

A PROMISING ANTIDIARRHOEAL, ANTIMICROBIAL AND ANTHELMINTIC EFFECT OF METHANOLIC EXTRACT OF *HYGRORYZA ARISTATA* LEAVES

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Abstract

Hygroryza aristata is the significantly most important traditional medicinal plant practitioners all over the country since long years. Traditionally it is used as diuretic, emollient, galactagogue, strangury, diarrhea, otopathy, fatigue, general debility, Seeds are cooling and astringent to urinary tract; useful in biliousness. By using this plant only two investigations were done like: anti-inflammatory and antioxidant activities. The purpose of the study was to determine the anti-diarrheal, anthelmintic and antimicrobial activity of *Hygroryza aristata* leaves extract. Phytochemical screening of the methanolic extract of *Hygroriza aristata* leaves indicates the presence of alkaloids, carbohydrates, flavonoid, glycoside and steroid. The antimicrobial activity was investigated by the disk diffusion method using microorganisms. The antibiotic discs of Ciprofloxacin were used as reference standard. The methanolic extract of the leaf of *Hygroriza aristata* (250µg/disc and 500 µg/disc) showed a promising anti-microbial activity against *Bacillus subtilis* and *Vibrio metschnikovii*. In anthelmintic assay the plant extract exhibited that animal were paralyzed and finally dead within long time as compared with the standard drug Albendazole, which assuming that the plant extract has a promising anthelmintic effect with a long duration of action. In the investigation of anti-diarrheal activity comparing with the standard drug Loperamide we found that the number of faeces was markedly reduced by both the standard drug Loperamide (72.73%) as well as the plant extract, with the concentration of 300mg/kg (36.37%) and 600mg/kg (54.55%) respectively. Percentage of faecal output was also reduced with the increase of doses of the extract. Thus, we can assume that, *Hygroriza aristata* plant have a promising effect of anti-diarrheal, anti-microbial and anthelmintic effect which showed a ways of inventing new lead compound having such type of pharmacological effects respectively.

Keywords: *Hygroriza aristata*, antimicrobial, antidiarrhoeal, anthelmintic, methanolic extract

Introduction

The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs [1]. In order to avoid the negative side effects of allopathic drugs, people, especially in developing countries like Bangladesh, choose the opposite option like: Traditional medicine, Herbals medicine, Unani and ayurvedic medicine. Different forms of traditional medicines have been used in this country as an essential means for the treatment of diseases and the management of various health problems since a long. In this country, this practice has flourished enormously in recent years along with that of modern medicine. As a result, even at this age of highly advanced allopathic medicine, a large majority of the population of this country, particularly in rural and semi-urban areas, still prefers to use traditional medicine in the treatment of the majority of their diseases despite the fact that modern medical facilities may be available in the neighborhood.

Microorganisms are the pathogens agents in many infectious diseases of humans. Bacteria cause diseases such as plague, tuberculosis, anthrax, ringworm, candidiasis, and AIDS [2]. For this disease we used antimicrobial agent such as: Penicillin, Aminoglycosides, Erythromycin, Tetracycline, Chloramphenicol. Plants are the one of the best source of antimicrobial agent.

Diarrhea is the state of having something like three free, fluid, or watery solid discharges every day. The most widely recognized reason is a contamination of the digestion tracts due to an infection, microorganisms, or parasite – a condition otherwise called gastroenteritis. Every year diarrhea killed around 525000 kids under five. Loperamide, diphenoxylate is a medication used to decrease the frequency of diarrhea.

Haemonchus contortus, a gastrointestinal nematode normally found in small ruminants, makes by causing appetite depression, harms in gastric function and changes in absolute protein substance, energy and mineral digestion [3]. The fundamental prophylactic strategy utilized against this parasite has been anthelmintic medicines. Nonetheless, the

broad and aimless organization of anthelmintics has brought about parasite resistance [4].

Hygroriza aristata has a remarkable place among the drug producing plants in terms of medicinal properties. It is a stoloniferous perennial plant widely distributed in tropical Asia. This plant grows in paddy fields and ponds at 400–800 meters above sea level, often forms floating clusters in lakes and slow flowing rivers. Traditionally it is used as a diuretic, emollient, galactagogue, stranguria, diarrhea, otopathy, fatigue, general weakness [5]. *Hygroriza aristata* can also be used in aquariums with water temperatures of 20 to 30°C. The water should be soft to hard, the pH-value should be between 6 to 8 [6,7]. Seeds are used as cooling and astringent to urinary tract and soothing of biliousness [8]. The aim in this study is to evaluate the anti-diarrheal, anti-microbial and anthelmintic properties of methanolic extract of *Hygroriza aristata*.

Materials and Methods

Plant material

The leaves of the plant *Hygroriza aristata* were collected from the Babukhan, Rangpur, Bangladesh and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka Bangladesh for its authentication.

Preparation of the extract

The harvested leaves of the plant were separated from undesirable materials. They were air dried for two weeks after cutting into small pieces. The parts of the plant were ground into a coarse powder with the help of a suitable grinder. The powder was put away in an airtight sealed compartment and kept in a cool, dim and dry spot. Then, the plant powder will dissolve in methanol solution in a flat bottom sealed container for several days. Then metabolic extract were evaporates by the rotary evaporator and plant extract were collect.

Animals

The examinations were done on Swiss Albino mice. Mice of 16 weeks old, weighing 30-35 gm were used. The rats were kept in gatherings of 5 in each polyvinyl confine. The animals were given standard mice feed and water kept in the laboratory

environment for several days. They were fasted overnight and weighed before the experiment.

Phytochemical screening

Various phytochemical tests which were performed under the heading of phytochemical screening are Benedict's Test and Fehling's Test for carbohydrates, general test for glycosides, tests for alkaloids by Mayer's reagent and Dragendroff's reagent, test for Saponins, test for Flavonoids, test for Steroids and test for gums [9].

Detection of carbohydrates

Extracts were dissolved independently in 5 ml refined water and separated. The filtrates were utilized to test for the presence of carbohydrates.

Benedict's Test

Filtrates were treated with Benedict's reagent and warmed tenderly. Orange red encourage shows the presence of reducing sugars.

Fehling's Test

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and warmed with Fehling's A and B solutions. Development of red precipitate shows the presence of reducing sugars.

Tests for Glycosides

2 ml solution of the extract was taken into a test tube. 1ml mixture of Fehling solution A and B then added into the test tube. The tube was set in a water-bath at 60° C. On the off chance that a brick red ppt. structure that demonstrates the presence of glycosides.

Test for alkaloids

Mayer's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. At that point 1 ml of Mayers reagent was included. Yellow color ppt. was shaped and that was shown as the presence of alkaloids.

Dragendroff's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. At that point 1 ml of Dragendroff's reagent was included.

Orange brown colored precipitate was framed and that was demonstrated as the presence of alkaloids.

Test for Steroids

Sulphuric acid test

1 ml solution of chloroform extract was taken and then added 1ml Sulphuric acid Red color indicates the presence of steroid.

Test for gums

5 ml solution of the extract was taken and after that molish reagent and sulphuric acid were included. Red violet ring created at the intersection of two fluids demonstrated as the presence of gums and carbohydrate.

Test for Flavonoids

Included a couple of drops of concentrated hydrochloric acid to a little measure of a alcoholic extract of the plant material. Prompt improvement of a red color demonstrates the presence of Flavonoids.

Test for Saponins

1 ml solution of the extract was diluted with refined water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of saponins.

Anthelmintic Activity

Phosphate-buffered saline (PBS) preparation

After cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.54 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na₂HPO₄ and 0.27 g KH₂PO₄ in 1 litre of distilled water at 37±1°C.

Preparation of sample

To prepare the suspension of methanol extract of *Hygroryza Aristata* the concentrations of 25, 50 and 100mg/ml; 0.25, 0.5, 1mg of extract were taken and triturated with 0.2% v/v of Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration with PBS. For the preparation of standard albendazole at concentrations of 15 mg/ml; 150 mg of albendazole powder were taken and triturated with 0.2% v/v of Tween 80 as a suspending agent and final volume

was made to 10 ml for respective concentration with PBS.

Methodology

10 ml of control, standard and extract of each concentration were taken in different petridishes. 3 parasites of both types were taken in each different petridishes. Time taken for paralysis for each parasite was recorded. Time taken for death for each parasite was recorded [10].

Anti-Diarrheal Activity

To prepare the suspension of the test samples at the doses of 300 & 600 mg/kg per body weight, 300 & 600 mg of crude methanolic extracts were measured respectively. The distilled water was slowly added. The final volume of the suspensions was made 10.0 ml (conc. became 30 & 60 mg/ml respectively). For the arrangement standard medication 3mg of Loperamide was taken and refined water was gradually added up to the last volume 10.0 ml (conc. became 0.3mg/ml).

Young Swiss-albino mice aged 4-5 weeks, average weight 25-32gm were used for the experiment. All mice were initially screened to give 1 ml of castor oil and only those showing diarrhea were selected for the final experiment. The test animals were randomly chosen and divided into three groups that had two mice in each; they were accurately weighed and the experimental groups were properly marked, group I or the control only received distilled water. Group-II or standard received standard antimotility drug, Loperamide at a dose of 3mg/kg-body weight as oral suspension. The test groups III were treated with suspension of plant extracts of *Hygroryza aristata* the oral dose of 3000mg/kg and 600mg/kg-body weight [11].

Antimicrobial activity

A total of 4 reference microbial strains (two Gram-positive and two Gram-negative) were used as the test organism for the antimicrobial screening of all the fruits and leaves extracts of *Hygroryza aristata*. The antimicrobial activity of the plant extracts against the test organisms was performed by disc diffusion method using standard disc (30 µg/disc) for comparison [12]. Ciprofloxacin was used as the standard disc for comparing antibacterial and antifungal activity, respectively. The test organisms

were inoculated on 10 mL previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile petri dish under an aseptic condition using a sterile loop. The paper discs containing the sample extract and standard disc were placed to the corresponding petri dish and were incubated for overnight at 37 °C. Clear zone of inhibition around the discs represented the presence of antimicrobial activity which was measured in millimeter (mm) [13,14].

Statistical analysis

The results were expressed as mean±SEM using Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA). We used a one-way analysis of variance (ANOVA), followed by Scheffe's post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 (p<0.05).

Results

Phytochemical screening

The phytochemical screening tests indicated that the different constituents such as alkaloids, carbohydrates, flavonoids, glycosides and steroids were present in the plant *Hygroryza aristata* which have pharmacological properties. The results of various qualitative chemical tests for the detection of chemical constituents of *Hygroryza aristata* is shown in the Table 1.

Anti microbial activity

Standard antibiotic discs of Ciprofloxacin were used for comparison as a standard drug. The table 2 showed that the methanolic extract of the leaf of *Hygroriza aristata* (250µg/disc and 500µg/disc) showed a promising anti-microbial activity against *Bacillus subtilis* and *Vibrio metschnikovii* as compared with the standard drug Ciprofloxacin.

Anti diarrheal activity

Castor oil administration to mice induced diarrhea after 2.3mins of administration which was observed up to 4 hrs in each group. The number faeces was higher in control group where as was a

markedly reduced result was found in standard drug Loperamide (72.73%) used group as well as in the extract groups. The effect of plant extract was observed at 300 mg/kg (36.37%) and 600 mg/kg (54.55%) as shown in Table 3. Percentage of faecal output was also reduced by the increases of the doses of the extract.

Anthelmintic activity

The results are presented in table 4 exhibited the animal were paralyzed and dead in a short time and by the use of standard drug where as by the use of plant extract that animal were paralyzed and finally dead in long time. It is culminated that the extracts of the plant have low anthelmintic activity due to the uses of crude extract but have long duration of action when compared with the conventionally used standard drugs.

Discussion

Plant polyphenols, a differing group of phenolic compound (flavonols, flavonoids, tannic acid, anthocyanins, phenolic acid etc.) have a perfect basic science with the expectation of complimentary radical searching action[15] and exhibits a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, thrombolytic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects [16, 17, 18].

Drug resistance is a greatest danger to human wellbeing this happens because of different variables to conquer this issue an elective treatment has been chosen. The plant materials are important to combat serious disease in the world. Several studies leaves of *A. undulatus* show Gram positive bacteria was found to be 7 ± 0.45 to 12 ± 0.65 mm in which the highest activity was shown against *Bacillus cereus* and Gram negative bacteria, *Escherichia coli* was to be more susceptible (zone of inhibition 10 ± 0.71 mm) where as *Salmonella paratyphi* showed more resistance to the extract (zone of inhibition 6 ± 0.29 mm) [19]. The present study of the methanolic extract of leaf of *Hygroriza aristata* anti-microbial activity against *Bacillus subtilis* (7.2 ± 0.37 mm and 13.8 ± 0.41 mm) and *Vibrio metschnikovii* (7.4 ± 0.43 mm and 13.4 ± 0.67 mm) at the concentration of 250 µg/disc and 500 µg/disc respectively (Table 2).

Diarrhoea results from an unevenness between the absorptive and secretory components in the intestinal tract, joined by rush, bringing about an overabundance loss of liquid in the faeces. The utilization of castor oil initiated looseness of the bowels show in our examination is intelligent on the grounds that the autocoids and prostaglandins are included these have been ensnared in the causation of diarrhoeas in man [20, 21]. Anti-dysentric and antidiarrheal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars. Several studies of *calotropis gigantea*. with doses of 200 or 400 mg/kg showed 47.67% and 57.05% where as markedly reduced by the intraperitoneal injection of atropine, 3 mg/kg (39.84%) [22]. The present study of the methanolic extract of leaf of *Hygroriza aristata* show the effect observed at 300 mg/kg (36.37%) and 600 mg/kg (54.55%) as well as markedly reduced by Loperamide (72.73%). Percentage of faecal output was also reduced by doses of the extract (Table 3).

Methanolic extract of *Hygroriza aristata* leaves was tested for anthelmintic activity on live parasites *P. cervi*. Standard Albendazole drugs were used for comparative study. The above discussion showed that methanolic extract of the leaves of *Hygroriza aristata* has an active compound that shows anthelmintic activity against helminthes. Death time of standard Albendazole is very short compared to the plant extract (Table 4). From this study, it was culminated that the extracts of the plant exhibited low anthelmintic activity but long duration of action in compared to the conventionally used standard drug. It may be due the crude extract which may contained low concentration of active ingredients. With the increase of amount of crude extract the death time was decreased as well (Table 4).

Conclusion

This study exhibited promising effect for anti diarrheal, anti-microbial and anthelmintic activities of *Hygroriza aristata*. On the basis of investigated results it can be concluded that the plant may be useful as anti-diarrheal, antimicrobial and anthelmintic drug (crude drug). However, further investigation is necessary to identify the lead compound.

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Table 1. The phytochemical constituents of the experimental plant *Hygroriza aristata* obtained by phytochemical screening tests.

Phytochemical constituents	Methanolic extract of <i>H. aristata</i> leaves
Alkaloids	+
Carbohydrates	+
Saponins	-
Flavonoid	+
Glycoside	+
Gums	-
Steroids	+

(+) = Present; (-) = Absent

Table 2. Result of anti microbial effect of *Hygroriza aristata* leaves extract.

Bacterial strains	Diameter of zone of inhibition in mm		
	Standard drug Ciprofloxacin	Methanol extract (250µg/disc)	Methanol extract (500µg/disc)
<i>Vibrio metschnikovii</i> (Gram negative)	28.2±0.37	7.4±0.43	13.4±0.67
<i>Bacillus subtilis</i> (Gram positive)	28.4±0.50	7.2±0.37	13.8±0.41

Values are mean±SEM, (n = 5); p<0.05, student's t-test compared to control.

Table 3. Effect of *Hygroriza aristata* leaves extract on castor oil induced diarrheal mice.

Treatment	Onset of diarrhea (min)	No. of faeces in 4 hours	% Inhibition of defecation
Group I	2.3±2.3	11.5±2.4	-
Group II	45±1.3	3.5±2.1	72.73
Group III	10.2±2.2	7.6±1.7	36.37
Group IV	40.4±1.5	4.4±2.1	54.55

Values are mean±SEM, (n = 5); p<0.05, student's t-test compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Loperamide 10 mg/kg body weight, Group III and Group IV were treated with 300 and 600 mg/kg body weight of the mice.

Table 4. Result of Anthelmintic effect by *Hygroriza aristata* leaves extract.

Group	Concentration(mg/ml)	Paralyzing time(min)	Death time(min)
Albendazole	25	15.4±2.4	17±2.34
	50	12.3±1.6	15.1±3.1
	100	10±1.3	12.2±1.8
Methanolic Leaf extract of <i>Hygroriza aristata</i>	25	90.4±2.5	100.5±3.6
	50	75±1.4	90.4±2.4
	100	40.3±2.6	65.3±2.7

Values are mean±SEM, (n = 5); p<0.05, student's t-test compared to control.