Preliminary Phytochemical Screening and Antidiarrheal Activity Study of Petroleum Ether Extract of *Hibiscus rosa sinensis*

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Abstract

Every year, many people die suffering from diarrhea. Therefore, one of the most prominent priority areas of modern science is the discovery of new sources of antidiarrheal drugs. Plants have been used since ancient times for the treatment of different diseases. Most current pharmaceuticals are extracted from plants. Traditionally Hibiscus rosa sinensis is usually used to treat different diseases. The goal of the current study was to test the existence of various phytochemicals and to determine the antidiarrheal activity of the petroleum ether extract of Hibiscus rosa sinensis. The antidiarrheal effect was evaluated using castor oil-induced diarrhea at 250 mg/kg and 400 mg/kg body weight in mice. It demonstrated defecation inhibition of 72.37 percent and 85.79 percent. The existence of alkaloids, flavonoids, phenols and tannins was revealed by phytochemical screening. These phytochemicals are responsible for demonstrating the plant's distinct pharmacological operation. This outcome suggests that petroleum ether extract of Hibiscus rosa sinensis contained different types of pharmacologically active phytochemicals which have potential as antidiarrheal agent for treating diarrhea.

Keywords: *Anitidiarrheal activity, Castor oil, H. rosa-sinensis, Phytochemical Screening*

Introduction

Diarrhea is characterized as a state of disease condition in which patient excretes watery stool three or more than that over a time period of twenty-four hours (WHO, 2014). Normal bowel movement changes during diarrhea, leading to a rise in the water content, length, or frequency of stools (Guerrant et al., 2001). It is considered as one of the major reasons of avoidable death especially in developing countries, affecting children and infants in particular. It is identified as the second major factor of death for children less than five years of age, according to the World Health Organization (WHO) (Mumtaz, 2014). Diarrhea is caused by different types of bacteria, viruses and parasites that affect the gastrointestinal tract. It can be distributed by food, drinking water, and unhygienic surroundings. They are also responsible for inducing diarrhea and other pathological state may also induce it. Four main mechanisms are usually responsible for this. They are - pathophysiology in the transport of electrolytes and water, such as the increase of luminal osmolarity and increase of electrolyte secretion, reduced absorption of electrolyte, and accelerated intestinal motility resulting in decreased transition time (Lutterodt, 1992). Some antibiotics are used to treat diarrhea, but often these medications show some adverse effects. Sometimes due to inappropriate and unnecessary uses of antibiotics microorganisms can develop resistance to them (Knecht et al., 2014). Therefore, an important area of active research has continued to be the hunt for safe and more powerful agents. In that case, the first priority is always to discover new agents of plant origin.

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h. rosa-sinensis is a herb of 'Magnoliophyta' phylum. This is an ornamental flowering plant with actual leaves, stems and roots (Pekamwar et al., 2013). The plant belongs to the genus 'Malvaceae'. Except in exceptionally cold regions, it is distributed in almost all geographical areas. The plant is endemic to tropical Asia. In Bangladesh, India, Malaysia, China and Indonesia, it is commonly found. The plant grows as small trees or shrubs and contains spiky pollen. *H. rosa-sinensis* is a member of the '*Hibiscus*' genus which encompasses more than two hundred fifty native species (Noman et al., 2017). The plant grows about four meters tall having branches with about ten cm long and fifteen cm wide stem (El-Sayed et al., 2012; Begum, 2015).

Different studies reported the presence of the different types of phytochemicals namely anthocyanins, alkaloid, flavonoids, cyanidin-3,5-diglucoside, quercetin, cyanidin-3-sophoroside-5-glucoside, riboflavin, quercetin-3,7-diglucoside, ascorbic acid, quercetin3-diglucoside, cyanidin chloride, hentriacontane and vitamins, thiamine etc. (Gupta et al, 2005; Khokhar & Ahmed, 1992; jadhav et al, 2009).

H. rosa-sinensis flowers and leaves are confirmed to have Hepatoprotective function, Anti-fungal, Anti-oxidant, Anti-cancer, Anti-diabetic, Anti-fertility, Neuroprotective, Anti-inflammatory, Cardioprotective, Anti-hyperlipidemic (Missoum, 2018).

Previous studies indicate that leaves and particularly flowers can function as promoters of hair growth. In healing ulcers and arterial hypertension, the plant also has a role. It has antifertility acivity as well (Jadhav et al, 2009).

No reports reporting conventional medicinal applications of H. rosa sinensis leaves have been found about treating diarrhea. The author of this study therefore chose to explore the presence of phytochemicals and to determine the effects of petroleum ether extract of this leaves by measuring the incidence of defecating wet waste, on castor oil induced diarrhea in mice.

Materials and Methods

Plant Materials and Extract Preparation

H. rosa sinensis leaves were collected from Mirpur at Dhaka, in September 2016 and identified by an expert taxonomist of National Herbarium of Bangladesh, Mirpur, Dhaka. The voucher of the identified plant signed by the Director of Bangladesh National Herbarium has been conserved in the Herbarium for further reference. The accession number was given is 41674. The leaves of *H. rosa-sinensis* L were washed well to remove dirt, dried well and ground into fine powder. This powdered leaf was soaked in petroleum ether for ten days with occasional stirring. Then it was filtered using cotton plug and then using Whatman number 1 filter paper. The obtained filtrate was taken in a rotary evaporator for evaporation of solvent and then kept at room temperature to get a semisolid extract. Then, the petroleum ether extract of H. rosa-sinensis leaves were collected and preserved for further use.

Drugs and Chemicals

Castor oil, 0.9% sodium chloride (NaCl) solution of Orion Infusions Ltd., Bangladesh as normal saline solution and loperamide, the standard drug collected from Square Pharmaceuticals Ltd., Bangladesh were used for antidiarrheal activity test. Other reagents, such as Hydrocholric acid, ammonia, ferric Mayer's reagent hydrochloric acid, Dragendorff's reagent, sulphuric acid, benzene was used in the to screen the phytochemicals present in the extract.

Experimental Animals

Swiss albino mice, weighing around 30-35 gm were being used as experimental model for study of the antidiarrheal function. We collected these mice from International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). All animals lodged at 25.0 ± 2.0 °C and 12 hours light under normal laboratory condition: dark period, acclimatized 10 days prior to experiment. The regular diet and water were constantly supplied. In accordance with the rules of animal handling ethical committee of the Department of Pharmacy, Southeast University, all experiments on mice were performed.

Grouping and Dosing of Animals

To conduct antidiarrheal activity in test model, 16 mice were used. The experimental mice were classified into four groups of four mice in each group. In the model, the negative control group was given the vehicle (saline water, 10 ml/kg) and the positive control was given loperamide 0.5mg/kg. The other groups (Groups 3 and 4) are treated with plant extracts having doses of 250 mg/kg and 500 mg/kg body weight.

Phytochemical screening

Plant extract was screened for phytochemical test compounds which include alkaloids, phenols, tannins, flavonoid and steroids according to the methods (WC, 2009; Trease, 1989).

Alkaloid Test

Approximately 0.5 gm of the extract was taken and stirred in a steam bath with 5 ml of 1 percent aqueous hydrochloric acid. For the treatment of 1 ml of the filter, a few drops of Dragendorff's reagent were used. Turbidity or precipitation of the solution was considered as indication of the existence of alkaloids with this reagent.

Saponin Test

In a test tube, precisely 0.5 gm of the extract was taken and dissolved well in distilled water. Frothing that persisted on warming specified the presence of saponins.

Tannins Test

Approximately 0.5 g of the extract was dissolved in distilled water and about ten ml of bromine water was added to identify the presence of tannins. The presence of tannins was demonstrated by the decolourisation of bromine water.

Anthraquinone Test:

For the detection of anthraquinone activity, the Borntrager test was used. Here, 0.5 gm of the plant extract was taken and shaken with a separated benzene layer. Then and 10 percent ammonia solution was applied to half of its own volume. The presence of pink, violet or red coloration in the ammonia phase specified the presence of anthraquinone.

Flavonoid Test:

With plant extract, 1% aluminum chloride solution in methanol, magnesium turnins, concentrated HCl, and potassium hydroxide solution were added to identify the existence of flavonoids. (Awoyinka et al., 2007).

Glycoside Test:

Liberman, Salkowski and Keller-Killani tests confirmed the existence of cardiac glycosides (Ciulei, 1982; Sofowora, 1996)

Castor Oil-Induced Diarrhea in Mice

This test was carried out with a few modifications depending on the process (Awouters et al, 1978). Sixteen mice were kept for fasting for 18 hours and divided into four groups, each group having four animals. Saline water (2ml/kg) was obtained by the first group and loperamide (5mg/kg) by the second group, acting as negative and positive controls respectively. Groups 3 and 4 obtained extracts of 250 and 500 mg/kg respectively. All the animals were given 0.5ml of castor oil orally after one hour. They kept the animals in different metabolic cages. For 6 hours, the severity of diarrhea was measured. The mean total counting of feces of mice (dry and wet droppings of diarrhea) was identified and compared to the negative control group. The average diarrheal feces result for the negative control group was considered to be 100%. The percentage of inhibition of total defecation and that of diarrhea were calculated using the following formulas:

Percentage of inhibition of defecation= Total no. of feces in the negative control − Total no. of feces in treated group/ Total no. of feces in the negative control × 100

Results

Hibiscus rosa sinensis L leaves contains alkaloids, saponins, tanins, anthraquinone, flavonoid, cardiac glycosides (Table1)

Table 1. Phytochemical constituents of *H. rosa-sinensis*

Phytochemicals	composition
Alkaloids	+
Saponins	++
Tannins	+
Anthraquinone	-
Flavonoid	++
Phenol	++
Cardiac Glycosides	+

'++'= present in large quantity
'+'= present in moderate quantity and
'-' = means absent

Effects on Castor Oil- Induced Diarrhea in mice

For all test doses of the extract relative to the negative control group, a substantial reduction in the number of defecation cases was observed. In the extract-treated groups, the mean total number of defecation cases was 3.5 ± 0.57 and 1.80 ± 1.25 at 250 and 500 mg/kg extract doses, respectively, while this value was 1.50 ± 1.10 in the loperamide-treated community. At 250 mg/kg and 500 mg/kg extract doses, respectively, the inhibition percentage of total defecation compared to the negative control group was 72.37 percent and 85.79 percent. The standard medication showed a more substantial decrease in the number of cases of defecation (88.16 percent) relative to the vehicle-treated community (Table 2).

Table 2: Effect of *Hibiscus rosa sinensis* leaves on castor oil-induced diarrhea in mice

Groups	Treatment	Total weight of hard stool	Total weight of wet stool	Total no of feces	% inhibition of defecation
I	castor oil+saline	0.004 ±0.008	0.205±0.023	12.97±1.15	-
II	castor oil+loperamide	0.022±0.003	0.020±0.003	1.50±1.10	88.16
III	castor oil+250 mg/kg extract	0.13±0.089	0.072±0.083	3.5±0.57	72.37
IV	castor oil+500 mg/kg extract	0.15±0.04	0.12±0.092	1.80±1.25	85.79

Data are showed as mean ± standard error of mean (SEM), n = 4

Discussion

Diarrhea is a disorder in which low-consistency feces of abnormally regular defecation occur. This disease occurs due to disorder occurrence in the transportation of water and electrolytes in the intestines. Some findings suggest that the diarrheal effect is responsible for nitric oxide in castor oil, while ricinoleic acid is shown to cause diarrhea via a hypersecretory reaction. This ricinoleic acid is considered as the most vital constituent of castor oil (Awouters et al, 1978; Racusen & Binder, 1979). Different mechanisms are suggested to elucidate the role of castor oil to produce diarrheal effect, including inhibition of the activity of intestinal Na+ K+ ATPase, which ultimately decreases usual fluid absorption (Vieira, 2000; Capasso,1994). Some mechanisms suggest activation of active secretion mediated by adenylate cyclase or mucosal cAMP (Imam et al., 2012) and invigoration of prostaglandin (PG) and platelet activating factor formation (Pinto et al., 1992). Castor oil is responsible for inducing diarrhea since it is metabolized in the intestine into ricinoleic acid, which induces intestinal mucosal discomfort and inflammation, contributing to the secretion of inflammatory mediators like as prostaglandins and histamine. Vasodilatation, contraction of smooth muscle and mucus secretion in the small intestines are initiated by these released prostaglandins. Prostaglandins from the E sequence are known to be good agents that can induce diarrhea in laboratory animals and in human beings too.

Our analysis showed that the total dose-dependent antidiarrheal activity. In our study, *Hibiscus rosa sinensis L* leaves extract showed notably less amount of feces in castor oil-induced mice. It indicated about 72.37 percent and 85.79 percent of defecation inhibition at 250 and 500 mg/kg doses, respectively. Such findings indicate that *Hibiscus rosa sinensis* L leaves hold such components which have antidiarrheal property. From these findings, it can also be assumed that reduced amount of water and electrolyte release into the small intestine can boost the absorption of electrolytes from the intestinal lumen compatible with hypersecretion inhibition (Mascolo, 1994). Hypermotility characterizes diarrhea in addition to various pathophysiological diarrhea disorders, where the secretory portion is not only the prime factor causing diarrhea but also castor oil contains ricinoleic acid, which causes irritation, intestinal mucosal inflammation and eventually causes diarrhea. The released prostaglandins invigorate gastrointestinal motility and causes release of water and electrolyte. It is also well known that loperamide has a significant function in inhibiting diarrhea caused by castor oil (Shah, 2004). With respect to gastrointestinal motility, the propulsive movement through the gastrointestinal tract has been suppressed by *H. rosa-sinensis* L, which indicates that the leaves extract will decrease the frequency of stool in diarrheal conditions. Phytochemical screening of Hibiscus rosa sinensis L leaves has revealed the presence of alkaloids, tannins, cardiac glycosides, saponins, flavonoids (Patel & Adhav, 2016). Previous studies indicate that the antidiarrheal activity properties of flavonoids and polyphenols are responsible for (Vaghasiya et al., 2011).

Some studies have also shown, however that flavonoids can hinder the intestinal motility and the secretion of water and electrolytes. In addition, some in vivo and in vitro studies have also shown that flavonoids can block prostaglandin E2-induced secretion of intestine and spasmogens-induced contraction. It also has the potential to hinder the secretion of autocoids and prostaglandins. Flavonoids are also deemed to postpone castor oil-induced diarrhea as prostaglandin biosynthesis inhibitors (Dosso et al., 2012; Di Carlo et al., 1993). By interacting with and inhibiting cytochrome P450 systems, polyphenols may also exhibit antidiarrheal properties (Brijesh, 2009; Anderson et al., 1991). So the antidiarrheal activity can be attributed due to the existence of flavonoids and phenols in the petroleum ether extract of the leaves of *H. rosa-sinensis.*

Conclusion

The result of the present study showed that petroleum ether extract of Hibiscus rosa sinensis leaves extract has potential antidiarrheal activity. This antidiarrheal effect can be due to presence of phenol and flavonoid which are found through phytochemical analysis. However, more chemical and pharmacological studies are needed to carry out to identify the bioactive compounds responsible for inhibition of diarrhea and to explain the precise mechanisms which are accountable for the observed pharmacological effect of the leaves.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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