work.

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