

*Research Article*

**ANTIMICROBIAL ACTIVITY AND PHYTOCONSTITUENTS SCREENING OF NOXIOUS INVASIVE ALIEN PLANTS, *AGERATINA ADENOPHORA* AND *CHROMOLAENA ODORATA* FROM MIZORAM, A PART OF INDO-BURMA BIODIVERSITY HOTSPOT IN INDIA**

Rabishankar Sengupta<sup>1</sup>, Arnab Kundu<sup>2</sup>, Bikram Dhara<sup>2</sup>, Arup Kumar Mitra<sup>2</sup>, Sudhansu Sekhar Dash<sup>1\*</sup>

*Received 01 December 2022, revised 08 August 2023*

**ABSTRACT:** *Ageratina adenophora* and *Chromolaena odorata* belonging to the family Asteraceae are the two most noxious invasive alien plant species in the natural forests of Mizoram, a part of the Indo-Burma biodiversity hotspot. These two noxious invasive species should either be controlled by eradication or managed by exploiting them as a resource for bioprospecting like antimicrobial medicines. The present study has explored the two abundantly available invasive species, for bioprospecting and investigating their antimicrobial potential. The obtained results revealed the presence of varying amounts of flavonoids, steroids, tannins, and alkaloids in the polar and non-polar solvent extracts of *A. adenophora* and *C. odorata*. The methanol extract of *A. adenophora* revealed promising antifungal activity against the test plant pathogenic fungi whereas petroleum ether extract of *A. adenophora* and *C. odorata* exhibited significant antibacterial potential against both Gram-positive and Gram-negative test bacteria. The results also revealed the solvent extracts of the studied invasive alien plants exhibited almost similar or more effectiveness with that of commonly used synthetic antifungals like Bavistin and broad-spectrum antibiotics like Gentamicin. Investigating the minimum inhibitory concentration of the plant extracts revealed their effectiveness even at minor concentrations. Therefore, the antimicrobial property of these two noxious invasive alien plant species can be recognized as a beneficial resource for medicinal as well as economic purposes for antibacterial and antifungal materials.

**Key words:** *Ageratina adenophora*, Antimicrobial, Bioprospecting, *Chromolaena odorata*, Invasive Alien Plants, Phytochemical.

## INTRODUCTION

The use of plants in healthcare is a very old practice and people living in remote areas still use plants for this purpose (Pradhan *et al.* 2021, Pattanayak 2021). Invasive Alien Plant species (IAPs), introduced to a new area, through intentional or unintentional means, cause detrimental impacts on the environment outside their native region and outcompete with native plants modifying the ecosystem equilibrium which leads to the loss of ecosystem function, reducing biodiversity richness and causing socioeconomic decline of rural communities (Vilà *et al.* 2011). Invasive alien

plants also compete with crops exerting allelopathy and toxicity to livestock (Mdee *et al.* 2009). Protected natural forest areas in Indian Himalayan regions, including Mizoram, a part of the Indo-Burma biodiversity hotspot, are highly susceptible to invasion by IAPs due to high anthropogenic disturbances. Recently, 163 alien plants were reported from Mizoram, including prioritized invasive species, such as *Ageratina adenophora*, *Chromolaena odorata*, *Mikania micrantha*, and *Ageratum conyzoides* with their ecological impact on natural vegetation and ecosystem (Sengupta and Dash 2020).

<sup>1</sup>Botanical Survey of India, CGO Complex, DF Block, Sector-1, Kolkata 700019, West Bengal, India.

<sup>2</sup>Post Graduate Department of Microbiology, St. Xavier's College, Kolkata 700016, West Bengal, India.

\*Corresponding author. e-mail: ssdash2002@gmail.com

Most plants naturally possess antimicrobial potential through secondary metabolite production, which protect them from pathogen attack (Górniak *et al.* 2019). The research for the development of laboratory-derived new antimicrobials is getting less funding recently (Pattanayak 2022), which may be due to the development of resistance to them by the microorganisms within a short period of use (Arun *et al.* 2022, Taib *et al.* 2022, Mandal *et al.* 2023) or even before coming to the field for widespread use (Srinivas *et al.* 2023). So, research for getting new antimicrobials from the plant origin got importance in recent years (Pattanayak 2018, Hassan *et al.* 2022).

The antimicrobial potential of various solvent extracts availed from plants including essential oils, alkaloids, tannins, etc. were reported by different researchers which are environmentally safe and economically feasible (Nigussie *et al.* 2021). Numerous phytotherapy manuals as well as ethnobotanical surveys reported medicinal plants for treating various infectious diseases, such as cutaneous infections, gastrointestinal disorders, urinary tract infections, and respiratory diseases (Omwenga *et al.* 2015). Invasive alien plants are successful competitors with the natural vegetation due to resistance towards different pathogens (Sahu and Devkota 2016). These noxious IAPs possess various active components to resist plant or animal or soil pathogens.

In recent times, multidrug-resistant (MDR) strains of various pathogenic microorganisms have emerged as a significant public health issue due to the frequent use of commercial synthetic antimicrobial drugs (Vivas *et al.* 2019, Mandal *et al.* 2023). Thus, it is important to explore natural broad-spectrum antimicrobial resources. A large number of medicinal plants are recognized worldwide, including traditional medicinal practices, as valuable resources of natural antimicrobial compounds (Chen *et al.* 2016). As a safe resource, plant extracts that are not toxic and possess a broad spectrum of antimicrobial activity could be considered as an alternative source of synthetic antimicrobials based on the abundant availability of resources. *Ageratina adenophora* and *Chromolaena odorata* have been frequently used by traditional healers in Mizoram locally for medicinal or antimicrobial purposes to combat various microbial diseases in humans (Sengupta and Dash 2022).

It is necessary to identify innovative and sustainable ways of invasive plants management transforming them from "environmental threat" to useful "resources". Phytochemical screening and antimicrobial potential

of noxious IAPs as bioprospecting resources have not been explored yet in Mizoram in particular and India in general. In this context, the present study focuses on the exploration of *Ageratina adenophora* and *Chromolaena odorata* for bioprospecting and also investigates the antimicrobial potential of use for medicinal purposes by traditional healers.

## MATERIALS AND METHODS

### Collection and identification of plant material

Mizoram, a north-eastern state of India, is neighboring Myanmar on the eastern side and Bangladesh on the western side and shares a common border with Assam, Manipur, and Tripura. The fresh vegetative parts of *Ageratina adenophora* (Spreng.) R.M. King and H. Rob. (South Vanlaihphai, Phawngpui national park, R. Sengupta & S.S. Dash 95404; Vapar, Murlen national park, R. Sengupta & S.S. Dash 95508) and *Chromolaena odorata* (L.) R.M. King and H. Rob. (Thaltang, Phawngpui national park, R. Sengupta & S.S. Dash 95430; Hnahlan, Murlen national park, R. Sengupta & S.S. Dash 95534) from protected areas in Mizoram including Phawngpui National Park (50 km<sup>2</sup>) and Murlen National Park (200 km<sup>2</sup>). Information regarding the socio-economic and ethnobotanical uses of the two IAPs was collected along with their geocoordinates and evaluated through a semi-structured interview with the locals with an open-ended questionnaire. The collected voucher specimens were preserved following the standard protocol (Jain and Rao 1977), then identified and deposited.

### Phytoconstituents screening

The dried powder of the plant samples was extracted with methanol using the maceration technique for three days followed by filtration (using Whatman filter paper, 110 mm). Then using the methanol extract, tested for the presence of bioactive compounds by following standard methods (Evans 2009).

To test for the presence of phenols and tannins; the extract was mixed with 2 ml of 2% solution of FeCl<sub>3</sub>, as a result, a blue-green or black color appeared, which indicated the presence of phenols and tannins.

To test for the presence of flavonoids; the extract was mixed with a few fragments of magnesium ribbon and concentrated HCl was added in drops. The pink scarlet color appeared after a few minutes, which indicated the presence of flavonoids.

To test for the presence of saponins; the extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable

foam was taken as an indication of the presence of saponins.

To test for the presence of glycosides; the extract was mixed with 2 ml of glacial acetic acid containing 1 or 2 drops of 2% solution of FeCl<sub>3</sub>. The mixture was then poured into another test tube containing 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interphase indicated the presence of cardiac glycosides.

To test for the presence of steroids; the extract was mixed with 2 ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> was added down the side of the tube. A red color produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing the crude extract with 2 ml of chloroform. Then 2 ml of each concentrated H<sub>2</sub>SO<sub>4</sub> and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

To test for the presence of terpenoids; the extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added and heated for about 2 minutes. The appearance of greyish color indicated the presence of terpenoids.

Test for the presence of alkaloids; the crude extract was mixed with 2 ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. The turbidity of the resulting precipitate was taken as evidence of the presence of alkaloids.

### Solvent Extraction

The collected leaves were first washed under running tap water and air-dried in the shade at room temperature for 15 days. The dried plant materials were then ground to a fine powder using a household grinder. In the case of each solvent (methanol/petroleum ether), 10 g leaf powder was extracted with 50 ml (w/v) solvent, respectively. The supernatant was filtered through Whatman filter paper and the filtrate was then evaporated at 50°C using a rotary evaporator to yield the crude extract (Manandhar *et al.* 2019).

Percentage Yield (%) = (Dry weight of extract / Dry weight of plant material) X 100

The resultant crude extract was then collected in a vial for further use.

### Test microorganisms

#### Bacterial strain

Four reference bacteria, where two Gram-negative, *viz.*, *Enterobacter hormaechei* (MT672707.1.), *Escherichia coli* (MTCC 443) and two Gram-positive,

*viz.* *Bacillus cereus* (MK774799), and *Streptococcus pyogenes* (MTCC 442) were used during the study as test bacteria. The tested strains were obtained from the Post Graduate Department of Microbiology, St. Xavier's College, Kolkata, India. The tested strains were cultured in beef extract peptone agar medium (PBA) at 27°C and stored in nutrient agar slants at 4°C.

#### Fungal strain

Two reference fungi, *Exserohilum rostratum* (MN 337265) and *Alternaria angustiovoidea* (MZ723052) were used during the study. The tested strains were obtained from the Department of Microbiology, St. Xavier's College, Kolkata, India. The fungi were incubated for 72 hr at 28°C in Potato Dextrose Agar (PDA) and stored in the plate at 4°C.

### Test microorganisms and antibacterial activity of plant extracts

Pure culture of the test bacteria was prepared and the antibacterial activity of the plant extracts was determined by the agar well diffusion method (Alghamdi 2021). For this, fresh (overnight) isolated colonies of test bacteria were suspended in sterile saline to get a turbidity of 0.5 McFarland standards. Then 0.1 ml of this suspension was spread aseptically on a sterile Muller Hinton agar medium (Hi media). Wells of 6 mm diam. were bored by sterile cork borer and 0.2 ml of each extract (100 mg/ml in 10% DMSO) was added to them, then it was allowed to diffuse by keeping in freeze for 20 min; 10% DMSO in one of the wells was used as a negative control. Gentamicin (10 mg/ml stock, converted to a working concentration of 50 µg/ml, HiMedia Laboratories Pvt. Ltd.) was used as a positive control (50 µl in each well) along the experimental setup on an agar plate to understand their zone of inhibition to compare with the extracts of interest. After extracts were diffused the plates were incubated at 37°C for 24 hr. Zones of inhibition were then measured in mm. For each extract, three replicates were maintained.

#### Antifungal activity assay

The antifungal activities of the compounds were tested using the hyphal growth inhibition method (Chuyong *et al.* 2019). The compounds were prepared using methanol and petroleum ether and diluted in Potato Dextrose Agar (PDA) medium. The PDA medium mixed with the compounds were dumped respectively into Petri dishes (9 cm diam.) as plating. An agar plug of fungal inoculums (6 mm in diam.)

was removed from a previous culture of the fungal strains tested and placed upside down in the center of the petri dishes. The same amount of methanol and petroleum ether, which were used to replace the compounds, were added respectively into the PDA mediums as the negative control. The efficacy of Bavistin 50% WP (Carbendazim 50% WP; CrystalTM), a fungicide, was assessed as a positive control for its antifungal properties against two target fungi. To achieve this, a solution of Bavistin 50% WP was first prepared. The necessary quantity of the fungicide powder was combined with PDA medium just before pouring, creating desired concentrations of 100 ppm. This mixture was gently agitated to ensure thorough blending. The PDA plate, now containing the fungicide, was then aseptically inoculated with the test fungi. This was accomplished by transferring a 6 mm diameter agar plug from recently cultivated cultures. Each treatment was done with three replicates. All growth mediums and inoculation instruments were subjected to autoclaving at 121°C for 15 min at 15 lbs/inch<sup>2</sup>. The mean diameter of the fungal colony was measured by the criss-cross method with calipers by incubating at 25°C. Following a five-day incubation period, the radial expansion of the test fungi on the treated plates was measured and juxtaposed against the control group.

#### **Determination of the minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration of the compounds was assessed using the broth microdilution method (Schwalbe *et al.* 2007). Inoculums of the microorganisms were prepared from 24 hr Mueller-Hinton Broth (MHB) cultures and suspensions were adjusted with turbidity equivalent to that of 108 CFU/mL using 0.5 McFarland standard. The compounds dissolved in methanol and petroleum ether; were first diluted to the highest concentration (200 mg/mL) to be tested, and then working dilutions were made in different concentrations, 0.25 mg/mL, 0.5 mg/mL, 1.0 mg/mL, and 2.0 mg/mL in 5 ml sterile test tubes containing the medium. Broth with 5 mL of solvents (methanol and petroleum ether) was used as blank. Plates were covered and incubated for 12 hr at 37°C. After incubation, the lowest concentration of tested samples, which did not show any visual growth after macroscopic evaluation, was determined as MIC.

#### **Data analysis**

The inhibition zone diameters (in mm) were represented in (Mean ± SE). The results were subjected

to a one-way analysis of variance (ANOVA) using the statistical package for SIGMAPLOT 14.5 (Systat Software Inc., USA).

### **RESULTS AND DISCUSSION**

Table 3 shows the results of the phytochemical screening of different solvent extracts of *Ageratina adenophora* and *Chromolaena odorata*. Results revealed the presence of phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids in varying amounts in the leaf extracts. The highest steroids, flavonoids, saponins, and terpenoids content were present (+ + +) in leaves of *C. odorata*. On the contrary, alkaloids, phenols, tannins, and glycosides were observed in the highest amount (+ + +) in *A. adenophora*. The study also exhibited the majority of the phyto-compounds observed in the leaves of *C. odorata*. The presence of flavonoids, alkaloids, and tannins in the tested invasive alien plants (*A. adenophora* and *C. odorata*) extracts in both polar and non-polar solvents was observed. The quantity of flavonoids, alkaloids, tannins, and other phytochemicals found in variable quantities may be due to the polarity of extraction solvents. Methanol is more polar than petroleum ether which is responsible for the observed variation. The presence of flavonoids and terpenoids (including volatile oils) in plants and their antimicrobial effects on fungi and bacterial growth was earlier documented by some workers (Ling *et al.* 2003, Subba and Kandel 2013). The detection of alkaloids, steroids, tannins, phenolics, flavonoids, and saponins present in the leaf extracts was also comparable to similar studies by different authors (Subba and Kandel 2013, Hridhya and Kulandhaivel 2018). The abundance of phytochemicals in these plant extracts may be attributed to their protective function and competitive ability to establish their invasion in areas of native plants (Zheng *et al.* 2015).

Amongst the yield percentage, it was observed that both *A. adenophora* (5.83%) and *C. odorata* (5.72%) exhibited maximum yield in methanol extract in comparison to petroleum ether extract (Table 2).

The antibacterial activity of different solvent extracts of *A. adenophora* and *C. odorata* is shown in Table 3. The antibacterial activities were compared to the broad-spectrum antibiotic Gentamicin. Petroleum ether extract of *A. adenophora* exhibited antibacterial activity against all four bacteria. But the methanol extract of *A. adenophora* exhibited antibacterial activity against all test bacteria except *Enterobacter hormochaei*. In comparison, petroleum ether extract of *C. odorata*

exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. The methanol extract of *C. odorata* showed antibacterial activity against all test bacteria except *Enterobacter hormochaei*. DMSO used as a negative control, did not exhibit any antibacterial activity in the applied concentration. It was observed that both gram-positive and gram-negative bacterial strains were more susceptible to petroleum ether extract of *A. adenophora* and *C. odorata* in comparison to methanol extracts. Petroleum ether and methanol extracts of *A. adenophora* showed comparatively higher zone of inhibition against all test bacteria (both Gram-positive and Gram-negative).

The antifungal activity of the plant extracts is shown in Table 3. The methanol extract of *A. adenophora* revealed promising antifungal activity against both *Exserohilum rostratum* and *Alternaria angustiovoidea*. *Exserohilum rostratum* is a pathogenic fungus with a broad ecological niche and causes disease symptoms in more than 30 plant species including some economically important crops, such as corn, rice, sugarcane, and wheat (Cardona and González 2007). In some cases, *E. rostratum* is known to cause severe yield losses (Lin *et al.* 2011) and is also known to cause diseases in both animals and humans in rare cases (Sharma *et al.* 2014). Similarly, *Alternaria angustiovoidea* was reported to be a severe leaf spot-causing pathogenic fungus (Zhou and Xu 2014). Results of the study also revealed that *A. adenophora* extracts exhibited almost similar or more effectiveness with that of commonly used antifungal Bavistin (Carbendazim 50% WP) against both test fungi. The solvents, used as a blank negative control, exercised no inhibitory effect against the microorganism, which confirms the effectiveness of the used methanol extract of *A. adenophora*.

The antimicrobial effectiveness of the IAPs (*A. adenophora* and *C. odorata*) extracts against the test microorganisms was determined by evaluating their MIC, which was determined particularly for only those microorganisms which exhibited a zone of inhibition being sensitive to the IAPs extracts in the antimicrobial assay. Among the two plant extracts tested, *A. adenophora* exhibited strong antimicrobial potential. Against petroleum ether extract of *A. adenophora*, the MIC of *Enterobacter hormochaei* was 1 mg / mL, and the MIC of *Escherichia coli*, *Bacillus cereus*, and *Streptococcus pyogenes* was 0.5 mg / mL each. *E. coli* exhibited MIC of 0.25 mg/ mL against methanol extract of *C. odorata*. MIC value of

*A. adenophora* is 6 mg/mL and 7 mg/mL for *E. rostratum* and *A. angustiovoidea* respectively. Unlike *A. adenophora*, the addition of *C. odorata* extracts only exhibits a zone of inhibition against *A. angustiovoidea* with a MIC of 7 mg/mL.

This implies that even at minor concentrations (0.25 - 1 mg/ mL), the plant extracts are very effective. Thus, it is recommended to use lower concentrations of the plant extracts. It is also noteworthy to say that *A. adenophora* and *C. odorata* could be used as strong antimicrobials since both their polar (methanol) and non-polar (petroleum ether) extracts were capable of inhibiting the growth of test microorganisms even in little amounts. Gram-positive bacteria, such as *Bacillus cereus* and *Streptococcus pyogenes* were found to be more susceptible to all plant extracts than Gram-negative bacteria like *Escherichia coli*. This difference in sensitivity between Gram-positive and Gram-negative bacteria could be explained by the morphological differences in their cell walls (El-Astal 2004). Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Mai-Prochnow *et al.* 2016). As an important outcome of the study, it could also be interpreted that the plant extracts can be more effective than broad-spectrum antibiotics like gentamicin since the extracts exhibited greater or equivalent zone of inhibition compared to the antibiotic against the test bacteria, significantly against *Enterobacter hormochaei* (Gram-negative) and *Bacillus cereus* (Gram-positive). The probable mechanism of antimicrobial activity of *A. adenophora* and *C. odorata* could be attributed to the presence of flavonoids and tannins exhibited in the phytochemical screening which bind to the cell walls of the bacteria, thereby inhibiting its growth. Similar observations were reported by *C. odorata* extracts against *E. coli* and *B. cereus* (Hanphanphoom and Krajangsang 2016) and *A. adenophora* against *E. coli* (Rajamani *et al.* 2014). The plant extracts from *A. adenophora* and *C. odorata* could be used instead of broad-spectrum antibiotics like gentamicin as there were multiple reports on the potency of test bacteria for widespread resistance to broad-spectrum antibiotics (Fiedler *et al.* 2019, Larsson and Flach 2022).

Similarly, the results confirm the possibility of using methanol extracts of *A. adenophora* as a new resource of antifungal agent against the pathogenic *E. rostratum* and *A. angustiovoidea*. The activity of the methanol extracts of *A. adenophora* and *C. odorata* was noticeably higher against the fungal organism

than against test bacteria (Table 3). The presence of alkaloids, flavonoids, and other phytochemicals (Table 1) might be attributed to the antifungal potential of the used plants (Hridhya and Kulandhaivel 2018). The ability of *A. adenophora* and *C. odorata* extracts to exhibit antimicrobial activities in this research study confirms their potential for alternative use of the IAPs as raw materials for medicine production to be used in

diseases caused by *Enterobacter hormochaei*, *Escherichia coli*, *Bacillus cereus*, *Streptococcus pyogenes*, *Exserohilum rostratum*, and *Alternaria angustiovoidea*. Thus, the use of these two IAPs for antimicrobial purposes by local villagers in Mizoram instead of using synthetic antibiotics and drugs is a sustainable way of bioprospecting as well as controlling the invasion of alien plants.

**Table 1. Qualitative phytochemical analysis of *A. adenophora* and *C. odorata* extracts.**

Samples	Phenols/Tannins	Flavonoids	Saponins	Glycosides	Steroids	Terpenoids	Alkaloids
AA	+++	++	++	+++	+	++	+++
CO	++	+++	+++	++	++	+++	++

[AA = *Ageratina adenophora*, CO = *Chromolaena odorata*]

**Table 2. Yield percentage of *A. adenophora* and *C. odorata* extracts used in the study.**

Samples	Methanol	Petroleum Ether
AA	5.83%	4.09%
CO	5.72%	3.91%

[AA= *Ageratina adenophora*, CO = *Chromolaena odorata*]

**Table 3. Antimicrobial activity of *A. adenophora* and *C. odorata* extracts.**

Test samples Experiments	Microorganisms						Fungi	
	Gram-negative		Gram-positive		Er	Aan		
	Eh	Ec	Bc	Sp				
Antibacterial (C)	ZOI (mm)	16±0.059 <sup>a</sup>	17±0.043 <sup>f</sup>	18±0.27 <sup>d</sup>	16±0.038 <sup>h</sup>	NA	NA	
	MIC (mg/mL)	1.5	1.0	1.0	1.5	NA	NA	
Antifungal (C)	ZOI (mm)	NA	NA	NA	NA	8.58±0.63 <sup>b</sup>	7.87±0.59 <sup>a</sup>	
	MIC (mg/mL)	NA	NA	NA	NA	8	7.5	
AA (PE)	ZOI (mm)	18.08±0.75 <sup>b</sup>	16.5±0.5b <sup>c</sup>	21.6±0.4 <sup>a</sup>	12.9±0.36 <sup>c</sup>	10.56±0.60 <sup>c</sup>	ND	
	MIC (mg/mL)	1	0.5	0.5	0.5	7	ND	
AA (M)	ZOI (mm)	ND	17.03±0.15 <sup>b</sup>	14±0.2 <sup>c</sup>	15.63±0.30 <sup>c</sup>	13.33±0.91 <sup>b</sup>	8.233±0.95 <sup>c</sup>	
	MIC (mg/mL)	ND	0.5	0.25	0.5	6	7	
CO (PE)	ZOI (mm)	16.66±0.61 <sup>c</sup>	15.26±1.41 <sup>c</sup>	11.8±0.2 <sup>e</sup>	16.16±0.55 <sup>c</sup>	ND	ND	
	MIC (mg/mL)	0.5	1	0.5	0.5	ND	ND	
CO (M)	ZOI (mm)	ND	16.23±0.25b <sup>c</sup>	15.5±0.3 <sup>d</sup>	12.43±0.20 <sup>c</sup>	ND	9.607±0.35b <sup>c</sup>	
	MIC (mg/mL)	ND	0.25	0.5	0.5	ND	7	
PE (C)	ZOI (mm)	5	5	5	5	-	-	
M (C)	ZOI (mm)	7	7	7	7	-	-	

[EH = *Enterobacter hormochaei*, EC = *Escherichia coli*, BC = *Bacillus cereus*, SP = *Streptococcus pyogenes*, ER = *Exserohilum rostratum*, AAN= *Alternaria angustiovoidea*, AA = *Ageratina adenophora*, CO = *Chromolaena odorata*; PE = Petroleum ether, M = Methanol. ZOI = Zone of inhibition in mm (means of triplicate ± standard deviation), MIC = Minimum inhibitory concentration in mg/mL, ND = Not detected, NA = not applicable, C = Control. Mean values within the same column not sharing a common letter (a, b) are significantly different at p ≤ 0.05 in one-way ANOVA analysis].

## CONCLUSION

The antimicrobial screening and phytochemical assessment of *Ageratina adenophora* and *Chromolaena odorata* against the selected pathogenic microorganisms revealed that the IAPs extracts possess various phytoconstituents with high antimicrobial properties which was confirmed by comparing with commercial antibiogram of broad-spectrum antibiotic and antifungal materials. As an important outcome of the study, the antimicrobial activity of *Ageratina adenophora* and *Chromolaena odorata* is confirmed and could be used as a bioprospecting resource. Since the drug-resistance nature of the microorganisms increases gradually against commercially available synthetic drugs, the selected IAPs could serve as an alternative medicine without adverse side effects. *Ageratina adenophora* and *Chromolaena odorata* are two noxious invasive weeds, which are readily spread by easily inhabiting any available space in the Indian Himalayan region, including Mizoram. The exploitation of these IAPs would also be a sustainable way of managing their invasion in natural forests and be used for discovering bioactive natural products for the development of new phytopharmaceuticals. The outcome of the present work will significantly enrich the knowledge on the usage of invasive alien plants in bioprospecting, both locally and globally, to control and manage them in a sustainable way attracting the general public and policymakers' attention. However, while the benefits of such invasive alien plants derived treatments are obvious and should be welcomed, biosafety-related experiments are also required in lieu.

## ACKNOWLEDGEMENTS

The authors are grateful to Dr. A.A. Mao, Director, Botanical Survey of India, Kolkata, India, for encouragement and facility. The authors' RS and SSD gratefully acknowledge the financial support from the Ministry of Environment, Forest and Climate Change, Government of India through the National Mission on Himalayan Studies (Project NMHS-2017/LG/01/475).

## REFERENCES

Alghamdi AA (2021) Phytoconstituents screening and antimicrobial activity of the invasive species *Nicotiana glauca* collected from Al-Baha region of Saudi Arabia. Saudi J Biol Sci 28(3): 1544-1547.

Arun A, Jaiswal U, Tripathi S, Singh AP, Choudhury S, Prabhu SN (2022) Surveillance of carbapenem-resistant Gram-negative bacteria from animal sources in Mathura region, Uttar Pradesh, India. Explor Anim Med Res 12(1): 91-98. DOI: 10.52635/eamr/12.1.91-98.

Cardona R, González MS (2007) First report of *Exserohilum rostratum* associated with rice seed in Venezuela. Plant Dis 91(2): 226.

Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A (2016) Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med 11(1): 1-10.

Chuyong GB, Duah I, Darkwa K (2019) The morphometric evidence and antifungal activity of *Chromolaena odorata* in Western Cameroon. J Herbs Spices Med 25(4): 401-413.

El-Astal Z, Younis KM, Strip-Palestinian G (2004) Bacterial pathogens and their antimicrobial susceptibility in Gaza strip, Palestine. Pak J Med Sci 20 (4): 365-370.

Evans WC (2009) Trease and Evans' pharmacognosy. Elsevier Health Sciences.

Fiedler G, Schneider C, Igbinosa EO, Kabisch J, Brinks E *et al.* (2019) Antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates from fresh vegetables from German retail markets. BMC Microbiol 19(1): 1-13.

Górniak I, Bartoszewski R, Króliczewski J (2019) Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev 18(1): 241- 272.

Hanphanphoom S, Krajangsang S (2016) Antimicrobial activity of *Chromolaena odorata* extracts against bacterial human skin infections. Math Models Methods Appl Sci 10: 159

Hassan AA, Iskander D, Oraby NH (2022) Evaluation of the synergistic antimicrobial activities of selenium nanoparticles and rosemary oil against *Aspergillus fumigatus* and *Klebsiella pneumoniae* recovered from respiratory infection in cattle in Giza Governorate, Egypt. Explor Anim Med Res 12(1): 24-32, DOI: 10.52635/eamr/ 12.1.24-32.

Hridhya KV, Kulandhaivel M (2018) Antimicrobial activity of *Chromolaena odorata* against selected pyogenic pathogens. Phytopathology 9(7): 1001-1007.

Larsson DJ, Flach CF (2022) Antibiotic resistance in the environment. Nature Reviews Microbiol 20(5): 257-269

Jain SK, Rao RR (1977) A Handbook of Field and Herbarium Methods. Today and Tomorrow's Printers and Publishers, New Delhi, India.

Lin SH, Huang SL, Li QQ, Hu CJ, Fu G *et al.* (2011) Characterization of *Exserohilum rostratum*, a new causal agent of banana leaf spot disease in China. Australas Plant Pathol 40(3): 246-259.

Ling B, Zhang M, Kong C, Pang X, Liang G (2003) Chemical composition of volatile oil from *Chromolaena odorata* and its effect on plant, fungi and insect growth. J Appl Ecol 14(5): 744-746.

Mai-Prochnow A, Clauson M, Hong J, Murphy AB (2016) Gram-positive and Gram-negative bacteria differ in their sensitivity to cold plasma. Sci Rep 6(1): 1-11.

- Manandhar S, Luitel S, Dahal RK (2019) *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med 2019: 1895340.
- Mondal T, Dey S, Isore DP, Samanta S, Banerjee A *et al.* (2023) Detection of drug-resistant extended-spectrum and AmpC $\beta$ -lactamases producing *Escherichia coli* from poultry faecal samples in West Bengal, India. Explor Anim Med Res 13(1): 31-38, DOI: 10.52635/eamr/13.1.31-38.
- Mdee LK, Masoko P, Eloff JN (2009) The activity of extracts of seven common invasive plant species on fungal phytopathogens. S Afr J Bot 75(2): 375-379.
- Nigussie D, Davey G, Tufa TB, Brewster M, Legesse BA *et al.* (2021) Antibacterial and antifungal activities of Ethiopian medicinal plants: A systematic review. Front Pharmacol 12: 633921.
- Omwenga EO, Hensel A, Shitandi A, Goycoolea FM (2015) Ethnobotanical survey of traditionally used medicinal plants for infections of skin, gastrointestinal tract, urinary tract and the oral cavity in Borabu sub-county, Nyamira county, Kenya. J Ethnopharmacol 176: 508-514.
- Pattanayak S (2021) Plants in healthcare: past, present and future. Explor Anim Med Res 11(2): 140-144, DOI: 10.52635/eamr/11.2.140-144.
- Pattanayak S (2018) Alternative to antibiotics from herbal origin - outline of a comprehensive research project. Current Pharmacogenom Personal Med 16: 9-62, DOI: 10.2174/1875692116666180419154033.
- Pattanayak S (2022) Research targeting business profits: impacts on health and environment. Explor Anim Med Res 12 (1): 1-7, DOI: 10.52635/eamr/ 12.1.1-7.
- Pradhan DK, Ghosh J, Lepcha N, Nandi A, Banerjee D *et al.* (2021) New ethnomedicinal information from Lepcha community of Dzongu, Sikkim. Explor Anim Med Res 11(2): 179- 187, DOI : 10.52635/eamr/11.2.179-187.
- Rajamani R, Kumar HK, Shanmugavadivu M, Kuppsamy S (2014) Antibacterial activity of leaf extracts of *Ageratina adenophora* L medicinal plant of Nilgiris Hill, Tamil Nadu against human pathogens. Int J Biosci Nanosci 1(1): 1-3.
- Sahu A, Devkota A (2016) Antimicrobial activity of leaf extracts of some invasive alien plant species of Aster family against clinical bacteria. Ecoprint 23: 1-12.
- Schwalbe R, Steele-Moore L, Goodwin AC (2007) Antimicrobial susceptibility testing protocols. CRC Press.
- Sengupta R, Dash SS (2022) Quantitative assessment of the traditional uses of invasive alien plants in Mizoram, an Indo-Burma Biodiversity hotspot region in India. Nelumbo 64(1): 337-362.
- Sengupta R, Dash SS (2020) A comprehensive inventory and ecological assessment of alien plant invasion in Mizoram, India. Indones J For Res 7(2): 135-154.
- Sharma K, Goss EM, Dickstein ER, Smith ME, Johnson JA *et al.* (2014) *Exserohilum rostratum*: characterization of a cross-kingdom pathogen of plants and humans. PloS one 9(10): e108691.
- Srinivas K, Ghatak S, Angappan M, Milton AAP, Das S *et al.* (2023) Occurrence of antimicrobial resistance genes prior to approval of antibiotics for clinical use: evidences from comparative resistome analysis of *Salmonella enterica* spanning four decades. Explor Anim Med Res 13(1): 71-84, DOI: 10.52635/eamr/13.1.71-84.
- Subba B, Kandel RC (2013) Chemical composition and bioactivity of essential oil of *Ageratina adenophora* from Bhaktapur district of Nepal. J Nepal Chem Soc 30: 78-86.
- Taib GA, Abdulrahman RF (2022) Molecular characterization of virulence and antibiotics resistance genes and genetic diversity of *Salmonella enteritidis* from raw chicken meat in Duhok city, Iraq. Explor Anim Med Res 12(2): 176-186, DOI: 10.52635/eamr/12.2.176-186.
- Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V *et al.* (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14: 702-708.
- Vivas R, Barbosa AAT, Dolabela SS, Jain S (2019) Multidrug-resistant bacteria and alternative methods to control them: an overview. Microb Drug Resist 25(6): 890-908.
- Zheng YL, Feng YL, Zhang LK, Callaway RM, Valiente-Banuet A *et al.* (2015) Integrating novel chemical weapons and evolutionarily increased competitive ability in success of a tropical invader. New Phytol 205(3): 1350-1359.
- Zhou LX, Xu WX (2014) First report of *Alternaria alternata* causing leaf spots of tea (*Camellia sinensis*) in China. Plant Dis 98(5): 697.

**\*Cite this article as:** Sengupta R, Kundu A, Dhara B, Mitra AK, Dash SS (2023) Antimicrobial activity and phytoconstituents screening of noxious invasive alien plants, *Ageratina adenophora* and *Chromolaena odorata* from Mizoram, a part of Indo-Burma biodiversity hotspot in India. Explor Anim Med Res 13(Ethnomed. Spl.): 133-140, DOI: 10.52635/eamr/13(S)133-140.