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Phytochemical Screening, Evaluation of Membrane Stabilizing and Antibacterial Activity of the Leaves of *Cephalandra Indica* Naudin Collected from Dibrugarh District, Assam, India.

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ABSTRACT

It was aimed to carry out phytochemical screening, membrane stabilizing and antibacterial activity of the leaves of *Cephalandra indica* Naudin. The petroleum ether and methanol extracts were used for phytochemical screening. Membrane stabilizing activity was studied using human erythrocyte against standard and the antibacterial activity of the methanolic extract of the leaves was carried out with five different strains. The phytochemical screening of the plant part (leaf) of *Cephalandra indica* revealed the presence of steroid. The extract of the leaves of *Cephalandra indica* showed human erythrocyte membrane stabilizing activity against heat and hypotonic induced lyses as the percentage membrane ranged between 50.78±.54 and 88±1.25. The plant also possesses significant antibacterial activity against five pathogenic bacteria. The study envisaged possibility of isolating compound from the methanolic and petroleum ether extract for membrane stabilizing and antibacterial activity and would provide ample opportunities for further investigation.
Keywords: *Cephalandra indica*, steroid, phytochemical, antibacterial, membrane stabilizing

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INTRODUCTION

Medicinal plants grow naturally around us. Over centuries, cultures around the world have heard how to use plant to fight illness and maintain health. These readily available and culturally important traditional medicines form the basis of an accessible and affordable health care regime and are an important source of livelihood for indigenous and rural populations. Now a day, increasingly, medicinal species that reside in natural areas have received scientific and commercial attention. Human use these plants as medecines at least to the Middle Paleolithic age some 60,000 years ago [1]. In the United States, of the top 150 prescription drugs, at least 118 are based on natural resource. Studies have revealed that several herbal derived drugs have been demonstrated to contain principles that possess ability to facilitate the stability of biological membranes when exposed to induced lyses [2]. There are various process for drug development. On the basis of traditional use plats are screened for phytoconstituents and for preliminary pharmacological activity. Membrane stabilization is a process of maintaining the integrity of biological membrane such as erythrocyte and lysosomal membranes against osmotic and heat induced lyses [3,4]. The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drug on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. The leaf extract of *Cephalandra indica* Naudin also has the ability to show this activity of membrane stabilization when exposed to induced lysis.

Cephalandra indica Naudin (Cucurbitaceae) is rapidly growing, perennial climber used as vegetable and known in the name of *Kunduli* in local language (Assamese language). Different parts of this plant namely root, leaves and fruits are traditionally used as medicine for several purposes like jaundice, diabetes, wound healing, stomach, skin disease, fever, asthma etc. The leaves have been reported to possess anti diabetic, anti- inflammatory, antipyretic, analgesic, antimicrobial, expectorant properties [5]. Recently, Elamathi *et al* reported the qualitative analysis of phytochemicals of this plant [5]. However, no work has been reported so far on membrane stabilizing and antibacterial activity or phytochemical screening of this plant collected from this region.

In our present study, isosaline extract of the leaves was evaluated for membrane stabilizing and the methanolic extract (MeEx) was evaluated for antibacterial activity. The results of the study are reported here.

MATERIAL AND METHODS

Collection and authentication of plant material

Fresh sample of leaf of *Cephalandra indica* was collected in the month of February 2012 from Dibrugarh, Assam, and was authenticated by Botanical Survey of India (BSI), Eastern Circle, Shillong, Meghalaya. A herbarium specimen (No. BTPS/HS/C-0126) of this plant was deposited in the Centre for Studies in Biotechnology, Dibrugarh University, Assam. The plant part was shade dried at room temperature (32-35ºC) to a constant weight over a period of 5 days and reduced to powdery form using an electric blender.

Preparation of extract

The powdered plant material (30.0 g) was extracted successively with 250.0ml of petroleum ether and methanol separately by using soxhlet extractor for 6 hours until the powdered materials became exhausted totally and refluxed. The extract was filtered and concentrated by heating on rotary evaporator. The yield of extract was calculated in percentage from the ratio of the weight of extract to the weight of raw material. Each extract was transferred to glass vials and kept at 4±1 ºC before use and both the extracts were subjected to phytochemical screening and the methanolic extract (MeEx) was used for antimicrobial activity study.

Phytochemical Screening

For phytochemical screening, 30 g of powdered plant material was extracted in soxhlet apparatus with petroleum ether and methanol successively. The extracts were concentrated in rotary evaporator and weighed. The presence of different phytoconstituents like steroids, triterpenoids, tannins, alkaloids, glycosides, flavonoid and phenols were detected as standard procedures given in the standard text. Quantitative analysis of total flavonoid and phenol content was also evaluated with standard protocols [6,7].

***In vitro* antimicrobial activity study**

Antimicrobial activity of the extracts was determined by disc diffusion method. For this study, overnight cultures of the Gram positive and Gram negative strains were prepared on nutrient agar plates. Paper disc (6mm) were soaked in 20.0 ml of the extract diluted in 25% of DMSO and were placed on the culture plates and incubated for 24 hours at 37º C. A disc loaded with 25% DMSO alone was served as a negative control. Antibiotic disc containing Ofloxacin (30.0µg/ml) served as a positive control. After the incubation period, the zone of inhibitions was measured in mm.

Membrane stabilizing activity

The membrane stabilizing activity of the leaf extract was assessed by using hypotonic solution- induced erythrocyte hemolysis designed by Shinde *et al*., 1999 [3]. For this action, saline leaves extract was prepared essentially as described by Oyedapo and Famurewa [4]. 5.0g of leaves were dried and extracted with 100.0 ml isosaline by stirring for 8 hours on a magnetic stirrer. The resulting suspension was filtered and the filtrate clarified by centrifugation. The pH of the extract was adjusted to 7.2 and kept at 4 ^{0}C.The blood was collected from one of the authors by vein puncture in to an EDTA coated container. The blood was centrifuged and supernatant was carefully removed with sterile pipettes. The packed cells were suspended in an equal volume of isosaline and centrifuged. The washing with isosaline was repeated until supernatant was clear. The effect of the leaf extract on the stability of human erythrocyte membrane was determined by the method of Oyedapo and Famurewa [4]. The extract was tested on human erythrocytes subjected to hypotonic and heat stress. Two drugs, aspirin and dichlorosodium phenac were used as positive controls while isosaline is used as negative control. The reaction mixture (4.5ml) is made up of 2.0ml

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hyposaline (0.25%w/v NaCl, 1.0ml 0.15 M sodium phosphate buffer, pH 7.4 and varying volume of extracts in isosaline to make the volume 4.0 ml, 0.5ml of 10% erythrocytes were added to this. For control test, 1.0 ml of isosaline was used instead of extract while drug control tests lacked erythrocytes. The mixture was incubated at 56 ^{0}C for 30 minutes and centrifuge and the absorbance of supernatants read at 560nm. The membrane stabilizing activity was calculated by the following formula [4].

$$100 - (OD\ drug\ test - OD\ control)/OD\ control \times 100\%$$

RESULTS

The phytochemical analysis of the petroleum extracts of leaves of *Cephalandra indica* shows the presence of steroid and triterpenoids where the methanolic leaf extracts contain reducing sugar, cardiac glycosides, steroid and triterpenoids (Table1). Table 2 shows the the zone of inhibition against five different pathogenic strains. The zone of inhibition for leaf of *Cephalandra indica* was found as 10 mm for *S. aureus*, 12 mm for *E. coli, B. Subtilis, S. faecal* and 14 mm for *P. Mirabilis*. The results of membrane stabilizing activity of leaf extract are as presented on Table 3. The result showed that the saline leaves extracts of *Cephalandra indica* has a very potent human erythrocyte membrane stabilizing activity against heat and hypotonic induced lyses. The percentage membrane ranged between 50.78±.54 and 88±1.25 at the extract concentration studied. Aspirin and dichlorosodium phenac had membrane stabilizing activity between 9.56±0.89 and 16.78±.112 and 11.05±0.23 and 19.5±0.34 respectively at a drug concentration of 0.2mg/ml to 1.0mg/ml (Table 3).

Table 1: Results of Phytochemical screening of *Cephalandra indica* leaf extracts

Sl No.	Plant Constituents	Observations*	
		Petroleum Ether	Methanol
1	Carbohydrate	_	_
2	Reducing sugar	_	+
3	Non-Reducing polysaccharides	_	_
4	Protein	_	_
5	Amino acid	_	_
6	Fats and Oils	_	_
7	Steroids and Triterpenoids	+	++
8	Cardiac glycosides	_	+
9	Saponin glycosides	_	_
10	Flavonoids	_	_
11	Tannins and Phenolic compounds	_	_
12	Alkaloids	_	_

* '-' absent; '+' present, '++' present (higher amount)

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Table 2: Antibacterial effect of the *Cephalandra. indica* leaf

Si No	Test Organism	Diameter of the zone (mm)		
		MeEx (1000µg/ml)	DMSO	Ofloxacin (30µg/disc)
1	*Bacillus subtilis* (ATCC 85900)	12	10	24
2	*Staphylococcus aureus* (ATCC 11576)	10	11	22
3	*Streptococcus faetal* (ATCC 18590)	12	10	36
4	*Escherichia coli* (ATCC 10586)	10	10	28
5	*Pseudomonas mirabilis* (ATCC 12453)	14	12	24

Table 3: Membrane stabilization activity of *Cephalandra indica* leaf and NSAIDS on human erythrocyte.

Drug volume	% Membrane Stabilization		
	C .indica(extract)	Aspirin	Dichlorosodium phenac
0.2	88±1.25	16.02±0.2	12.98±0.7
0.4	82±2.23	9.56±0.89	11.05±0.23
0.6	76±0.66	12.67±0.5	16.98±0.45
0.8	50.78±.54	14.78±.112	12.94±0.56
1	85.89±54	11.85±.234	17.5±0.34

DISCUSSION

Previous work done on methanolic extract of leaf of *Cephalandra indica* reported the presence of maximum numbers of secondary metabolites such as alkaloids, tannins, steroids, Terpenoids and flavonoids which are known to exhibit as medicinal activities. In our present study, the leaf of *Cephalandra indica* revealed the presence of phytochemicals such as steroids, triterpenoids and cardiac glycoside (Table1). Steroids are very important compounds and they have been reported to have antibacterial activity [8]. As in our study both the extracts petroleum ether and methanol clearly showed the presence of steroidal compounds, the leaf of *Cephalandra indica* demonstrated significant antibacterial activity. The zone of inhibition for leaf was found as 10 mm for *S. aureus*, 12 mm for *E. coli, B. Subtilis, S. faecal* and 14 mm for *P. Mirabilis* (Table 2).

Earlier studies have shown that various herbal drugs are capable of stabilizing the red blood cell membrane [3,8]. Omale *et al* showed that the leaf extract of *Ipoma aquatic* are highly potent on human erythrocyte protecting it against heat and hypotonic induced lyses [9]. Membrane stabilizing profiles of various extracts of *Lantana camara* on bovine red blood cell exposed to both heat and hypotonic induced lyses were reported previously [2]. Previously work also reported that the saline leaves extracts of *Hibiscus rosa* has a very potent human erythrocyte membrane stabilizing activity [10]. To check the use of this plant part as a herbal drug, we carried out the membrane stabilizing activity assay on human erythrocyte leaf of *Cephalandra indica* and the leaves extracts showed high potent membrane stabilizing activity. The observed membrane stabilization of leaf of *Cephalandra indica* may also be due to the presence of anti-haemolytic compound such as steroid [3,4],

as phytochemical analysis showed that steroid was present in *Cephalandra indica* leaves. However, the beneficial effects of plants have been reported to result from an additive or synergistic effect of a complex mixture of phytochemical constituents and the bioactivity is affected when the components are isolated [11, 12].

CONCLUSION

On the basis of the results obtained in the present study, it could be inferred that the leaf of *Cephlandra indica* indicated presence of steroids and triterpeniods in the extracts and exhibited antibacterial and membrane stabilizing activity. The antibacterial as well as anti- haemolytic properties may be attributed to these constituents. The plant therefore could be considered as a natural source for biological activities and further studies would specifically identify the compounds responsible for these activities.

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