

Comparative Diuretic Study of Medicinal Plants in Individual and Combination Form

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ABSTRACT

In the present study, the hydroalcoholic extracts of *Nycatanthes arbortristis* Linn. (Parijatha) and *Hygrophila spinosa* T. Anders (Kokila) and combination of these extracts were compared for their diuretic activity using Lipschitz test. Standard drug used was furosemide (100mg/kg body weight) in 0.9% sodium chloride solution. All the extracts significantly increased urine elimination and Na⁺, K⁺, and Cl⁻ excretion as compared to normal saline treated groups. The combination of hydroalcoholic extracts of *Nycatanthes arbortristis* and *Hygrophila spinosa* in 1:1 ratio showed better diuretic activity. Comparatively the hydroalcoholic extracts of *Nycatanthes arbortristis* showed more significant activity than *Hygrophila spinosa* extracts. The result revealed that *Nycatanthes arbortristis* is having better diuretic activity than *Hygrophila spinosa* which supports the traditional use of the plant.

Key words: *Nycatanthes arbortristis* Linn., *Hygrophila spinosa* T. Anders, Diuretic activity, Lipschitz test, Flame photometry.

INTRODUCTION

Drugs that induce diuresis (enhances urine outflow) are known as diuretics¹. Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnea. They decrease plasma volume and subsequently venous return to the heart. This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure. Thus, diuretics play an important role in hypertensive patients². Plants medicine is commonly used in the traditional treatment of some renal diseases and many plants are reported to possess significant diuretic activity. The diuretic activity of number of plants used in ethnomedicine as diuretic agents has been confirmed in experimental animals³. *Nycatanthes arbortristis* Linn. (Oleaceae), a widely growing plant has been reported to possess number of medicinal properties. In the traditional system of medicine the leaves of *Nycatanthes arbortristis* are said to possess diuretic property. *Nycatanthes arbortristis* commonly known as night jasmine or parijatha is a large shrub or small tree up to 10 meters high which is cultivated throughout Central

India, Maharashtra and Himalaya ranges from Chenab to Nepal, Assam, Burma, Southwards to Godavari. According to the traditional uses leaves are bitter, acrid, thermogenic, antibacterial, anodyne, antiinflammatory, digestive, Cholagogue, anthelmintic, depurative, sudorifuge, expectorant, diuretic, laxative, trichogenous and tonic. Decoction of leaves is used in sciatica. Leaves are antibilious, expectorant, rheumatism and fever. The leaves are used as an antidote to reptile venom⁴.

Hygrophila spinosa T. Anders (Acanthaceae) is described in Ayurvedic literature as Ikshura, Ikshugandha and Kokilasha "having eyes like Kokila or Indian cuckoo", common in moist places on the banks of tanks, ditches, paddy fields etc., widely throughout India, Srilanka, Burma, Malaysia, Nepal. Leaves, roots, seeds and ashes of the plants were used traditionally as diuretic. Decoction of the whole plant and aqueous extracts of ashes of *Hygrophila spinosa* showed diuretic action in rats, which was attributed to presence of potassium salts in high concentration⁵.

MATERIALS AND METHODS

Plant Materials:

The leaves of *Nycatanthes arbortristis* and *Hygrophila spinosa* were collected from the local area of Solapur during summer. The leaves were authenticated at Shri Chandrshekhar Vidhyalay and Mahavidhyalay, Shreepur, Solapur, Maharashtra. The fresh leaves were washed and cleaned with water to remove adhered dirt, shade dried and pulverized to get coarse powder.

Preparation of Extracts:

The leaves of *Nycatanthes arbortristis* and *Hygrophila spinosa* were dried under shade, crushed and pulverized to get coarse powder and extracted in soxhlet assembly successively with 50% ethanol and 50% water. The extracts was concentrated by distilling off the solvent and then evaporated to dryness on water bath. The obtained extracts were subjected to preliminary phytochemical investigation and pharmacological screening for diuretic activity⁶.

Preliminary Phytochemical Screening:

All the extracts were screened for the presence of various secondary metabolites like alkaloids, glycosides, carbohydrates, steroids, flavonoids, proteins, tannins, saponins and amino acids using the standard established methods^{4,5}.

Procurement and Selection of Animals:

Wistar albino rats of either sex weighing between 100-150g. These animals were used for acute toxicity and diuretic activity studies. The animals were stabilized for 1 week; maintained in standard environmental conditions of temperature ($21 \pm 2^{\circ}$), $60 \pm 5\%$ relative humidity and 12h light dark cycle. They were given standard pellet diet and water *ad libitum* throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output. The study was approved by Institutional Animal Ethics Committee and all the experiments were carried out between 9:00-16:00 hours.

Acute Toxicity Studies:

The acute toxicity study was carried out in adult albino rats by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No. 240. Fixed dose method as in Annex 2nd: Test procedure with a starting dose of

2000 mg/kg body weight was adopted. The animals were fasted overnight and next day extracts of the plants *Nycatanthes arbortristis* and *Hygrophila spinosa* were administered orally at dose level 2000 mg/kg body weight. Then the animals were observed continuously for three h for general behavioral, neurological, autonomic profiles and then every 30 min for next three h and finally for mortality after 24 h till 14 days^{7,8}.

Diuretic Activity:

Albino rats of either sex weighing 100-150g were divided into sixteen groups of six animals each. The animals were fasted for 15 h, deprived of food and water. All the animals received priming dose of 0.9% sodium chloride solution (25mL/kg b. w.). The first group served as control and the second group received the standard drug furosemide (20 mg/kg b. w.) in 0.9% sodium chloride solution. The other fourteen groups received hydroalcoholic extracts of *Nycatanthes arbortristis* at dose levels of 200, 400, 600, 800, 1000 mg/kg; *Hygrophila spinosa* at dose levels of 200, 400, 600, 800, 1000 mg/kg; combination of *Nycatanthes arbortristis* and *Hygrophila spinosa* (1:1 and 1:2); at dose levels of 200 and 400 mg/kg body weight suspended in 0.9% sodium chloride solution (p. o.). Immediately after the respective treatments the animals were placed in metabolic cages (3 animals per metabolic cage) and urine was collected in a measuring cylinder till 6 h. During this period, no food and water was made available to animals. Then the volumes of urine and Na^+ , K^+ and Cl^- in urine were estimated for assessing diuretic activity⁹⁻¹². Na^+ and K^+ concentrations were determined by flame photometer (Model-381 E) and Cl^- concentration was estimated by titration with silver nitrate solution (0.17 N) using 2 mL of ferric alum solution as indicator¹³.

Statistical Analysis:

The data were expressed as mean \pm SEM. The data of diuretic activity were analyzed by one way analysis of variance (ANOVA) followed by "Dunnett's test". p value less than 0.05 was considered as statistically significant.

RESULT AND DISCUSSION

The preliminary phytochemical screening of all the hydroalcoholic extracts indicate the presence of

alkaloids, glycosides, carbohydrates, steroids, flavonoids, proteins, tannins, saponins and amino acid.

In acute toxicity studies, it was found that all the extracts in individual form and in combination form induced diuresis at a dose of 2000 mg/kg. However, there was no mortality till the end of 14 days of observation.

Comparatively the hydroalcoholic extract of *Nycatanthes arbortristis* showed better diuretic activity than hydroalcoholic extract of *Hygrophila spinosa*. In case of combination form the combination of hydroalcoholic extracts of *Nycatanthes arbortristis* and *Hygrophila spinosa* in (1:1) ratio showed better diuretic activity than the combinations of *Nycatanthes arbortristis* and *Hygrophila spinosa* in (1:2) ratio. *Nycatanthes arbortristis* and *Hygrophila spinosa* (1:1) at the dose of 200 and 400 mg/kg showed better activity (synergistic) than that of the extracts in individual form at these doses. When the ratio of *Nycatanthes arbortristis* and *Hygrophila spinosa* is 1:2, antagonistic effect was observed. When the activity was compared among individual and combination form of extracts, the hydroalcoholic extract of *Nycatanthes arbortristis* showed more significant activity just at the dose level of 200 mg/kg (Table I).

In the present study, the diuretic effect of orally administered hydroalcoholic extracts of *Nycatanthes arbortristis* (Parijatha) and *Hygrophila spinosa*

(Kokila) in individual form and in combination form were evaluated in normal rats at multiple doses for diuretic activity. Furosemide, a widely used diuretic in clinical practices was used as standard drug to compare the pharmacological response.

Diuretics relieve pulmonary congestion and peripheral edema. The excess water and electrolyte was given to stimulate edema. Diuresis has two components: increase in urine volume (water excretion) and a net loss of solutes (i.e. electrolytes) in the urine. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream¹⁴.

In case of all the extracts, the urine output started after 2 h of administration while in case of furosemide it started just after 30 min of administration. The difference in the time of onset of the diuretic action these substances may be related to the active principle(s).

CONCLUSION

We conclude that *Nycatanthes arbortristis* has significant effects on urinary excretion of electrolytes than the *Hygrophila spinosa* in individual form and in combination form that is necessary in case of edema and cardiovascular diseases. Comparatively the hydroalcoholic extract of *Nycatanthes arbortristis* showed better diuretic activity than hydroalcoholic extract of *Hygrophila spinosa*.

Table I: Effect of oral administration hydroalcoholic extracts of *Nycatanthes arbortristis* and *Hygrophila spinosa* in individual form and in combination form on urine volume and electrolytic concentration.

Treatment	Dose mg/kg	Total Urine Volume(mL/6h)	Total Na ⁺ (ppm)	Total K ⁺ (ppm)	Total Cl ⁻ (ppm)	Na ⁺ /K ⁺ ratio
Normal Saline	25mL/kg	0.12±0.00	215.00±0.58	242.00 ±1.31	254.50 ±0.83	0.79
Furosemide	20mg/kg	1.13±0.04**	355.50 ±0.62**	280.00 ±0.75**	295.50 ±0.43**	1.27
<i>Nycatanthes arbortristis</i>	200mg/kg	0.25±0.01**	224.67 ±1.66**	185.00 ±1.46**	250.83 ±1.64**	1.21
	400mg/kg	0.40±0.00**	331.00 ±0.36**	252.67 ±0.80**	255.42 ±0.39**	1.31
	600mg/kg	0.54±0.01**	258.67 ±1.62**	309.50 ±1.76**	324.66 ±1.66**	0.83
	800mg/kg	0.73±0.04*	1122.7 ±0.71**	1850.8 ±0.60**	1886.3 ±0.66**	0.60
	1000mg/kg	1.25±0.07**	1075.5 ±0.99**	1874.0 ±0.47**	1897.8 ±0.70**	0.57
<i>Hygrophila spinosa</i>	200mg/kg	0.16±0.00	240.21 ±0.01**	258.13 ±0.08**	275.45 ±0.00**	0.94
	400mg/kg	0.20±0.02	243.00 ±0.00**	260.43 ±0.10**	285.20 ±0.04**	0.93
	600mg/kg	0.24±0.01**	280.34 ±0.02**	295.00 ±0.12**	310.65 ±0.00**	0.95
	800mg/kg	1.2±0.04**	335.17 ±1.51**	375.33 ±1.08**	388.66 ±1.02**	0.89
	1000mg/kg	0.43±0.04**	301.67 ±1.05**	313.67 ±0.91**	315.16 ±0.70**	0.96

<i>Nycatanthes arbortristis:</i>	200mg/kg	0.24±0.00*	234.57 ±1.63**	252.00 ±1.32**	270.33 ±1.54**	0.92
<i>Hygrophila spinosa(1:1)</i>	400mg/kg	0.53±0.01**	260.07 ±1.61**	310.35 ±1.66**	328.36 ±1.56**	0.83
<i>Nycatanthes arbortristis:</i>	200mg/kg	0.23±0.00*	240.53 ±1.44**	255.00 ±1.36**	273.72 ±1.45**	0.94
<i>Hygrophila spinosa(1:2)</i>	400mg/kg	0.14±0.01	252.63 ±1.55**	295.32 ±1.62**	310.52 ±1.65**	0.85

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