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PHYTOCHEMICAL INVESTIGATION, DIURETIC ACTIVITY AND ANTHELMINTIC ACTIVITY OF *TRICHOSANTHES CUCUMERINA* LINN

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ABSTRACT

Keywords:

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Trichosanthes cucumerina linn is used in the traditional medicine as diuretic. In the present study, the diuretic activity of Petroleum ether, Chloroform, Methanol extract of *Trichosanthes cucumerina* linn was studied and the activity was compared with furosimide as standard. The methanolic extract exhibited significant diuretic activity as evidenced by increased total urine volume and the urine concentration of Na⁺, K⁺ and Cl⁻. The results thus support the *Trichosanthes cucumerina* linn use of as diuretic agent. *Trichosanthes cucumerina* Linn produced a potent anthelmintic activity against the pheretima posthuma when compared with reference standard Albendazole (P<0.001). The methanolic extract showed significant activity than pet ether and chloroform extracts. These results clearly indicate that *Trichosanthes cucumerina* linn is effective against free radical mediated diseases.

INTRODUCTION

Trichosanthes cucumerina linn (Cucurbitaceae) is a well known plant, the fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato. The fruit is usually consumed as a vegetable due to its good nutritional value. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. Belonging to family Cucurbitaceae is an annual climber and widely distributed in southern parts of India. Traditionally, decoction of the stem, leaves and aerial parts were used in the treatment of diabetes and inflammatory diseases. The major active constituents of the drug are triterpenoid saponins viz., cucurbitacins. It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxic, anti-inflammatory, larvicidal effects^{1,2,3,4,5,6}. A perusal of literature revealed that its diuretic effects remain to be studied. Here in we report the diuretic effect of the *Trichosanthes cucumerina* linn in Petroleum ether, Chloroform, Methanol extract of in albino rats.

MATERIALS AND METHODS

The plant materials were collected from Madurai District, Tamilnadu, India and authenticated by Madurai during May 2008. It was authenticated by Dr. Stephen, Department of Botany, The American College, Madurai-2. The voucher specimen was kept at Department of Pharmacognosy in our laboratory for future reference.

Preparation of the Extract

About 500gms of dried coarse powder was soaked with petroleum ether (3000ml) for two days. After this, materials were extracted with petroleum ether (40°C – 60°C) by continuous hot percolation method for 72 hrs. The petroleum ether extract were filtered and concentrated under reduced pressure. A green-black residue was obtained (25gms). The marc left after the petroleum ether extraction were dried and extracted with chloroform (3000ml) for 72hrs. The chloroform extract were also filtered and concentrated under reduced pressure. A dark black residue was obtained (20gms). Then marc left after the chloroform extraction were dried and extracted with methanol

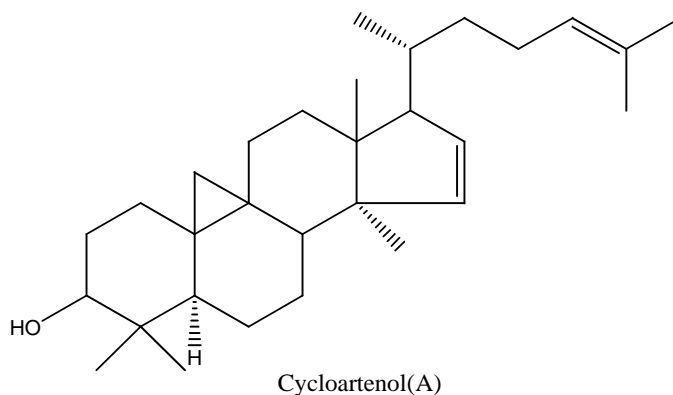
(3000ml) for 72hrs. The methanolic extract were also filtered and concentrated under reduced pressure. A darkgreen residue was obtained (15gms).Table 1. TLC studies were carried out using Hexane: Ethylacetate using UV lamp. R_f values were calculated at different spots. The preliminary phytochemical analysis^{7,8,9,10} were carried out to find out the phyto constituents present in the crude extracts Table 2.

Preparation of column chromatography

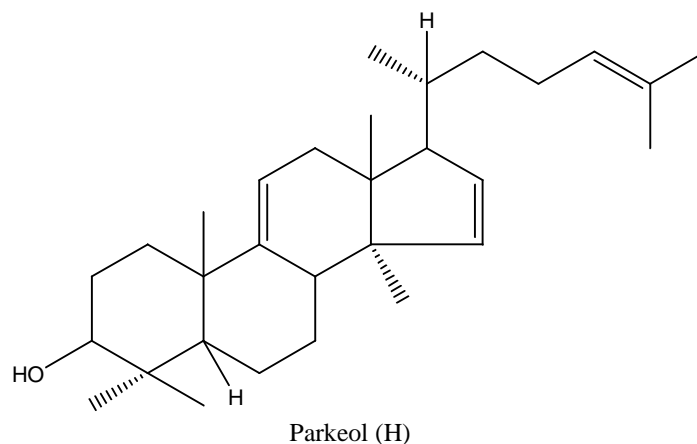
Chloroform extract obtained from the aerial parts of *Trichosanthes cucumerina* linn was adsorbed on silica gel (60-120 mesh) for column chromatography. The slurry was air dried to remove any adsorbed moisture on surface and loaded on the top of the column of silica gel packed with disappearance or appearance of the existing new spot, visualized on TLC^{11,12}. Various compounds isolated from the extract are listed below along with their spectral data.

Spectral Analysis^{13,14,15}

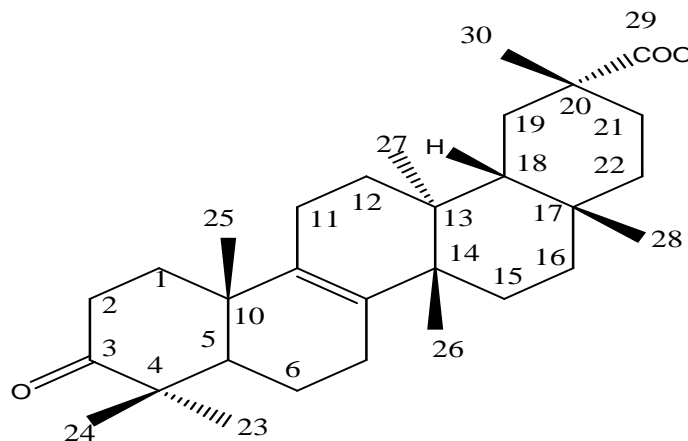
Compound A



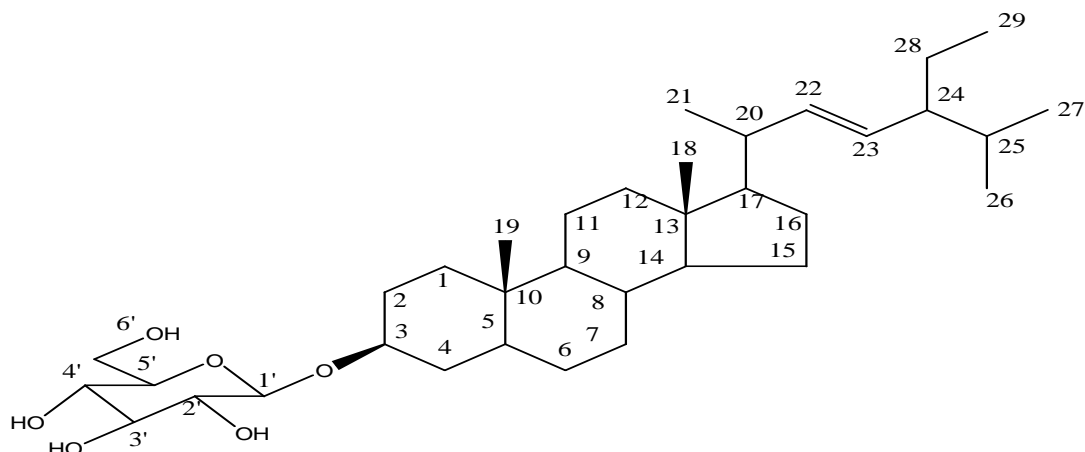
The compound showed green colour which is semisolid in state. The melting point was 180-220°C which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using ethyl acetate : methanol (9:1) having the UV absorbance of 270 to nm. The R_f value was found to be 0.6932. The IR data showed the frequency at 3853,3430,2925,1713,1443,1220,1091,767 cm^{-1} and $^1\text{HNMR}$ showed the signals at 0.993,1.470,1.525,1.895,2.266,3.179,5.462,7.202 δppm .

Compound B

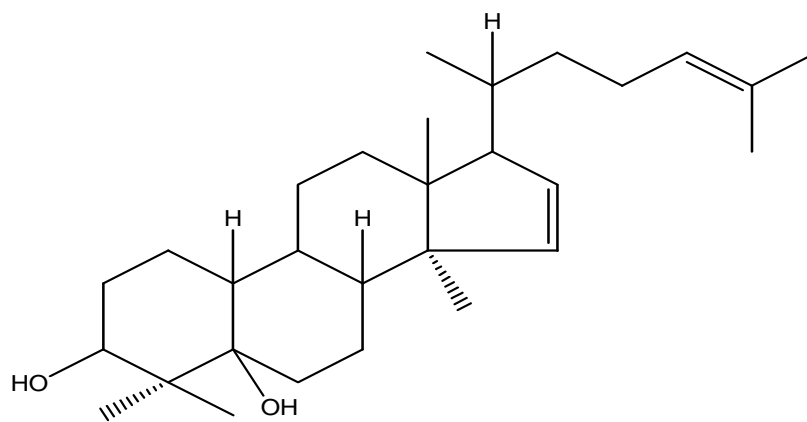
The compound showed greenish violet colour which is semisolid in state. The melting point was 190-230°C which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using ethyl acetate : hexane (4:6) having the UV absorbance of 260 nm. The R_f value was found to be 0.4210. The IR data showed the frequency at 3946, 3423, 2865, 1710, 1453, 1219, 666 cm⁻¹ and ¹H NMR showed the signals at 0.916, 1.599, 2.018, 2.277, 3.269, 3.743 δppm and ¹³C NMR showed the value at 12.08, 12.98, 14.29, 19.37, 22.63, 25.62, 29.10, 32.62, 36.64, 39.36, 127.78, 130.25.

Compound C

The compound showed yellowish brown in colour which is semisolid in state. The melting point was 140-160°C which is soluble in absolute alcohol and double chloroform. The TLC showed the single spot using hexane : ethanol (9.5:0.5), R_f value was found to be 0.6931 having the UV absorbance of 280nm. The IR data showed the frequency at 3931, 3414, 2923, 1715, 1445, 1222, 764 cm⁻¹.

Compound D

The compound showed yellowish brown colour which is semisolid in state. The melting point was 120-140°C which is soluble in chloroform. The TLC showed a single spot using chloroform : ethanol (8:2), R_f value was 0.4528 having the UV absorbance of 265 nm. The IR data showed the frequency at 3958, 3420, 3094, 2920, 2467, 1643, 1453, 1219, 1092 and 770 cm⁻¹.

Compound E

Litsomentol (G)

The compound showed yellowish brown in colour which is semisolid in state. The melting point was 145-155°C which is soluble in absolute alcohol and chloroform. The TLC showed a single spot using chloroform : ethanol (9:1) having the UV absorbance of 280 nm. The R_f value was found to be 0.5579. The IR data showed the frequency at 3369, 2918, 1734, 1463, 1260, 1022, 801 cm⁻¹ and ¹HNMR showed the signals at 0.763, 1.229, 1.529, 3.212, 3.981, 5.294, 7.196 δppm.

Diuretic Activity^{16,17,18}

Male rats (wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz et al^{7,8} was employed for the assessment of diuretic activity. The experimental protocols have been approved by the Institutional Animal Ethical Committee. Four groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received normal saline(10ml/Kg,p.o.); the second group received furosemide (25mg/Kg,i.p.) in saline; the third, fourth, fifth groups received the Pet ether, Chloroform, Methanol extract at the doses of 100 mg/Kg, respectively, 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 room temperature of 25± 0.5°C through out the experiment. The urine was collected in measuring cylinders up to 3 hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na⁺, K⁺, and Cl⁻ in the urine. Na⁺, K⁺ concentrations were measured by Flame photometry⁹ and Cl⁻ concentration was estimated by titration¹⁰ with silver nitrate solution(N/50)using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20mg/Kg of furosemide per kg body weight in series of supportive experiments. Results are reported as mean ± SD, the test of significance p<0.01 was stastically Table 3.

Anthelmintic activity^{19,20,21,22,23,24}

The anthelmintic activity was evaluated on earth worms by the method of Mathew et.al and Dash et.al was followed. The assay was performed on adult Indian earth worm due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings. Equal sized (8 ±1cm) worms were selected for the study. The worms were washed with normal saline to remove all the extraneous matter. Eight groups of approximately equal size Indian earth worms consisting of six earth worms in each group were released into 50ml of desired formulation. Each group was treated with one of the following. The first group served as control (received 1% gum acacia in normal saline),

second group served as standard (received Albendazole 10mg/ml), third, fourth and fifth groups were (received pet ether, chloroform and methanolic extracts of 10mg, 25mg, in 1% gum acacia in normal saline respectively). The above prescribed dose were prepared and poured into respective labeled Petri plates and the volume was made up to 50ml with normal saline. The standard Albendazole (10mg/ml) and the test pet ether, chloroform and methanolic extracts (10mg, 25mg /ml) were evaluated for anthelmintic activity.

Treatment protocol

Group I

A control group received orally 10ml/kg body weight of normal saline.

Group II

The standard group received orally 10mg/ml of Albendazole

Group III

The treatment group received orally 10mg/ml of petroleum ether extract of *Trichosanthes cucumerina* Linn.

Group IV

The treatment group received orally 25mg/ml of petroleum ether extract of *Trichosanthes cucumerina* Linn.

Group V

The treatment group received orally 10mg/ml of chloroform extract of *Trichosanthes cucumerina* Linn.

Group VI

The treatment group received orally 25mg/ml of chloroform extract of *Trichosanthes cucumerina* Linn.

Group VII

The treatment group received orally 10mg/ml of methanolic extract of *Trichosanthes cucumerina* Linn.

Group VIII

The treatment group received orally 25mg/ml of methanolic extract of *Trichosanthes cucumerina*Linn.

*Statistical analysis*²⁵

Diuretic activity All the results are expressed as mean \pm standard error. The data was analyzed statistically using ANOVA followed by student 't' test at a probability level of $P < 0.01$.

Anthelmintic activity

Results are expressed as mean \pm S.E.M were evaluated by one way ANOVA followed by Newman Kew's multiple range tests. Values of $P < 0.001$ were considered statistically significant.

RESULTS

Diuretic activity

The preliminary phyto chemical analysis showed the presence of flavanoids, saponins, carbohydrates, terpenoids and alkaloids in all the extracts.. The methanol extract 100mg/Kg p.o. showed significant increase in excretion of sodium, potassium and chloride ions in the urine in a dose dependent manner. The obtained effect was comparable to that of furosemide (25mg/Kg). Increase in urine output a sufficient index for assessing the diuretic effect through estimating the urinary concentration of Ion like Na^+ , K^+ , Cl^- etc., may reveal in specific the Ion responsible for the diuretic activity. The results reveals that electrolyte excretions and diuretic activity of various extract of *Trichosanthes cucumerina* linn treatment possess significant diuretic activity at $P < 0.01$.

Anthelmintic activity

Trichosanthes cucumerina Linn produced a potent anthelmintic activity against the pheretima posthuma when compared with reference standard Albendazole ($P < 0.001$). The methanolic extract showed significant activity than pet ether and chloroform extracts. This activity was Concentration dependent. The Potency was found to be inversely proportional to the time taken for paralysis and death of the worms.

DISCUSSION

Diuretics relive pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure²⁶. Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ethanol, aqueous

and chloroform extract may produce diuretic effect by increasing the excretion of Sodium, Potassium and Chloride. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles²⁷. The regulation of Sodium, Potassium balance is also intimately related to renal control of acid-base balance. The Potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended²⁸. In present study chloroform and alcohol extracts showed elevated levels of Potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavonoids, saponins and terpenoids are known to be responsible for diuretic activity^{29,30,31}. Results of present investigation showed that alcohol is most effective in increasing urinary electrolyte concentration of all the ions i.e Sodium, Potassium and Chloride followed by chloroform and pet ether extracts while other extracts did not show significant increase in urinary electrolyte concentration.

TABLE 1 Extractive Values

S.NO	SOLVENT	COLOUR OF THE EXTRACT	PERCENTAGE OF YIELD
1	Petroleum ether	Dark Brown	3.7422
2	Chloroform	Dark Brown	2.5124
3	Methanol	Dark Brown	2.4620

TABLE 2 Preliminary phytochemical screening of *Trichosanthes cucumerina linn*

S.NO.	CONSTITUENTS	PET.ETHER EXTRACT	CHLOROFORM EXTRACT	METHANOL EXTRACT
1	CARBOHYDRATE	-	+	+
2	GLYCOSIDES	+	+	+
3	ALKALOIDS	-	+	-
4	FLAVANOIDS	-	+	+
5	FLAVONES	-	-	+
6	STEROIDS	+	+	-
7	PROTEINS&AMINOACIDS	-	+	+
8	TANNINS	+	+	-
9	SAPONINS	-	+	+
10	COUMARINS	-	-	+

+ → indicates positive test results, - → indicates negative test results

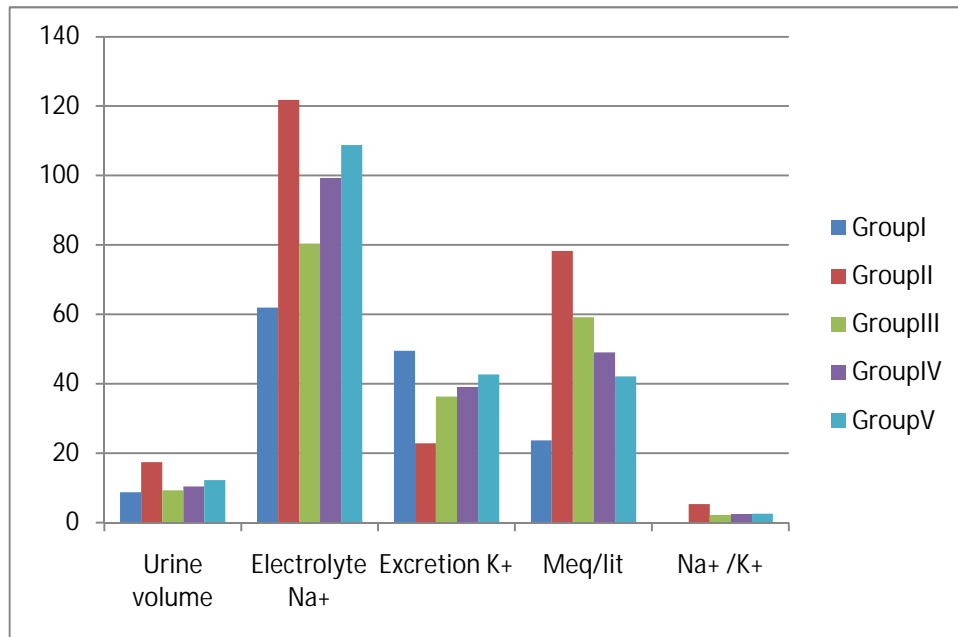
TABLE 3 Electrolyte excretion and Diuretic activity of various extracts of *Trichosanthes cucumerina* Linn.

Groups	Treatment	Dose	Urine volume	Electrolyte Na ⁺	Excretion K ⁺	Meq/lit	Na ⁺ /K ⁺
Group I	Normal Control	10ml/kg	8.8 ± 2.30	61.93 ± 3.14	49.50 ± 2.30	23.70±2.31	0.012
Group II	Standard Control	25mg/kg	17.4 ± 3.40	121.80 ± 5.60	22.86 ± 1.40	78.26±3.96	5.32
Group III	Treatment Control	100mg/kg Pet.ether	9.3 ± 2.16	80.40 ± 3.90	36.30 ± 1.95	59.21±2.60	2.21
Group IV	Treatment Control	100mg/kg Chloroform extract	10.4 ± 2.40	99.26 ± 4.10	39.10 ± 2.05	49.05±1.95	2.53
Group V	Treatment Control	100mg/kg Methanol extract	12.3 ± 2.90	108.80 ± 4.46	42.66 ± 2.15	42.11± 1.30	2.55

Values are expressed as Mean ± SEM

Values are found by using one way ANOVA followed by Neuman Keul's multiple range test.

Values were significantly different from normal control at P < 0.01



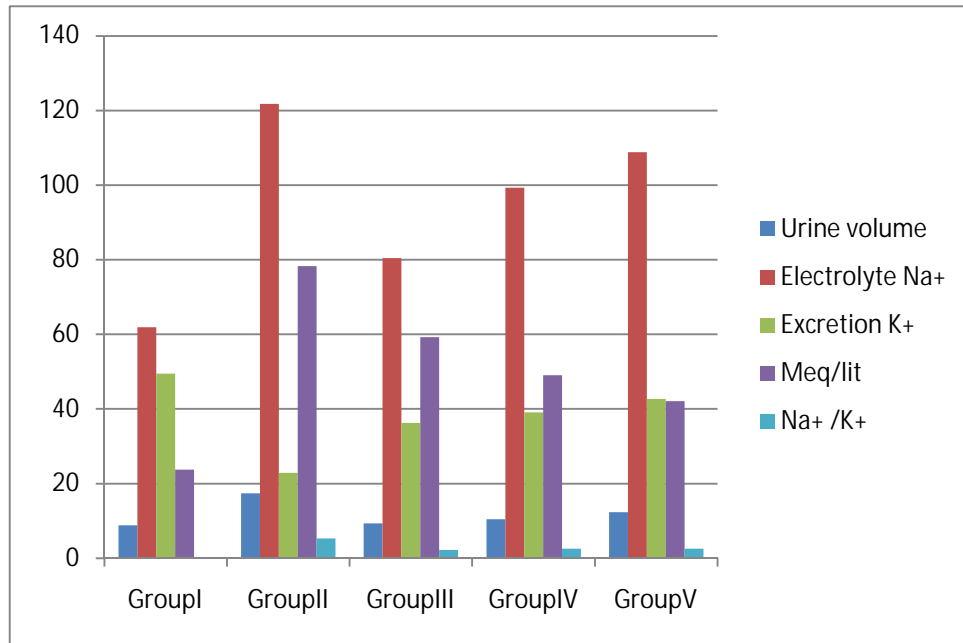
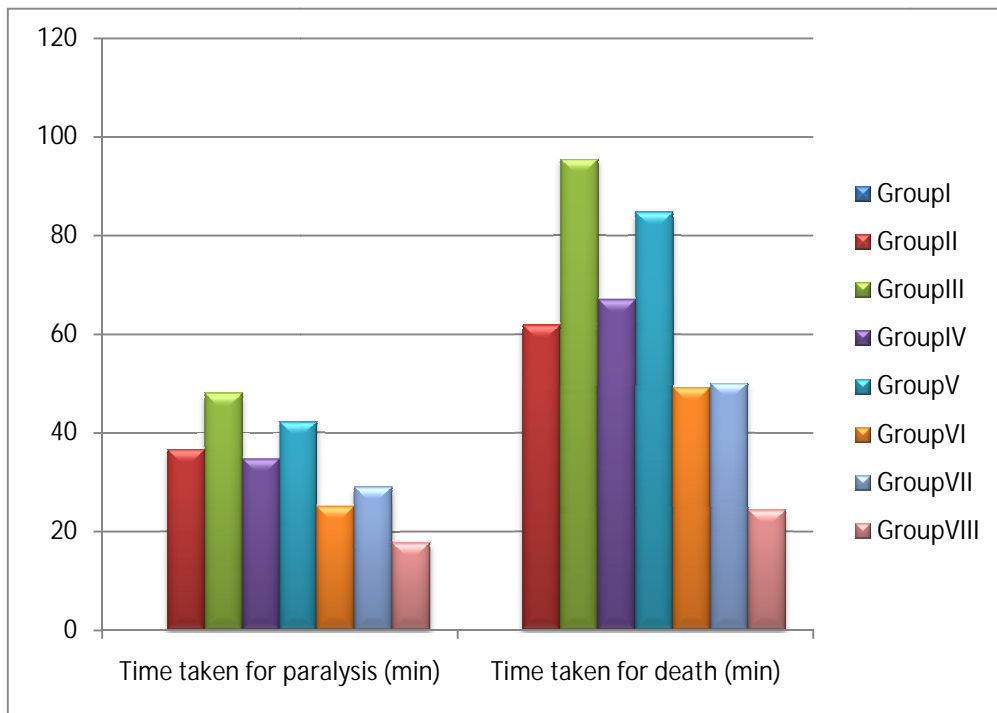
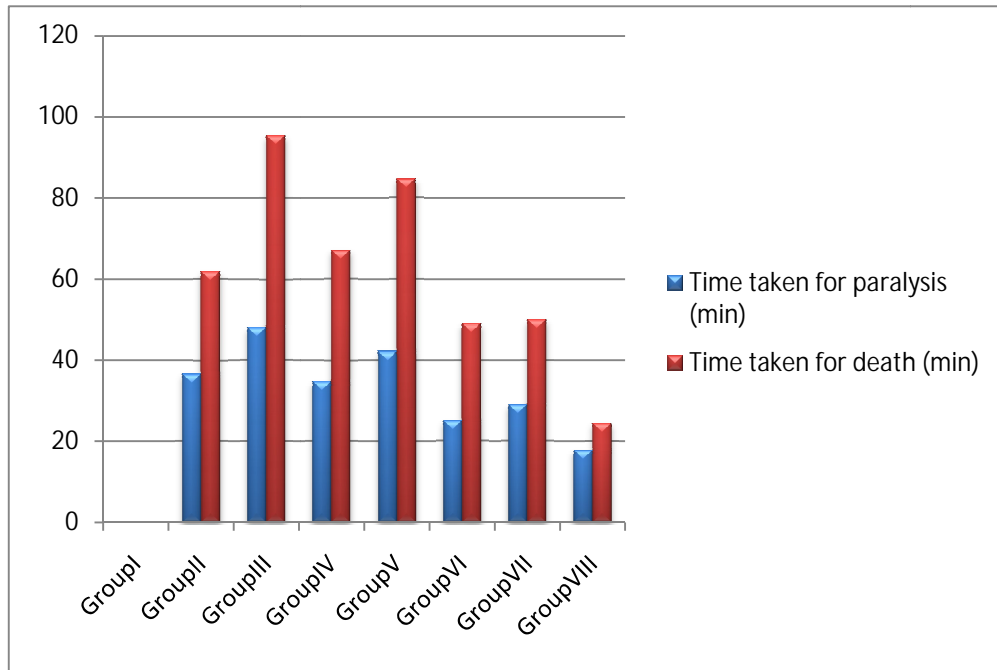


TABLE 4 Anthelmintic activity of various extracts *Trichosanthes cucumerina* Linn

Groups	Treatment	Conc. used (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
I	Control	1% Acacia in Normal Saline	-	-
II	Albendazole	10	36.60±0.64	61.92±1.66
III	Pet.Ether	10	48.09±0.45	75.46±1.13
IV	Pet.Ether	25	34.75±0.41	67.07±2.05
VI	Chloroform extract	10	46.16±0.46	70.73±2.08
VII	Chloroform extract	25	30.04±0.47	50.11±1.40
VII	Methanol extract	10	40.01±0.51	63.06±1.19
VIII	Methanol extract	25	23.75±0.77	38.44±1.48

Values are expressed as mean ± S.E.M (n=6) Control worms were alive up to 24 hours of the experiment.



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