

**Diuretic and Laxative Activity of *Cassia Sophera* Linn,
a Prevalent Western Ghat Species**

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Summary

This study aims to pharmacological evaluation of the diuretic activity and acute toxicity study of different extract of *Cassia sophera* Linn. in laboratory rats. Water excretion rate, pH, density, conductivity, and content of Na⁺, K⁺ and Cl⁻, diuretic index, saluretic index were measured in the urine of rats subjected to hypersaline conditions. Methanol extract showed a significant diuretic effect compared with non-treated controls, with notable increases in the rates of water and sodium excretion. Ethanolic extract of *Cassia sophera* was found to produce significant laxative activity; in a dose dependent manner up to 8h of drug administration. Along with this result *cassia sophera* was found safe and failed to exhibit any toxicity within rodent model. The results justify the use of *Cassia sophera* as a diuretic agent in folk medicine of western ghat region.

Keywords: Diuretic, Laxative Ethnomedicine, *Cassia sophera*, acute toxicity, Saluretic Index

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Introduction

Cassia sophera Linn. (Casealpiniaceae), known as “Kasmard” in Ayurveda. It is reported to be used in homoeopathy decoction of plant is antidiuretic. The juice made from the paste of sandal wood and lime juice is considered specific for ring worm. An infusion of leaves is given with sugar in jaundice, and in sub acute stage in gonorrhoea. Internally it is used to as febrifuge in rheumatic and inflammatory fever¹. In folk literature it is used as expectorant in asthma, GIT disorder, rheumatic disorders² and remedy for various Skin ailments. Triterpenoids, Crysophanol, dianthraquinone, anthraquinone³ and various sterols moieties were previously isolated from *Cassia sophera* as well as others *cassia* species⁴.

Materials and Methods

Plant material

The Plant material (leaves) was collected from Pune district region of Maharashtra, India and was authenticated at Botanical Survey of India in Pune (MS) India and a voucher specimen has been preserved in research laboratory for future reference. The fresh leaves were washed to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of Extract and Isolation

The leaves were extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a brownish-black colored sticky residue (yield- 21.49 % w/w on dried material basis). Aqueous extract was obtained by cold maceration (28.89% w/w)

An anthraquinone moiety having similar analytical characters to that of emodin was isolated from ethanolic extract of *Cassia sophera* Linn. Extract was treated with 5 % Potassium hydroxide, hydrochloric acid systematically for better isolation and extracted several time with solvent ether⁵. Then treated extract is applied on TLC (Benzene: Methanol, 9:1) for detection that revealed one prominent fluorescent spot with other spots under the UV-225nm^{6,7}. After detection extract was concentrated to solid material and eluted by column chromatography using solvent system Benzene: methanol (9:1). Typical orange colored fraction was isolated and evaporated to dry followed by recrystallisation with toluene⁸.

Animals

Male Swiss albino mice weighing 20–25 g used for acute toxicity study and Wistar albino rats, 120-150 g were used to evaluate laxative effect and diuretic activity. Animals were housed in suitable environmental conditions and fed with standard diet and water *ad libitum*. Protocols for all the experimental work was approved by the Institutional Animals Ethics Committee.

Acute toxicity study

Acute toxicity of extract was determined using female albino mice. Animals were fasted 3 h prior to experiment as per OECD guideline 423 and were observed for next 48 h and up to 14 day of oral administration of extract⁹.

Diuretic activity

Diuretic activity was carried out according to reported method by Lipschitz *et al*, 1943 with slight modification^{10,11}. 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, 13}; other groups received doses of extract (200 and 400mg/kg) or extract fractions (200mg/kg each), in normal saline. Immediately after administration, the animals were placed in metabolic cages designed specially to separate urine and fecal material. Each cage contains two rats after placed in to metabolic cage supplement of food and water was withdrawn for next five hours. The volume of urine collected was measured at the end of 5 h. The urine collected was subjected to determine the concentration of Na⁺, K⁺ and Cl⁻ in the urine by flame photometer¹⁴ and titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator¹⁵.

Laxative activity

The test was performed according to Capasso *et. al.* using rats of either sex, fasted for 12 h before the experiment, but with water provided ad libitum¹⁶. 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-Sennoside tablet or doses of extract (200 mg/kg)¹⁷. Immediately after dosing, the animals were separately placed in cages suitable for collection of faeces¹⁸.

Statistical Analysis

All results are expressed as mean \pm standard error. The data was analyzed using two ways of analysis of difference of the means was evaluated by Student's 't' test.

Results

In acute toxicity study, it was found that the extract induced 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 observation. The ethanolic extract was found to produce significant increase in excretion of sodium, potassium and chloride ions at the higher dose tested (400 mg/kg p.o.).

In the evaluation of laxative activity (Table no 3), the Ethanolic extract was found to produce significant activity at the tested level of dose (200 mg/kg, p.o.). The effect was nearly similar to that of the standard tested.

Table No.1- Effect of Furosemide, aqueous, and Methanolic Extracts on Urinary volume, Sodium, Potassium and Chloride Concentration in normal rats.

Compound	Dose (mg/kg)	Concentration of ions (mEq/l)			Saluretic Index			Na ⁺ /K ⁺ Ratio
		Na	K	Cl	Na	K	Cl	
Control	--	24.60±0.07	8.89±0.16	13.48±0.02	--	--	--	2.76
Furosemide	10	51.16±3.01*	30.56±0.02*	32.23±1.91*	2.07	3.43	2.39	1.67
Aqueous Ext	400	38.81±0.73	20.26±0.08	25.27±0.69	1.57	2.27	1.87	1.91
Methanolic Ext	400	59.8±0.05*	39.03±0.13	41.61±0.08*	2.43	4.39	3.08	1.53

Dose: per mg / Kg of animal body weight

Values are expressed as mean ± S.E. (n = 6) after 24 hrs observation. * P<0.05 compared with vehicle control

Saluretic index = mEq problem group / mEq control group

Table No. 2- Effect of Furosemide, aqueous, and methanolic Extracts on urinary volume, pH, Conductivity and density in normal rats.

Compound	Dose(mg /kg)	Urine volume (ml)	pH	Conductivity	Density
Control	--	3.16 ± 0.10	6.14	14.95	0.9470
Furosemide	4mg	9.20 ± 0.32*	7.20	18.01	0.9636
Aqueous Ext	200 mg	3.22 ± 0.49	7.26	17.96	0.9861
	400 mg	6.61 ± 0.14*	7.04	17.90	0.9735
Methanolic Ext	200 mg	4.19 ± 1.24	7.40	18.02	0.9755
	400 mg	9.53 ± 0.69*	7.18	18.22	0.9978

Dose: Per Kg of Body weight.

Values are expressed as mean ± S.E. (n = 6) after 24 hrs observation. * P<0.05 compared with vehicle control

Table No.3 – Laxative effect of *Cassia sophera* Linn leaf extracts and isolated compound

Oral treatment	Mean defecation per animal	Frequency of wet feces
Vehicle control	1.9 ± 0.0224	1.9 ± 0.0224
(2% aqueous tween 80)	3.5 ± 0.0615*	3.1 ± 0.0731
Sennoside Tablet	2.8 ± 0.0833	2.7 ± 0.0820
(7.5mg/ kg)	3.2 ± 0.0543*	3.0 ± 0.0532
Aqueous extract	3.5 ± 0.0419*	3.2 ± 0.0591
Methanolic extract		
Isolated compound		

p value was calculated by comparing with control by student 't' test, there is a statistically significant difference between the input groups (*p=<0.001). Values are mean ± SEM.

Discussion

The present study revealed that, ethanolic extract of *Cassia sophera* significantly increased the urinary output as well as urinary electrolyte concentration at a higher dose tested (400mg/kg, p.o.). The aqueous extract significantly potentiates the activity. The ethanolic 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 extracts at the low concentration (200mg/Kg) was found to be least potent hence results were not mentioned. Determination of urinary electrolyte concentration revealed that, ethanolic extract was most effective in increasing urinary electrolyte concentration for all the three ions tested (Na⁺, K⁺, Cl⁻). Both the extracts caused similar type of increase of urinary electrolyte concentration, but to a lesser extent.

Aqueous extract although did not increase urinary electrolyte concentration significantly, it increased urinary output significantly. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extracts 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.

Ethanolic extract of *Cassia sophera* was found to produce significant laxative activity, in a dose dependent manner up to 8h of drug administration. The effect was found to be superior to that of the standard drug. The extracts and isolated compound potentiate the activity. Ethanolic extract was found to be most active than aqueous. Presence of phytoconstituents like terpenoids, flavonoids and anthraquinone and constituents found in phytochemical investigation of ethanolic extract and isolated emodin like constituent of *Cassia sophera* may be responsible for the observed diuretic and laxative activities. In summary, *Cassia sophera* produces a notable effect of the saluretic type, not due to an osmotic mechanism related to the salts contained within the plant, and with a diuretic profile different from the furesamide, due to the interesting potassium-saving effect showed 200 mg/kg methanol extract. Also, *Cassia sophera* was very safe, and within the rodent model, failed to exhibit any toxicity.

It is known that diuretics are the one of the part of management of diabetes. This be considered as a corroboration of Ayurvedic claims for the plant. Ayurveda has recommended leaves, seeds and roots for the diuresis. In the current project, plant has also been evaluated for laxative activity indicating that the leaves have pronounced diuretic and laxative effects.

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