International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 3, Issue 12 - 2016

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2016.03.12.028

Antibacterial screening on phytochemical extracts of *Hygrophila auriculata* (Neer mulli) on MRSA(methicillin resistant *Staphylococcus aureus*)-an *in vitro* study.

S.Savitha[1] and N.Yogananth[2]
[1]Department of Microbiology, Sri Muthukumaran Medical College Hospital & Research Institute, Chikkarayapuram, Chennai - 600 069 Tamil Nadu, India
[2]PG& Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Chennai, Tamilnadu, India
*Corresponding author: *savithaknc@gmail.com*

Abstract

Acetone, hexane, ethyl acetate, butanol, methanolic extract of *Hygrophila auriculata* (stem, leaf, root) were screened for their antibacterial activity against a clinical isolate of Methicillin Resistant *Staphylococcus aureus* (MRSA)from throat infected patients. 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 auriculata* 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 auriculata*, 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 The plant *Hygrophila auriculata* (K. Schum) Heine (Acanthaceae) has been traditionally used for the treatment of inflammation, pain, urinary infection, edema, gout and as a diuretic. It is described in ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha having eyes like the Kokila or Indian Cuckoo. It is classified in the ayurvedic system of medicine as Seethaveryam, Mathuravipaka and is used for the treatment of a number of conditions including diabetes and dysentery9, 10.The plants are widely distributed throughout India, Sri Lanka, Burma, Malaysia and Nepal. Following various folk claims as a cure for numerous diseases, efforts have been made by researchers to verify the efficacy of the plant by scientific biological screening. The plant contains saponins, alkaloids, steroids, tannins, flavonoids and triterpenoids are the main phytoconstituents. A scrutiny of literature revealed some notable pharmacological effects like anti-nociceptive11, antitumor12,13, antioxidant14, hepatoprotective15-18, hypoglycemic19, haematinic, diuretic, free radical scavenging, anthelmintic, anti-inflammatory, antipyretic and antimotility activities . The present review is an attempt to highlight the various ethanobotanical and traditional uses as well as phytochemical and pharmacological reports about *Hygrophila auriculata* (K. Schum) Heine. The aim of the present work is to carryout phytochemical studies on the leaves of the medicinal plant, *Hygrophila auriculata* (Heine.) and to study the in-vitro antibacterial activities of the leaf, stem,root-extract of the plant *in vitro.*

Materials and Methods

Collection of Plant Material

The plant material *Hygrophila auriculata* was collected from paddy fields in and around mylapur village and chikkarayapuram area in Thiruvalluvar District on different days. 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 Microbiology, Sri muthukumaran medical college, Chennai. They were standardized to a final concentration of 5g/100ml.

Microorganism used

The clinical strain of MRSA was isolated at the Department of Microbiology, Sri muthukumaran medical college , hospital &RI, Chennai from throat infected patients 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 106 CFU/ml18-22.

The wells 6mm wide were prepared with the help of a cork borer and the drug (50µl) 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^{o}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 106 organisms was added. The plates were incubated at 37oC 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,stem and root of *H. auriculata* extracted with different solvents (acetone, Butanol, ethanol, ethyl acetate, and methanol) at 50µg 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 &3 . Butanol extract of *H.auriculata* (leaf extract) is effective against MDR *S.aureus* with ZOI 23mm diameter compared with all tested solvent form. 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 auriculata* is found to have low MIC & MBC values of 0.5mg/ml. Hence, the result of the present study suggests that leaves of *H.auriculata* 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 *Hygrophila auriculata* (50µg concentration)leaf extract.

	Butanol	Ethanol	Ethyl acetate	Methanol
11mm	23mm	13mm	14mm	11mm

Table 2: Agar well diffusion method *H.auriculata* (stem extract) 50µg concentration.

	Butanol	Ethanol	Ethyl acetate	Methanol
18mm	15mm	20mm	15mm	17mm

Table 2: Agar well diffusion method *H.auriculata* (Root extract) 50µg concentration.

	Butanol	Ethanol	Ethyl acetate	Methanol
13mm	19mm	22mm	14mm	17mm

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 extract.

MIC and MBC in(mg/ml)					
Hygrophila auriculata					
	1.0	1.0	0.6	0.5	2.0
MBC	2.0	4.0	1.5	2.0	1.5

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 leaf extract of *Hygrophila auriculata* exhibit antimicrobial properties against MRSA a resistant strain. The potential antimicrobial activity of leaf extract of *Hygrophila auriculata* towards the infectious microorganism explains the basis for its use in future in combating the disease caused by such harmful bacteria.

References

1. Barrett FF, McGehee RF Jr, Finland M. Methicillin-resistant Staphylococcus aureus at Boston City Hospital: bacteriologic and epidemiologic observations. N Engl J Med 279:1968; 441-448.
2. Herold BC, Immergluck LC, Maranan MC. Community acquired methicillin resistant Staphylococcus aureus in children with no identified predisposing risk. JAMA 1998; 279:593-598.
3. Fridkin SK, Hageman JC, Morrison M. Methicillin-resistant Staphylococcus aureus disease in three communities. N Engl J Med 352:2005;1436-1444. [Erratum, N Engl J Med 2005; 352:2362]
4. Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections -- Los Angeles County, California, 2002-2003. MMWR Morb Mortal Wkly Rep 52:2003; 88-88.
5. Kazakova SV, Hageman JC, Matava M, et al. A clone of methicillin-resistant Staphylococcus aureus among professional football players. N Engl J Med 352:2005; 468- 475.
6. Zinderman CE, Conner B, Malakooti MA, LaMar JE, Armstrong A, Bohnker BK. Community-acquired methicillin- resistant Staphylococcus aureus among military recruits. Emerg Infect Dis 10:2004; 941-944.
7. 7.Gonzalez BE, Martinez-Aguilar G, Hulten KG, et al. Severe staphylococcal sepsis in adolescents in the era of community-acquired methicillin-resistant Staphylococcus aureus. Pediatrics 115:2005; 642-648.
8. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M and Khan AU: Antimicrobial Activity of Five Herbal Extracts Against MultiDrug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin: Molecules 14: 2009; 586-597.
9. Chahal K. J., Sarin R., and Manvimalwal: Efficacy of clerodendrum inerme l. (garden quinine) against some human pathogenic strains: International Journal of Pharma and Bio Sciences 1(4): 2010; 219 - 223.
10. Cos P, Vlietinck AJ, Berghe DV, Maes L: Anti-infective potential of natural products: How to develop a stronger in vitro proof-of-concept‘: Journal of Ethnopharmacology 106: 2006; 290 – 302
11. Bandow JE, Brotz H, Leichert LIO, Labischinski H and Hecker M: Proteomic approach to understanding antibiotic action: Amicro. Agents. Chemother. 47:2003; 948 – 955.
12. 12. Kumar VR, Kumar S, Shashidhara, Anitha: Modulation of CNS Activity By Traditional Medicines: A Review On Neuro Pharmacognosy: International Journal of Current Research and Review, 3(1):2011; 39- 48.
13. Hygrophila plant available at en.wikipedia.org/wiki/ Hygrophila_(plant)
14. Erdatman n, g., Pollen morphology and plant taxonomy, 1. Angiosperms. Almqvist and wiksell, Stockholm, 1952. 539.
15. Webstar G. l. and Elias, J. R., Cytotaxonomic Studies in the Euphorbiaceae, Subtribe phyllanthinae. 1962.
16. Betty A.Forbes. Daniel F.Sahm., Alice S.Weissfeld. Bailey & Scott’s Diagnostic Microbiology 11th edition Mosby, 229 – 257.
17. National Committee for Clinical Laboratory Standards: Methods for Disc Susceptibility Tests for Bacteria that Grow Aerobically: NCCLS Document M2-A7: National Committee for Clinical Laboratory Standards: Wayne, USA: 2000; 7.
18. Ananthanarayan R and Paniker‘s: Textbook of Microbiology: 8th edition: Publishers University Press: Hyderabad 2009: 618.
19. Jennifer MA: Determination of Minimum Inhibitory Concentrations: Journal of Antimicrobial Chemotherapy 48, 2001; (SI): 5 -16.
20. J.G.Colle., A.G.Faster., B.P.Marmion., A.Simmons. Practical Microbiology [Mackie & Mc Cartney] 14th edition, 851 – 852.

Access this Article in Online	
	Website: www.ijarbs.com
	Subject: Medicinal Plants
Quick Response Code	
DOI: 10.22192/ijarbs.2016.03.12.028	

How to cite this article:
S.Savitha and N.Yogananth. (2016). Antibacterial screening on phytochemical extracts of *Hygrophila auriculata* (Neer mulli) on MRSA(methicillin resistant *Staphylococcus aureus*)-an *in vitro* study. Int. J. Adv. Res. Biol. Sci. 3(12): 196-199.
DOI: http://dx.doi.org/10.22192/ijarbs.2016.03.12.028