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Studies on Diuretics and Laxative Activity of the *Hibiscus Tiliaceus* Linn. Bark Extracts

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Abstract: The main objective of the study was to investigate diuretic and laxative activity activity of *Hibiscus tiliaceus* bark. The extracts obtained in soxhalation process were investigated for diuretic and laxative activity in albino rats. The responses of test extracts were compared with standard drugs furosemide (10mg/kg, p.o.) for diuretic and agar agar (300mg/kg, p.o.) for laxative activity respectively. The *Hibiscus tiliaceus* bark extracts were found to produce significant diuretic as well as laxative activity in dose dependant manner as compared to standard drugs. These activities may be contributed to the phytoconstituents present in the bark. Isolation of these important phytoconstituents may be taken up in further studies.
Keywords: Agar-Agar, diuretics activity, furosemide, *Hibiscus tiliaceus*, laxative activity.

Introduction

The extracts of many plants used in traditional medicine contain curative agents that are used in many modern medicines. The pharmacological action of crude drug is determined by the nature of its constituents. Thus the plant species may be consider as a biosynthetic and for the chemical compounds example proteins, carbohydrates and fats that are utilized as food by the animals and humans, but also for a huge number of compounds including alkaloids, terpenoids, flavonoids, glycosides etc. which exert definite physiological effects. These chemical compounds are mostly responsible for the desired beneficial properties [1]. Natural products extracted from plants which belong to the Malvaceae family are used in the treatment of many diseases worldwide. One important genus in this family is Hibiscus with more than 220 species distributed in tropical and subtropical regions [2]. *Hibiscus tiliaceus* L. is a typical plant of tropical climates found in the regions of mangroves in significant quantities [3]. An aqueous extract of wood and fresh flowers is a registered treatment for skin disease [4,5]. Recently it was shown that methanolic flower extract exerts an antioxidant effect on the yeast *Saccharomyces cerevisiae*, protecting against hydrogen peroxide (H_2O_2) and tert-butylhydroperoxide (t-BHP) cytotoxicities. In addition, the extract was not mutagenic in *Salmonella typhimurium* or *S. cerevisiae* and showed a significant antimutagenic action against oxidative mutagens in *S. cerevisiae* [2]. It is also reported traditionally, where the leaves are used to treat fevers and soothe coughs, the bark to treat dysentery, and the flowers aid in treating ear infections and abscesses [6]. Previous pharmacological investigations of the genus Hibiscus plants indicated the presence of species with useful biological activities. Thus, the Hibiscus genus deserves additional evaluation as a provider of chemopreventive agents. Indeed, there is a current need for availability of new plant-derived bioactive molecules; for the development of new drugs and may provide a cost-effective mean of treating cancers and other diseases in the developing world [7, 8].

Materials and Methods

Plant material

Hibiscus tiliaceus Linn. bark was collected from Costal side of Ratnagiri district of Maharashtra and authenticated By Dr. J. Jayanthi, Scientist "C" for Director, Botanical Survey of India, Pune vide letter No. BSI/WRC/TECH/2010 Voucher No. HIBCA1VIT. The herbarium is kept at BSI, Pune for further reference.

Preparation of extracts

For the present study, the extracts were obtained by continuous hot extraction method using soxhlet apparatus. 500 gm of bark powder was passed through sieve no. 60 and packed in soxhlet apparatus and extracted using petroleum ether, ethyl acetate and methanol as solvents. The filtrate was concentrated in rotary evaporator. The extracts were calculated for their percentage yields.

Table no 1. Extractive value determination

Plant name	Plant Part used	Extractive value (% w/w)		
Hibiscus tiliaceus Linn.(Malvaceae)	Bark	Petroleum ether extract	Ethyl acetate extract	Methanol extract
		11.22	6.40	9.62

Phytochemical Screening

Preliminary phytochemical analysis was carried out to find out nature of chemical constituents present in the extracts. Qualitative chemical test were carried out for all the extracts. It revealed presence of carbohydrates, proteins, steroids, alkaloids, saponins, tannins, glycosides and amino acids (Table 2). Phytochemical screening of the extract was carried out according to the standard methods.

Table no 2. Preliminary Phytochemical screening of *Hibiscus tiliaceus* Linn. bark extracts

Chemical test for constituent	Petroleum ether extract	Ethyl acetate extract	Methanol extract
Alkaloids	--	++	++
Glycosides	--	++	--
Tannins	++	++	--
Terpenoids	--	++	++
Flavonoids	--	++	++
Proteins	++	--	++
Saponins	++	++	++
Steroids	--	++	--
Amino acids	++	++	++
Carbohydrates	++	--	++

"++" indicates presence "--"indicates absence

Animals used

Male Swiss albino mice, weighing 20–25 g and Wistar albino rats weighing 120-150 g, were used for acute toxicity study and evaluation of pharmacological studies respectively. The animals were housed in polypropylene cages and maintained under standard environmental conditions: 25±2^{o}C, 12:12 h light: dark cycle and 45-55% humidity, with free access to food and water *ad libitum*. The Institutional Animals Ethics Committee approved all the experimental protocols.

Acute toxicity study

The test was carried out as suggested by Ganapaty et. al. [9]. Swiss albino mice of either sex weighing between 25–30 g were divided into different groups comprising six animals each. The control group received

normal saline (2 ml/kg, p.o.). The other groups received 100, 200, 300, 600, 800, 1000, 2000, 3000 and 4000 mg/kg of the test extract respectively. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. Thereafter, they were then kept under observation up to 14 days after drug administration to find out the mortality if any.

Diuretic activity

The method of Lipschitz et. al, [10,11] was employed for the assessment of diuretic activity. In this method, male albino rats weighing between 120- 150 g, deprived of food and water for 18 hours prior to the experiment, were divided in eight groups of six rats in each. The first group of animals, serving as control, received normal saline (25 ml/kg, p.o.); the second group received furosemide (10 mg/kg, p.o.) in saline [12]. Other groups received doses of petroleum ether extract, ethyl acetate extract and methanol extract (200 and 400 mg/kg) in normal saline. Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and faeces, kept at $20^0C \pm 0.5^0C$. The volume of urine collected was measured at the end of 5 hr. During this period, no food and water was made available to animals. The parameters taken were total urine volume, concentration of Na^+, K^+ and Cl^- in the urine. Na^+ and K^+ concentrations were determined by flame photometer [13] and Cl^- concentration was estimated by titration [11,14,15] with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator.

Laxative activity

The test was performed according to Capasso et. al. [16] on rats of either sex, fasted for 12 hr before the experiment, but with water provided *ad libitum*. The animals were divided into eight groups of six in each. The animal groups were administered orally either with vehicle (1% Tween-80 solution in normal saline, 25 ml/Kg), reference standard drug, agar-agar (300 mg/ kg, p.o.) in saline [9] and other groups received doses of petroleum ether extract, ethyl acetate extract and methanol extract (200 and 400 mg/kg) in normal saline. Immediately after dosing, the animals were separately placed in cages suitable for collection of faeces. After 8 hr of drug administration, the faeces were collected and weighed. Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16 hr.

Results

Preliminary phytochemical screening of various extracts of *Hibiscus tiliaceus bark* are shown in Table 2. In acute toxicity study, it was found that the extract induced sedation, diuresis, purgation and temporary postural defect at all tested doses. However, there was no mortality at any of the tested doses till the end of 14 days of the observation. The methanol extract was found to produce significant increase in excretion of sodium, potassium and chloride ions at the higher dose tested. The order of activity of increase of urinary output was methanol extract > ethyl acetate extract > petroleum ether extract. Also the order of activity of increase of urinary electrolyte excretion was found to be in same pattern (Table 3).

In the evaluation of laxative activity, all the extracts were found to produce significant dose dependant activity at both the tested level of doses (200 and 400 mg/kg, p.o.). The effect of extracts was superior to that of the standard drug used at 400 mg/kg, p.o. dose level (Table 4).

Discussion

The present study revealed that, extracts of *Hibiscus tiliaceus* bark linn. significantly increased the urinary output as well as urinary electrolyte concentration in a dose dependent manner. The methanol extract was found to be the most potent in increasing the urinary output. The effect was comparable to that of the standard drug. Whereas, the petroleum ether extract was found to be least potent. Determination of urinary electrolyte concentration revealed that, was most effective in increasing urinary electrolyte concentration for all the three ions determined (Na^+, K^+ and Cl^-). The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the methanol extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect. Methanol extract of *Hibiscus tiliaceus* bark linn. was found to produce significant laxative activity, in a dose dependent manner up to 8 h of drug administration. The effect was found to be superior to that of the standard drug. Presence of phytoconstituents like terpenoids, saponins, flavonoids have been previously found to be

responsible for diuretic and laxative activities in plants [17, 21]. The presence of the said constituents in methanolic extract of *Hibiscus tiliaceus* barks linn. may be responsible for the observed diuretic and laxative activities.

Table no 3- Diuretic activities of various extracts of *Hibiscus tiliaceus* Linn. Bark

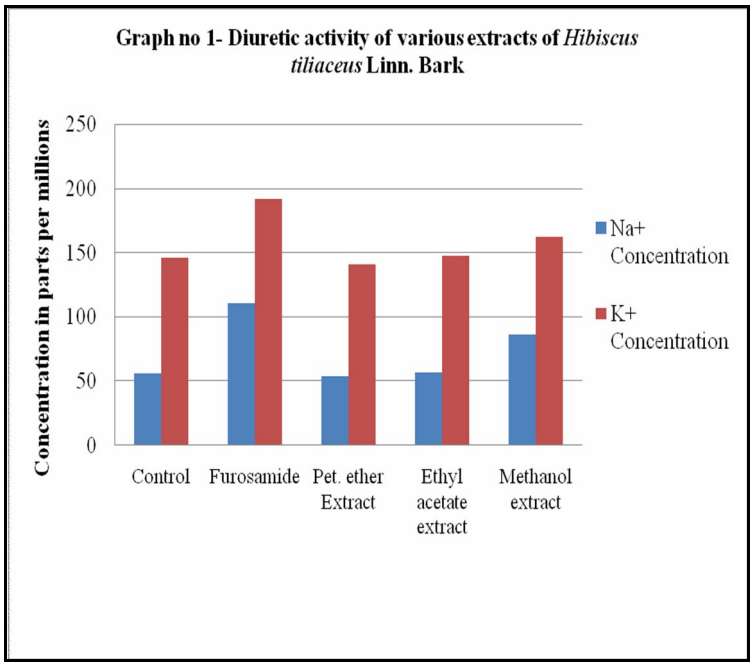
Treatment	Dose	Urine volume (ml)	Conc. of ions (ppm)			Na^+/K^+ ratio
			Na^+	K^+	Cl^-	
Control	25 ml/kg	2.90±0.12	56.18±2.43	146.08±1.49	97.50±3.22	0.38
Furosemide	10 mg/kg	10.62±0.26	110.55±2.28	192.07±1.30	127.00±4.37	0.57
Petroleum ether extract	200 mg/kg	3.03±0.29	54.17±3.91	141.07±3.50	96.95±3.96	0.38
	400 mg/kg	3.12±0.38	71.10±2.84	152.01±2.99	103.07±4.38	0.46
Ethyl acetate extract	200 mg/kg	4.64±0.48	57.10±4.27	147.50±4.02	92.83±4.32	0.39
	400 mg/kg	4.82±0.75	74.25±4.17	158.12±2.65	107.18±4.38	0.47
Methanol extract	200 mg/kg	7.96±0.65	86.02±2.88	161.95±2.49	124.05±5.26	0.53
	400 mg/kg	7.98±0.85	105.05±4.39	186.00±3.33	170.55±5.78	0.56

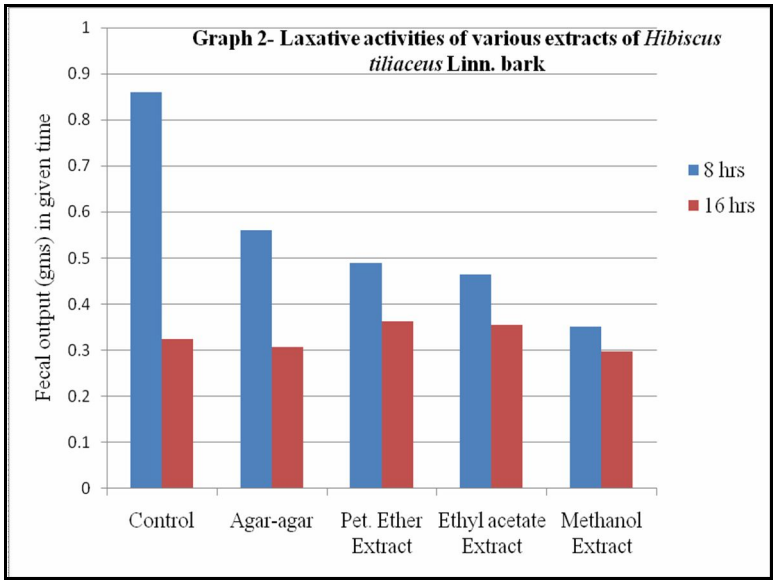
All values are express as mean ± SEM, n=6 (ANOVA followed by Dunnet t-test)

Table no 4- Laxative activities of various extracts of *Hibiscus tiliaceus Linn.* Bark

Treatment	Dose	Fecal output in gms	
		8 hours	16 hours
Control	-	0.86±1.05	0.325±1.043
Agar-Agar	300 mg/kg	0.056±0.087	0.308±0.075
Petroleum ether extract	200 mg/kg	0.490±0.092	0.362±1.020
	400 mg/kg	1.035±0.063	0.858±1.097
Ethyl acetate extract	200 mg/kg	0.465±0.091	0.355±2.029
	400 mg/kg	0.398±1.086	0.302±2.038
Methanol extract	200 mg/kg	0.352±1.480	0.298±1.685
	400 mg/kg	0.303±1.281	0.292±1.426

All values are express as mean ± SEM, n=6 (ANOVA followed by Dunnet t-test)





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