



ANTIBACTERIAL SCREENING OF PHYTOCHEMICAL EXTRACTS AGAINST MRSA (METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS) - AN *IN VITRO* STUDY

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ABSTRACT

Acetone, hexane, ethyl acetate, butanol, methanolic extract of *Hygrophila quadrivalvis* and *Phyllanthus multiflorus* were screened for their antibacterial activity against a clinical isolate of Methicillin Resistant *Staphylococcus aureus* (MRSA). Agar well Diffusion method and Broth Serial Dilution method were used. It was compared with the Standard drug-Rifampicin (positive control). The Resistance pattern of the strain was checked by the agar well diffusion method and confirmed to be a MRSA strain. The prepared plates were incubated and results were evaluated by measuring the Zone of Inhibition - ZOI (in mm.) of drug extract. MIC and MBC were also determined for the resistant strain. All the experiments were conducted in triplicates and in sterilized conditions. Present investigation indicates that *Hygrophila quadrivalvis* and *Phyllanthus multiflorus* possesses antibacterial activity and hence can be used for future natural plant based antimicrobial agents. Hence our present study provides an *in vitro* evidence of its antibacterial activity against MRSA. However further investigations are needed to find out the active compound responsible for its pharmacological activity for the welfare of mankind where it can be used against the infectious diseases caused by the resistant strain of *Staphylococcus aureus* safely and effectively.

Keywords: *Hygrophila quadrivalvis*, *Phyllanthus multiflorus*, MIC, MBC, Zone of inhibition.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged in the 1960s as a cause of infection among patients exposed to the bacteria in health care level.¹ It is resistant to a large group of antibiotics like beta-lactams, which include the penicillin and the cephalosporins. Recently, MRSA infections have been reported among persons without such exposure (community-associated MRSA).^{2, 3} Community-associated outbreaks of MRSA infection have occurred among intravenous-drug users, athletes, military trainees, and men who have sex with men.⁴⁻⁷ Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms.⁸

The worldwide emergence of resistant strains of *Staphylococcus aureus* has become a major therapeutic problem. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.⁹ Researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multidrug resistant microbe strains.^{10,11} A wide group of medicinal plant preparations are available that have been used over the centuries almost exclusively because of the safety and economic value. The use of

Herbal medicine has been found to be increasing in the developed and developing countries in recent years.¹²

Hygrophila quadrivalvis commonly known as the temple plants or hygros and *Neermulli* in Tamil is a genus of flowering plants in the family *Acanthaceae*.¹³ The leaves are either homomorphic or heteromorphic. When they are homomorphic, they are always simple and entire. When heteromorphic, the basal or submerged leaves are pinnately divided with either filiform (i.e. feather-like) or linear segments that can be either simple or forked, while the cauline leaves (i.e. those on the stem) or emergent leaves are entire. The whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for various ailments. It possess good antibacterial activity.

Phyllanthus multiflorus commonly known as *Neerpoolan* in Tamil is the largest and most diversified genus among the genera in the subfamily phyllanthoideae of the euphorbiaceae.^{14, 15} *Phyllanthus* is a small shrub with dense fine foliage and wide spreading weeping habit. Its botanical features includes evergreen, exotic¹⁶ and foliage, it comprises approximately 1,270 species, making it one of the world's largest plant genera. Molecular data so far are of limited value for understanding *phyllanthus* because of the sheer size of the genus. They can, however, help sort-out cryptic species and provide information on the origin of suspected introduced species.¹⁷ It also possess potent antibacterial activity.

Our literature survey revealed that there are no scientific studies carried out regarding the antibacterial activity against MRSA on *Hygrophila quadrivalvis* & *Phyllanthus*



multiflorus to substantiate their therapeutic claim. Hence in our present study the Ethanolic, Ethyl acetate, Butanol, Hexane and Methanolic extract of aerial parts and roots were examined for its anti-bacterial property against MRSA.

MATERIALS AND METHODS

Plant material extraction

The leaves were collected from the Karungalakudi (forest area) hills near Madurai. The shade dried leaves were subjected to solvent extraction in ethyl acetate, acetone, Butanol, ethanol and methanol using the Soxhlet apparatus. This was performed in the Dept of Botany and Microbiology, Lady Doak College, Madurai. They were standardized to a final concentration of 5g/100ml.

Microorganism used

The clinical strain of MRSA was isolated at the Department of Microbiology, Saveetha Dental College from pus in a 50 year old diabetic patient and compared with control strains ATCC 25923 and Mu 50 MRSA (Methicillin resistant *Staphylococcus aureus*) The bacterial cultures were grown in Nutrient Broth (M002 Himedia Labs, Mumbai, India) and then plated on to Nutrient agar to obtain pure cultures. They were maintained in pure culture until use on nutrient agar slant.

Methodology

Antimicrobial assay (agar well diffusion technique)

Antimicrobial assay of the leaf extracts was performed against pathogenic strains by the Agar well diffusion method. The broth culture of the clinical isolate was prepared and compared to 0.5 McFarland's standard corresponding to 10^6 CFU/ml¹⁸⁻²².

The wells 6mm wide were prepared with the help of a cork borer and the drug (50 μ l) was poured in the respective well with the help of a micropipette. Finally, rifampicin discs (Himedia Labs, Mumbai, India) were placed on the prepared plates with sterile forceps. These plates were kept at room temperature for 30 minutes (Pre-diffusion time) and then incubated at 37°C for 24 hours.

Rifampicin discs were used as Positive Control while the solvents acetone, Butanol, ethyl acetate, methanol and ethanol were used as the Negative Control. The diameter of the inhibition zone was measured in mm.

Determination of minimum inhibitory concentration (MIC & MBC)

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method²¹. A series of two- fold dilution of each extract (0.25 to 4mg/ml) was made to which 1ml of the standardized bacterial suspension containing 10^6 organisms was added. The plates were incubated at 37°C for 24h and observed for visible growth. The minimum inhibitory concentration (MIC) is taken as the lowest

concentration of the extracts at which there is turbidity after incubation, the values are given in table 3. Minimum Bactericidal Concentration (MBC) was further determined from the same isolates MBC is the minimal concentration of drug needed to kill most (99.9%) of the viable organisms after incubation for 24 hours.

RESULTS AND DISCUSSION

The antibacterial activity of the leaves of *H. quadrivalvis* and *Phyllanthus multiflorus* extracted with different solvents (acetone, Butanol, ethanol, ethyl acetate, and methanol) at 50 μ g concentrations was screened by agar well diffusion technique and the diameter of the zone of inhibition was measured in mm. The results are given in the table 1 & 2. Butanol extract of *H. quadrivalvis* is effective against MDR *S. aureus* with ZOI 23mm diameter compared with all tested solvent form and Ethyl acetate extract of *P. multiflorus* is effective against MDR *S. aureus* with ZOI 22 mm in diameter. The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] were also determined for the extracts and the results are given in table 3. The butanol extract of *Hygrophila quadrivalvis* is found to have low MIC & MBC values of 0.5mg/ml and the ethyl acetate extract of *Phyllanthus multiflorus* is also found to show low MIC&MBC values of 0.5mg/ml respectively against MRSA. Hence, the result of the present study suggests that leaves of *H. quadrivalvis* and *P. multiflorus* are a potent natural source of biologically active compounds from herbal medicines, which may potentially prove to be efficient natural antimicrobial agents. The active component of the leaf extracts have to be further characterised to make them useful in therapeutic medicine.

Table 1: Agar well diffusion method

Hydrophila Quadrivalvis 50 μ g concentration				
Acetone	Butanol	Ethanol	Ethyl acetate	Methanol
11mm	23mm	12mm	14mm	12mm

Table 2: Agar well diffusion method

Phyllanthus Multiflorus 50 μ g concentration				
Acetone	Butanol	Ethanol	Ethyl acetate	Methanol
18mm	15mm	18mm	22mm	15mm

Zone of inhibition of positive control (Rifampicin): 20 mm

Zone of inhibition of negative controls (all solvents used for extraction): 0 mm.

Table 3: MIC and MBC of the herbal extracts

MIC and MBC (mg/ml)					
Hydrophila Quadrivalvis					
	Acetone	Butanol	Ethanol	Ethyl acetate	Methanol
MIC	1.0	0.5	2.0	4.0	1.5
MBC	1.0	0.5	2.0	4.0	1.5
Phyllanthus Multiflorus					
	Acetone	Butanol	Ethanol	Methanol	Ethyl acetate
MIC	1.0	2.0	1.0	2.0	0.5
MBC	1.0	4.0	2.0	2.0	0.5



CONCLUSION

With the emergence and widespread occurrence of multi drug resistant bacteria focus has now been shifted in exploring natural compounds that may combat drug resistance problem. Our study suggests that *Hygrophila quadrivalvis* and *Phyllanthus multiflorus* exhibit antimicrobial properties against MRSA a resistant strain. The potential antimicrobial activity of leaf extract of *Hygrophila quadrivalvis* and *Phyllanthus multiflorus* towards the infectious microorganism explains the basis for its use in future in combating the disease caused by such harmful bacteria.

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