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Anti-diarrheal, Analgesic and Anti-microbial activities of the plant Lalmesta (*Hibiscus sabdariffa*): A review

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Abstract

The aim of this study was to phytochemically investigate the ethanolic extract of *Hibiscus sabdariffa Linn. calyces and* to evaluate the analgesic, anti-microbial and anti-diarrheal activities of this. The calyces of *Hibiscus sabdariffa* were separated from the other plant parts and sun dried and extracted using ethanol and phytochemically and pharmacologically evaluated.Different Phytochemical tests were performed for phytochemical screening and for determining the functional groups. Different methods like Disc diffusion method for antimicrobial activity determination, Castor oil induced diarrhea in mice method for the anti-diarrheal study and Acetic acid induced writhing test for analgesic activity were employed. Phytochemical screening of the calyces of *Hibiscus sabdariffa* ensured the presence of alkaloid**,** flavonoids, saponins, tannins in the crude ethanolic extract. The peripheral analgesic activity was evaluated by acetic acid induced Writhing method. The extract produced 66.85% ($p< 0.001$) inhibition of writhing in mice at the dose of 500-mg/kg body weight, which is comparable to diclofenac sodium (78.45% ($p<0.001$) at the dose of 25mg/kg). The anti-diarrheal activity of the crude extract of *Hibiscus sabdariffa* was evaluated using the model of castor oil induced diarrhea in mice. The crude etanolic extract of *Hibiscus sabdariffa (calyces)* showed a marked antidiarrhoeal activity at dose of 500 mg/kg-body weight as compared to the standard antidiarrhoeal agent loperamide (dose:50mg/kg-body weight). *Hibiscus sabdariffa* caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation. Anti-microbial activity was tested using a number of micro-organisms.The peripheral analgesic activity of the ethanolic extract of the calyces of *Hibiscus sabdariffa* against acute inflammatory pain was significantly high as compared to potent inhibitory activity of Diclofenac (25mg/kg). Therefore, it is likely that the ethanolic extract at a dose of 400mg/kg might suppress the formation of these substances or antagonize the action of these substances and thus exerts its analgesic activity in acetic acid-induced writhing test. Anti-diarrheal activity was present in the ethanolic extract (500mg/kg) which indicate that the drug in decreased intestinal motility. This plant sample didn't show any antimicrobial activity.

Keyword: *Hibiscus sabdariffa,* phytochemical screening, Antidiarrhoeal screening, castor oil, analgesic activity, writhing, disc diffusion method.

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1. Introduction

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone (Ahmad *et al., 1992*). All of these drugs present well known side and toxic effects. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. Diarrheal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year (Carlos and Saniel, 1990). Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs (Ojewole *et al., 2010*). Therefore, the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases treatment depending on traditional medical practices (Atta and Mouneir, 2004). Plants also represent a rich source of antimicrobial agent (Mahesh and Satish, 2008). Plants generally produce many secondary metabolites which constitute an important source of microcides, anti-oxidants. Many natural substances having anti-oxidant and anti-microbial properties have been used in health foods for medicinal and preservative purposes. *Hibiscus sabdariffa* is an annual or perennial herb or woody-based sub shrub, growing to 2–2.5 m tall. It is distributed in the Indian subcontinent, Bangladesh, Myanmar, Thailand, Senegal, Mali, Niger, Congo, France, Gambia, Nigeria, Egypt, Sudan, Namibia, Caribbean Panama, Indonesia and Malaysia. The plant is considered to have antihypertensive properties. In East Africa, the calyx infusion, called "Sudan tea", is taken to relieve coughs. Roselle juice, with salt, pepper, asafetida and molasses, is taken as a remedy for biliousness.

The heated leaves are applied to cracks in the feet and on boils and ulcers to speed maturation. A lotion made from leaves is used on sores and wounds. The seeds are said to be diuretic and tonic in action and the brownish-yellow seed oil is claimed to heal sores on camels. In India, a decoction of the seeds is given to relieve dysuria, strangury and mild cases of dyspepsia. Brazilians attribute stomachic, emollient and resolutive properties to the bitter roots.

2. Materials and method

2.1 Collection of the plant sample

The calyces of the plant *Hibiscus sabdariffa* was collected from hatazari under Chittagong district, Bangladesh in December 2008 at day time. During collection process the calyces were not washed or cleaned by water due to chance of hydrolysis, oxidation and other types of chemical degradation. Dusty calyces were cleaned by shaking and other type of chemical degradation and any type of adulteration was strongly prohibited. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and tagged with the accession number- 33858. A voucher specimen is deposited.

2.2 Preparation of plant extract

The calyces were sun dried for 10 days. After drying, the calyxes were ground into coarse powder with the help of a grinder. Then the plant powder was stored in an airtight vessel and kept in cool and dry place. One hundred gram of the dried powder was taken in a washed jar. After that 95% ethanol (500 ml) was poured into the jar up to 1 inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover was closed properly with aluminum foil to resist the entrance of air into the jar. The extraction was carried out for ten days with occasional stirring and shaking. Then the plant extract was filtered by a piece of clean white cotton material two times and then through Whitman paper. The filtrate was collected in a beaker. After filtration, the residue was taken for re extraction in jar and it was extracted with 250ml 95% ethanol for 10 days. The jar was shaken several times during the process to get better extraction. Then filtration was performed in the same way as described earlier. The filtrate thus obtained was evaporated under ceiling fan until dried. It rendered a gummy concentrate of deep red color.

2.3 Drugs and Chemicals

Diclofenac Sodium BP and Loperamide BP were obtained from ACI pharmaceuticals. Morphine was obtained from Gonoshastho Pharmaceuticals Ltd., Dhaka, Bangladesh and acetic acid and other reagents were from Merck, Germany.

2.4 Experimental animal

Swiss albino mice (25-30 g) were obtained from the Animal Research Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). The animals were housed in polyvinyl cages and received feed, formulated by ICDDR, B and water *ad libitum* and proper laboratory housing conditions were maintained to keep the hydration rate constant, food and water were stopped 12 hours before the experiments. The ethics for use of experimental animals were followed carefully.

2.5 Phyto-chemical screening

2.5.1 Composition of Reagents used for the different chemical group test

The following reagents were used for the different chemical group test (Trease and Evans, 2009).

2.5.1.1 Mayer’s reagent

1.36 gm mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm of potassium iodide in 20 ml of water.

2.5.1.2 Dragendroff’s Reagent

1.7 gm basic bismuth nitrate and 20 gm tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm potassium iodide and 40 ml water.

2.5.1.3 Fehling's solution A

34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

2.5.1.4 Fehling's solution B

17.6 gm of sodium potassium tartarate and 7.7 gm of sodium hydroxide were dissolved in sufficient water to produce 100 ml. Equal volume of above solution were mixed at the time of use.

2.5.1.5 Benedicts Reagent

1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

2.5.1.6 Molish Reagent

5 gm of pure α-naphthol was dissolved in 50 ml of ethanol.

2.5.2 Tests procedure for identifying different chemical groups

The following tests were performed for identifying different chemical groups present in the plant extract (Trease and Evans, 2009).

2.5.2.1 Tests for Reducing sugar

a) Benedict's Test

0.5 ml of aqueous extract of the plant material is taken in a test tube. 5ml of benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. The formation of a red color precipitate of cuprous oxide indicates the presence of a reducing sugar.

b) Fehling's Test

2ml of an aqueous extract of the plant material is added 1ml of a mixture of equal volumes of Fehling's solutions A and B and boiled for few minutes. The formation of a red or brick red color precipitate was formed which indicates the presence of a reducing sugar.

2.5.2.2 Tests for Tannins

a) Ferric Chloride Test

5 ml solution of the extract is taken in a test tube. Then 1 ml of 5%Ferric chloride solution is added. **G**reenish black precipitate and indicated the presence of tannins.

b) Potassium dichromate test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added. The formation of a yellow precipitate indicates the presence of tannins.

2.5.2.3 Test for Flavonoids

A few drops of concentrated hydrochloric acid is added to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of Flavonoid.

2.5.2.4 Test for Saponins

1 ml solution of the extract is diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of one centimeter layer of foam indicates the presence of saponins.

2.5.2.5 Test for Gums

5 ml solution of the extract is taken and then molish reagent and sulphuric acid are added. Formation of red violet ring at the junction of two liquids indicate the presence of gums and carbohydrate

2.5.2.6 Test for Steroids

Sulphuric acid test

1 ml solution of chloroform extract is taken and then added1ml Sulphuric acid. Red color indicates the presence of steroid.

2.5.2.7 Test for alkaloids

a) Mayer's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid are taken in a test tube. Then 1 ml of Mayer's reagent is added. Formation of yellow color precipitate indicates the presence of alkaloids.

b) Dragendroff's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid are taken in a test tube. Then 1 ml of Dragendroff's reagent is added. Formation of orange brown precipitate indicates the presence of alkaloids.

c) Wagner's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid are taken in a test tube. Then 1 ml of iodine solution (Wagner's reagent) is added. The formation of reddish brown precipitate indicates the presence of alkaloids.

D) Hager's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid is taken in a test tube. Then 1 ml of picric acid solution (Hager's reagent) is added. The formation of yellowish precipitate indicates the presence of alkaloids.

2.6 Acetic acid induced writhing test

The peripheral analgesic activity of whole plant *Boerhavia repens* was measured by the acetic acid induced writhing test as described earlier by Saha *et al*.,(2007). Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Diclofenac Sodium at oral dose of 25mg/kg was used as standard analgesic agent. Intraperitonal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhing was calculated for 20 min, 5 min after the application of acetic acid.

2.7 Antidiarrhoeal Activity Screening

Antidiarrheal test of the ethnolic extract was conducted by using castor oil induced diarrhea in swiss albino mice by the method followed by Nwodo and Alumanah (Nwodo, OFC., Alumanah, EO., 1991). The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Of the experimental groups, group-I or the control received only distilled water containing 1% Tween-80. Group-II or the positive control received standard antimotility drug, Loperamide at a dose of 50mg/kg-body weight as oral suspension. The test groups were treated with suspension of calyces extract of *Hibiscus sabdariffa* at the oral dose of 500mg/kg-body weight. The mice were fed with the samples 1 hour prior to the oral administration of castor oil. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in four hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 4-hour period and were noted for each mouse. The latent period of each mouse also counted. At the beginning of each hour new papers were placed for the old ones (Chatterjee, 1993).

2.8 Antimicrobial Screening

Antimicrobial screening was performed using disc-diffusion method (Rizvi *et al., 2011*). 8 mg of samples from different extract were dissolved in methanol to obtain desired concentration in aseptic condition. Sterilized filter paper discs were taken in a blank Petridis under laminar hood. Then discs were soaked with solutions of test samples and dried. Standard Kanamycin (30 µg/disc) discs were used as positive control and blank discs were used as negative controls. The sample discs, standard antibiotic discs and control discs were placed gently on marked zones in the agar plate's pre-inoculated with test bacteria, protozoa and fungi. The plates were then kept in a refrigerator at 4^0C for about 24 hours to allow sufficient diffusion of materials from discs to surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours. Both gram positive and gram-negative organisms were taken for the test and they are listed in Table 1.

Table 1: List of micro-organisms used for the anti-microbial screening

Gram negative	Gram positive
1. *Escherichia coli*	1. *Staphylococcus aureus*
2. *Shigella dysenteriae*	2. *Staphylococcus epidermidis*
3. *Shigella sonnei*	3. *Staphylococcus saprophyticus*
4. *Salmonella typhi*	4. *Streptococcus pyogenes*
5. *Salmonella paratyphi*	5. *Streptococcus agalactiae*
6. *Vibrio cologet*	6. *Enterococcus faecalis*
7. *Shigella boydii*	
8. *Shigella flexneri*	
9. *Pseudomonas spp.*	
10. *Proteus spp.*	

2.9 Statistical analysis

The students t-test was performed and the p-value calculated for the tests to determine the statistical significance of the results.

3. Results and discursion

3.1 Results of Phytochemical screening

The chemical group tests were performed and the results are mentioned in the following table-1. Results indicates that alkaloids, flavonoids, saponins and tannins were detected in the ethanol extract of calyex of *H.sabdariffa.* The results of the test are given in Table 2.

Table 2: Results of the different chemical tests performed

Chemical Group test	Specific tests	Observation	Inference
Test for Alkaloids	a)Mayer's test	positive	Presence of Alkaloids.
	b)Dragendroff's test	positive	
	c)Wagner's test	positive	
	d)Hager's test	positive	
Test for Steroid	a) Sulphuric acid test	Negative	Absence of steroid
Test for Flavonoids	-	positive	Presence of Flavonoids.

Test for Saponins	-	positive	Presence of saponins.
Test for Tannins	a)Ferric Chloride Test	positive	Presence of Tannins.
	b)Potassium dichromate test	positive	
Test for Gums	-	Negative	Absence of gums
Test for Reducing Sugars	a) Benedict's Test	Negative	Absence of reducing sugar
	b) Fehling's Test	Negative	

3.2 Acetic acid induced writhing test

Analgesic activity of the ethanolic extract of *H.sabdariffa* calyxes was tested by acetic acid induced writhing model in mice. The extract produced 33.15% of writhing (dose 500mg/kg) and diclofenac sodium produced 21.55% of writhing (dose 25 mg/kg). So, the extract produced 66.85% ($p< 0.001$) acetic acid induced writhing inhibition in mice at the dose of 500-mg/kg body weight, which is comparable to diclofenac sodium (78.45% ($p<0.001$) at the dose of 25mg/kg). The results are shown in Table 3.

Table 3: % inhibition of writhing of the different test samples

Group	Number of Writhing (Mean ± SEM)	% of Inhibition of Writhing
1) Control	36.2±2.04	-
2)Standard	7.8±1.11*	78.46
3)Crude ethanolic Extract 500 mg/kg	12± 0.89*	66.85

The student's t-test was carried out and the P values calculated. * indicate $P<0.05$. All values are means of individual data obtained from five rats ($n = 5$)

3.3 Anti-diarrheal study:

Hibiscus sabdariffa (calyxes) showed a marked anti-diarrhoeal activity in castor oil induced test in mice at the dose of 500 mg/kg-body weight as compared to the standard antidiarrhoeal agent loperamide (dose:50mg/kg-body weight). *Hibiscus sabdariffa* caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation. The results are noted in Table 4.

Table 4: **Mean latent period and the average number of defecation per hour**

Group	Mean latent period	Average number of defecation ±SEM*				
		1st hour	2nd hour	3rd hour	4th hour	5th hour
1) Group **I** (Control)	0.72±0.038	4.4±0.67	6.4±0.85	5.2±0.6	3.4±0.94	2.2±0.61
2) Group **II** Standard loperamide (50mg/kg)	1.66±0.087	0.6±0.27	2.2±0.35**	3±0.65*	1±0.35*	0.4±0.27*
3) Crude ethanolic fraction (500mg/kg)	1.22±0.045	2±0.35	4±0.54*	4.4±0.89	2±0.61	1±0.5

The t-test was done and the P-value calculated to find the statistical significance. * indicate P<0.01 and * indicate P< 0.05.

4.4 Anti-microbial screening

Experiment showed that the ethanolic extract of the calyx of *Hibiscus sabdariffa* (L.) (500 µg/disc) had no anti-microbial activity against above bacterial strains. The results are shown in Table 5.

Table 5: **In vitro anti-microbial activity of ethanol extract**

Serial No	**Bacterial Strains**	**Type of Bacterial Strains**	**Diameter of Zone of Inhibition in mm**		
			Blank	**Kanamycin (30 µg/disc)**	**Extract of *Hibiscus sabdariffa* calyx (500µg/disc)**
1	*Salmonella typhi*	Gram(-)	**-**	18	-
2	*Salmonella paratyphi*	Gram(-)	**-**	17	-
3	*Escherichia coli*	Gram(-)	**-**	18	-

4	*Staphylococcus epidermidis*	Gram(+)	-	18	-
5	*Vibrio cologet*	Gram(-)	-	17	-
6	*Shigella flexneri*	Gram(-)	-	19	-
7	*Enterococcus faecalis*	Gram(+)	-	18	-
8	*Streptococcus agalactiae*	Gram(+)	-	19	-
9	*Shigella sonnei*	Gram(-)	-	17	-
10	*Shigella boydii*	Gram(-)	-	17	-
11	*Streptococcus pyogenes*	Gram(+)	-	19	-
12	*Shigella dysenteriae*	Gram(-)	-	18	-
13	*Proteus spp.*	Gram(-)	-	17	-
14	*Pseudomonas spp*	Gram(-)	-	18	-
15	*Staphylococcus saprophyticus*	Gram(-)	-	19	-
16	*Staphylococcus aureus*	Gram(-)	-	17	-

4. Conclusion

The peripheral analgesic activity of the ethanolic extract of the calyx of *Hibiscus sabdariffa* against acute inflammatory pain was significantly high as compared to potent inhibitory activity of Diclofenac (25mg). Diclofenac offer relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process (Hirose *et al*). Therefore, it is likely that the ethanolic extract at a dose of 400mg/kg might suppress the formation of these substances or antagonize the action of these substances and thus exerts its analgesic activity in acetic acid-induced writhing test. Anti-diarrheal activity was present in the ethanolic extract (500mg/kg) which indicate that the drug decreased intestinal motility.

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