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Screening of Phytochemical and Antibacterial Property of Some Local Herbs of Meghalaya

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ABSTRACT

The present study was conducted to evaluate the antibacterial properties of four plant species, selected on the basis of folklore medicinal reports practiced by the tribal people of Meghalaya. In-vitro antibacterial properties of alcoholic (Ethanolic and methanolic) and aqueous extracts of leaves of four plant species, were assayed against four bacteria *viz.* Gram positive *(Staphylococcus aureuss and Listeria monocytogenes)* and Gram negative (*Escherichia coli* and *Salmonella* Typhimurium) using both agar well diffusion and disc diffusion method. All four plants *viz. Centella asiatica, Eupatorium cannabium, Galinsoga parviflora* and *Clerodendrum serratum* showed control of growth. The maximum inhibitions were observed in *Galinsoga parviflora* against *Staphylococcus* and *Listeria monocytogenes,* followed by. *Clerodendrum serratum, Centella asiatica* and *Eupatorium cannabium.* The different extracts differed significantly in their antibacterial properties with the methanolic extract being more effective followed by ethanolic extract. Aqueous extract showed very least activity. The results highlight that some of the studied plants had good antibacterial properties.

1. Introduction

Traditional use of indigenous medicinal plants for primary human and animal health care has been an integral part of the inhabitants of Meghalaya *viz.* Khasi, Jaintia and Garo predominantly in the rural remote areas, inaccessible to the modern health care system. Meghalaya is bestowed with a wealth of medicinal plants of which above 30% has been used in local traditional medicine practices (Lakadong and Barik, 2006). Efforts to conserve the vast medicinal resources have been undertaken by the tribal communities of the region by protecting the scattered sacred groves except for medicinal purpose and not allowing undue exploitation to take place. Based on the folklore practices four plants *viz. Centella asiatica*, *Eupatorium cannabinum, Galinsoga parviflora* and *Clerodendrum serratum* were selected traditional uses in Table 1.

Centella asiatica (L) belongs to the family Apiaceae. Scientific reports include aqueous extract showed wound healing, however in another study, the alcoholic extract of *Centella asiatica* (oral and topical) was also found to improve wound healing property (Arora *et al.,* 2002). Seventy percent ethanol extract was observed to exhibit anticonvulsant activity (Sudha *et al.,* 2002). *Centella asiatica* extracts was also found to show imipramine like antidepressant effect (Kalshetty *et al.,* 2012). Anti-stress and anti-anxiety activity was found to be exhibited by extracts of *Centella asiatica* (Hemamalini and Muddanna, 2013). Anti-ulcer activity was also found to be reported in various extracts of *Centella asiatica* extracts (Abdulla *et al.,* 2010).
Eupatorium cannabinum is a perennial growing belongs to the family. As a medicinal plant, it has been traditionally used as febrifuge, cathartic, diuretic and scorbutic properties (Hendriks *et al.,* 1983). Infusion of the fresh herb acts as a strong purgative and emetic. Recent research showed that the plant might have anti tumor activity (Rucker *et al.,* 2001).

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Table 1. Traditional uses of selected plants

SL. No.	Plant species	Part used	Traditional uses
1	*Centella asiatica,*	Whole plants	Prevent infection
2	*Eupatorium cannabium,*	Leaves	Cuts & wound
3	*Galinsoga parviflora*	Whole plants	Diarrhoea
4	*Clerodendrum serratum*	Leaves & Flowers	Diarrhoea

Galinsoga parviflora belong to the family Asteraceae, in Japan known as -khavu, is a weed found in cultivated and vacant places in the Himalaya at the altitude of 4 - 8000 ft. Its leaf and inflorescence decoction is used as a remedy for diarrhoeal disorders in the folklore medicine practice of Naga tribes. Scientific reports on its insecticidal activity and antidiarrhoeal activity (Macedo *et al.,* 1997, Yadav and Tangpu, 2008).

The plant *Clerodendrum serratum*, Linn belong to the family Verbenaceae commonly known as "Bharngi" in the ayurvedic medicine of Indian system. Ethanol extract of root exhibited antibacterial, wound healing activity, aqueous extract and methanol extract of root in Dalton's Lymphoma Ascites showed anti-cancer activity, anticarcinogenic activity of *C. serratum* leaf extract on liver and kidney, polyphenolics of plant is responsible for antioxidant, vasorelaxant and antiangiogenic have reported (Chinchali *et al.,* 2011, Mohamed, 2012, Edeoga *et al.,* 2005).

Thus, the present study focuses on the selected four plants with the objective to evaluate the constituents of phytochemicals and preliminary screening for antibacterial property against both Gram negative and Gram positive bacteria.

2. Materials and Methods

2.1 Collection and preparation of plant materials

For the present study, four plants were collected, three of which (*Eupatorium cannabinum, Galinsoga parviflora* and *Clerodendrum serratum)* from umiam and nearby area and *Centella asiatica* was procured from the local market of Shillong, Meghalaya. Both the leaves and stems parts were placed in a polyethylene bag to prevent loss of moisture during transportation to the laboratory. A part of the collected plants were carefully cleaned and dried to prepare herbarium sheets for further identification. The plant species were initially recorded based on the vernacular names and identification of the plants species was done in consultation with taxonomist from Botanical Survey of India, Shillong, Meghalaya. The remaining plant samples were shade dried, ground into uniform powder using a blender, weighed, and stored in air tight container until further processing.

2.2 Preparation of plant extracts

- ***Alcoholic extraction***

Four grams of powdered sample was soaked in 40 ml of methanol or ethanol in a conical flask and kept for 72 hrs. Filtrate was obtained after filtering through Whatman filter paper No. 1 and the solvent was evaporated by rotary evaporator under reduced pressure at 45°C. Further the solvent was air dried and stored in air tight container at 4°C.

- ***Aqueous extraction***

Four grams of powdered sample was dissolved in 40 ml of distilled water in a conical flask and kept for 72 hrs. Filtrate was filtering through muslin cloth and filter papers. The filtrate collected was evaporated by rotary evaporator under reduced pressure at 45°C. Further the solvent was air dried and stored in air tight container at 4°C.

2.3 Test organisms and preparation of inoculum

The tested organisms included *Escherchia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 49416 and *Listeria monocytogenes*. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient broth were incubated for 24 hrs at 37°C. The cultures were diluted with sterile distilled water achieve optical densities of 0.5 corresponding to 1.0×10^6 colony forming units (CFU/ml) for bacteria using a nephlometer (BD).

2.4 Phytochemical screening

- ***Test for alkaloids***

A total of 50 mg of powdered sample was dissolved in 5 ml of methanol and then filtered. Then 2 ml of filtrate was mixed with 5 ml of 1% aqueous HCl. One milliliter of mixture was taken separately in two test tubes. Few drops of Dragendorff's+ reagent were added in one tube and occurrence of orange-red precipitate was taken as positive (Mohamed *et al.,* 2010).

Table 2. Phytochemical analysis of plant extracts

Plants	***Alkaloid***	***Flavanoids***	***Anthraquinones***	***Tannin***	***Saponin***
Centella asiatica	+	+	-	+	-
Eupatorium cannabium	+	+	-	+	+
Galinsoga parviflora	+	+	-	+	+
Clerodendrum serratum	+	+	-	+	+

+ Indicates presence, - Indicates absence

- ***Test for tannins***

To 0.5 ml extract solution, added 1 ml distilled water and 1-2 drops of ferric chloride solution to it and observed for blue black coloration which indicates presence of tannins (Palombo, 2006).

- ***Test for phenol***

To 2ml of extract solution, added 2ml of alcohol and few drops of ferric chloride solution and observed for coloration (Mohamed, 2010).

- ***Test for saponins***

One gram of powdered sample was boiled in 10 ml of distilled water and then filtered. 3 ml of distilled water was added to filtrate and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins (Palombo, 2006).

- ***Test for flavonoids***

Dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids (Mohamed, 2010).

- ***Test for anthraquinones***

A total of 0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes (Palombo, 2006).

2.5 Antimicrobial assay

- ***Agar well diffusion***

Kirby-Bauer method was followed for agar well diffusion assay (Bauer *et al.,* 1966). Twenty millilitres of sterilized Nutrient Agar was poured into sterile petriplate, after solidification, 100 µl of fresh culture of test microorganism were swabbed on the plates. The wells were punched over the agar plates using sterile gel puncher and sealed. Thereafter 100 µl of each plant extract at 200mg/ml and 20mg/ml concentration were added to the wells. The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each discs were measured in mm and recorded. The standard drug used was cefotaxime (30µg).

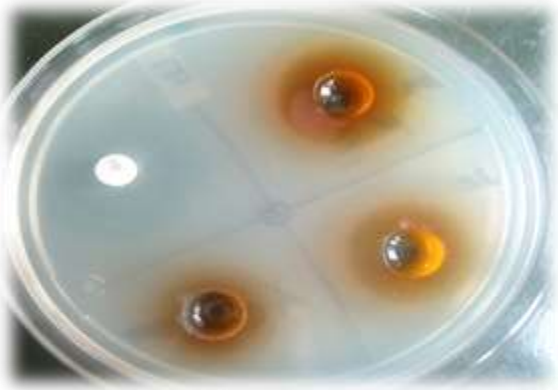

Figure 1. Antibacterial Property of Plant extract (Agar well diffusion)

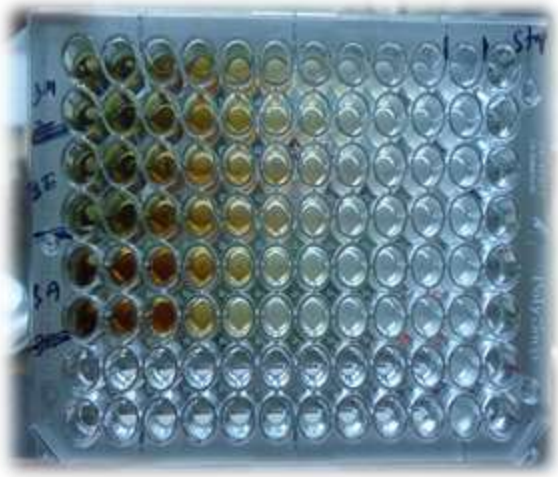

Figure 2. Mic of Plant extract (microbroth dilution)

Table 3. Antibacterial property of plant extracts

Plants	*Staphylococcus aureus*	*Listeria monocytogenes*	*E. coli*	*Salmonella Typhimurium*
Centella asiatica	+	+	-	-
Eupatorium cannabium	+	+	-	-
Galinsoga parviflora	++++	+++	+	+
Clerodendrum serratum	+++	++	-	-

+ Activity, - No activity, ++++ Very good activity, +++ Good activity, ++ Fair

- ***Minimum Inhibitory Concentration (MIC) Asssay***

The MIC method (microdilution in liquid medium) as per NCCLS, 1990 was applied on extracts that proved their high efficacy against microorganisms by the agar/disk diffusion method (Perez *et al.,* 1990). The highest dilution of a plant extract that still retains an inhibitory effect against the growth of a microorganism is known as MIC. Selected plant extracts were subjected to a serial dilution (20 mg/ml to 0.16 mg/ml) using sterile nutrient broth medium as a diluent. In a 96-well titre plate 20 µl of an individual microorganism and 20 µl of selected plant extract were loaded and inoculated at 37^0C for 24 h. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract. A control experiment was run without plant extracts and another without culture on growth of the two test organisms *(Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes)*.

3. Results and Discussion

Phytochemical analysis of selected plant samples are summarised in Table 1. Alkaloids, tannins and flavanoids were found in all samples. Saponin was found in all samples except *Centella asiatica* and anthroquinones was not found in any of the plants. Analysis of plant extracts revealed the presence of coumarins, flavonoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antimicrobial property (Bonja, 2004). These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Tannins bind to proline rich proteins and interfere with the protein synthesis. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999). Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes (Epand, 2007). All the 4 plants tested showed antibacterial activity by inhibiting one or more microorganisms. The results of the antimicrobial activity of plant extracts tested against microorganisms are shown in Table-3. Among the plants screened, the methanolic extract of *Galinsoga parviflora* and *Clerodendrum serratum* showed significant inhibition of all tested bacteria followed by *Centella asiatica* and *Eupatorium cannabium* against few pathogens. The agar well diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. *Galinsoga parviflora* and *Clerodendrum serratum* (20mg) exhibited the prominent antibacterial activity all the four bacteria but was more susceptible against *S. aureus* and *Listeria monocytogenes,* further the methanolic extract of the respective plants showed better activity than ethanol followed by aqueous extract (Table 4). Pino *et al.,* 2010 had studied the essential oil of *Galinsoga parviflora* to evaluate the chemical composition and demonstrated antimicrobial activities against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*. Vidya *et al.,* 2010, had also studied the antibacterial activity of *Clerodendrum serratum,* which is reported to have shown a wide range of activity against gram positive as well as gram negative bacterial strains (Jagtap *et al.,* 2009). The discs containing 7.5mg of root extract showed maximum activity against all the tested bacteria, including *Staphylococcus aureus.* Jagtap *et al.,* 2009 demonstrated the antibacterial property of *Centella asiatica* both against gram positive and gram negative bacteria and also observed that the ethanolic extract of *Centella asiatica* has higher antimicrobial activity than petroleum ether and water extract. Senatore *et al.,* 2001 also reported the Chemical composition and antibacterial activity of *Eupatorium cannabinum.* Methanol extracts of *Galinsoga parviflora* and *Clerodendrum serratum* that showed maximum antimicrobial activity against *S. aureus* and *Listeria monocytogenes* was taken for MIC assay.

Table 4. Zone of inhibition (Agar well diffusion in mm)

Plants	*Staphylococcus aureus*			*Listeria monocytogenes*			*E. coli*			*Salmonella spp.*		
	E	*M*	*A*	*E*	*M*	*A*	*E*	*M*	*A*	*E*	*M*	*A*
Centella asiatica	12	21	10	-	-	-	-	-	-	-	-	-
Eupatorium cannabium	-	-	-	-	-	-	-	-	-	-	-	-
Galinsoga parviflora	27	26	17	27	25	13	-	-	-	-	-	-
Clerodendrum serratum	21	25	17	12	14	15	-	-	-	-	-	-

E-Ethanol extract, M-Methanol Extract, A- Aqueous extract, - < 6mm

The result of MIC assay is shown in Table-5. *Galinsoga parviflora* exhibited the highest antibacterial efficacy against *S. aureus* at 0.625 mg/ml concentration followed by *Listeria monocytogenes* at 1.25 mg/ml concentration.

Table 5. Minimum inhibitory concentration against Methanol extract

Plants	***Staphylococcus aureus* (mg/ml)**	***Listeria monocytogenes (mg/ml)***
Galinsoga parviflora	0.625	1.25
Clerodendrum serratum	2.5	5

Conclusion

All the four plants tested showed a significant control of the growth. The maximum inhibitions were observed in *Galinsoga parviflora* against *Staphylococcus aureus* and *Listeria monocytogenes* followed by. *Clerodendrum serratum, Centella asiatica* and *Eupatorium cannabium.* In this study, *S. aureus* was found to be sensitive to all the four plant extracts. Our results suggest that gram-positive bacteria are generally more sensitive to the herb extracts. The results highlight that some of the studied plants had good antibacterial properties.

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