INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-8, Issue-4, Oct-Dec-2017 Coden IJABFP-CAS-USA Copyrights@2017

*Received: 14th July 2017* *Revised: 27th Aug- 2017* *Accepted: 30th Aug-2017*

*DOI: 10.21276/Ijabpt, http://dx.doi.org/10.21276/ijabpt* *Research article*

# ANTIBACTERIAL ACTIVITY OF *MIKANIA SCANDENS* (L.) WILLD. AGAINST MULTIDRUG RESISTANT BACTERIAL PATHOGENS ISOLATED FROM CLINICAL SAMPLES

Rimashree Baishya[1], Ankita Singh[1], Bhoomika Basu Mallik[1], Asmita Dewanjee[1], Prasanta Kumar Maiti [2],Kumkum Bhattacharya[2], Soma Banerjee[1*]

[1]Department of Biotechnology, Heritage Institute of Technology, Chowbaga Road, Anandapur, P.O. East Kolkata Township, Kolkata – 700107. Ph: +91 8420864992, Fax: + 91 33 24430455.

[2]Department of Microbiology, I.P.G.M.E.R. Medical College & Hospital, 244 A.J.C.Bose Road, Kolkata-70020, West Bengal.

**ABSTRACT**

Objective: To evaluate the antibacterial activity of the leaf extracts of *Mikania scandens* (L.) Willd. The antibacterial activity was tested against sixty two multidrug resistant clinical isolates from human wounds which consist of two categories of bacteria: *Staphylococcus aureus* (19) and *Pseudomonas aeruginosa* (43) along with their corresponding reference strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853.

Methods: The antibacterial sensitivity multi-drug resistant bacteria to the ethanolic, methanolic and aqueous extract of leaves of medicinal plant *Mikania scandens* were studied by the agar-well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each bacteria were also determined.

Results: *S. aureus* isolates were found to be sensitive to ethanol, methanol and aqueous extract of the plant sample with 94.7%, 94.7% and 26.3% respectively. It was noted that all organic extracts are comparatively more effective than aqueous extracts.

Conclusion: It can be concluded that the leaf extracts of *Mikania scandens* possess antibacterial activity against human wound pathogens.

**Key words**- Agar-well diffusion method, Antibacterial activity, *Mikania scandens*, Plant extracts.

*Corresponding author: Soma Banerjee, Department of Biotechnology, Heritage Institute of Technology, Chowbaga Road, Anandapur, P.O. East Kolkata Township, Kolkata – 700107. Ph: +91 8420864992, Fax: + 91 33 24430455 soma.banerjee@heritageit.edu



## INTRODUCTION

In recent years, it is found that due to emergence of drug-resistant pathogenic bacteria or a long-standing infection several premature death occurred. (Singh A, Singh PK. (2009). This may be due to the existence of multidrug resistant (MDR) strains of pathogens, whose emergence is a natural process another reason might be the random use of broad-spectrum antibiotics which induce the development of MDR bacterial strains (De Silva T et al, 2009). In healthy individuals, in whom infection has been controlled, the appearance of these may not be a major issue of concern but in patients with immune-suppression, these mutants emerge as a new population. MDR strains are resistant to available antimicrobial agents and this issue has become a major global health problem. The frequencies of bacterial resistance to the antimicrobial agents are increasing worldwide, posing a serious threat to public health (Norrby S.R et al, 2005, Levy S.B., Marshall B. 2004). Hence, an alternative antimicrobial strategy of making use of plants and plant-based products has led to a reassessment of the therapeutic use of ancient remedies (Mandal S et al, 2009, Mandal S et al, 2010).

The use of traditional medicine to treat infection has been practiced since the origin of mankind, as plants have been an integral part of human society since the beginning of civilization. The World Health Organization (WHO) estimated that about 80% population of developing countries rely on traditional medicines, mostly plant-based products, for their primary health care needs (Basualdo C et al, 2007). Despite of modernisation in the medicinal system, tribes of India, particularly, are still practicing the art of the use of crude herbal products as medicines. *Mikania scandens* (L.) Willd. (Asteraceae), a climbing weed with long-petioled opposite leaves and small homogenous flower-heads, grown as a common weed throughout the plains of India and Bangladesh. It is reported that this plant have been used to treat stomach ulcers in folk medicine. Traditionally, affected area of body having wounds and bruises were treatment with its leaf juice. (Sangita Chandra et al, 2012). The present study was carried out to evaluate the antibacterial activity of leaves of *Mikania scandens,* a common weed against bacterial pathogens isolated from clinical samples.

## MATERIALS AND METHODS

### Preparation of plant extracts

### Plant Material Collection and Extraction:

Whole plant was collected from the nearby College campus in Kolkata, West Bengal, India. The plant was identified by Dr Nanda Paria, Department of Botany, University of Calcutta, India. A voucher specimen has been submitted in the University of Calcutta Herbarium with Accession number: 20007-CUH. The leaves were separated, washed The leaves were shade dried. 20gm of leaves were crushed using a mortar pestle then 70% ethanolic, 70% methanolic and aqueous extract of 200ml each were prepared and kept at 4°C for 48 hours. These extracts were then filtered and were kept in -18ºC for further use.

### Microbial strains and culture media

Sixty two clinical isolates of two test organisms were used for the assay of the antimicrobial property from *Mikania scandens.* These isolates were obtained from clinical samples from a tertiary health care hospital, Kolkata. Out of these different clinical samples (wound swap and pus) 19 samples were found to be positive for *Staphylococcus aureus* and 43 samples for *Pseudomonas aeruginosa*. Apart from those, reference strain of *Staphylococcus aureus* 25923 ATCC and *Pseudomonas aeruginosa* 27853 ATCC were taken. All the isolates were maintained on nutrient agar (HiMedia) slants at 5°C.

### Antibacterial activity tests

Antibacterial activity tests were performed by standardized agar-well diffusion method (Bazerque P et al, 1990). For this technique, bacterial pathogens prepared in saline water (0.85% NaCl) and adjusted to a turbidity of 0.5 McFarland standards ($10^8$ CFU/ml) were spread on the solid plates with a sterile swab. Wells (6 mm depth) were punched and the wells were filled with ethanolic, methanolic and aqueous extracts of leaves of *Mikania scandens* along with the solvents used for preparing extracts, as negative control according to standard protocol**.** Standard disc of ciprofloxacin (5 µg/disc), ampicillin/sulbactam (10/10 µg/disc), levofloxacin (5 µg/disc), chloramphenicol (30 µg/disc), nitrofurantoin (300 µg/disc), nalidixic acid (30 µg/disc), ceftazidime (30 µg/disc), piperacillin/tazobactam (100/10 µg/disc), co-trimoxazole (25 µg/disc) and oxacillin (1 µg/disc) (HiMedia) were used against all isolates for sensitivity test by disc diffusion method. Plates were incubated at 37°C for 24 hours. Antibacterial activities were evaluated by measuring the diameter of zone of inhibition and comparing results from interpretative standards.

### Determinations of MIC and MBC

Original stock solutions of plant extracts prepared with methanol and ethanol (70%). An aliquot of 80 µl of a suitable dilution of a solvent-extract was released to a well on a 96 well micro-titer plate along with an aliquot of 100 µl nutrient broth, an aliquot of 20 µl bacterial inoculum ($10^9$ cfu/ml) and a 5 µl-aliquot of 0.5% 2,3,5-triphenyl tetrazolium chloride (TTC). The microplate was incubated at 37$^o$C for 18 h. A pink colouration due to TTC in a well indicated bacterial growth and the absence of any colour was taken as inhibition of bacterial growth (Brown S.A. 1987). The MIC value was noted at the well, where no colour was manifested. Further, bacteria from each well of the microplate were sub-cultured on a nutrient agar plate; the level of dilution, where no bacterial growth on the nutrient agar plate was observed, was noted as the MBC value.

## RESULTS

### Antibacterial activity of plant extracts and antibiotics

In this study two reference strains *Staphylococcus aureus* 25923 ATCC and *Pseudomonas aeruginosa* 27853 ATCC were taken in parallel to 62 clinically isolated samples. In the study of monitoring antibacterial activities 10 different types of antibiotic discs were tested against individual clinical isolates and also with reference strains, i.e., ciprofloxacin, levofloxacin, chloramphenicol, ampicillin/sulbactam, nalidixic acid, ceftazidime, nitrofuratoin, piperacillin/tazobactam, co-trimoxazole and oxacillin along with aqueous, 70% ethanolic and 70% methanolic extracts of leaves were tested.

All the reference strains exhibited sensitivity to all the ten antibiotics and in all the plant extracts. In *Pseudomonas aeruginosa* clinical isolates (43) they were found to be maximum resistant to the following antibiotics ampicillin/sulbactam, oxacillin, and nitrofurantoin 69.8% (30), 67.4% (29), and 65.1% (28) respectively.
For other antibiotics like nalidixic acid, chloramphenicol, ceftazidime showed moderate resistance (Table 1). In case of ethanolic extracts of leaves antibacterial activity was shown in 72.1%, followed by methanolic and aqueous extract of leaves 62.8%, 23.26% respectively (Table 2).

Among the 19 *Staphylococcus aureus* clinical isolates they showed moderate resistance to oxacilin in nine strains (Table 1). In case of both ethanolic and methanolic plant extracts of leaves 94.7% of isolates were sensitive, followed by 26.3% of aqueous extract of leaves (Table 2).

## MIC and MBC

Ethanolic and methanol extracts showed MIC at 10 mg of *Mikania scandens* leaves extracts which is about 95%. The MIC values of both ethanol and methanol extracts were found to be same. The aqueous extracts have shown 60% MIC in 15 mg concentration. MBC values were found to be 15mg for ethanol and methanol extracts and 20 mg for aqueous extracts (Table 3).

**Table 1: Resistant patterns of clinical isolates with standard antibiotics**

| Antibiotics (µg/disc) | Antibiotic sensitivity pattern of 62 isolates | | | |
|---|---|---|---|---|
| | *Pseudomonas aeruginosa* | | *Staphylococcus aureus* | |
| | Number of sensitive isolates | Number of resistant isolates | Number of sensitive isolates | Number of resistant isolates |
| **Ampicillin/Sulbactam (10/10)** | 13 | 30 | 16 | 3 |
| **Oxacillin (1)** | 14 | 29 | 10 | 9 |
| **Nitrofurantoin (300)** | 15 | 28 | 19 | 0 |
| **Co-Trimoxazole (25)** | 21 | 22 | 17 | 2 |
| **Chloramphenicol (30)** | 26 | 17 | 19 | 0 |
| **Nalidixic Acid (30)** | 28 | 15 | 16 | 3 |
| **Ceftazidime (30)** | 29 | 14 | 17 | 2 |
| **Piperacillin/Tazobactam (100/10)** | 35 | 8 | 17 | 2 |
| **Ciprofloxacin (5)** | 39 | 4 | 19 | 0 |
| **Levofloxacin (5)** | 39 | 4 | 19 | 0 |

**Table 2: Resistant patterns of clinical isolates with plant extracts of *Mikania scandens***

| Leaf and flower extracts (mg) | Sensitivity pattern of 62 isolates | | | |
|---|---|---|---|---|
| | *Pseudomonas aeruginosa* | | *Staphylococcus aureus* | |
| | No of sensitive isolates | No of resistant isolates | No of sensitive isolates | No of resistant isolates |
| **Leaf ethanolic extract (10)** | 31 | 12 | 18 | 1 |
| **Leaf methanolic extract (10)** | 27 | 16 | 18 | 1 |
| **Leaf aqueous extract (10)** | 10 | 33 | 5 | 14 |

**Table 3: MIC of different extracts of *Mikania scandens***

| Solvent | Conc. (mg) | *Pseudomonas aeruginosa* | | | | | | | | | | *Staphylococcus aureus* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **Aqueous** | **10** | + | + | + | - | + | + | - | + | + | - | + | + | - | + | - | - | + | + | - | + |
| | **15** | - | + | + | - | - | - | - | + | - | - | + | - | - | + | - | - | - | - | - | - |
| | **20** | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Methanol** | **10** | - | - | + | - | + | - | - | + | + | - | - | - | - | + | - | - | + | + | - | - |
| | **15** | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | **20** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Ethanol** | **10** | - | - | + | - | + | - | - | + | - | - | - | - | - | + | - | - | + | + | - | - |
| | **15** | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | **20** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**+ = positive growth, - = negative growth**

## DISCUSSION

The leaves extract of *Mikania scandens* showed varied antibacterial efficacies against both the reference strains of bacteria. Both ethanolic and methanolic extracts of leaves showed better antibacterial activity against clinical sample than aqueous extracts. The extracted showed better antibacterial activity against *Staphylococcus aureus*. Aqueous extracts showed less activity as the availability of phytochemicals are less. This result suggests that this plant shows more antibacterial activity against gram-positive bacteria which than gram-negative bacteria (Table 1).

## CONCLUSION

Thus, the study suggests that this plant can be used for the treatment of wound infections caused by MDR bacteria. Additionally, the potential use of these plants must be explored to use it as an alternate therapy for the treatment of infections caused by antibiotic-resistant bacteria.

## ACKNOWLEDGEMENT

The authors are grateful to Department of Higher Education and Science and Technology and Biotechnology, Government of West Bengal for funding the project (9G-38/13).

## REFERENCES

Basualdo C., Sgroy V., Finola M.S., Juam M. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet Microbiol, 124: 375-381.

Bazerque P., Perez, C., Pauli, M. (1990). An antibiotic assay by agar-well diffusion method, *Acta Biologiae et Medecine Experimentaalis*, 15:113-115.

Brown S.A. (1987). Minimum inhibitory concentrations and post-antimicrobial effects as factors in dosage of antimicrobial drugs. J Am Vet Med Assoc, 19: 871-872.

De Silva T., Bahorun T., Sahu M., Le Mai H. (2009). Traditional and alternative medicines, research and policy Perspectives. Delhi: Daya Publishing House.

Levy S.B., Marshall B. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. Nature Med, 10: S122-S129.

Mandal S., Deb Mandal M., Pal N.K. (2010). Synergistic anti-*Staphylococcus aureus* activity of amoxicillin in combination with *Emblica officinalis* and *Nymphae odorata* extracts. Asian Pac J Trop Med, 3: 711-714.

Mandal S., Pal N.K., Chowdhury I.H., Deb Mandal M. (2009). Antibacterial activity of ciprofloxacin and trimethoprim alone and in combination, against *Vibrio cholerae* O1 biotype El Tor serotype Ogawa isolates. Polish J Microbiol, 58: 57-60.

Norrby S.R., Nord C.E., Finch R. (2005). Lack of development of new antimicrobial drugs: A potential serious threat to public health. Lancet Infect Dis, 5: 115-119.

Sangita Chandra, Protapaditya Dey, Sanjib Bhattacharya. (2012). Preliminary in vitro assessment of anti-inflammatory property of *Mikania scandens* flower extract. J. of Advanced Pharmacy Education & Research 2 (1): 25-31.

Singh A, Singh PK. (2009). An ethnobotanical study of medicinal plants in Chandauli district of Uttar Pradesh, India. J Ethnopharmacol: 12, 324-329.

ISSN : 0976-4550

# INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com