Research Article

Anti-bacterial Potential and Qualitative Phytochemical Analysis of an Invasive Alien Plant *Mikania micrantha* kunth found in Dhenkanal District of Odisha, India.

Sandeep Kumar Nayak, Monalisha Maharana, Sasmita Jagat, Akhtari Khatoon, Kunja Bihari Satapathy*
P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.
***Corresponding author's E-mail:** kbsbotuu@gmail.com

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ABSTRACT

The objective of the present investigation was to evaluate the antibacterial potential of n-hexane and methanolic extracts of an invasive alien plant *Mikania micrantha* Kunth. The antibacterial potential of the plant was tested against 13 human pathogens such as *Enterococcus faecalis* (MTCC- 459), *Escherichia coli* (MTCC- 614), *Bacillus circulans* (MTCC- 490), *Salmonella paratyphi* (MTCC-3220), *Vibrio choleriae* (MTCC- 3906), *Salmonella enterica typhimurium* (MTCC- 98), *Pseudomonas aeruginosa* (MTCC- 8076), *Shigella flexneri* (MTCC-9543), *Salmonella enterica ser typhi* (MTCC-733), *Bacillus subtilis* (MTCC-1305), *Streptococcus mitis* (MTCC-2897), *Klebsiella pneumoniae* (MTCC-109) and *Staphylococcus aureus* (MTCC-1430) using agar well diffusion method. The results of the study revealed that n-hexane extract of sample was highly effective against *Pseudomonas aeruginosa* whereas *Staphylococcus aureus* and *Klebsiella pneumoniae* showed no response and other test pathogens under study responded moderately. It was observed that methanolic extract had high inhibition potential against *Pseudomonas aeruginosa* whereas *Salmonella paratyphi* and *Shigella flexneri* showed no response and other test pathogens under study responded moderately. Phytochemical analysis revealed presence of alkaloids, steroids, terpenoids, glycosides, tannins, proteins, carbohydrates and phenolic compounds in methanolic extract whereas n- hexane extract contains steroids, terpenoids and phenolic compounds.

Keywords: Agar well diffusion, Antibacterial activity, Dhenkanal, *Mikania micrantha*, Odisha, Phytochemical analysis.

INTRODUCTION

Plants have been a source of food, fibre and medicine since the beginning of the human civilization. In India, Ayurvedic system of medicine has used many herbs such as turmeric possibly as early as 1900 B.C. Many other herbs and minerals used in 'Ayurveda' were later described by ancient Indian herbalists including 'Charaka' and 'Sushruta' in 'Charaka Samhita' and 'Sushruta Samhita' respectively which describes 700 medicinal plants. Plant products play a key role in the human health care. According to (WHO, 1993) 80% of the world populations rely on the use of traditional medicine based on plant materials for their primary healthcare[1]. *Mikania micrantha* Kunth. is an invasive alien plant species which was introduced into Indonesia as ground cover in the 1940's where it then spread to the Pacific Islands and South-east Asia.[2] It was introduced into India during World War II to camouflage air fields or as ground cover for tea plantations.

This weed may compete for nutrients, light and soil moisture with nearby plant species and then kill the plant species.[3] In addition, its rampant growth characteristics and potential allelopathic effects can devastate most native species populations[4] and cause substantial damage to natural ecosystem and biodiversity.

Hence, *M. micrantha* is listed as one of the world's worst weeds. It is reported that, sesquiterpenoids, flavonoids, polyphenols, sesquiterpene, lactones have been isolated from *M. micrantha*.[5] The leaves of *M. micrantha*, commonly known as 'Guaco', are used to make a poultice for snake bites and scorpion stings; decoction of the leaves is used to bath rashes, skin itches, athlete's foot and as wound dressings in Jamaica.[6]

More particularly, the extracts from leaves and stem of the plant are known to be active against a wide variety of microorganisms, including Gram-negative bacteria and Gram-positive bacteria.[6]

Several reports on the antimicrobial activity of *M. micrantha* against some plant pathogens are available in the literature.[8,9]

The antimicrobial activity of *M. micrantha* is assigned to a number of sesquiterpene lactone compounds, which also in pure form have been shown to exhibit antibacterial or antifungal activity.[10]

Ethnomedicinally, *M. micrantha* is used to treat fever, rheumatism, influenza and respiratory diseases.

In the present investigation, n-hexane and methanolic extracts of *M. micrantha* Kunth have been evaluated for their antibacterial activities.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant *Mikania micrantha* Kunth was collected from Kapilash in the district of Dhenkanal, Odisha in the month of March 2014.

Identification of the voucher specimen was done by following 'Flora of Orissa'.[11]

The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University,

Vani Vihar, Bhubaneswar.

The samples were collected in bulk amount, washed in running tap water, dried under shade and made to coarse powder form.

Processing of Plant Material and Preparation of Extract

The collected plant material which was shade dried and ground to form course powder and had been successively extracted with the solvent n-hexane and methanol by Soxhlet apparatus[12,13] and the extract was recovered under reduced pressure in a rotatory evaporator.

The extracts were kept in desiccators for further use.

Evaluation of the Extracts for Antibacterial Activity

The *in vitro* antibacterial screening was carried out against selected bacterial pathogens causing various dreadful diseases in human.

The bacterial pathogens were *Enterococcus faecalis* (MTCC-459), *Escherichia coli* (MTCC-614), *Bacillus circulans* (MTCC-490), *Salmonella paratyphi* (MTCC-3220), *Vibrio choleriae* (MTCC-3906), *Salmonella enterica typhimurium* (MTCC-98), *Pseudomonas aeruginosa* (MTCC-8076), *Shigella flexneri* (MTCC-9543), *Salmonella enterica ser typhi* (MTCC-733), *Bacillus subtilis* (MTCC-1305), *Streptococcus mitis* (MTCC-2897), *Klebsiella pneumoniae* (MTCC-109) and *Staphylococcus aureus* (MTCC-1430).

These species were procured from Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India.

These organisms were identified by standard microbial methods.[14]

The antibacterial screening of the extracts were carried out by determining the zone of inhibition using agar well diffusion method.[11]

Ciprofloxacin was taken as reference antibiotic.

Agar Well Diffusion Assay

Agar well diffusion method[12,13] was followed to determine the zone of inhibition of microbes in Nutrient Agar (NA, HiMedia Laboratories Ltd., Mumbai) plates which were swabbed (sterile cotton swabs) with 8 hr old broth culture of bacteria.

Wells (8 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer.

Stock solution of plant extracts were prepared at a concentration of 3 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 hours.

Control experiments comprising inoculums without plant extract were set up.

The plates were incubated at 37 °C for 24 hours for bacterial pathogens.

Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and the data were statistically analysed.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary phytochemical investigation reports of the various extracts indicated that the n-hexane extract of *Mikania micrantha* was found to contain steroid, terpenoid and phenolic compounds, whereas the methanol extract showed the presence of alkaloids, terpenoid, phenolic compounds, steroids, glycoside, tannins, proteins and carbohydrate (Table 1).

Table 1: Preliminary phytochemical analysis of different extracts of *Mikania micrantha* Kunth

Phytoconstituents	n-hexane extract	Methanol extract
Alkaloid	-	+
Steroids	+	+
Terpenoid	+	+
Glycosides	-	+
Tannins	-	+
Phenolic compounds	+	+
Carbohydrate	-	+
Proteins	-	+

'+' indicates the presence and '– 'indicates absence

Antibacterial Screening

The extract of whole plant subjected to antibacterial screening against three gram-positive (*Bacillus subtilis, Streptococcus mitis* and, *Staphylococcus aureus*) and three gram-negative (*Pseudomonas aeruginosa*, *Salmonella enterica ser typhi, Escherichia coli, Salmonella enterica typhimurium, Shigella flexneri, Vibrio choleriae* and *Klebsiella pneumoniae*) bacteria causing various diseases.

The results indicated that n-hexane extract of the plant sample exhibited highest zone of inhibition against *Pseudomonas aeruginosa* (16.03 ± 0.12), least against *Salmonella enterica typhimurium* (10 ± 0.08) while *S. aureus* and *K. pneumoniae* were resistant.

This extract exhibited moderate activity against *Streptococcus mitis* (14.1 ± 0.08).

Whereas methanolic extract exhibited highest zone of inhibition on *Pseudomonas aeruginosa* (20 ± 0.08), least against *Vibrio choleriae* (10.06 ± 0.04) and moderate against *B. subtilis* (17 ± 0.08) followed by *B. circulans* (12 ± 0.08), *S. mitis* (12.96 ± 0.04) and *E. faecalis* (11.96 ± 0.04).

Methanolic extract of *M. micrantha* showed comparatively high antibacterial activity as compared to n-hexane extract (Table 2).

Table 2: Antibacterial activity of different extracts of *Mikania micrantha* Kunth

Name of bacteria	Zone of inhibition (mm)								
	n-hexane extract				Methanol extract				Ciprofloxacin
	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.5 µl/ml
Bacillus circulans	10.4 ± 0.29	-	-	-	12 ± 0.08	11.03 ± 0.04	10.1 ± 0.08	-	30
Bacillus subtilis	11.06 ± 0.16	10.03 ± 0.20	10.1 ± 0.08	10.06 ± 0.09	17 ± 0.08	14.93 ± 0.04	14.03 ± 0.04	10 ± 0.08	25
Enterococcus faecalis	11 ± 0.08	10.06 ± 0.16	10.1 ± 0.08	10.13 ± 0.12	11.96 ± 0.04	11 ± 0.00	10.1 ± 0.08	-	23
Escherichia coli	11 ± 0.08	10.16 ± 0.12	10.1 ± 0.08	9.9 ± 0.08	11.1 ± 0.08	10.06 ± 0.04	-	-	29
Klebsiella pneumoniae	-	-	-	-	10.96 ± 0.04	10.03 ± 0.04	-	-	34
Pseudomonas aeruginosa	16.03 ± 0.12	14 ± 0.08	12.96 ± 0.12	12.1 ± 0.08	20 ± 0.08	15.03 ± 0.04	14.06 ± 0.09	12.96 ± 0.04	31
Salmonella enterica ser typhi	13.03 ± 0.04	10.96 ± 0.04	11.03 ± 0.04	11.03 ± 0.124	11 ± 0.08	10.96 ± 0.04	10 ± 0.08	-	30
Salmonella enteric typhimurium	10 ± 0.08	-	-	-	11.13 ± 0.12	10.06 ± 0.09	-	-	28
Salmonella paratyphi	11 ± 0.08	10.03 ± 0.04	9.93 ± 0.04	-	-	-	-	-	34
Shigella flexneri	13 ± 0.08	12.1 ± 0.08	10.93 ± 0.04	10.06 ± 0.04	-	-	-	-	32
Staphylococcus aureus	-	-	-	-	11.06 ± 0.04	10.96 ± 0.04	10.1 ± 0.08	10.1 ± 0.08	27
Streptococcus mitis	14.1 ± 0.08	13 ± 0.08	11 ± 0.00	10.03 ± 0.04	12.96 ± 0.04	12 ± 0.08	11.03 ± 0.04	9.93 ± 0.04	28
Vibrio choleriae	11.06 ± 0.16	10.1 ± 0.08	10.13 ± 0.12	-	10.06 ± 0.04	10 ± 0.08	-	-	31

Results expressed as mean ± S.D. of three determinations

DMSO did not show any zone of inhibition.

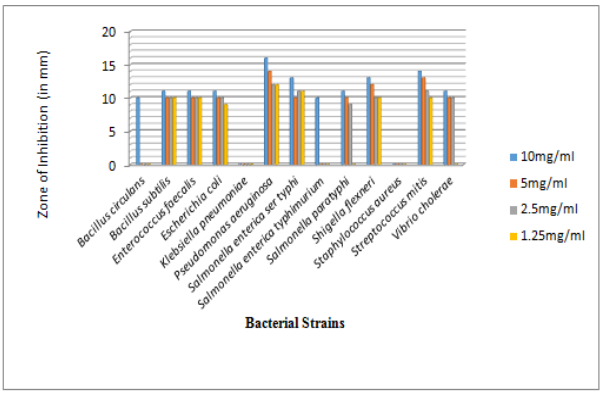

Figure 1: Antibacterial inhibition zone of n-hexane extract of *Mikania micrantha* Kunth

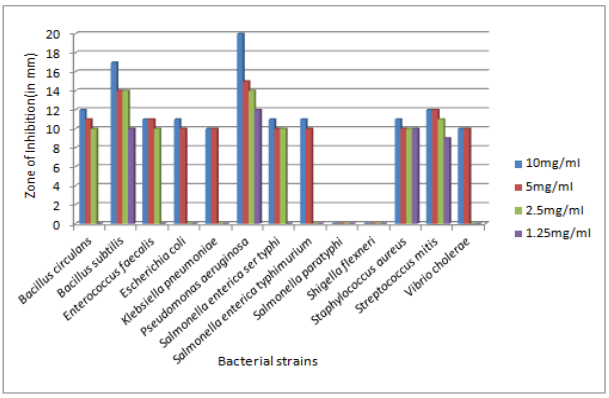

Figure 2: Anti-bacterial inhibition zone of methanol extract of *Mikania micrantha* Kunth

CONCLUSION

It was found that *Mikania micrantha* Kunth. had a high inhibitory effect against bacterial strains studied in the present investigation. The methanolic extracts of this p8lant showed more antibacterial activity than n-hexane extract. The findings of the present study to control the pathogenic bacterial strains can further be explored in order to discover new drug molecules to combat human diseases. The peculiar event which was evident during the investigation that the n-hexane extract of the plant was having more inhibitory activity against *Pseudomonas aeruginosa* whereas its methanolic extract had still more effect on *Pseudomonas aeruginosa*. The antibacterial activity exhibited by the extracts might be due to the presence of various phytoconstituents identified during the preliminary phytochemical screening of the extracts. This plant can further be exploited for isolation of important chemical constituents to formulate new drugs.

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