

Evaluation for Diuretic Activity of *Abutilon indicum* and *Amaranthus spinus* Leaves Extracts

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Abstract

The diuretic activity of *Abutilon indicum* and *Amaranthus spinus* leaves was studied. The fractions of ethanol and methanol showed significant increase in the urine elimination, while ethyl acetate fraction does not show diuretic activity. All the groups were compared to the control group; frusemide was used as reference standard drug in this screening activity. The phytochemical screening study showed the presence of flavonoids, saponins, terpenoids, and steroid content in both the plant extracts. The diuretic effect in all the extracts may be due to phytochemicals present in the fractions except benzene fraction of *A. indicum*.

Keywords: *A. indicum*, titremetric, ethyl acetate, traditional, frusemide

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INTRODUCTION

Various parts of traditional plants *Abutilon indicum* and *Amaranthus spinus* are used in treating various ailments of humans. The *Abutilon indicum* belongs to family malvaceae. Roots of this plants are useful in treating uterine haemorrhagic discharges, seeds are used in the treatment of bronchitis, piles and gonorrhoea, leaves are used in lumbago, toothache, and different kinds of inflammation, and bark of this plant is used as an emollient [1].

It is erect velvety tomentose under shrub, stem round, frequently tinged with purple (Figure 1). Leaves ovate to orbicular-cordate, flowers solitary on jointed peduncles, orange-yellow or yellow, capsules hispid, hardly larger than the calyx, erect, seeds 3–5, reniform tubercled or minutely stellate-hairy, black or dark brown. Whole plant is used as a febrifuge, has anthelmintic and anti-inflammatory properties and its use is also reported in urinary tract problems, and as bark astringent and diuretic [2].

Amaranthus spinus is another traditionally used medicinal plant and belongs to family *Amaranthaceae* (Figure 2). It is commonly known as *kate wali chaulai* in Hindi, used as vegetable and cultivated throughout India and other tropical countries. Its leaves are used to cure jaundice, as anti-oxidant, the roots are

used as expectorant, lower menstrual flow, and leprosy [3]. The juice of whole plant is used to prevent inflammation, as laxative, diuretic, digestible, in diabetes, piles and gonorrhoea [4].



Fig. 1: Leaves Twig of *Abutilon indicum*.



Fig. 2: Whole Plant of *Amaranthus spinus*.

Amaranthus spinus is an erect glabrous herb with hard stem; the herb is often reddish with

many grooved branches with spines. Leaves are 3.2–7.5 cm long, 1.3 to 3.8 cm wide, ovate, obtuse, spinous apiculate, glabrous, petioles 2, 6–3 cm long. Flowers numerous, sessile, in dense axillary clusters and in terminal interrupted spikes, bracteaules, linear, bristle pointed, usually longer than the sepals [5].

Although a number of studies have been performed on these well-known traditional plants, diuretic activity, wound healing, analgesic, anti-oxidant and antipyretic activity [6–9]. But no work reported on its diuretic effect on fractions of leaves. Hence this is an attempt to investigate the diuretic effect of these plants.

MATERIALS AND METHODS

The leaves of *Abutilum indicum* and *Amaranthus spinosus* were collected in the month of Aug–Sep from the local area and surroundings of Shivamoga, Karnataka. The authentication was done by a botanist from Sahyadri Science College, Shimoga. A voucher specimen (No.NCP/PCG/06/2016) has been deposited at the museum of National College of Pharmacy, Shivamogga.

Preparation of Extracts

The shade dried, leaves were coarse powdered and packed in to soxhlet column and extracted with ethanol (70%). The extract was fractioned with ethyl acetate and methanol for *Amaranthus spinosus*, ethyl acetate and methanol for *Abutilum indicum* leaves, and the extracts were concentrated under reduced pressure (bath temp 50°C) and the yield of the extract was calculated. The dried extract was stored in airtight container in refrigerator below 10°C. The solution of methanol, ethanol and ethyl acetate were prepared using 1% gum acacia and methanol solution was prepared using distilled water and used for the diuretic activity.

Animals Used

Albino mice weighing 20–25 g and albino rats of Wister strain weighing 150–200 g were used for studying acute toxicity and diuretic activity respectively. Animals were maintained under standard laboratory conditions. Study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Acute Toxicity Study

The acute toxicity of ethyl acetate, methanol and ethanol extracts of *Abutilum indicum* and *Amaranthus spinosus* were determined in female albino mice. Animals were fasted overnight prior to the experiment. Fixed dose (Annexure-2d) method of CPCSEA, OECD guideline No. 420, was adopted for the study [10]. 1/10th of LD₅₀ cut off values were taken as screening dose [10].

Diuretic Activity [11]

The *A. spinosus* ethyl acetate and ethanol extract at the doses of 100, 200 mg/kg, and *A. indicum* ethyl acetate and methanol extracts at 100, 400 mg/kg p.o., were evaluated for the diuretic activity, according to Lipschitz *et al.* on the either sex rats [11]. The animals were fasted and deprived of water for 18 h prior to the experiment. Albino rats were divided into six groups of six animals each. Group I treated as control, receives normal saline (25 ml/kg) orally. Group II as standard, receives frusemide 20 mg/kg orally, groups III, IV and Vth received the extract at doses of 100, 200 and 400 mg/kg p.o. Immediately after dosing, the animals were separately placed in metabolic cages which were attached with graduated measuring cylinder, the volume of urine was collected up to 5 h. The Na⁺ and K⁺ ion concentration in the samples were determined using flame photometer and the Cl⁻ ion concentration was found using titrimetric method against AgNO₃ solution. Results obtained were compared with that of control and analyzed by student's 't' test.

Six groups of six rats in each group were fasted and deprived of water for 18 h prior to the experiment.

Group I: Control (Normal saline 25 ml/kg p.o.)

Group II: Standard (Furosemide (20 mg/kg)

Group III: *A. spinosus* Ethyl acetate fraction (100 mg/kg p.o.)

Group IV: *A. spinosus* Ethanol fraction (200 mg/kg p.o.)

Group V: *A. indicum* Ethyl acetate fraction (100 mg/kg p.o.)

Group VI: *A. indicum* Methanol fraction (400 mg/kg p.o.)

Statistical Analysis

The results were subjected to statistical analysis using ANOVA and the values of significance were determined at p<0.001.

Table 1: Diuretic Activity of *Amaranthus spinosus* and *Abutilon indicum* Leaves Extracts.

Treatment	Dose (mg/kg)	Vol. (ml)	Concentration of Ions (meq/l)			pH
			Na ⁺	K ⁺	Cl ⁻	
Control	25	1.95	68.4±.81	25.98±0.61	43.11±0.67	6.7
Standard	20	4.26	121.52±1.13***	51.96±0.53***	76.18±0.32***	6.4
<i>A. spinosus</i> Ethyl acetate fraction	100	2.93	74.43±1.5***	30.01±1.4*	36.50±0.47***	7.6
<i>A. spinosus</i> Ethanol fraction	200	3.45	86.46±0.26***	37.28±0.17**	44.16±0.83*	7.1
<i>A. indicum</i> Ethyl acetate fraction	100	1.80 ^{ns}	71.14±0.55*	38.05±0.96**	56.80±1.7**	7.0
<i>A. indicum</i> Methanol fraction	400	3.80	91.35±1.16	45.60±1.21***	63.15±0.96***	6.8

Values are mean± S.E.M (n=6); ***p<0.001, **p<0.01, *p<0.05, Student's 't' test.

RESULTS

The phytochemical screening of the extracts of *A. spinosus* and *A. indicum* leaves revealed the presence of saponins, flavonoids, steroid, terpenoids and glycosides. Due to presence of these phytoconstituents, it may show significant diuretic effect, at the doses of 100 and 200 mg/kg of ethyl acetate and methanol leaves extract of *A. spinosus*. In *A. indicum* leaves, extract of methanol showed significant diuretic effect at the dose of 400 mg/kg, but ethyl acetate fails to show the effect. But this extract moderately increases the Na⁺, K⁺ and Cl⁻ ion excretion when compared to control group (Table 1).

DISCUSSION

The ethyl acetate and methanol extracts of *A. spinosus* and *A. indicum* increase the Na⁺, K⁺ and Cl⁻ excretion, causing alkalisation of urine, showed strong diuretic activity and carbonic anhydrase inhibition activity. These effects were observed predominantly at 100, 200 and 400 mg/kg doses and there was no dose response relationship. This study strongly suggests that the above two traditional plants are acting as a thiazide like diuretic with a carbonic anhydrase inhibitory activity which restates the claim as a diuretic herb [12].

CONCLUSION

From the above result we can conclude that the ethyl acetate, ethanol and methanol fractions of *A. spinosus* and *A. indicum* at the 100, 200 and 400 mg/kg p.o. body weight possess more significant diuretic activity by increasing the total urine output and increased excretion of sodium and potassium salts, that are comparable to the control group, and also increased excretion of potassium salts as compared to standard frusemide drug.

These experimental results have established pharmacological evidence for the traditional claim of the plants to be used as diuretic agent. Further studies are necessary to better evaluate its safety and modes of action.

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