



Phytochemical and Antimicrobial Activity of Some Medicinal Plants of North East India

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In present study, we examined the presence of phytochemical constituents in the hexane, ethyl acetate and methanol extracts of 6 medicinal plants of north east India. The methanol extracts of all the 6 plants were further analyzed for antibacterial activity by following standard agar well method against four bacterial strains *viz.* *Staphylococcus aureus*, *Bacillus subtilis* as Gram-positive bacteria and bacterial strains of *Escherichia coli*, *Proteus vulgaris* as Gram-negative bacteria and were assessed by the MIC (minimum inhibitory concentration) values. The results revealed the presence of alkaloid, polyphenols, flavonoids, terpenoids and carbohydrates in all the 6 plant species.

Keywords: Medicinal plants, Phytochemicals, Antimicrobial activity, Minimum inhibitory concentration.

INTRODUCTION

Now a days, increase in antibiotic resistant strains of clinically important pathogens have led to the emergence of multi resistant bacterial strains¹⁻². The high cost and non-availability of new generation antibiotics resulted in increase in mortality. Therefore, the search for more effective antimicrobial agents from various other sources such as materials of plant origin that can serve as a source and template for synthesizing new antimicrobial drugs has become absolute necessary³. Recent evidence from various pharmaceutical companies shows that natural products, because of their privileged mechanism over a millions of years still represent an extremely valuable source for the production of chemical entities for the treatment of some complex diseases.

In many parts of the world, various human ailments are being treated using traditional medicine. Moreover, 80 % of the world's population is relied upon medicinal plants² and in Indian traditional medicinal system the use of the plants as therapeutic agents remains an important component⁴⁻⁵.

However, scientifically only a few plants have been studied for the assessment of their quality, safety and efficacy. In modern medicine, the importance of natural products can be assessed by the rate of introduction of new chemical entities of wide structural diversity that can serve as templates for synthetic modification, by the number of diseases treated or prevented by these substances and also by their frequency of use for the treatment of diseases⁶.

Current researches involve a multi faced approach in drug discovery from medicinal plants that combines botanical, phytochemical, biological and molecular techniques. Discovery of drug from medicinal plants continues to provide new and important leads against various diseases *viz.* cancer, HIV/AIDS, Alzheimer, malaria and pain *etc.*⁷ Considering the vast potentiality of plants as a source for antimicrobial drugs with references to antibacterial agents a systematic investigation was undertaken to screen a number of local flora for antibacterial activity namely *Bryophyllum pinnatum* Linn., *Bacopa monnieri* Pennell, *Leucas cephalotes* Spreng, *Vitex negundo* Linn., *Caesalpinia pulcherrima* Linn. and *Azadirachta indica*.

EXPERIMENTAL

Immense climate endemic and altitudinal variation in India have resulted in a great range of ecological habitats and North Eastern region take the pride place (located between 87°32 to 97°52 'E latitude and 21°34 to 29°50 'N latitude and finds place as one of the two biodiversity hotspots in India. North East India is thus the geographical "gateway" for much of India's flora and fauna⁸. This region is however one of the most threatened area and recent studies estimated that these valuable germplasm will disappear in the next 15-20 years⁹.

It has been observed that this region is still unexplored and remains data poor for a wide variety of plant taxa¹⁰. However efforts are on in terms of taxonomic identification¹¹. But works on evaluation of therapeutic properties of these plants are still very less. The aim of this work is to explore and highlight the

diverse natural products that have potential to lead more effective and less toxic antimicrobial drugs. So we have selected six plants species, leaves of which have been used by local people of this region as medicinal plant.

The six plant species were collected from nearby areas especially from villages around Simaluguri, Dist. Sivasagar, Assam, India. The leaves collected were washed thoroughly 2-3 times with running water and once with sterile distilled water and cut by knife into small pieces, then air dried in the shade and crushed to obtain fine powder.

Preparation of the extracts: The dried and powdered materials (100 g) were extracted successfully with hexane, ethyl acetate and methanol for 48 h using Soxhlet apparatus and extract was reduced to dryness in a rotary evaporator and preserved at 5 °C in an airtight bottle until required.

Phytochemical screening of the plant extracts: Hexane, ethyl acetate and methanol extracts prepared from the powdered material of the six plants were used for the preliminary phytochemical studies by following standard prescribed methods¹².

Test organism used in the antibacterial study: The antimicrobial activity of the methanol extracts of the different plants species were tested by standard microbiological agar well method against four bacterial strains viz., *Staphylococcus aureus* (ATCC 18590), *Bacillus subtilis* (TCC 8590) as Gram-positive bacteria and bacterial strains of *Escherichia coli* (ATCC 10586), *Proteus vulgaris* (ATCC 12453) as Gram-negative bacteria. All the bacterial cultures were obtained from Hi-media Pvt. Ltd and maintained at 40 °C in nutrient agar media (Himedia). The subculture was done in regular interval of 2 months.

Antibacterial activity: A suspension of each bacterium with the cell density of approximately 1×10^7 colony forming units (CFU)/mL was prepared in nutrient broth media pre-sterilized at 121°C for 20 min and was used as bacterial inoculums (BI). About 1 mL of BI from each test organisms was transferred to different conical flask containing 50 mL pre-sterilized nutrient agar medium (temperature = 40 °C). After proper mixing of the two, about 20 mL of the culture media in the conical flasks was distributed in two pre-sterilized Petri dishes and then allowed to settle for solidification of the media. Wells measuring the diameter of 6 mm were bored at equidistant places in the nutrient agar media and 50 µL of the samples in DMSO (10 mg/mL) were transferred to these wells and incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) values of the methanol extracts of the three plants was determined by preparing solutions of varying concentration (5, 2.5, 1.25 and 0.625 mg/mL). By observing the dishes where colony growth was inhibited it was possible to characterize the antibacterial activity of each plant extract. The lowest concentration of each plant extract that did not allow any colony growth in the solid medium after the incubation period was regarded as the minimum inhibitory concentration¹².

RESULTS AND DISCUSSION

The results of the qualitative screening of the phytochemical components of the hexane, ethyl acetate and methanol extracts are shown in Table-1. The results revealed the presence of alkaloids, polyphenols, flavonoids, terpenoids, carbohydrates in all the six plant species. Steroid was found to be present in the extracts of all the plants except *Bryophyllum pinnatum*. Glycoside was found to be present in leave extracts of *Leucas*

TABLE-1
PRELIMINARY PHYTOCHEMICAL STUDIES ON HEXANE, ETHYLACETATE AND
METHANOL EXTRACTS OF DIFFERENT PLANT MATERIALS

Comp.	<i>Vitex negundo</i> Linn.			<i>Azadiracta indica</i>			<i>Caesalpinia pulcherrima</i> Linn.			<i>Leucas cephalotes</i> Spreng			<i>Bacopa monnieri</i> Pennell			<i>Bryophyllum pinnatum</i>		
	H	E	M	H	E	M	H	E	M	H	E	M	H	E	M	H	E	M
Alkaloid	+	-	+	+	-	+	+	+	+	+	-	-	+	+	-	+	+	-
Poly phenols	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
Glycosides	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	+	+
Proteins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	-
Carbohydrates	+	+	+	-	-	+	+	-	+	+	-	+	-	+	+	-	+	+
Steroids	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-
Reducing sugar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

H- Hexane; E- Ethyl acetate; M- Methanol

TABLE-2
MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE METHANOL EXTRACTS
OF THE THREE PLANTS AGAINST TESTED BACTERIA

Organism	MIC (mg/mL)					
	<i>Vitex negundo</i> Linn.	<i>Azadiracta indica</i>	<i>Caesalpinia pulcherrima</i> Linn	<i>Leucas cephalotes</i> Spreng	<i>Bacopa monnieri</i> Pennell	<i>Bryophyllum pinnatum</i>
<i>S. aureus</i>	0.625	0.625	0.625	1.25	2.50	1.25
<i>B. subtilis</i>	2.500	1.250	1.250	2.50	1.25	1.25
<i>E. coli</i>	5.000	5.000	5.000	2.50	2.50	1.25
<i>P.vulgaris</i>	0.625	0.625	1.250	1.25	2.50	2.50

cephalotes Spreng, *Bacopa monnieri* Pennell, *Bryophyllum pinnatum*. Proteins and reducing sugar were found to absent in all the extracts of all the plants under investigation.

The results of the antibacterial activity of the six plants are shown in Table-2. The antibacterial activity was investigated only for the methanol extracts of the six plants and was assessed by the MIC values against two gram positive bacteria viz. *Staphylococcus aureus*, *Bacillus subtilis* and two gram negative bacteria viz. *Escherichia coli* and *Proteus vulgaris*. The methanol extracts of all the six plants showed antibacterial activity against all the tested bacteria with varied activity.

The methanol extract of *Vitex negundo* Linn. showed MIC value of 0.625 mg/mL for *S. aureus*, 2.5 mg/mL for *B. subtilis*, 5 mg/mL for *E. coli* and 0.625 mg/mL for *P. vulgaris*. The methanol extract of *Azadirachta indica* showed MIC value of 0.625 mg/mL for *S. aureus*, 1.25 mg/mL for *B. subtilis*, 5 mg/mL for *E. coli* and 0.625 mg/mL for *P. vulgaris*. The methanol extract of *Caesalpinia pulcherrima* Linn. showed MIC value of 0.625, 1.25, 5 and 1.25 mg/mL for *S. aureus*, *B. subtilis*, *E. coli* and *P. vulgaris*, respectively. The methanol extract of *Leucas cephalotes* Spreng showed MIC value of 1.25, 2.5, 2.5 and 1.25 mg/mL for *S. aureus*, *B. subtilis*, *E. coli* and *P. vulgaris*, respectively. The methanol extract of *Bacopa monnieri* Pennell showed MIC value of 2.5, 1.25, 2.5 and 2.5 mg/mL for *S. aureus*, *B. subtilis*, *E. coli* and *P. vulgaris*, respectively. The methanol extract of *Bryophyllum pinnatum* showed MIC value of 1.25, 1.25, 1.25 and 2.5 mg/mL for *S. aureus*, *B. subtilis*, *E. coli* and *P. vulgaris*, respectively.

Phytochemical constituents such as alkaloids, flavonoids, terpenoids steroids and several other aromatic compounds are the secondary metabolites of plants and recent years witnessed a considerable attention due to their diverse pharmacological activities. In plants, these phytoconstituents are involved in defence mechanism against microorganisms, insects and herbivores¹³. The results of our phytochemical tests conform to the earlier reports.

Literature review shows a large number of works on the evaluation of zone of inhibition of all the six plants, but very little information is available on the literature regarding the determination of minimum inhibitory concentration (MIC) for all the six plants¹⁴⁻¹⁷, particularly of this region. The present study showed that *Vitex negundo* Linn. and *Azadirachta indica* had the highest activity against *S. aureus* and *P. vulgaris* (0.625 mg/mL). *Caesalpinia pulcherrima* Linn. had the highest activity against *S. aureus* (0.625 mg/mL). *Leucas cephalotes* Spreng showed the highest activity against *S. aureus* and *P. vulgaris* (1.25 mg/mL). *Bacopa monnieri* Pennell showed the highest activity against *B. subtilis* (1.25 mg/mL) and *Bryophyllum pinnatum* showed the highest activity against three bacteria, *S. aureus*, *B. subtilis* and *E. coli* (1.25 mg/mL). However *Vitex negundo* Linn., *Azadirachta indica* and *Caesalpinia pulcherrima* Linn. were found to have better antibacterial activity than the other three plants as evidenced by the MIC values. The presence of the phytochemicals in the various extracts explains the antibacterial activity of the investigated plants.

Conclusion

In this research, phytochemical constituents in the hexane, ethyl acetate and methanol extracts of six medicinal plants of north east India were evaluated. The methanol extracts of all the six plants were further analyzed for antibacterial activity by following standard agar well method against four bacterial strains viz., *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria and bacterial strains of *Escherichia coli* and *Proteus vulgaris* as Gram-negative bacteria and were assessed by the MIC (minimum inhibitory concentration) values. The results revealed the presence of alkaloid, polyphenols, flavonoids, terpenoids, carbohydrates in all the six plant species. Phytochemical screening and analysis will be useful in the presence and quantification of the bioactive compounds. Our study revealed the presence of medicinally important constituents in the studied six species. Therefore, this study confirm the potential of the six plants as a valuable source of bioactive compounds and could be potential alternatives for synthetic antimicrobial drugs and their quantified values can be used as a major tool for obtaining a quality control profile for a drug.

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