

Research Article

Phytochemical Constituents and Diuretic Activity of Leaf Extracts of *Andrographis Echioides*-L-Nees

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ABSTRACT

Andrographis echioides *Andrographis echioides* is used in the traditional medicine as diuretic. In the present study, the diuretic activity of Petroleum ether, Chloroform extract of *Andrographis echioides* was studied and the activity was compared with furosimide as standard. The Chloroform 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 use of as diuretic agent. When compared with reference standard the value is P<0.01. These results clearly indicate that *Andrographis echioides* is effective against free radical mediated diseases.

Keywords: Diuretic activity, *Andrographis echioides*, Flavanoids.

INTRODUCTION

Andrographis echioides (L) Nees, Acanthaceae - Acanthus Family. *Andrographis echioides* have been reported for their analgesic, anti-inflammatory and antipyretic activity, hepato-protective activity, anti-oxidant and anti-microbial activities. Flavones and flavanoids are the responsible active constituents for mentioned activities¹⁻⁵. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. A perusal of literature revealed that its diuretic effects remain to be studied. Here in we report the diuretic effect of the extract of *Andrographis echioides* in Petroleum ether, Chloroform extract of in albino rats.

MATERIALS AND METHODS

Plant material

The plant materials were collected from Madurai District, Tamilnadu, India and authenticated by Madurai during May 2009. It was authenticated by Dr. Stephen, Department of Botany, The American College, Madurai-2. The voucher specimen

was kept at Dept .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). Table 1. TLC studies were carried out using Hexane: Ethylacetate using UV lamp. R_f values were calculated at different spots.

Preliminary phytochemical screening

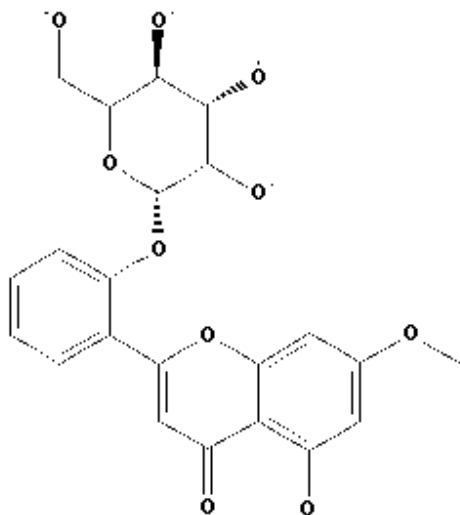
The preliminary phytochemical analysis⁶⁻⁹ were carried out to find out the phyto constituents present in the crude extracts Table 2

Acute toxicity studies¹⁰⁻¹²

Animals were starved over night and divided into 5 groups (n=6). They were fed orally with the leaf extracts of *Andrographis echioides* in increasing doses levels of upto 2000mg/Kg body weight.

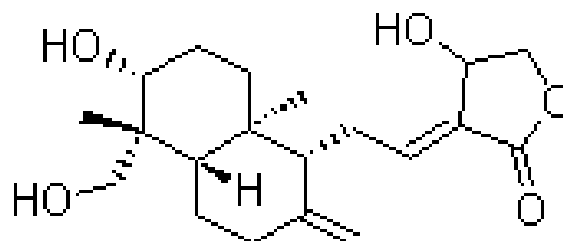
Preparation of column chromatography

Chloroform extract obtained from the aerial parts of *Andrographis echioides* 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^{13,14}. Various compounds isolated from the extract are listed below along with their spectral data.

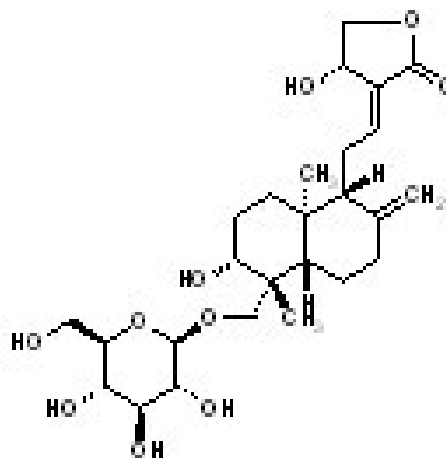
Spectral Analysis¹⁵⁻¹⁷**Compound A**

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.

Compound B

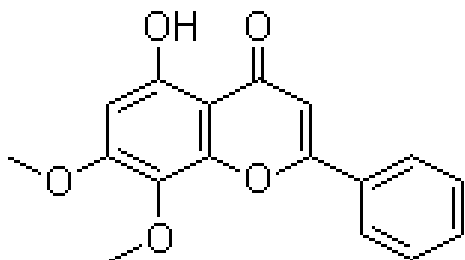
The compound showed yellowish brown colour which is semisolid in state. The melting point was 120-140°C which is soluble in chloroform. The TLC showed a single spot using chloroform : ethanol (8:2), R_f value was 0.4528 having the UV absorbance of 265 nm. The IR data showed the frequency at 3958, 3420,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

Compound C

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 δ ppm

Compound D



The compound showed green colour which is semisolid in state. The melting point was 180-220 $^{\circ}\text{C}$ 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 ^1H NMR showed the signals at 0.993,1.470,1.525,1.895,2.266,3.179,5.462, 7.202 δ ppm.

Diuretic Activity¹⁸⁻²⁰

Male rats (wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz et al 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 $25 \pm 0.5^{\circ}\text{C}$ 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 photometry and Cl^- concentration was estimated by titration 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 \pm SD, the test of significance $p < 0.01$ was stastically Table 3.

Statistical analysis²¹

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 $P < 0.01$.

RESULTS

Acute toxicity studies

The behaviour of the animal was carefully monitored for 1 day.

1. CNS activity : Tremors, Convulsions
2. CVS activity : Palpitation, increase in pulse rate.
3. Respiratory tract activity : Bronchial constriction, difficult in breathing
4. Reflex action : Loss of writhing reflex.

The number of deaths, if any were recorded after 24 and 72 h.

Preliminary phytochemical screening

Preliminary phytochemical screening of the plant extract showed the presence of flavanoids, tannins, aminoacids, glycosides and carbohydrates.

Diuretic activity

The preliminary phyto chemical analysis showed the presence of flavanoids,

saponins, carbohydrates, terpenoids and alkaloids in all the extracts. The chloroform extract 200mg/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 (20mg/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 *Andrographis echiooides* treatment possess significant diuretic activity at P < 0.01.

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 pressure²². 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 muscles. 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 hypokalemia²³. For this reason, generally potassium-sparing diuretics are recommended²⁴. 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 activity²⁵⁻²⁷. 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

Table 2: Preliminary phytochemical screening of *Andrographis echiooides*

S. No.	CONSTITUENTS	PET.ETHER EXTRACT	CHLOROFORM EXTRACT
1	CARBOHYDRATE	-	+
2	GLYCOSIDES	+	+
3	ALKALOIDS	-	+
4	FLAVANOIDS	+	+
5	FLAVONES	+	+
6	STEROIDS	+	+
7	PROTEINS& AMINO ACIDS	-	+
8	TANNIINS	+	+
9	SAPONINS	-	+
10	COUMARINS	-	-

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

Table 3: Electrolyte Excretion and Diuretic Activity of Various Extracts of *Andrographis Echioides*

Groups	Treatment	Dose	Volume of urine (ml)	Electrolyte excretion		
				Na ⁺ m.eq/L	K ⁺ m.eq/L	Cl ⁻ m.eq/L
Group-I	Normal control	10ml/kg	7.6 ±0.60	63.85 ±3.93	12.15 ±0.68	52.98 ±2.95
Group-II	Positive control. frusemide	20mg/kg	14.2 ±1.06	126.26 ±6.24	17.36 ±1.10	110.6 ±4.10
Group-III	Treatment control. Pet. Ether extract	200mg/kg	8.8 ±0.78	85.93 ±4.26	13.76 ±0.88	73.33 ±3.68
Group-IV	Treatment control. chloroform extract	200mg/kg	12.50 ±0.98	103.45 ±5.50	14.96 ±0.96	94.9 ±3.95

Values are expressed as Mean ± SEM

Values were found out by using one way ANOVA followed by Newman Keul's multiple range tests

Values were significantly different from normal control at P<0.01

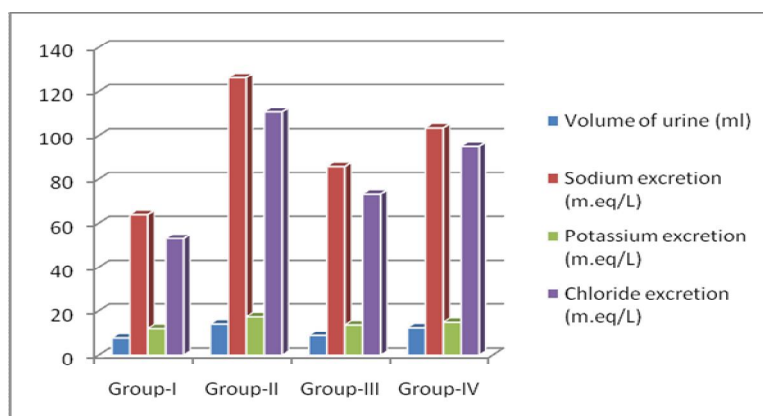


CHART 1

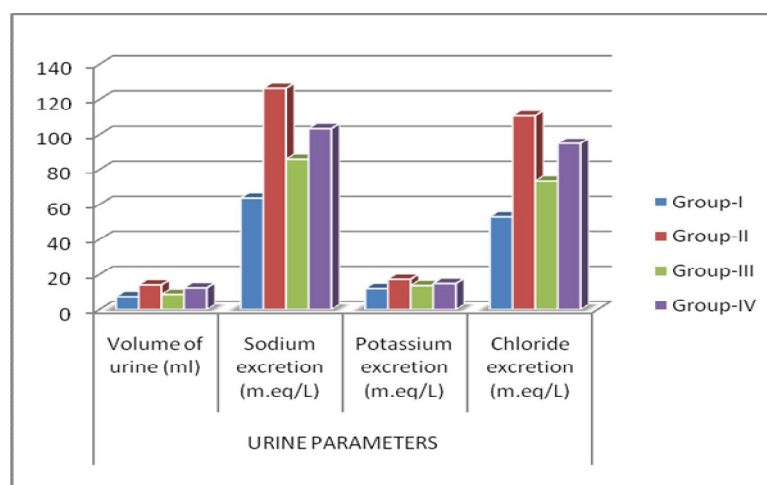


CHART 2

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