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ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.2, pp 149-152 , April-June 2009

Diuretic and laxative activity of ethanolic extract and its fractions of *Euphorbia Thymifolia* Linn.

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Abstract: Crude ethanolic extract and fractions of *Euphorbia Thymifolia linn.*(family: Euphorbiaceae) was investigated for diuretic and laxative activity in albino rats that was compared with standard drugs Furosemide (10mg/kg, p.o.) and Agar agar (300mg/kg, p.o.), respectively. The extract was found to produce significant diuretic as well as laxative activity in dose dependant manner. Fractions of the extract potentiated the observed activities. The activities may be contributed to the phytoconstituents present.

Keywords: *Euphorbia Thymifolia linn*; Acute toxicity study; Diuretic activity; Laxative activity.

Introduction

The extracts of many plants used in traditional medicine contain curative agents that is used in many modern medicines. As part of the quest for potentially valuable Plants of medicinal value. In Sanskrit Euphorbia thymifolia means "Laghu dudhika or Raktavindachada". The Euphorbia thymifolia widely used in the Ayurveda to cure many remedies. The leaves and seeds are given in worm case and in certain bowel affection of children. In India they are considered as stimulant and laxative also use in many disease due to presence of different constituents. The reference to medicinal plants as therapeutic agents in traditional there are many other species of euphorbia, though all possess widely diverse characteristics from older medicinal plants .In the dominions of the Mauritanian despot the plants abound as large succulent trees or brushes resembling cacti, but differing from them in having a milky juice, which exudes on the slightest puncture.neary all the Euphorbias are more or less poisonous, and all exude this acrid milky fluid when broken. Like their fellow of the same natural order, the Ricinus commanis, or castor oil plant, most of them have cathartic powders, through some astringent. In hot countries the milk of a certain pieces is employed as acautic, while another furnishes the natives with an"arrow poison" by simply dipping the weapon in the milky juice. Anthelmentic properties are possessed by the Euphorbia thymifolia Linn India. (1, 2)

Materials and Methods

Plant material

Euphorbia thymifolia Linn. is one of the important multipurpose species of desert and arid regions of the Indian subcontinent. It provides vegetative cover in dry, hot, sandy desert areas where little else grows and is an extremely hardy species. Altitude range: 300-1200 mm, Mean annual rainfall: 100-750 mm, Mean annual temperature: 25-31^{o}C, Soil, it prefers alkaline, sandy and gravelly soils, thriving on shallow, hard soils and rocky outcrops.The whole plant material was collected from Sangli region,and Authentification of Plant Material was done by Dr U. S. Yadav, Dept. of Botany, Willingdon College Sanglis, authenticated the plant material.

Preparation of Extract

The plant were extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black coloured sticky residue (yield- 11.6% w/w on dried material basis). A portion of dried ethanolic extract was suspended in water and fractionated successively with petroleum ether (40-60^{o}C), diethyl ether, ethyl acetate and n-butanol. All the fractions were dried by distillation under reduced pressure. Standard methods (3,4) were used for preliminary phytochemical screening of the ethanolic extract and its fractions to know the nature of phytoconstituents present in it (Table 1).

Table No 1 Phytochemical screening of Euphorbia thymifolia linn

Extract	Phytoconstituents present
Ethanolic extract	steroids, tannins, flavonoids, sugars
Pet-ether extract	steroids
Diethyl ether fraction	steroids, flavonoids
Ethyl acetate extract	tannins, flavonoids
n-butanol fraction	tannins, flavonoids

Animals

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. Animals were housed in standard environmental conditions and fed with standard rodent diet and water. The Institutional Animals Ethics Committee approved all the experimental protocols.

Acute toxicity study

The test was carried out as suggested by Ganapaty *et. al*. (5). 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, as well as, extract fractions up to 2000 mg/kg, in a similar manner. 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*, 1943 (6,7) 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 (8); other groups received doses of extract (200 and 400mg/kg) or extract fractions (200mg/kg each), in normal saline. Immediately after admistration, 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 h. 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 (9) and Cl^- concentration was estimated by titration (7, 10,11) 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*. (12) 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, p.o.) in saline (5) or doses of extract (200 and 400mg/kg) or extract fractions (200mg/kg each). Immediately after dosing, the animals were separately placed in cages suitable for collection of faeces. After 8h 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 h.

Statistical Analysis

All results are expressed as mean ±standard error. The data was analyzed using two ways of analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Dunnet's test.

Results

The results of the preliminary phytochemical screening of the ethanolic extract and its fractions are given in Table 1. 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 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.). The order of activity of increase of urinary output was diethyl ether fraction > n-butanol fraction > ethyl acetate fraction > petroleum ether fraction. The order of activity of increase of urinary electrolyte excretion was found to be n-butanol fraction > diethyl ether fraction > ethyl acetate fraction > petroleum ether fraction. In the evaluation of laxative activity, the ethanolic extract was found to produce significant dose dependant activity at both the tested level of doses (200 and 400 mg/kg, p.o.). The effect was superior to that of the standard tested at 400mg/kg, p.o. dose level. The order of activity for the ethanolic extract fractions was diethyl ether fraction > ethyl acetate fraction > n-butanol fraction > petroleum ether fraction.

Discussion

The present study revealed that, ethanolic extract of *Euphorbia Thymifolia linn.*significantly increased the urinary output as well as urinary electrolyte concentration at a higher dose tested (400mg/kg,p.o.). The fractionation of ethanolic extract potentiated the activity. The diethyl ether fraction 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 fraction was found to be least potent. Determination of

urinary electrolyte concentration revealed that, n-butanol fraction was most effective in increasing urinary electrolyte concentration for all the three ions tested (Na+, K+, Cl-). All fractions except petroleum ether fraction caused similar type of increase of urinary electrolyte concentration, but to a lesser extent. Petroleum ether fraction did not increase urinary electrolyte concentration. Ethyl acetate fraction although did not increase urinary output significantly, it increased urinary electrolyte concentration significantly. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the 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. Ethanolic extract of *Euphorbia Thymifolia linn.*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. Fractionation of the extract potentiates
the activity. Diethyl ether fraction was found to be most active and petroleum ether fraction was found to be least active. Presence of phytoconstituents like terpenoids, saponins, flavonoids have been previously found to be responsible for diuretic and laxative activities in plants (13-17). The presence of the said constituents in ethanolic extract and its fractions of *Euphorbia Thymifolia Linn.* may be responsible for the observed diuretic and laxative activities.

Table no 2- Diuretic activity of ethanolic extract and its fractions of Euphorbia thymifolia linn.

Treatment	Dose	Urine volume (ml)	Conc. of ions			Na/K ratio
			Na^+	K^+	Cl^-	
Control	25 ml /kg	2.85+0.14	52.12+2.86	141.72+2.68	87.85+3.88	0.38
Furosemide	10 mg/kg	10.5+0.25	108+4.13	187.55+1.25	130.61+4.26	0.58
Ethanolic extract	200 mg/kg	2.97+0.26	51.14+4.25	139.55+3.56	94.88+5.28	0.37
	400 mg/kg	5.1+0.45	67.85+2.36	147.50+2.65	104.95+1.59	0.46
Pet-ether fraction	200 mg/kg	4.18+0.68	54.30+3.45	143.35+1.15	85.70+4.32	0.37
Diethyl ether fraction	200 mg/kg	9.14+0.85	72.13+1.98	151.07+2.12	108.87+3.25	0.48
Ethyl acetate fraction	200 mg/kg	4.15+0.69	78.35+3.12	152.01+2.65	123.05+2.56	0.51
n-butanol fraction	200 mg/kg	6.22+0.75	101.65+2.58	182.55+2.85	164.83+3.54	0.55

Values are expressed as +- SE n=6 compared with vehicle control (ANOVA followed by Dunnet t-test)

Table no 3 Laxative activity of ethanolic extract and its fractions of Euphorbia thymifolia linn.

Treatment	Dose	Fecal out put	
		8 hours	8-16 hours
Control	-	0.87+0.014	0.313+1.025
Agar-Agar	300 mg/kg	1.087+-0.08	0.303+0.025
Ethanolic extract	200 mg/kg	0.37+0.389	0.200+1.256
	400 mg/kg	10285+0.079	0.306+2.154
Pet-ether fraction	200 mg/kg	0.278+0.048	0.295+6.124
Diethyl ether fraction	200 mg/kg	1.148+0.112	0.180+1.246
Ethyl acetate fraction	200 mg/kg	0.779+0.158	0.400+1.854
n-butanol fraction	200 mg/kg	0.553+0.049	0.107+0.145

Values are express as means +- SE n=6(ANOVA followed by Dunnet t-test)

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