Pharmacological Screening for Evaluation of diuretic activity of Medicinal plants

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ABSTRACT:
The Diuretic activity of *Abutilon indicum* and *Amaranthus spinusus* was studied. The fractions of ethyl acetate, methanol showed significant increase in the urine elimination, while benzene fraction doesn't show diuresis. All the groups were compare to 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 steroidcontentin both the plants extract. The diuretic effect in all the extract may be due to phytochemicals present in the fractions except benzene fraction of *A. indicum*.

KEYWORDS: *A maranthus spinusus*, expectorant, Ethylacetate, traditional, frusemide.
DOI Number:10.48047/nq.2021.19.1.NQ21039 NeuroQuantology2021;19(1):292-295

INTRODUCTION:
Traditional plants *Abutilon indicum* and *Amaranthus spinusus* various parts used in treating various ailments of human. The *Abutilo nindicum* belongs to family malvaceae. Roots of this plants are useful intreating uterine heamorrhagic discharges, seeds used in the treatment of bronchitis, piles and gonorrhoea, leaves used in lumbago, toothache, different kinds of inflammation, bark of this plant is used as a nemollient[1].

It is Erectvelety temntoseunder shrubs, stems round, frequently tinged with purple. Leaves ovate to orbicular-cordate, flowers solitary on jointed penduncles, orange-yellow or yellow, capsules hispid, hardly larger than thecalyx, erect, seeds 3-5, reniform tubercled or minutely stellate-hairy, black or dark brown. Whole plant used as a febrifuge, anthelminiticandan anti-inflammatory properties and it also reported urinary tract problems, bark a string entanddiuretic[2].

Amaranthus spinusus is another traditionally used medicinal plant, otbelongs to family Amaranthaceae. It is commonly known a skate walichaulaiin hindi, used as vegetable and cultivated throught India and other tropical countries. leaves used to cure jaundice, as anti-oxidant, the roots seed as expectorant, lower menstrual flow, leprosy.[3]The juice of whole plant usd to prevent inflammation, laxative, diuretic, digestible, indiabetes, piles and gonorrhoeal.[4]

Amaranthus spinusus is an erect glabrous herb with hardstem, the herbis often red dish with many grooved branches with spines. Leaves 3.2-7.5 cm long, 1.3 to 3.8cm width ovate, obtuse, spinousapiculate, glabrous above, petioles 2,6-3cmlong. Flowers numerous, sessile, indense axillary clusters and interminal interrupted spikes, bracteaoles, linear, bristle

pointed, usually longer than the sepals.[5]

Although a number of studies have been performed on these well known traditional plants[6,7,8,9]. 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 spinusus* were collected in the month of Aug-Sep from the local area and sourrindings of Shivamogsga, Karnataka.

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 Benzene and methanol for *Amaranthus spinusus,.* 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 Benzene, ethyl acetate were prepared using 1% gum acacia and methanol solution were 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 benzene extracts of *Abutilum indicum* and *Amaranthus spinusus* 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]. $1/10^{th}$ of LD_{50} cut off values taken as screening dose.[10]

Diuretic activity[11]:

The *A spinusu* benzene and methanol extract at the doses of 100, 200 mg/kg, and *A.indicum* ethyl acetate and methanol extracts at 100,400mg/kg p.o., Evaluated for the diuretic Activity according to Lipschitz et.al (1943) on the either sex rats. The animals fasted and deprived of water for 18 hr prior to the experiment. Albino rats were divided into six groups of 6 animals each. Group I treated a control receives normal saline (25ml/kg) orally. Group II as standard, receives frusemide 20mg/kg orally, groups III, IV and Vth were received the extract at doses of 100,200 and 400mg/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 collected up to 5hrs. the Na^+ and K^+ ion concentration in the samples were determined using flame photometer, the Cl^- ion concentration was found titremetric 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 hours prior to the experiment.

Group I : Control (Normal saline 25ml/kg p.o)
Group II : Standard (Furosemide (20mg/kg i.p)
Group III: *A. spinusus* Ethyl acetate fraction (100mg/kg p.o)
Group IV:*A. spinusus* Methanol fraction (200mg/kg p.o) **Group V:** *A. indicum* Benzene fraction (100mg/kg p.o) **Group VI:** *A. indicum* Methanol fraction (400mg/kg p.o)

Statistical Analysis:

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

RESULTS:

The phytochemical screening of the extracts of *A spinusus* and *A.indicum* leaves revealed presence of saponins, flavonoids, steroid, terpenoids and glycosides. Due to presence of these phytoconstituent may be showed significant diuretic effect, at the doses of 100

and 200mg/kg o ethyl acetate and methanol leaves extract of *A spinusus.* In *A.indicum* leaves extract of methanol showed significant diuretic effect at the dose of 400mg/kg, but benzene fails to show the effect. But benzene extract moderately increases the Na+, K+, Cl^- ion excretion when compared to control group. (Table No.1)

Table.1Diuretic activity of *Amaranthus spinusus* and *Abutilonindicum* leaves extracts

Treatment	Dose(mg/kg)	Vol	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
A.spinusu Ethyl acetate fraction	100	2.93	74.43±1.5***	30.01±1.4*	36.50±0.47***	7.6
A.spinusus Methanol fraction	200	3.45	86.46±0.26***	37.28±0.17**	44.16±0.83*	7.1
A.indicum Benzene fraction	100	1.80ns	71.14±0.55*	38.05±0.96**	56.80±1.7**	7.0
A. indicum 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

DISCUSSION:

The ethyl acetate and methanol extracts of *A. spinosus* and *A.indicum* increases the Na+, K+, Cl^- excretion, caused alkalinisation 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, methanol fractions of *A spinusus* and *A.indicum* at the 100,200 and 400mg/kg p.o body weight posses 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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