



## Evaluation of diuretic activity of aerial parts of *Phyllanthus longiflorus* Heyne ex Hook

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### ABSTRACT

Medicinal plants have proved to be good resource for medicines; many species of the genus *Phyllanthus* are used in folk remedies. In the present study aerial parts of *Phyllanthus longiflorus* Heyne ex Hook belonging to the family Phyllanthaceae was investigated for its possible diuretic activity. Aqueous and methanol extract of *Phyllanthus longiflorus* at the doses 100 mg/kg and 200 mg/kg were administered orally and the activity was ascertained by different parameters such as total urine volume, urine concentration of sodium, potassium and chloride were accounted for establishing the activity. The sodium, potassium concentrations were determined using flame photometer and the concentration of chloride was determined by titration with silver nitrate. Both the extracts exhibited a significant ( $P < 0.01$ ) rise in urine volume,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentration of urine in a dose dependant manner. From the findings of the present study, it was concluded that the aerial parts of *Phyllanthus longiflorus* possess significant diuretic activity.

**Key words:** Diuretic activity, *Phyllanthus longiflorus*, Nallapulati, Phyllanthaceae, aerial parts.

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### INTRODUCTION

Diuretics are the drugs used in the treatment of many life threatening diseases such as heart failure, liver cirrhosis, hypertension and kidney disease [1]. Diuretics are used for diseases that necessitate to get free of fluid retention, such as obesity, arthritis, gout etc. Plants and plant based drugs are used, since ancient times, to treat various urinary ailments, still most of the peoples, especially in developing countries depends on medicinal plants for their basic health care rations.

*Phyllanthus longiflorus* (PHL) Heyne ex Hook, Syn- *Reidia longiflora* Gamble; *Reidia ovalifolia* wright, (Tamil-Nallapullati; Malayalam-Malenkizhanelli), is a small bush with obliquely obovate – oblong leaves, pink flowers and small capsular fruits (0.4cm long). It belongs to the family Phyllanthaceae and distributed in Western Ghats southern India [2]. Genus *Phyllanthus* plays an important role in folklore medicine and holds wider biological activities which include anti-inflammatory [3], anti tumor and anti angiogenic [4], hepatoprotective [5,6,7], antioxidant [8,9], antidiabetic [10], anti bacterial and anti fungal activities [11] activities. Some of the species such as *Phyllanthus*

*amarus* [12], *Phyllanthus sellowianus* [13] have been established scientifically for diuretic activity. In this view, the present study was carried out to investigate aerial parts of *Phyllanthus longiflorus* for diuretic activity.

## EXPERIMENTAL SECTION

### Plant collection and authentication

The plant material was collected from the Western Ghats, Tamilnadu, India during February 2008. It was authenticated by Dr.V.Chelladurai, Govt. Research officer, Botany CCRAS, Govt. of India, (Retired), Tirunelveli, Tamilnadu, India. A voucher specimen (PHL001) has been deposited for future reference.

### Preparation of extracts

The plant material was dried under shade for fifteen days, coarsely powdered using a mechanical grinder and then extracted exhaustively with methanol by continuous hot extraction method [14] at 40-50°C for 18-20 hrs. Aqueous extract was prepared by a cold maceration process. The solvent was removed under reduced pressure, dried using rotary evaporator and a green solid mass was obtained. It was then preserved in desiccators until further use. Preliminary phytochemical analysis was done using standard procedures [15] to identify the phytoconstituents present in the extracts.

### Animals

Male albino rats (130-150g) of Wister strain were procured and housed in standard polypropylene cages. They were kept under controlled room temperature ( $24 \pm 20^\circ\text{C}$ ; relative humidity 60-70%) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. The protocol for the present study was approved by institutional animal ethics committee (Approval no. 509/02/C/CPCSEA).

### Acute toxicity study

Graded doses technique was employed for studying acute toxicity. The extracts were administered in graded doses of 100 to 2000 mg/kg body weight by intraperitoneal route. They were observed continuously for the first 1 h, intermittently for the next 4 h for any behavioral changes like sedation, loss of righting reflex, hyper activity, convulsion and periodically for first after 24 h for mortality and the study was continued for fourteen days [16].

### Diuretic activity

The method described by Lipschitz *et al* [17], was used for the evaluation of diuretic activity. The animals were divided in to five groups of six animals each, deprived of food and water for 18h prior to the experiment. On the day of experiment, the Group I animals received normal saline (20 mg/kg. p.o.), the Group II animals received Frusemide (20 mg/kg. i.p.), the Group III and IV received aqueous extract of PHL at 200 mg/kg and 400 mg/kg respectively, Group V and VI received methanol extract of PHL at 200 mg/kg and 400 mg/kg respectively. Immediately after the administration, the animals were kept in metallic cages (two per cage) specially designed to separate urine and faecal matter, maintained at room temperature ( $20 \pm 0.5^\circ\text{C}$ ). The total volume of urine was collected at 5h. During this period no water and food was made available to the animals. The diuretic activity was assessed by total volume of urine and the urine concentration of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ . The  $\text{Na}^+$  and  $\text{K}^+$  were measured by flame photometry [18] and  $\text{Cl}^-$  concentration was estimated by titration [19] with silver nitrate solution (N/50) using 3 drops of potassium chromate as indicator.

### Statistical analysis

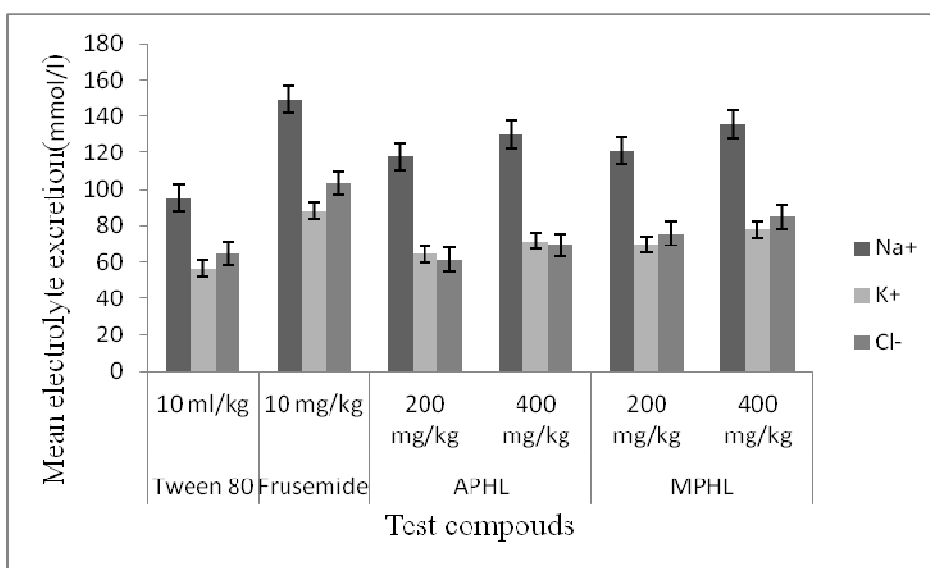
The data obtained were analyzed by “t” test, one-way ANOVA followed by Dunnett test using GraphPad InStat v. 3.0.10.0 (GraphPad Software, Lajolla, CA, U.S.A) for statistical significance. The values expressed are Mean  $\pm$  S.E.M.  $P < 0.001$  or  $P < 0.01$  were considered significant.

## RESULTS

Methanol and aqueous extract of PHL at the doses 100 and 200 mg/kg produced a significant ( $p < 0.01$ ) diuretic activity, as shown by the increase in total urine volume and urine concentration of sodium, potassium and chloride ions. The activity was found to be dose dependant (Figure 1&Table 1).

Aqueous and methanol extract of *Phyllanthus longiflorus* have increased the urine volume by 58.31% and 71.32% at 200mg/kg respectively while the reference drug has shown 81.68%. There was also a significant ( $p < 0.01$ ) increase in  $\text{Na}^+$  level ( $134.1 \pm 0.52$  and  $136.5 \pm 0.72$  by APHL and MPHL at 200 mg/kg, respectively),  $\text{K}^+$  level ( $67.6 \pm 0.45$  and  $67.9 \pm 0.79$  by APHL and MPHL at 200 mg/kg, respectively) and  $\text{Cl}^-$  level ( $69.27 \pm 0.25$  and  $84.7 \pm 0.15$  by APHL and MPHL at 200 mg/kg, respectively) in urine of treated animals. The values obtained were comparable to that of reference drug, which showed  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  levels as  $157.4 \pm 0.73$ ,  $73.2 \pm 0.17$  and  $103.5 \pm 0.63$  respectively. Also, APHL attained the  $\text{Na}^+/\text{K}^+$  ratio of 1.98 at 400 mg/kg, MPHL attained the  $\text{Na}^+/\text{K}^+$  ratio of 2.01 at 200 mg/kg while control and standard exhibited 2.00 and 2.15 respectively. This result has revealed a significant ( $p < 0.01$ ) normalization of  $\text{Na}^+/\text{K}^+$  ratio (aldosterone secretion index) which is essential for the plasma aldosterone level.

**Figure 1. Effect of aqueous and methanol extract of aerial parts of *Phyllanthus longiflorus* on sodium, potassium and chloride concentration of urine**



$n = 6$ ,  $p < 0.001$  vs. control, measured using GraphPad InStat v. 3.0.10.0 soft ware, Bars represent mean value with standard error. Where, APHL stands for aqueous extract of *Phyllanthus longiflorus*; MPHL stands for methanol extract of *Phyllanthus longiflorus*.

**Table 1. Effect of aqueous and methanol extract of aerial parts of *Phyllanthus longiflorus* on urine volume, diuretic index and PH**

Drug	Dose (mg/kg)	Urine volume (ml)	Diuretic index	PH	$\text{Na}^+/\text{K}^+$
Tween 80	10 ml/kg	$4.15 \pm 0.09$	-	7.45	2.00
Frusemide	10	$7.54 \pm 0.57$	1.816	7.39	2.15
	100	$5.11 \pm 0.46$	1.228	7.27	1.83
APHL	200	$5.92 \pm 0.61$	1.426	6.71	1.98
	100	$6.86 \pm 0.19$	1.653	6.70	1.95
MPHL	200	$7.21 \pm 0.06$	1.737	6.43	2.01

$n = 6$ ,  $p < 0.01$  vs. control, measured using GraphPad InStat v. 3.0.10.0 soft ware. Diuretic index = volume of drug treated group/volume of control group. Where APHL – Aqueous extract of *Phyllanthus longiflorus*; MPHL – ethanol extract of *Phyllanthus longiflorus*.

## DISCUSSION

Diuretics are the drugs which increase urine flow. Diuretics plays an important role in managing various pathological conditions which includes hypertension, edema associated with congestive cardiac failure, hypercalcaemia, hyperkalaemia, acute pulmonary edema, cerebral edema, acute renal failure, poisoning by barbiturates and halides etc., The control of plasma sodium is essential in the regulation of blood volume and blood pressure [20], the control of plasma potassium is important for normal physiology of cardiac and skeletal muscle and the sodium and potassium balance is important for proper renal functions [21].

PHL showed a significant increase in urine volume confirming its diuretic activity. Increase in the rate of excretion of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  ions, evident for its Natriuretic, hypokalaemic and hypocalcaemic potential. PHL also normalizes

Na<sup>+</sup> and K<sup>+</sup> ratio, which is essential for the regulation of aldosterone concentration. Mostly the diuretics increase the excretion of water, Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> through vasodilatation [22] or increasing tubular reabsorption [23] The reference drug frusemide is a loop diuretic, act mainly by inhibiting the transport of Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> in the luminal membrane in the ascending limb of loop of Henle.

Flavonoids, saponins, organic acids from various plant sources were reported in various studies to possess diuretic activity [24,25,26,27]. Preliminary phytochemical analysis of PHL showed the presence of alkaloids, flavonoids, phenolic compounds, tannins and steroids in the methanol extract and flavonoids, phenolic compounds and tannins in the aqueous extract. The diuretic activity of PHL may also be due to the presence of flavonoids, saponins, phenolic compounds, possibly by the same mechanism.

### CONCLUSION

The present study has revealed that the aerial parts of *Phyllanthus longiflorus* possess notable diuretic activity. The activity could be due to individual or combined action of phytoconstituents present in it. However, it needs to perform advanced toxicological studies in other animals, further phytochemical investigation to identify active constituent responsible and to know exact mechanism of diuretic activity exhibited by aqueous and methanol extract of *Phyllanthus longiflorus*, for its suggestion to the medical use.

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