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Analgesic, Antidiarrhoeal and Antimicrobial Activities of *Rhyncholechum ellipticum* (Gesneriaceae) Stem and Leaf Extracts

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Abstract

Aims: Bangladeshi rural and hilly areas people have long tradition to use various medicinal plants for treating different diseases. Phyto pharmacology is always a vital way for drug discovery. Antibiotic resistance is rising as a challenging threat day by day. Diarrhea and pain are always common problems. Present study was designed to evaluate Analgesic, Antidiarrhoeal and Antibacterial activities of acetone extracts of stem and leaf of *Rhyncholechum ellipticum* by different methods.

Methods: The analgesic activity of the samples was studied using acetic acid- induced writhing model in mice. Castor oil-induced antidiarrheal activity was observed by Thomas method. And antimicrobial activity was monitored by disc diffusion method.

Results: At higher dose (500 mg/kg), *Rhyncholechum ellipticum* inhibited 65.79 % and 63.16 % writhing of stem as well as leaf acetone extracts, respectively, compared to standard drug Diclofenac Na inhibited 78.07 % writhing. Further study needed to find possible active ingredients and mechanism of action as well. At higher dose (500 mg/kg) of the acetone stem and leaf extracts, significant inhibition 50.58 and 52.87 % of characteristic diarrhoeal feces was observed, respectively, as well as at lower dose (250 mg/kg) of the both extract, inhibition 41.37 and 40.22 %. Moderate zone of inhibition was observed at 250µg/disc and 500µg/disc compare to zone of inhibition at average 38mm of ciprofloxacin at 50µg/disc.

Conclusion: From the above results, it is clear that *Rhyncholechum ellipticum* showed significant pharmacological potentiality in different study model. So, it will be very much possible source for an isolating lead compound for curing the numerous disorders.

Keywords: Analgesic activity, Antibacterial, Antidiarrhoeal, *Rhyncholechum ellipticum*

INTRODUCTION

Plants have been a valuable source of medication and gradually becoming popular throughout the world. Plant secondary metabolites play an important role in medical care for a good percentage of world population. Thus, emphasis is now given on the standardization of herbal medicines by screening of biological activities of medicinal plants and isolation active principles from them. [1]. *R. ellipticum* the medicinal plant which is locally known as Cheodhima, Paicha, Sattari belongs to family Gesneriaceae. It is an erect undershrub, 0.6-1.2 m high with thickish stems. Leaves are opposite, 16.5 cm or longer, broadly elliptic or obovate acute, minutely dentate, base cuneate, whitened beneath, above tawny as well as silkily wooly. Afterwards flowers are rose-purple, in umbellate cymes in the lower axills and berry is 6 mm diameter. It is available in Chittagong and Sylhet, Bangladesh. Traditionally it is used as alleviates coughs in children. [2]

Diarrhoea is the major problem for developing countries especially in infants and children causing of morbidity and mortality [3]. Distribution of the entire world diarrhoea accounts for more than 5-8 million deaths each year who are below 5 years old [4]. Diarrhea is a changing normal bowel movement which characterized by increased frequency of bowel sound and movement, watery stool and abdominal pain [5]. Diarrhoea could be responsible for dehydration and electrolyte imbalance through loss of fluids [6]. Oral rehydration salts and zinc tablets are the prime concern of treatment and have been enumerated to

have saved 50 million children in the last 25 years [7]. But typically, diarrhoea are treating in various ways such as loperamide, diphenoxylate and atropine [8].

Analgesics are agents which selectively relieve pain by acting in the CNS and peripheral pain mediators without changing consciousness. The study of pain in animals raises ethical, philosophical, and technical problems. Both peripheral and central pain models are included to make the test more evident for the analgesic property of the plant [9]. Medicinal plants have been suggested to presence natural effective substances for prevention or treatment of pain related conditions. Drugs with herbal origin have attracted attention of reviews and people by having minimum or no side effects. [10, 11]

In antique times mankind invented the existence of microbes, the idea indicated certain plants had healing potential, indeed, that they contained antimicrobial properties [12]. Medicinal plants represent an abundant origin of antimicrobial agents [13]. Antimicrobial is the term which referring to a group of drugs such as antibiotics, antifungals, antiprotozoals as well as antivirals [14]. Antimicrobial agents that are used for treating diseases include synthetic chemicals and metabolic products made by microorganisms as well as chemical substances originated from plants [15]. The antimicrobial compounds from natural sources that may be inhibited bacteria by a various mechanism than the presently used antibiotics as well as have pharmacological value in treatment of resistant microbial strains [16].

MATERIAL & METHOD

Chemicals and Reagents

Diclofenac sodium, loperamide, ciprofloxacin, acetic acid and castor oil were used.

Plant Materials

Rhynchoetechum ellipticum (Gesneriaceae) leaves and stems were collected from Sylhet, Bangladesh and identified by experts at Bangladesh National Herbarium, Dhaka, Bangladesh. A voucher specimen (DACB 47042) has been submitted there for future reference.

Preparation of acetone extract

At first, a clean flat flat-bottomed glass container was taken and added about 300 gm of powdered leaves and 290 gm stems were taken in separate clean, flat-bottomed glass container. Then 1800 ml of acetone added into the container and soaked the powder into the methanol. Afterwards, the container was sealed with its contents and kept for a period of 14 days accompanying occasional shaking and stirring. After that, the coarse part of the leaf and stem were separated from the mixture by using white cotton. Then the liquid portion was also filtered three times with the help of white cotton. Then again, it was filtered through whatman filter paper. Then the filtrate was kept in Rotary evaporator machine which separates solvent and desirable crude extract was obtained.

Experimental animals

Swice albino mice (21-25g) were purchased from Jahangirnagar University, Dhaka, Bangladesh and their ages five to six weeks and were housed in animals' cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University, Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

Test Microorganisms

Five pathogenic bacterial strains were used to evaluate antibacterial activity. Three of them were Gram negative (*Klebsiella Oxytoca*, *Vibrio metschnikovii*, *Escherichia coli*) and two was gram positive (*Bacillus subtilis*, *Staphylococcus aureus*). All of the bacterial strains were collected from Microbiology Lab of Department of Pharmacy, Dhaka University Dhaka, Bangladesh

Analgesic activity

For analgesic test all mice were divided into six groups. Each group comprises of 4 mice. Control group (received 0.5% methyl cellulose, per oral), Standard Group (received Diclofenac-Na10mg/ kg intraperitoneally), group III and IV were treated with Leaf extracts of *R. ellipticum* at the doses of 250 and 500 mg per kg of body weight, respectively and), group V and VI were treated with Stem extracts of *R. ellipticum* at the doses of 250 and 500 mg per kg of body weight, respectively. The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples and vehicle

were administered orally 30 mins before intraperitoneal administration 10ml/kg of .7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 minutes before the acetic acid injection, the mice were observed for specific contraction of body referred to as "writhing" for the next 10minutes [17]. Percentage protection of acetic acid induced writhing was calculated by the formula. Percentage protection = $(Wc-Wt)/Wc \times 100$; Where, wc is the mean values of control group and Wt is the mean values of treated group.

Castor oil-induced diarrhoea

24 mice were allowed to fast for 18 h and divided into six groups of four animals each. All groups received castor oil at a dose of 1 ml/animal orally (p.o.). 30 min after castor oil administration, group I (control group) received vehicle (1% CMC in distilled water), Group III and Group IV orally received the acetone extract of leaf of *R. ellipticum* at 250 mg/kg and 500 mg/kg doses, respectively and), Group V and Group VI orally received the acetone extract of Stem of *R. ellipticum* at 250 mg/kg and 500 mg/kg doses, respectively. Group II received the reference drug, loperamide (3 mg/kg p.o.). Then the animals were placed separately in cages with filter papers underneath, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h and the characteristic diarrhoeal droppings were recorded [18].

Test of antimicrobial activity by disc diffusion method

In this method-measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentration ($\mu\text{g/ml}$). Then sterile materiel filter paper discs are impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried are used as positive and negative control, respectively. These discs are then placed in petri dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial screening. The plates are then kept at 40°C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator (37° C) for 12-18 hour to allow the growth of the microorganisms. If the test material has any anti-microbial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter. The experiments are carried out three times and the mean of the reading are recorded [19].

Statistical Analysis

The results are presented as Mean \pm SEM. Data were analyzed by one-way ANOVA followed by Dunnet's test and *P* values <0.001 were considered statistically significant.

RESULT AND DISCUSSION

Table 1 Results of Analgesic effect of *R. ellipticum* leaves and stems extracts on acetic acid-induced writhing in mice.

Treatment	Writhing counting (Mean±SEM)	% Inhibition
Control	28.5±0.77	-
Standard (Diclofenac Na)	6.25±0.22***	78.08
Leaf 250mg	10.25±0.43***	63.16
Leaf 500mg	9.5±0.58***	66.67
Stem 250mg	11.75±0.43***	58.78
Stem 500mg	9.75±0.43***	65.79

Values are presented as Mean ± SEM (n = 4), ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett test).

R. ellipticum hindered 66.67 % and 65.79 % writhing of leaf as well as stem extracts, respectively, In the acetic acid induced writhing test the extract of showed a significant ($p < 0.001$) reduction in the number of writhes compared to standard drug Diclofenac Na inhibited 78.08 % writhing. Strong prominent effects were observed with both extracts group (500 mg/kg) (Table 1). Pain is an unpleasant sensation as well as emotional touching which is linked to tissue damage. Its aim is to allow the body to react and prevent tissue damage as well. The cause of pain may also be damage to the nervous system, both the peripheral nerves, brain and spinal cord. Pain can be also occurred without damaging tissues, although the patient refers to it (psychogenic pain). The mode of pain is a perplexing phenomenon. Experience of pain depends on the strength of the stimulus, as well as individual resistance to pain. Pain receptors are sensitive to mechanical, thermal or chemical stimuli. The operation of harmful stimulus to these receptors resulting in the process into an electrical signal [20].

Table 2: Effect of leaf and stem extracts of the *R. ellipticum* on castor oil-induced diarrhoea in mice.

Treatment	No. of faecal droppings in 4 h	% Inhibition of defaecation
Control	21.75±1.18	-
Standard (Loperamide)	7.0±0.37***	67.82
Leaf 250mg	13.0±0.37***	40.22
Leaf 500mg	10.25±0.43***	52.87
Stem 250mg	12.75±0.77***	41.37
Stem 500mg	10.75±0.57***	50.58

Values are presented as Mean ± SEM (n = 4), ***P < 0.001, which is significant compared with the control group (one-way ANOVA followed by Dunnett test).

Table 3: In vitro antibacterial activity of *R. ellipticum* leaf and stem extracts.

Bacterial Strains	Type of bacteria	Diameter of zone of inhibition					
		Blank	Ciprofloxacin (50µg/disc)	Leaf (250 µg/disc)	Leaf(500 µg/disc)	Stem(250 µg/disc)	Stem(500 µg/disc)
<i>Klebsiella Oxytoca</i>	Gram (-)	-	35mm	11mm	9mm	10mm	7mm
<i>Escherichia coli</i>	Gram (-)	-	37mm	16mm	18mm	13mm	15mm
<i>Vibrio metschnikovii</i>	Gram (-)	-	40mm	12mm	15mm	14mm	13mm
<i>Bacillus subtilis</i>	Gram (+)	-	37mm	16mm	13mm	11mm	15mm
<i>Staphylococcus aureus</i>	Gram (+)	-	41mm	13mm	16mm	14mm	13mm

Gram (-): indicates Gram Negative Bacteria and Gram (+): Gram Positive Bacteria

The extract at the doses of 250 and 500 mg/kg, produced a dose dependent decrease in the number of faecal matters passed by the mice in castor oil-induced diarrhoeal model (Table 2). In superior dose (500 mg/kg) of the leaf and stem extracts, significant inhibition 52.87 % and 50.58 % of characteristic diarrhoeal feces was observed, respectively, as well as in poorer dose (250 mg/kg) of the both extract, inhibition 40.22 and 41.37% of diarrhoeal feces was found, respectively. The castor oil metabolite ricinoleic acid activated intestinal and uterine smooth muscle cells via prostaglandin E2 receptor 3 (EP3) prostanoid receptors which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. That's why delay diarrhoea induced with castor oil [21,22].

The antimicrobial effects of acetone leaf and stem extracts against different test organisms are shown (Table 3). Both extracts were showed moderate inhibitory activity against all of these organisms.

CONCLUSION

R. ellipticum possesses significant Antidiarrhoeal, Antibacterial and Analgesic activities that support to the ethnopharmacological uses of this plant. This species can be promoted for the large scale cultivation and marketing for the benefit of the local communities.

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Compliance with Ethical Standards: The handling and use of animals were in accordance with the National Institute for Health Guide for the Care and Use of Laboratory Animals. Our study was approved by a Research Ethics Committee for animal house of department of pharmacy, Faculty of Allied Health Science, Daffodil International University.

Conflict of Interest: The authors declared that they have no conflict of interest.

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