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Research Article

EVALUATION OF *LEUCAS ASPERA* WHOLE PLANT EXTRACTS FOR DIURETIC AND LAXATIVE PROPERTY

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ABSTRACT

Objective: This study was undertaken to investigate diuretic and laxative potency of *Leucas aspera* whole plant.

Methods: The dried whole plant (leaves, stems, and flowers) material was subjected to extraction by continuous hot percolation method. In evaluation of diuretic activity male albino rats were used as the experimental animals. The first group of animals, serving as control, received normal saline (25 ml/kg, post-operative); the second group received furosemide (10 mg/kg, post-operative) in saline. Other groups received doses of extract (200-400 mg/kg) in normal saline. The parameters determined were total urine volume, the concentration of Na^+(sodium), K^+(potassium), and Cl^- (chloride) in the urine. Na^+ and K^+ concentrations were determined by flame photometer, and Cl^- concentration was estimated by titrimetric method. Laxative activity was also studied using male albino rats. 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, post-operative) in saline or doses of extract (200-400 mg/kg). After 8 h of drug treatment, the feces were collected and weighed.

Results: This study revealed that *L. aspera* whole plant extracts possesses significant diuretic and laxative activity in comparison with the standard drugs. The activity may be due to the chemical constituent present in the plant parts. The further studies may be taken up to isolate these active constituents.

Conclusion: *L. aspera* whole plant possesses diuretic and laxative property since it contains a variety of phytoconstituents.

Keywords: *Leucas aspera*, Diuretics activity, Lipchitz method, Laxative activity, Furosemide, Agar-agar.

INTRODUCTION

Leucas aspera belonging to the family labiate is well-known for its wide medicinal applications (Fig. 1). It is used traditionally as anti-inflammatory, stimulant, in the treatment of jaundice, cough, asthma, conjunctivitis, diabetes, malaria, skin diseases, snakebite, toothache, and wound healing. *L. aspera* is scientifically evaluated for anti-inflammatory activity, analgesic activity, cobra venom induced mortality in mice, anti-parasitic activity, antibacterial activity against *M. pyrogenes, V. aureus,* and *Escherichia coli.* It is toxic to the filarial vector mosquito, antinociceptive, antioxidant and cytotoxic activity [1-10].

The plants of *L. aspera* revealed the presence of triterpenoids, oleanolic acid, ursolic acid, and 3-sitosterol [11,12]. Aerial parts are reported to contain nicotine, sterols, two new alkaloids (α-sitosterol and β-sitosterol), reducing sugars (galactose), and glucoside [13]. This study was undertaken to evaluate the diuretic and laxative property of *L. aspera* whole plant extracts.

METHODS

Collection and authentication of plant material

L. aspera Family Labiate plants were collected from local areas around the Nashik, Maharashtra, India. The plant material was authenticated by Dr. R. H. Autade, Head Department of Biotechnology, College of Agricultural Biotechnology, Loni, Tal-Rahata, Ahmednagar Vide Letter No. CABT/9/2010 Voucher Specimen No. LUAS9. The herbarium is being maintained in the department for future references.

Preparation of extracts

In this study, the extracts for whole plant material (leaves, stems, and flowers) of *L. aspera* were obtained by continuous hot extraction method in soxhlet apparatus. 500 g of whole plant material powder was passed through sieve No. 60 and packed in soxhlet apparatus. Extraction was carried out using petroleum ether, ethyl acetate and ethanol as solvents in succession. Extracts were concentrated to dryness under reduced pressure and controlled temperature using flash evaporator. All the extracts were calculated for their extractive values (Table 1).

Preliminary phytochemical investigation

Preliminary phytochemical analysis was carried out to find out nature of chemical constituents present in the extracts. Qualitative chemical test was carried out for all the extracts. It revealed the 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.

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°C, 12:12 hr 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 with permission Letter Vide No. PRCOP/AEC/2015-16/11 dated 20.11.2015.

Acute toxicity study

The study was carried out as suggested by Ganapaty *et al.* [14]. Swiss albino mice of either sex weighing between 25 and 30 g were divided into different groups comprising six animals each. The control group received normal saline (2 ml/kg, post-operative). The other groups received 100, 200, 300, 600, 800, 1000, 2000, 3000 and 4000 mg/kg of the test extracts, respectively. Immediately after dosing, the animals were observed continuously for the first 4 hrs 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

Assessment of diuretic activity was performed using the method as described by Lipschitz *et. al,* [15,16]. In this method, male albino rats weighing between 120 and 150 g, deprived of food and water for 18 hrs before the experiment, were divided into eight groups of six rats in each. The first group of animals, serving as control, received normal saline (25 ml/kg, post-operative); the second group received furosemide (10 mg/kg, post-operative) in saline [17]. Other groups received doses of petroleum ether extract, ethyl acetate extract and ethanol extract (200-400 mg/kg) in normal saline. Immediately after administration, the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and feces, kept at 20±0.5°C. The volume of urine collected was measured at the end of 5 hrs. During this period, no food and water was made available to animals. The parameters taken were total urine volume, the concentration of Na^+, K^+ and Cl^- in the urine. Na^+ and K^+ concentrations were determined by flame photometer [18] and Cl^- concentration was estimated by titration [16,19,20] with silver nitrate solution (N/50) using three drops of 5% potassium chromate solution as indicator.

Fig. 1: *Leucas aspera* whole plant

Table 1: Extractive values

Group	Plant part	Extractive value (% w/w)		
		Petroleum ether extract	Ethyl acetate extract	Ethanol extract
Leucas aspera	Whole plant	13.06	8.52	9.14

Table 2: Preliminary phytochemical investigation of *Leucas aspera* whole plant extracts

Groups	Name of extract		
	Petroleum ether extract	Ethyl acetate extract	Ethanol extract
Alkaloids	++	--	++
Glycosides	--	++	--
Carbohydrates	++	--	--
Proteins	++	++	--
Saponins	--	++	++
Steroids	--	--	++
Tannins	++	--	++
Terpenoids	++	++	++
Flavonoids	--	++	--
Amino acids	++	++	++

"++": Indicates present, "--": Indicates absent

Laxative activity

The test was performed according to Capasso *et al.* [21] on rats of either sex, fasted for 12 h 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, post-operative) in saline and other groups received doses of petroleum ether extract, ethyl acetate extract and ethanol extract (200-400 mg/kg) in normal saline. Immediately after dosing, the animals were separately placed in cages suitable for collection of feces. After 8 h of drug administration, the feces were collected and weighed. Thereafter, food and water was given to all rats and fecal outputs were again weighed after a period of 16 hrs.

Statistical analysis

The values are expressed as mean±standard error mean from six animals in each group. Results were statistically analyzed using one-way ANOVA followed by Tukey–Kramer test for individual comparisons. *p<0.05, **p<0.01, ***p<0.001 considered to be statistically significant.

RESULTS

Preparation of extract

The extracts were obtained using different solvents as petroleum ether, ethyl acetate and ethanol; The percentage yield of these extracts was found to be 13.06% w/w, 8.52% w/w and 9.14% w/w, respectively.

Preliminary phytochemical screening

The preliminary phytochemical analysis of the extracts of *L. aspera* revealed the presence of alkaloids, tannins, carbohydrate, proteins, steroids, saponins, phenolic compound, and flavonoids.

Acute toxicity studies

Acute toxicity studies for all the extracts of *L. aspera* were conducted as per Organization for Economic Co-operation and Development guidelines 423 using albino swiss mice. The animals were observed for any changes continuously for the first 4 hrs and up to 24 hrs for

Table 3: Diuretic activities of various extracts of *Leucas aspera* whole plant

Groups	Dose	Urine volume (ml)	Concentration of ions (mmol/L)			Na^+/K^+ ratio
			Na^+	K^+	Cl^-	
Control	25 ml/kg	3.00±0.12	53.19±2.43	140.70±1.49	92.07±3.22	0.37
Furosemide	10 mg/kg	10.60±0.26	108.5±2.28	190.20±1.30	122.07±4.37	0.57
Petroleum ether extract	200 mg/kg	3.04±0.29	50.72±3.91	138.09±3.50	93.50±3.96	0.37
	400 mg/kg	3.10±0.38	68.60±2.84	147.60±2.99	101.20±4.38	0.46
Ethyl acetate extract	200 mg/kg	4.61±0.48	55.62±4.27	140.02±4.02	90.76±4.32	0.40
	400 mg/kg	4.90±0.75	72.70±4.27	153.60±4.02	103.40±4.32	0.48
Ethanol extract	200 mg/kg	7.98±0.65	90.06±2.88	168.22±2.49	126.02±5.26	0.53
	400 mg/kg	8.05±0.85	109.80±4.39	190.91±3.33	171.08±5.78	0.57

Values are expressed as mean±SEM, from six mice. Significant at **p<0.01 as compare to control using oneway ANOVA, followed by Tukey–Kramer test for individual comparisons. SEM: Standard error mean

mortality. There were no mortality and noticeable behavioral changes in all the groups tested. The extracts were found to be safe up to 2000 mg/kg body weight. Since no death was observed at 2000 mg/kg, it was thought that 2000 mg/kg was cutoff dose and 1/5th and 1/10th of this dose were selected for evaluation of diuretic and laxative activity.

Effect on urine volume

The order of activity of increase of urinary output was ethanol extract >ethyl acetate extract >petroleum ether extract. Furthermore, the order of activity of increase of urinary electrolyte excretion was found to be in the same pattern (Table 3).

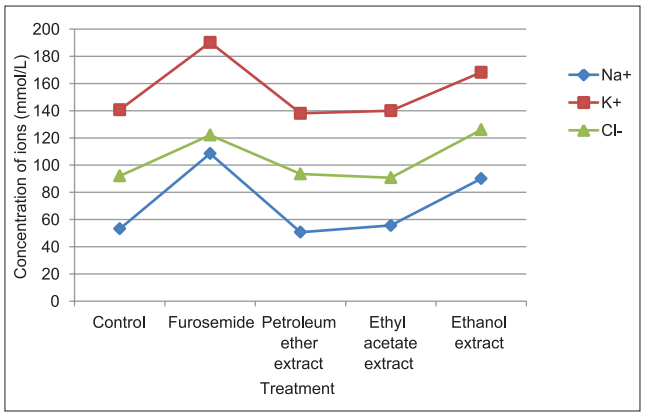

Fig. 2: Diuretic activites of various extracts of *Leucas aspera* whole plant

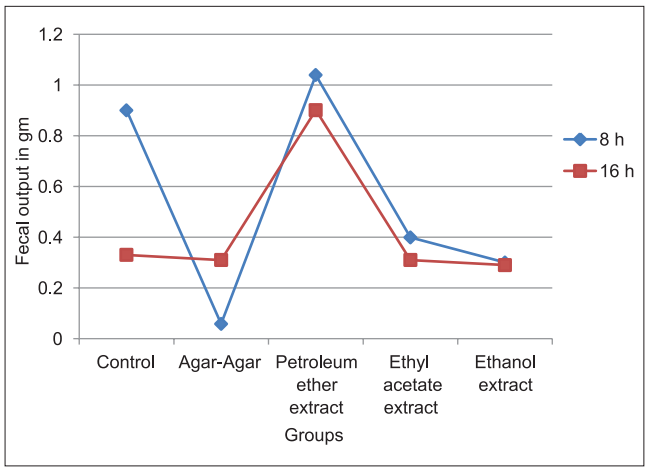

Fig. 3: Laxative activites of various extracts of *Leucas aspera* whole plant

Table 4: Laxative activities of various extracts of *Leucas aspera* whole plant

Groups	Dose	Fecal output in gm	
		8 h	16 h
Control	-	0.90±1.06	0.330±1.042
Agar-Agar	300 mg/kg	0.058±0.074	0.310±0.078
Petroleum ether extract	200 mg/kg	0.510±0.099	0.382±1.030
	400 mg/kg	1.039±0.042	0.901±1.090
Ethyl acetate extract	200 mg/kg	0.470±0.098	0.359±2.023
	400 mg/kg	0.399±1.097	0.310±2.032
Ethanol extract	200 mg/kg	0.350±1.489	0.296±1.542
	400 mg/kg	0.300±1.485	0.290±1.562

Values are expressed as mean±SEM, from six mice. Significant at **p<0.01 as compare to control using oneway ANOVA, followed by Tukey–Kramer test for individual comparisons. SEM: Standard error mean

Effect on urinary electrolyte

The ethanol extract was found to produce significant increase in excretion of sodium, potassium, and chloride ions at the higher dose tested. As shown in Table 3 test compound shows a significant increase in the excretion of all electrolytes when compared to the control group and less than the standard group. Sodium: Potassium ratio of test compound is more than that of standard group (Fig. 2).

Laxative activity

In the evaluation of laxative activity, all the extracts were found to produce significant dose dependent activity at both the tested level of doses (200 and 400 mg/kg, post-operative) (Fig. 3). The effect of extracts was superior to that of the standard drug used at 400 mg/kg, post-operative dose level (Table 4).

DISCUSSION

Diuretics are the agents extremely valuable in the treatment of mild to moderate hypertension and also in enhancing the effect of other antihypertensive agents. Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing volume overload and relieve orthopnea and paroxysmal nocturnal dyspnea conditions in congestive cardiac failure and acute left ventricular failure. This study revealed that extracts of *L. aspera* whole plant significantly increased the urinary output as well as urinary electrolyte concentration in a dose-dependent manner. The increase in the ratio of the concentration of excreted sodium and potassium ions indicates that the ethanol extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalemia side effect. Ethanol extract of *L. aspera* whole plant 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. The presence of phytoconstituents such as terpenoids, saponins, and flavonoids have been previously found to be responsible for diuretic and laxative activities in plants [22-26]. The presence of these constituents in ethanol extract of *L. aspera* whole plant may be responsible for the observed diuretic and laxative properties.

CONCLUSION

The traditional use of *L. aspera* plant in folk medicine as a diuretic and laxative has been justified by this research work. The whole plant showed significant diuretic and laxative property.

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