



STUDY OF PHYTOCHEMICAL AND DIURETIC POTENTIAL OF METHANOL AND AQUEOUS EXTRACTS OF AERIAL PARTS OF *PHYLA NODIFLORA* LINN.

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ABSTRACT

The dried aerial parts of *Phyla nodiflora* Linn. were subjected to successive extraction using the solvents (Petroleum ether, chloroform, methanol and water) in increasing order of polarity. The prepared extracts were then subjected to preliminary phytochemical analysis. It was found that the plant possesses steroids, alkaloids, carbohydrates, flavonoids, essential oil, tannins and salts of potassium. The methanol and aqueous extracts were selected for further study. The diuretic potential of methanol and aqueous extracts of the aerial parts was assessed in albino rats using *in-vivo* Lipschitz test model. The volumes of urine, urinary concentration of sodium and potassium ions were the parameters of the study. Frusemide was used as standard. The results indicate that methanol and aqueous extract at 500 mg/kg body weight shows a significant ($p < 0.05$) increase in the urine volume and electrolyte excretion ($p < 0.001$) when compared to control. Both the extracts show significant diuretic activity. From the present study it may be concluded that the constituents present in methanol and aqueous extracts may be responsible for diuretic activity.

Key words : *Phyla nodiflora*, Aerial parts, Diuretic, Methanol and aqueous extract.

INTRODUCTION

Phyla nodiflora Linn. (Verbenaceae) is commonly called as Bhujokra in Hindi, Ratoliya in Gujarati and Jalpippali in Sanskrit¹⁻⁴. It is found throughout warmer parts of India ascending up to 900m in the hills. It is common in wet places, along irrigation channels, canal edges and river banks⁵.

It is a creeping, prostrate, much branched perennial herb with branches spreading profusely and rooting at the nodes. The stem is woody at the base, light violet if exposed to the sun on drying; it remains straight, pale creamy or white brown in color, shriveled, glabrate forming furrows with vertical channels. The leaves are simple, small, obtuse-ovate, deeply and sharply serrate

towards the apex; both surfaces are shiny, hairy with medifixed white strigose hairs. The roots of this plant are tough, knotty with fibrous fracture and whitish wood⁶⁻¹³. In literature review it was found that the aerial parts are used as anodyne, antibacterial, diuretic, emmenagogue, parasiticide, refrigerant, febrifuge and cooling^{14, 15}. According to traditional uses and Unani system of medicine the plant is acrid, hot and dry; diuretic, maturant, useful in fevers and cold, astringent to bowels, stomachic, used in lack of bowel movements, pain in knee joints and in lithiasis¹⁶⁻¹⁹. *Phyla nodiflora* contains flavonoids, sugars, sterol, an essential oil, resin, non-glucosidal bitter substance, tannin, large amount of potassium nitrate and other constituents²⁰. Several workers have reported many pharmacological properties including antispasmodic²¹, hair affections²², anti-inflammatory, analgesic and antipyretic²³, antibacterial²⁴, anti *Helicobacter pylori* activity²⁵, hypotensive activity²⁶, antinociceptive²⁷ and antifungal²⁸. However, there are no reports on the diuretic activity of the plant. Hence, the present study was designed to verify the claims of traditional and use in Unani system of medicine. Elemental detection of powdered plant drug was also carried out to quantify the potassium content.

MATERIAL AND METHODS

Fresh aerial parts of *Phyla nodiflora* were collected during the month of September 2007, from Dakor, Dist.Kheda, Gujarat. The plant was identified and authenticated at Bioscience Department, Sardar Patel University, Vallabh Vidyanagar, and herbarium specimen deposited at A.R.College of Pharmacy, Vallabh Vidyanagar. The fresh plant material was then dried under shade. Dried plant material was powdered using mechanical grinder and passed through 60 # sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preparation of extract

Dried powder was defatted by maceration process with petroleum ether. This defatted material was taken and extracted with chloroform and methanol using a soxhlet extractor. After extraction the solvent was distilled off using a rotary vacuum evaporator and concentrated to a syrupy mass (MEPN). The marc was finally extracted with water to get the water (aqueous) extract (AEPN). These extracts were subjected to preliminary phytochemical screening and diuretic activity.

Preliminary phytochemical screening

All the extracts were screened for the presence of various secondary metabolites like steroids, alkaloids, carbohydrates, flavonoids, essential oil and tannins using standard methods²⁹.

Elemental detection in powder drug was carried out by Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Powder was digested with 10 ml HNO₃ for 10 minutes and then to it added 10 ml HCl and again heated for 10 minutes. It was then diluted with 20 ml double distilled water. This solution was filtered and filter paper washed thoroughly with water. The volume of the clear solution was made up to 100 ml and stored in a tightly capped plastic bottle. The clear solution of powder drug prepared was used directly for determination of mineral elements.

Animals

Male albino rats weighing 200-300 gm of either sex were procured from Cadila Health Care Ltd., Dholka, Ahmedabad. All the animals were kept in standard polypropylene cages and maintained at 27°C ± 2°C under 12 h. dark/light cycle. The animals were fed with standard rat feed and water was given *ad libitum*. Ethical clearance for handling the animals and the procedures used in the

study was obtained from the institutional animal ethical committee.

Diuretic Activity

The diuretic activity of MEPN, AEPN and frusemide was carried out by using *in-vivo*, Lipschitz test method³⁰⁻³². The rats were divided into 6 groups of 6 animals each and deprived of food and water for 18 hours. All the rats received priming dose of normal saline (25ml/kg) orally. Both the extracts and frusemide (Standard) were dissolved in a normal saline. Group I served as control in which only normal saline (25ml/kg) was administered through intraperitoneal route. Group II served as standard received frusemide (100mg/kg). Rest of the groups served as treated groups. Group III and Group IV received MEPN at the dose levels of 250 and 500 mg/ kg i.p., respectively. Group V and Group VI received AEPN at the dose levels of 250 and 500 mg/kg, respectively. Immediately after administration, the rats (one in each cage) were placed in metabolic cages specially designed to separate urine and faeces and kept at room temperature of 25±0.5°C. The urine was collected in a measuring cylinder upto 6 h. During this period, no food or water was made available to

animals. The volume of urine collected was measured for all the groups. The parameters taken for each individual rat were body weight before and after test period, urine volume (concentrated for water intake during the test period), concentration of Na⁺ and K⁺ in urine. The content of Na⁺ and K⁺ in the urine

RESULTS

The phytochemical tests revealed the presence of flavonoids, saponins, carbohydrates in methanol and aqueous

was estimated by ICP-OES (Inductive Coupled Plasma-Optical Emission Spectroscopy.)

Statistical analysis

All the results are expressed as mean ± standard error. The data was analyzed statistically using ANOVA followed by Dunnett's Multiple Comparison Test.

extract and phenolics in methanol extract. The results of phytochemical screening are given in Table 1.

Table 1: Preliminary phytochemical screening of aerial parts of *Phyla nodiflora*

Type of constituents	Pet ether extract	Chloroform extract	Methanol extract	Aqueous extract
Flavonoids	--	--	+	+
Carbohydrates	--	--	--	+
Sterols	+	--	--	--
Essential oil	+	--	--	--
Phenolics	--	--	+	+

+, -- represent presence and absence of phytoconstituents respectively

ICP-OES analysis of the powder showed the presence of 6652.5 ppm of potassium.

The results of diuretic activity of *Phyla nodiflora* obtained from the urine samples of the rats are shown in Table 2.

Table 2: Effect of methanolic and aqueous extracts of *Phyla nodiflora* on urine volume and Na⁺, K⁺ concentration in rats.

Group	Urine volume (ml/kg)	Concentration of excreted ions		
		Na+ (mEq/L)	K+ (mEq/L)	Na+/K+ Ratio
I	2.65±0.076	62.45±0.043	50.61±0.010	1.234
II	12.65±0.085*	153.25±0.170	134.51±0.052 [#]	1.138 [#]
III	2.28±0.047	96.18±0.0645	61.77±0.025 [#]	1.557 [#]
IV	5.85±0.042*	123.33±0.068	138.50±0.028 [#]	0.890 [#]
V	2.68±0.047	72.23±0.040	149.63±0.030 [#]	0.483 [#]
VI	8.43±0.033*	135.42±0.100	194.78±0.033 [#]	0.695 [#]

Statistical analysis by ANOVA and Dunnett's Multiple comparison Test. Results are expressed as mean ± standard error, n = 6 in each group. *Significantly difference compared to control group at p < 0.05. [#]Significant difference compared to control group at p<0.001

The methanol and aqueous extracts increased urine volume significantly ($p < 0.05$) at 500 mg/kg body weight and lower dose i.e. 250mg/kg body weight failed to do so. The excretion of sodium and potassium ions is significantly increased ($p < 0.001$) in a dose dependant manner.

DISCUSSION

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They increase plasma volume and subsequently venous return to the heart. This decreases cardiac work load, oxygen demand and plasma volume, thus decreasing blood pressure. Thus diuretics play an important role in hypertensive patients. On the basis of the results of the present investigations, we can conclude that the methanol and aqueous extracts are potent natriuretic but weak diuretic. That means the natriuretic effect of lower dose may not be sufficient to induce diuresis. However, the natriuretic effect at higher dose is sufficient to cause diuresis. The diuretic activity of the plant may be due to the presence of the salts of potassium or the constituents responsible for hypotensive activity which is already

studied³³. However, the contribution of polyphenolic compounds to diuretic activity can not be ruled out. Further studies like isolation and characterization of the diuretic principle from the aerial parts of the plant is needed to confirm the activity. From the present study it may be concluded that the claim of the native practitioners that, aerial parts of *Phyla nodiflora* possesses diuretic activity is justifiable.

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