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Evaluation of antibacteriial and diuretic activity of ethanolic extract of dried leaves of *Bryophylum calycinum* Salisb.

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Abstract

The ethanolic extracts of dried leaves of Bryophylum calycinum were tested for antibacterial and diuretic activity. The diuretic activity was tested in rats at the dose of 125 and 250 mg/Kg, orally and compared with furosemide (20mg/Kg, intraperitoneally) as the standard.The antibacterial activity was assessed by disc diffusion method against Bacillus subtilis ,Staphylococcus aureus,,Escherichia coli and Salmonella typhi at the concentrations of 100, 250 and 500 µg/disc respectively. Amikacin,a standard drug was used as reference control for the screening of antibacterial activity.The extract was found to possess significant diuretic activity with respect to the dose and was found effective against both gram positive and gram negative bacteria in a concentration dependent manner.

Key-Words: Antibacterial activity, Diuretic activity, Amikacin, *Bryophylum calycinum.*

Introduction

In spite of technological and conceptual developments in the present practice of medicines, the formation and growth of renal calculi continues to afflict mankind. The recognition of different types of urinary calculi resulted in more options of medical treatment[1]. Regardless of these advances, recurrence rates continue to be high and one of every two patients will develop other renal calculi within 5 years of the initial incident[2,3] conforming a need to develop new drugs and treatments in order to prevent the recurrence of kidney stones. High urinary oxalate is a major risk factor for human idiopathic CaOx stone disease1. Studies indicate that oxidative stress mediates the pathogenesis of kidney stone disease[4, 5]. Though noninvasive and minimal invasive methods facilitated the treatment of kidney stone disease, recurrence is a common problem and makes it difficult and expensive to manage. *Bryophylum calycinum* is naturalized throughout the hot and moist part of India. The leaves and bark extract is bitter tonic, astringent, carminative, analgesic, used in treatment of diarrhoea, vomiting[6] and antiulcer[7]. It also has anti-inflammatory[8-9] and antimicrobial activity[10].In the present study diuretic and antibacterial activity of ethanolic extract of the entire plant of *Bryophylum calycinum* has been reported.

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Material and methods

Plant Material

Fresh leaves of *Bryophylum calycinum* salisb were collected locally from Wagheshwar temple near Wagholi, Pune. during July 2010 and authenticated by Dr .P. G. Diwakar, Joint Director, Botanical Survey of India (BSI), Pune, India. A voucher specimen of the plant was deposited in the JSPMs Charak College of Pharmacy and Research, Wagholi, Pune as an herbarium under the voucher number (TTSBC-1).

Preparation of extracts: The leaves were washed thoroughly, dried in shade and coarsely powdered with the help of mesh (size - 40). The ethanolic extract of dried leaves was prepared using 70% (v/v) ethanol by soxhlet method at a temperature of 60-70 ºC. The extract was then filtered, concentrated under vacuum and then freeze-dried. A 10% w/v aqueous suspension was prepared from the concentrated extract just before dosing the rats. The extract was subjected to preliminary phytochemical testing.

Preliminary Phytochemical Screening

Methods given in standard books[11, 12] were used for preliminary phytochemical screening of the extract.

Screening for Diuretic activity

Male Wistar rats weighing 180 to 200gm were maintained under standard condition of temperature and humidity. The method of Lipschitz et al., [13,14] was employed for the assessment of diuretic activity. The experimental protocols have been approved by the

Institutional Animal Ethical Committee. The rats are divided into four groups. Each group containing six rats and were fasted and deprived of water for eighteen hours prior to the experiment. Group-I animals serving as control, received normal saline (25ml/kg, p.o.); Group-II received Furosemide (20mg/kg, i.p.) in saline; Group-III and Group-IV received the ethanolic extract at the doses of 125,250 mg/kg, respectively, in normal saline. Immediately after drug administration, the animals were placed in metabolic cages, kept at room temperature of 25 ± 0.5º through out the experiment. The urine was collected in measuring cylinders up to 5 hrs after dosing. During this period, no food or water was made available to the animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na^+, K^+ and Cl^- in the urine. Na^+, K^+ concentrations were measured by Flame photometry[15] and Cl^- concentration was estimated by titration[16] with silver nitrate solution using three drops of 5% potassium chromate solution as an indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20 mg/kg of Furosemide per kg body weight in series of supportive experiments. Results are reported as mean ± S.D and Significance was observed at $p<0.01$ (Table 1).

Screening for Antibacterial Activity

The disc diffusion method was used to perform the anti bacterial activity of the extract on nutrient agar plates[17].Three to five similar colonies were selected and transferred to 5ml broth with a loop and the broth cultures were incubated for 24 hr at 37°C. For screening, sterile 6-mm diameter filter paper discs were impregnated with 100-500 µg of the ethanolic extract of *Bryophylum calycinum* and then placed in Muller Hinton Agar Medium.The inoculum for each organism was prepared from broth cultures. The concentration of cultures was 1×105 colony forming units/ ml. The results were recorded by measuring the zones of growth inhibition surrounding the disc indicate the presence of antibacterial activity. All the data regarding antibacterial activity are the average of triplicate analyses were recorded. (Table 2). The antibacterial amikacin (10 µg/ml) was used as reference standards as recommended by the National Committee for clinical laboratory standards. The selected microorganisms included *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella typhi* at concentrations of 100, 250 and 500 µg/disc respectively.

Results and discussion

The preliminary phytochemical screening of the extract showed the presence of Steroids, Alkaloids, Saponins, Carbohydrate, Amino acids, Polyphenols, Flavonoids, Tannins and Glycosides in the ethanolic extracts of *Bryophylum calycinum*.The ethanolic extract 250 mg/kg p.o. showed significant increase in excretion of Na^+, K^+ and Cl^- ions in the urine in a dose dependent manner. This effect was comparable to that of furosemide (20mg/Kg). At the same time the ethanolic extract was unable to produce significant actions at dose of 125 mg/Kg but at a high dose of 250 mg/Kg extract showed significant increase in volume of urine. The antibacterial activity of the extract was found to be concentration dependent on the test organisms. The preliminary study supported the presence of effective diuretic constituents in the ethanolic extract of *Bryophylum calycinum.* Active principles such as Steroids, Saponins, Flavonoids, Tannins and Glycosides are known to be responsible for diuretic activity[18,19]. The data (Table1) allow to conclude that the extract is most effective in increasing urinary electrolyte concentration of all ions. The extract increases sodium ion excretion to a greater extent than potassium. As reported earlier, secondary metabolites like saponins,glycosides and flavonoids are responsible for the observed antibacterial activity of plant[20]. The presence of said constituents in the ethanolic extract of *Bryophylum calycinum* as found in phytochemical test may be responsible for the antibacterial activity.

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Table 1: Diuretic activity of ethanolic extract of *Bryophylum calycinum* Salisb.

Experimental Group	Dose	Urine volume (ml/kg)	Total Na^+ (mEq/l)	Total K^+ (mEq/l)	Total Cl^- (mEq/l)
Control (Normal Saline)	25ml/kg, p.o.	0.80 ±0.20	99.6 ± 11.4	53.5 ± 9.4	103.7 ±9.54
Standard (furosemide)	20mg/kg, i.p.	3.9 ± 1.5*	138 ± 0.30*	98.68 ± 3.2*	154 ± 0.51*
EEBC	125 mg/kg, p.o	1.5 ± 0.20	100.8 ± 9.1	114.6±10.6	124 ±13.7*
EEBC	250 mg/kg, p.o	1.72 ± 0.36*	110.9 ± 3.8*	132.0 ± 8.5*	158.6 ± 18*

Values are mean ± SEM, n=6,*p<0.01 when compared to normal saline (control), EEBC: Ethanolic extract of *Bryophylum calycinum*

Table 2: Antibacterial activity of ethanolic extract of *Bryophylum calycinum* Salisb.

Organism	Diameter of zone of inhibition (mm)			
Bacteria (10µg/ml)	**Ethanolic extract**			**Standard (Amikacin)**
Gram (+ ve)	**A** (100µg/disc)	**B** (250µg/disc)	**C** (500µg/disc)	
Bacillus subtilis	8.51±0.64	9.24±0.49	14.9±0.98	23.62±0.6
Staphylococcus aureus	10.25±0.7	12.21±1.5	16.42±0.5 *	21.6±1.2
Gram (-ve)				
Escherichia coli	NI	8.48±1.0	10.27±1.2	17.5±0.8
Salmonella typhi	7.18±0.6	8.7±0.8	10.5 ±1.3	21.29±0.4

All the values indicate mean ±SD (n=3). Statistical value *p<0.05 when compared to standard.
The concentrations were A: 100µg/disc, B: 250µg/disc, C: 500µg/disc., I- No Inhibition Zone.