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Original Article

DIURETIC EFFECT OF THE ETHANOL EXTRACTS OF *PHYLLANTHUS ACIDUS* L (SKEELS) LEAVES IN *WISTAR* RATS

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ABSTRACT

Objective: The objective of this study is to test diuretic effect of ethanol extract of *P. acidus* leaves in female *Wistar* rats using modification of Lipschitz method.

Methods: The doses of the extract were 22.5, 45 and 90 mg/kg bw. Parameter of diuretic effect was cumulative urine volume during 6 and 24 hours after administration of the extract. Sodium and potassium level in urine were analyzed using atomic absorption spectrophotometry (AAS). The results were analyzed with Student-t test.

Results: The ethanol extracts of *P. acidus* at doses of 22.5, 45, and 90 mg/kg bw could accelerate the urinary in rats since 60 minutes after oral administration compared to the control and furosemide 3.6 mg/kg bw ($P<0.05$). The level of sodium on 24 hour urine of the ethanol extracts of *P. acidus* at doses 22.5 and 45 mg/kg bw significantly higher than control group ($P<0.05$), but significantly lower than furosemide 3.6 mg/kg bw ($P<0.05$). The level of potassium on 24 hour urine of the ethanol extracts of *P. acidus* at doses 45 and 90 mg/kg bw significantly higher than control group ($P<0.05$), but significantly lower than furosemide 3.6 mg/kg bw ($P<0.05$).

Conclusion: It can be concluded that the ethanol extract of *P. acidus* at doses of 22.5 mg/kg bw, 45 mg/kg bw and 90 mg/kg bw had significantly diuretic and saluretic effect compared to control group ($P<0.05$), but significantly less than furosemid 3.6 mg/kg bw ($P<0.05$).

Keywords: *Phyllanthus acidus*, Ceremai, Diuretic, Saluretic, Sodium, Potassium.

INTRODUCTION

One of traditional medications that accepted by WHO is herbal medicines. Herbal medicines usually use in developing countries for primary health care to treat various diseases based on long historical use of the herbal medicines itself. The term herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products, which contain as active ingredients parts of plants, or other plant materials, or combinations [1]. A review of herbal medicine as diuretic by Wright *et al.* (2007) stated that the most promising herbs are *Foeniculum vulgare*, *Fraxinus excelsior*, *Hibiscus sabdariffa*, *Petroselinum sativum, Spergularia purpurea*, species from the genuses *Cucumis* (*Cucumis melo* and *Cucumis trigonus*), *Equisetum* (*Equisetum bogotense*, *Equisetum fluviatile*, *Equisetum giganteum*, *Equisetum hiemale* var. *affine* and *Equisetum myriochaetum*), *Lepidium* (*Lepidium latifolium* and *Lepidium sativum*), *Phyllanthus* (*Phyllanthus amarus*, *Phyllanthus corcovadensis* and *Phyllanthus sellowianus*) and *Sambucus* (*Sambucus mexicana* and *Sambucus nigra*) [2].

Traditionally several *Phyllanthus* sp. is used as diuretic. Udupa *et al.* (2010) have tested the diuretic effect of aqueous extract of *Phyllanthus niruri*, the result showed the significant increase in the volume of urine and excretion of sodium, potassium and chloride after the extract administered in hydrated albino rats [3]. An evaluation of the diuretic activity of methanolic extracts of aerial parts of *P. fraternus* showed that the extract increased the volume of urine and ionic concentration of sodium and potassium levels in urine as compared with control group [4]. Diuretic effect of an aqueous extract (5% w/v) of the stem bark of *P. sellowianus* (400 mg/kg bw) also tested on animals, which showed that after 8 h of the single oral administration, the extract increased a significant in the urinary excretion [5]. Even, Srividya and Periwal (1995) have observed the potential effect of *P. amarus* as diuretic, hypotensive, and hypoglycaemic on human subject [6]. Another *Phyllanthus* sp. that easily found in Indonesia is *Phyllanthus acidus* Linn.

Phyllanthus acidus Linn has been used traditionally to treat inflammation, hypertension, diabetic and hyperlipidemia [7]. One of the mechanisms of antihypertension is diuretic, which work by increasing the excretion of urine from the body as well as the amount of sodium in urine [2], and *P. acidus* is suspected has diuretic effect. The objective of this study is to test diuretic effect of ethanol extract of *P. acidus* (L.) Skeels leaves.

MATERIAL AND METHODS

Plant collection and determination

The leaves of *P. acidus* were collected from Purwakarta, West Java, Indonesia and determination of the plant was done by School of Life Science and Technology, Institut Teknologi Bandung.

Preparation of extract

The leaves of *P. acidus* L. Were dried and then were powdered. The powder of *P. acidus* was extracted with 96% ethanol using Soxhlet apparatus. The ethanol was removed with a rotary evaporator at reduced temperature to obtain a solid mass of extract. The extract were dried at 60^0C (yield: 7.948 % w/w) and used for phytochemical analysis and diuretic test at doses of 22.5, 45, and 90 mg/kg bw.

Phytochemical analysis

Phytochemical analysis of the extract was done to evaluate the presence of alkaloids, tannins, saponins, flavonoids, steroid, and terpenoid. Alkaloid assay was done using Dragendorf and Mayer test. Evaluation of tannins was done using Ferric chloride test and saponins assay was done using Foam test.

Animal and diuretic experimental design

Diuretic effect of ethanol extracts of *P. acidus* leaves have been examined in female *Wistar* rats using modification of Lipschitz method [8]. All animal experiments were conducted under institutional ethical guidelines.

Female *Wistar* rats (150-250g) were used for the diuretic test. The animals were obtained from Center of Life Sciences, Institut Teknologi Bandung, Bandung, Indonesia and housed under standard laboratory conditions. The animals fed with standard rat feed and water *ad libitum*. Female rats were used in order to give more comprehensive results. Craft *et al* (2000) has been evaluated the diuretic effect of bremazocine, pentazocine, and furosemide in females and males rats and the result showed that when data were corrected for individual difference in body weight, furosemide produced nearly equivalent diuresis/kg in females and males rats [9]. Prior the experiment, the animals were fasted for 18 hours except water was given ad libitum. The animals were divided into five groups (five in each). The extract was suspended in 0.5% sodium carboxymethyl cellulose (Na CMC) for oral administration. The doses of the extract were 22.5, 45 and 90 mg/kg bw. Furosemide (3.6 mg/kg bw p. o) in 0.5% Na CMC was used as the reference diuretic agent. In the beginning of the experiment, the animals were given NaCl 0.9% 20 mL/kg bw as loading dose. After 30 minutes, the animals groups were treated as follows:

Group I (control): received 0.5% Na CMC orally at a dose of 10 ml/kg bw.

Group II (standard): received furosemide orally at a dose of 3.6 mg/kg bw.

Group III (test 1): received ethanol extract of *P. acidus* orally at a dose of 22.5 mg/kg bw.

Group IV (test 2): received ethanol extract of *P. acidus* orally at a dose of 45 mg/kg bw.

Group IV (test 3): received ethanol extract of *P. acidus* orally at a dose of 90 mg/kg bw.

After treatment, the animals were kept in a metabolic cage. The urine was collected and measured every 30 minutes for 6 hours and 24 hours after the dose was administered. Parameter of diuretic effect was total urine output during 6 and 24 hours after administration of the extract and the percentage of total urine output compared to the volume of NaCl 0.9% which is given as loading dose. The diuretic effect is stated as the positive result if the percent of total urine output is higher than 80%. Sodium and potassium level in urine was analyzed using atomic absorption spectrophotometer (AAS).

Statistical analysis

All the values expressed are Mean ± S. E. M. Statistical evaluations were performed by Student's t-test at the 95% confidence level using an SPSS program for Windows 17.0. Values of $p < 0.05$ were considered significant.

RESULTS

Phytochemical analysis

The ethanol extract of *P. acidus* leaves contain flavonoids, triterpene, monoterpene and sesquiterpene as shown in table 1.

Diuretic effect

Diuretic effect of the ethanol extract of *P. acidus* leaves is characterized using urinary output (volume of urine) and ionic concentration of electrolytes (sodium and potassium).

Effect on urinary output

Urinary profile every 30 minutes for 6 hours after administered of the extract can be seen in fig. 1, while total urine output after 6 hours and 24 hours can be seen in table 2.

Table 1: It shows phytochemical analysis in ethanol extract of *P. acidus* leaves

Parameter	Result
Alkaloid	–
Tannin	–
Saponins	–
Flavonoids	+
Steroid	–
Triterpene	+
Monoterpene and sesquiterpene	+

(+) = present; (–) = absent

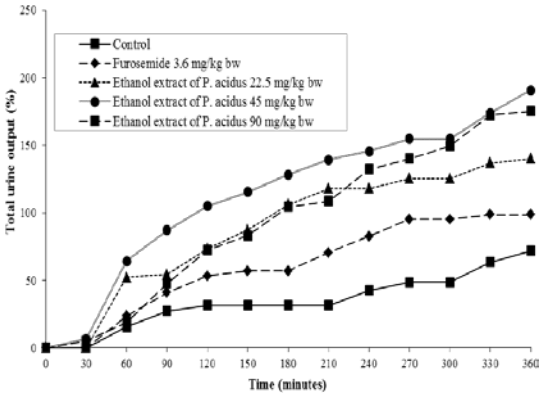

Fig. 1: It shows time course of diuresis in rats treatment with ethanol extract of *P. acidus* at doses 22.5, 45 and 90 mg/kg bw, vehicle and reference drug furosemide 3.6 mg/kg bw.

The diuretic assay showed that the ethanol extracts of *P. acidus* at dose 22.5, 45, and 90 mg/kg bw could accelerate the urinary in rats since 60 minutes after oral administration compared to the control and furosemide 3.6 mg/kg bw, especially total urine output of ethanol extract of *P. acidus* at dose 22.5 and 45 mg/kg bw significantly higher than control group (P<0.05).

The ethanol extracts of *P. acidus* at dose of 22.5 mg/kg bw had diuretic effect on 150th minute with percent of total urine output by 80.09%; ethanol extract of *P. acidus* at dose of 45 mg/kg bw had diuretic effect on 90th minute with percent of total urine output by 80.03%; ethanol extract of *P. acidus* at dose of 90 mg/kg bw had diuretic effect on 150th minute with percent of total urine output by 89.36%; whereas furosemide at dose of 3.6 mg/kg bw showed a diuretic effect on 240thminute with percent of total urine output by 84.08%.

Table 2: It shows total urine output in rats at 6 and 24 hours after oral administration of ethanol extract of *P. acidus* at doses of 22.5, 45 and 90 mg/kg bw, vehicle and reference drug furosemide 3.6 mg/kg bw

Groups	Total urine output (mL)	
	6 hours	24 hours
Control	2.45±1.04	8.62±3.44
Furosemide 3.6 mg/kg bw	3.23±0.58	17.45±3.10*
Ethanol extract of *P. acidus 22.*5 mg/kg bw	4.93±1.16*	16.65±4.35*
Ethanol extract of *P. acidus* 45 mg/kg bw	5.96±2.04*	16.06±1.25*
Ethanol extract of *P. acidus* 90 mg/kg bw	5.49±3.23	16.11±5.17*

n = 5, *P<0.05, compared to control group using Student t-test

Table 3: It shows sodium and potassium level on 24 hours urine in rats after oral administration of the ethanol extract of *P. acidus* at doses of 22.5, 45 and 90 mg/kg bw, vehicle and reference drug furosemide 3.6 mg/kg bw

Groups	Parameter (gram)		Ratio Na/K
	Sodium	Potassium	
Control	34.13±9.68	46.64±14.59	0.732
Furosemide 3.6 mg/kg bw	68.38±10.02*	87.56±11.30*	0.781
Ethanol extract of *P. acidus 22*.5 mg/kg bw	47.18±4.82*	57.44±4.51	0.821
Ethanol extract of *P. acidus* 45 mg/kg bw	47.46±4.89*	63.49±5.43*	0.748
Ethanol extract of *P. acidus* 90 mg/kg bw	47.20±10.22	65.47±10.56*	0.721

n = 5, *P<0.05, compared to control group using Student t-test

Total urine output at 6 hours after oral administration of the ethanol extract of *P. acidus* leaves at doses of 22.5 and 45 mg/kg bw significantly higher than control group (P<0.05), and also higher than a reference drug furosemide 3.6 mg/kg bw. However, total urine output at 24 hours after oral administration of the ethanol extract of *P. acidus* leaves at doses of 22.5, 45, and 90 mg/kg bw significantly higher than control group (P<0.05), but slightly lower than the furosemide 3.6 mg/kg bw.

Effect on urinary electrolytes

Sodium and potassium level on 24 hours urine is shown in table 3.

The result showed that the ethanol extract of *P. acidus* leaves at doses 22.5, 45 and 90 mg/kg bw could increase sodium and potassium level on 24 hour urine. The level of sodium of the ethanol extracts of *P. acidus* leaves at doses 22.5 and 45 mg/kg bw significantly higher than control group (P<0.05), but significantly lower than furosemide 3.6 mg/kg bw (P<0.05). The level of potassium of the ethanol extracts of *P. acidus* leaves at doses 45 and 90 mg/kg bw significantly higher than control group (P<0.05), but significantly lower than furosemide 3.6 mg/kg bw (P<0.05).

DISCUSSION

Diuretic can be define as an agent which produces an increased flow of urine or as an agent that capable of removing excess of water from the body. Diuretic can be divided into water diuresis, mercurial diuretics, xanthine diuretics, and osmotic diuretics [10]. Based on the diuretic assay, the ethanol extract of *P. acidus* leaves at doses 22.5, 45, and 90 mg/kg bw increased the flow of urine. These indicate that the ethanol extract of *P. acidus* leaves probably have an effect as water diuresis.

In the assay of water diuresis in rats, water (in this study is replaced by isotonic saline) is completely absorbed in less than 75 minutes and urine flows raised peak at about 90 minutes after oral administration. Water diuresis will influence the role of antidiuretic hormone (ADH) in the regulation of urinary output. The ethanol extract of *P. acidus* may reduce the liberation of ADH. Inhibition of ADH could produce diuresis by stimulating the release of endogenous natriuretic peptides, which promote sodium and water secretion [10,11]. Water diuresis, in Europe known as aquaretics, describes some herbs which increase urine output. These herbs act on the glomerulus to increase water excretion without affecting renal handling of electrolyte. Aquaretics may work by causing dilation of glomerular arterioles, thereby increasing glomerular filtration rate [12].

Effect of the ethanol extracts of *P. acidus* leaves is dose dependent, higher dose cause Na+/K+ ratio decrease. The ethanol extracts of *P. acidus* leaves at doses of 45 and 90 m/kg bw excreted potassium higher than sodium, made the ratio Na+/ K+ smaller. It's seem that the ethanol extract of *P. acidus* leaves is probably acting like loop diuretics which inhibits Na+, K+ and Cl- co transport at thick ascending loop of Henle. K+ excretion was increased perhaps due to high Na+ load reaching the distal tube [13].

Based on phytochemical analysis, the ethanol extract of *P. acidus* leaves contained flavonoids, triterpene, monoterpene and sesquiterpene. The recent study state that *P. acidus* contain triterpene (lupeol, ß-amyrin, phyllanthol), lignan (phyllantoside), and sesquiterpene (phyllanthusol A and B) [14, 15]. Lupeol is flavones that have activity as diuretic [16]. This compound may contribute for diuretic effect of the ethanol extract of *P. acidus* leaves. *P. niruri* contain astragalin, a flavonone, which has a diuretic activity [17], so it suggested that if the ethanol extract of *P. acidus* leaves contain astragalin. This compound may also contribute for diuretic effect.

CONCLUSION

It can be concluded that the ethanol extract of *P. acidus* leaves at doses of 22.5, 45, and 90 mg/kg bw had significantly diuretic and saluretic effect compared to control group (P<0.05), but significantly less than furosemide 3.6 mg/kg bw (P<0.05). Further study of the component that involved in diuretic effect and the exact site of action are needed.

CONFLICT OF INTEREST

The authors declare no conflict of competing interest.

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