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Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats

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ABSTRACT

Aim of the study: Orthosiphon stamineus (Labiatae) is a traditional folk medicine widely used in Southeast Asia for the treatment of several kidney disorders, gout and as a diuretic. This study was conducted to examine the diuretic and hypouricemic effects of *Orthosiphon stamineus* leaf extracts.
Materials and methods: The diuretic effect of different methanol extracts was examined by treating different groups of Sprague–Dawley rats with single (2 g/kg) oral doses of methanol and methanol:water (1:1) extracts. Hydrochlorothiazide (10 mg/kg) was used as positive control in acute study. Methanol and methanol water (1:1) extracts at 0.5 g/kg were administered for a period of 7 consecutive days. Cumulative urine volume and electrolytes (Na^+ and K^+) concentrations at different time intervals were measured. On the other hand, hypouricemic activity of methanol:water extract (1:1) was experimented using different oral single doses (0.25, 0.5, 1 and 2 g/kg). Allopurinol was used as positive control. Uric acid concentration in serum was analyzed by using RP-HPLC at 280 nm.
Results: Sodium and potassium excretion increased significantly ($p < 0.05$ and <0.01) in the first 8 h of treatment with a single dose (2 g/kg) of the extracts in a pattern comparable to that of the known diuretic hydrochlorothiazide. Meanwhile, repeated administration of 0.5 g/kg methanol:water (1:1) extract showed a significant increase in urine volume (from day 3 to day 7) ($p < 0.01$) and electrolytes excretion (Na^+ and K^+) from day 2 to day 7 ($p < 0.05$ and <0.01). On the other hand, 0.5, 1 and 2 g/kg of methanol:water (1:1) extract and the standard allopurinol reduced the serum urate level in hyperuricemic rats at hour 6.
Conclusion: These results provided an evidence of the high tendency of methanol:water (1:1) extract of *Orthosiphon stamineus* towards diuretic and hypouricemic effects in rats.

1. Introduction

Orthosiphon stamineus Benth. (Labiatae), popularly known in Malaysia as 'Misai kucing', is one of the most popular traditional folk medicines used in Southeast Asia for treating kidney stone and other urinary tract diseases (Dat et al., 1992; Tezuka et al., 2000; Awale et al., 2001). In Indonesia, it is known as Java Tea and the decoction of its leaves was used as diuretic, where the daily prescribed dosage was 6–12 g of the herb as infusion (2–3 g/150 ml 2–3 times daily) or its equivalent preparation (Blumenthal et al., 1998; Bames et al., 2002). In view of that, *Orthosiphon* has gained the interest of many researchers in different parts of the world and was subjected to extensive phytochemical and few pharmacological studies since the 1930s. In Malaysia, the popularity of its use in treating stone disease and gout increased the potential of developing it into modern drug. These studies were an attempt to identify the constituents of *Orthosiphon stamineus* and investigate its pharmacological activities. Terpenoids (diterpenes and triterpenes), polyphenols (lipophilic flavonoids and phenolic acids) and sterols have been isolated from *Orthosiphon stamineus* leaf extract and their structures have been elucidated (Masuda et al., 1992; Hollman and Katan, 1999; Awale et al., 2002, 2003). Studies on the leaves of local *Orthosiphon stamineus* (Malaysian) led to the isolation and identification of several components, such as betulinic acid, 16-β-hydroxybetulinic acid and caffeic acid derivatives mainly rosmarinic acid, the most abundant polyphenol in the aqueous methanol extract of *Orthosiphon stamineus* leaves (Amzad and Zhari, 2003; Akowuah et al., 2004; Hossain et al., 2006). Moreover, 69 compounds representing 97.6% and 97.4% of the total leaves and stem oils of *Orthosiphon stamineus*, respectively, were identified, and the major components were, β-caryophyllene, caryophyllene

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oxide, α-humulene, β-pinene, limonene, β-elemene, and 1-octen-3-ol (Hossain et al., 2008). However, these extensive phytochemical studies were accompanied with only a little pharmacological evaluation for the biological properties of *Orthosiphon stamineus* constituents.

A few studies have been carried out to investigate the biological effects of *Orthosiphon stamineus*, such as diuretic, anti-oxidant and alleviating hyperglycemia and improving lipid profile in diabetic rats (Dat et al., 1992; Englert and Harnischfeger, 1992; Olah et al., 2003; Akowuah et al., 2004; Sriplang et al., 2007). These properties have partially been attributed to the polyphenolic compounds in *Orthosiphon stamineus* (Schut and Zwaring, 1993; Akowuah et al., 2004). Nevertheless, it is hard to find studies which explain the anti-lithiatic property of *Orthosiphon stamineus* and the correlation with its diuretic and anti-oxidant pharmacological properties. The common use of *Orthosiphon stamineus* in treating kidney stone and other disorders in the urinary tract deserves special attention from pharmacologists. Fifty percent methanol extract of *Orthosiphon stamineus* was found to inhibit the growth of calcium oxalate crystals, one of the major components of kidney stone, using modified gel slide method (Dharmaraj et al., 2006). Studies have shown that most of the herbs with anti-lithiatic activity exhibit a pronounced diuretic property (Selvam et al., 2001; Al-Ali et al., 2003; Afzal et al., 2004). The contribution of diuresis to the anti-lithiatic property of these herbs can be explained by their ability to increase urinary volume which will increase the solubility of calcium oxalate and other crystallizing salts, such as uric acid, which are known for their ability to induce epitaxical deposition of calcium oxalate. This increase in the urinary rate and volume (diuresis) will facilitate the removal of small crystals and reduce the chance of these crystals from growing or aggregating. Diuretics have been introduced and widely been accepted as anti-lithiatic agents. For example, thiazides, a potent group of diuretics, which have effectively been used for the treatment of hypercalciuric nephrolithiasis and prevention of renal calcific stone reported to produce a significant decrease in urinary oxalate excretion during long-term therapy in patients with calcium oxalate nephrolithiasis (Cohanim and Yendt, 1980). Only a few studies have been carried out to investigate the diuretic properties of *Orthosiphon stamineus* (Englert and Harnischfeger, 1992; Olah et al., 2003). These studies focused on aqueous alcoholic extract of *Orthosiphon stamineus* using a single dose (700 mg/kg of tincture) at a single time only (24 h) and comparing it with furosemide. However, no detailed study or clear cut quantitative data has been found on a single dose of *Orthosiphon stamineus* and its late diuretic effect by comparing it with the standard synthetic diuretic, hydrochlorothiazide.

The flushing away of crystals does not explain all that might be happening. Lithiasis is a complicated disease that involves complex pathophysiological steps where oxidation plays a key role (Resnick and Persky, 1995; Selvam, 2002). For example, formation of uric acid, the end product of purine metabolism in humans, is a result of oxidation processes including a series of enzymatic reactions involving xanthine oxidase at the final step. Studies show that uric acid plays a significant role in urinary lithiasis (Shekarriz and Stoller, 2002). In animals, uric acid is subsequently converted into allantoin by the action of uricase. Therefore, uric acid production can be controlled by two types of drugs, uricosuric agents and xanthine oxidase/dehydrogenase inhibitors. Inhibiting xanthine oxidase, the enzyme that converts xanthine and hypoxanthine to uric acid, or administering uricosuric agents, can be utilized in treating hyperuricemia, and also gout and lithiasis. Allopurinol, a xanthine oxidase inhibitor, is one of the drugs used to treat nephrolithiasis and other uric acid-associated disorders. The conversion of allopurinol to oxypurinol via the catalytic activity of xanthine oxidase forms the mechanism for its activity. However, these drugs have adverse effects which differ from one to another in term of severity.

The aims of the present study were first, to evaluate the diuretic effect of a methanol extracts of *Orthosiphon stamineus* in normal rats after a single oral administration of the extracts followed by examining the diuresis behavior of the most active extract upon prolonged administration. Second, as it was planned, to investigate the effect of methanol:water (1:1) extract at different doses on uric acid level in hyperuricemic rats. The two solvents were used because methanol alone would extract the less polar flavonoids and caffeic acid derivatives, whilst the 50% of methanol would be more useful to extract the glycosidal derivatives (Sumaryona et al., 1991; Schut and Zwaring, 1993). The daily recommended dose in traditional medicine for *Orthosiphon* species as Java Tea was 6–12 g per day, which is equivalent to 200 mg per kg specimen or 60 mg of the extract per kg. In rats this dose should be seven times higher as the metabolism rate in rat is seven times higher than in humans. Therefore, the recommended dose will be 420 mg extract per kg of rats. In our study, 0.5 g per kg rat's weight was used as the daily dose to be administered in the chronic model. Meanwhile, 2 g of extracts per kg was chosen for the single dose study and this lead to a dose ration rat:man of about 10:1 which is corresponding to the traditional use of *Orthosiphon*. The role of different constituents in *Orthosiphon stamineus* and the possible mechanisms of action are also discussed.

2. Material and methods

2.1. Plant material

Plants were grown from cuttings using standard agronomic practices at Kepala Batas, Penang, Malaysia. The leaves were collected in the late afternoon from white-flowered plants. Leaf specimen was labeled and annotated with the date of collection and locality. Voucher specimen (no. 027) was deposited at the herbarium, School of Biological Sciences, Universiti Sains Malaysia.

2.2. Preparation of extracts

The dried powdered leaves of *Orthosiphon stamineus* (1 kg) were extracted with methanol for 36 h in a Soxhlet extractor. After removal of methanol under reduced pressure, the remaining extract was freeze-dried. The resultant yield after freeze-drying process was 30% (w/w). Another batch of 1 kg of dried powdered leaves of *Orthosiphon stamineus* was macerated with methanol:water (1:1) at 60 °C for 20 h. The methanol was then removed under reduced pressure and the remaining liquid freeze-dried to yield 37% (w/w) of dried methanol:water (1:1) extract. For administration to the animals, each dried extract was dispersed in a mixture of saline and Tween-80 (9:1).

2.3. Chemical reagents

Methanol, ethyl acetate solvents (analytical grade) and perchloric acid were purchased from Merck, Germany. Allopurinol and potassium oxonate were purchased from Sigma-Chemical Co., USA. Markers, sinensetin (SIN), eupatorin (EUP), 3′-hydroxy-5,6,7,4′-tetramethoxyflavone (TMF), rosmarinic acid (RA) and caffeic acid (CA) were purchased from Indofine Chemical Co. (Hillsborough, USA).

2.4. High Performance Thin Layer Chromatography (HPTLC) profile of Orthosiphon stamineus extract

Chromatography was performed on pre-activated (100 °C) silica gel $60F_{254}$ HPTLC plates (20 cm × 10 cm; 0.25 mm layer thickness; Merck). CAMAG densitometry (Camag Model-3 TLC scanner equipped with Camag CATS 4 software) with a reflectance spectrometer of monitoring range 190–700 nm was employed for the analysis. The slit was set to 8 mm × 0.4 mm and data acquisition and processing were performed using the software winCATS. The samples and markers (20 μl) were applied to the layers as 10 mm wide bands, positioned 10 mm from the bottom of the plate, using a CAMAG (Mutten, Swizterland) Linomat IV automated TLC applicator with nitrogen flow providing delivery from the string at a speed of 4 μl/s which was maintained for all analyses. TLC plate development was performed using a CAMAG twin-trough glass tank, which had been pre-saturated with the mobile phase chloroform:ethyl acetate (30:70), for 2 h. Solvent was allowed to run up the plate to a height of 8 cm. TLC analyses were made under room temperature. After development, the layers were dried and the components were visualized under UV light at 365 nm.

2.5. Electrolyte contents of Orthosiphon stamineus methanol extracts

The methanol (MeOH) and methanol:water (1:1) (MeOH:water) extracts of *Orthosiphon stamineus* were dissolved in methanol and deionized distilled water, respectively. The content of sodium and potassium in g/l of both extracts was measured using flame photometer Corning M410 (Ciba Corning, UK).

2.6. Experimental animals

Adult male Sprague–Dawley rats (150–250 g) were used in this study. The animals were bred and housed in the animal house, School of Pharmaceutical Sciences, Universiti Sains Malaysia. Rats were housed individually in metabolic cages for several days with access to food and water *ad libitum*. The handling and use of animals was in accordance with the institutional guidelines. An approval was obtained from the Animal Ethic Committee, School of Pharmaceutical Sciences, Universiti Sains Malaysia.

2.7. Diuretic activity

The rats were randomly divided into four groups (n = 9–10 in each group) and housed individually in the metabolic cages for three days to accommodate with the environment. The rats were fasted overnight and were administered by gavage as follows: group I, control animals received normal tap water (25 mL/kg); group II, positive control animals received hydrochlorothiazide (10 mg/kg in water, 25 mL/kg); group III, treated animals received MeOH extract (2 g/kg in water, 25 mL/kg); and group IV, treated animals received MeOH:water (1:1) extract (2 g/kg in water, 25 mL/kg). The cages were equipped with bottles for drinking water and food *ad libitum*. The urine was collected at 2, 4, 6, 8 and 24 h. The volume of urinary output was measured for every single rat. The results were graphically illustrated as cumulative urine output per 100 g of body weight per rat. The urine pH was measured daily, whereas, sodium and potassium concentration were analyzed by a flame photometer.

For the chronic model conducted to examine the effect of MeOH:water (1:1) extract on diuresis parameters, the rats were randomly divided into three groups (n = 8 in each group) and housed individually in the metabolic cages for three days. Test solutions were administered to rats by gavage once daily as follows: group I, control animals received normal tap water (25 mL/kg); group II, treated animals received MeOH extract (0.5 g/kg in water, 25 mL/kg); group III, treated animal received MeOH:water (1:1) extract (0.5 g/kg in water, 25 mL/kg), every morning for seven consecutive days. The 24 h urine samples were collected daily, and the volume was measured for every single rat. The pH and electrolyte concentrations were determined for daily collected urine as mentioned above.

2.8. Hypouricemic effect in rats injected with uricase inhibitor

This experiment was performed according to the methodology of Osada et al. (1993). Hyperuricemia was induced in groups of six rats for each group by injecting the uricase inhibitor, potassium oxonate (250 mg/kg; intraperitoneally). An hour later, the rats were treated orally with MeOH:water (1:1) extract of *Orthosiphon stamineus* (2, 1, 0.5 and 0.25 g/kg), allopurinol 50 mg/kg and saline, respectively. Blood samples were collected from the tail veins at 0, 2, 4, 6 and 8 h after oral treatment. The blood samples which were allowed to clot for 0.5–1 h at room temperature were then centrifuged. The sera were stored at −20 °C until assay. The uric acid concentration in serum was analyzed using Reverse Phase High Pressure Liquid Chromatography (RP-HPLC).

2.8.1. Measurement of uric acid concentration by RP-HPLC

2.8.1.1. Sample preparation. Serum samples were thawed at room temperature and then mixed with the equivolume (200 μl) of 0.6 M perchloric acid. The samples were then centrifuged and diluted (1:4) with 0.02 M phosphate buffer (pH 3.7).

2.8.1.2. Marker preparation and calibration curve. A stock solution of uric acid (1 mg/ml) was accurately prepared by dissolving 50 mg of uric acid and 30 mg of lithium chloride in 15 ml of distilled water and then stirred at 60 °C for few minutes until a clear solution was obtained. The solution was then topped up to 50 ml with distilled water to get the final concentration of 1 mg/ml. A calibration curve was then established using five different concentrations (2, 4, 6, 8 and 10 μg/ml). The calibration curve was obtained by plotting the peak area (y) of the marker versus the concentration (x) and the linear regressions and correlation coefficients were computed off-line. The regression equation was obtained in the form

$$Y = mx + b$$

where y is the peak area, m the slope of the line generated by standard curve, x the concentration of analyte (μg/ml) and b is the y-intercept of line generated by standard curve.

2.8.1.3. Chromatographic condition and HPLC analysis. Uric acid level in serum samples was measured by using RP-HPLC. The HPLC system consisted of a Rheodyne 7125 manual injector valve with a 20 μl loop, a SPD-6A UV detector (Shimadzu, Japan) and a Hitachi D-2500 chromato integrator. Separation was carried out on a 250 mm × 4.6 mm i.d. LiChrosorb RP-18 column (5 μm particle size) at a flow rate of 1 mL/min, the mobile phase used was 0.02 M phosphate buffer (pH 3.7). Detection wavelength was 280 nm.

2.9. Data and statistical analysis

All data was expressed as mean ± standard error of mean (S.E.M.). Statistical analysis was performed using one-way ANOVA followed by Dunnett multiple comparison test. The statistical analysis was done using the computer program SPSS (Release 11.5, SPSS Inc., 2001). p-Value less than 0.05 was considered as significant.

Table 1
Effect of oral administration of hydrochlorothiazide 10 mg/kg, methanol (MeOH) and methanol:water (1:1) extracts 2 g/kg on pH, cumulative urinary volume and cumulative urinary excretion of sodium and potassium in rats

Time (h)	Control	Hydrochlorothiazide	MeOH	MeOH:water
pH				
2	8.4 ± 0.4	8.2 ± 0.1	9.0	8.7 ± 0.1
4	7.4 ± 0.2	7.8 ± 0.2	8.3 ± 0.3	7.5 ± 0.3
6	7.3 ± 0.3	7.5 ± 0.2	7.8 ± 0.3	7.8 ± 0.3
8	7.7 ± 0.3	7.5 ± 0.5	7.9 ± 0.1	8.4 ± 0.4
24	8.0 ± 0.1	8.0 ± 0.1	8.7 ± 0.3*	8.9 ± 0.1*
Cumulative urine volume (ml/100 g body weight)				
2	0.5 ± 0.1	1.9 ± 0.2*	0.8 ± 0.2	0.9 ± 0.2
4	1.0 ± 0.2	3.1 ± 0.3*	1.6 ± 0.5	1.4 ± 0.3
6	1.6 ± 0.3	4.1 ± 0.4*	1.9 ± 0.5	1.7 ± 0.3
8	1.8 ± 0.3	4.5 ± 0.4*	2.3 ± 0.5	2.3 ± 0.4
24	4.4 ± 0.4	7.2 ± 0.8	5.0 ± 1.5	4.9 ± 0.8
Na^+ excreted (mmol/100 g body weight)				
2	37.7 ± 12.8	200.6 ± 25.7*	111 ± 33.5	99.5 ± 23.2
4	96.1 ± 18.6	344.2 ± 35.9*	235.3 ± 52.2*	230.9 ± 35.4*
6	165.9 ± 23.2	476.4 ± 42.6*	313.4 ± 63.9	326.9 ± 45.6*
8	169.6 ± 21.6	485.5 ± 44.3*	364.5 ± 63.2*	380.6 ± 47.9*
24	555.3 ± 61.8	708 ± 51.5	605.4 ± 79.5	636.9 ± 48.6
K^+ excreted (mmol/100 g body weight)				
2	29.5 ± 9.8	79.1 ± 9.4*	85.9 ± 22.1*	117.9 ± 16.5*
4	71.3 ± 15.8	145 ± 10.9	167.8 ± 34.9*	185.4 ± 26.1*
6	115 ± 23.1	208.5 ± 23.9	208.3 ± 35.2	237.7 ± 40*
8	131.4 ± 21.8	213.9 ± 24.4	236.9 ± 31.4	293.2 ± 44.9*
24	501.2 ± 101.2	587.3 ± 117.3	459.9 ± 117.2	782.2 ± 128.5

Mean ± S.E.M. $n = 9$. Na^+: Sodium; K^+: potassium.
* $p < 0.05$ vs. control.

3. Results

3.1. HPTLC profile of the extracts

The presence of the markers (SIN, EUP, TMF, CA and RA) in *Orthosiphon stamineus* MeOH extract was confirmed by the HPTLC densitometric method. Marker compounds were spotted on plate in parallel to the MeOH and MeOH:water extracts of *Orthosiphon stamineus*. The chromatograms of the samples and markers were visualized under UV light at 365 nm. The chromatograms of the samples showed the presence of spots of the same colour and at the same R_f values as the markers. The similarity in the densitograms and R_f values between the methanol extracts and the markers confirmed the presence of these components in *Orthosiphon stamineus* extracts.

3.2. Electrolyte contents of Orthosiphon stamineus methanol extracts

The methanol:water extract of *Orthosiphon stamineus* was found to have a high potassium content (32 mmol/g). The methanol extract contained 14 mmol/g of potassium but no sodium was detected. Sodium content was found to be low (5 mmol/g) in the more polar MeOH:water (1:1) extract.

3.3. Acute diuretic activity

A single dose of MeOH and MeOH:water extracts of *Orthosiphon stamineus* exhibited no significant increase in urinary output over the first 24 h of treatment (Table 1). Meanwhile, the standard hydrochlorothiazide increased the urinary output at an early time (2 h) which was persisted until 8 h. However, an increase in sodium excretion as a result of single dose of MeOH and MeOH:water extracts of *Orthosiphon stamineus* was detected at hour 4 up to hour 8. This increment in sodium excretion although was not as much as that shown by the standard hydrochlorothiazide which started as early as hour 2 and persisted until hour 8.

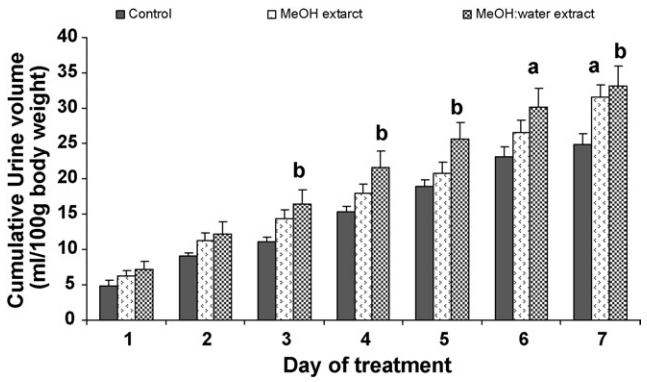

Fig. 1. Effect of MeOH and MeOH:water extracts of *Orthosiphon stamineus* at 0.5 g/kg on cumulative urine volume per 100 g of body weight per rat. Mean ± S.E.M., $n = 8$, (a and b) $p < 0.05$ and <0.01 vs. control, respectively.

On the other hand, the increase in potassium excretion was more predominant for MeOH:water extract than MeOH extract. Electrolytes excretion elevation declined after hour 8 for all extracts except for methanol extract which was after hour 4 for potassium excretion. The pH was significantly increased by MeOH extracts after 24 h (Table 1).

3.4. Effect of Orthosiphon stamineus methanol extracts on diuresis parameters upon repeated administration

3.4.1. Urine volume

The effects of repeated administration of 0.5 g/kg MeOH and MeOH:water (1:1) extracts of *Orthosiphon stamineus* on the cumulative urinary output (urine volume) are shown in Fig. 1. Chronic administration of MeOH:water extract at a dose of 0.5 g/kg increased the urinary output significantly from the third day compared to the negative control ($p < 0.01$). Meanwhile, the methanol extract at a dose of 0.5 g/kg showed a significant increase in cumulative urinary volume only at the seventh day of treatment ($p < 0.05$).

3.4.2. Urinary electrolytes

The effects of the MeOH and MeOH:water extracts of *Orthosiphon stamineus* on urinary electrolyte (Na^+ and K^+) excretion are shown in Table 2. Repeated oral administration of MeOH:water extract of *Orthosiphon stamineus* leaves significantly enhanced urinary excretions of sodium and potassium. The increase in sodium excretion was minor during the first three days but from the fourth day the augmentation of sodium excretion was clear ($p < 0.05$). The pattern was similar for potassium excretion, but the increase in potassium excretion was marked from the second day of treatment ($p < 0.05$) and continued until day 7 ($p < 0.01$).

3.4.3. Urine pH

The urine pH in MeOH extract treated rats increased on the second day ($p < 0.01$). After a fall on the third day, it increased again on the fifth and sixth days (Table 2). On the other hand, MeOH:water extract slightly increased the pH on the first day and again significantly on the fifth day ($p < 0.05$) and sixth day. Although the effect on urinary pH was not consistent, it still indicated that the changes could be related to the *Orthosiphon stamineus* extract.

Table 2
Effect of methanol (MeOH) and methanol:water (1:1) extracts at 0.5 g/kg on pH and cumulative urinary excretion of sodium and potassium in rats for seven consecutive days

Day	Control	MeOH	MeOH:water
pH			
1	7.1 ± 0.1	7.3 ± 0.1	7.3 ± 0.1
2	8.9 ± 0.1	9.5 ± 0.1*	9.2 ± 0.1
3	9.1 ± 0.1	8.9 ± 0.1	8.7 ± 0.3
4	8.6 ± 0.1	8.7 ± 0.1	8.8 ± 0.1
5	8.5 ± 0.1	8.7 ± 0.1	8.7 ± 0.1*
6	8.6 ± 0.1	8.8 ± 0.1	8.9 ± 0.1
7	8.5 ± 0.1	8.4 ± 0.1	8.4 ± 0.1
Na^+ excreted (mmol/100 g body weight)			
1	904.1 ± 144	1248.4 ± 124.3	1240.8 ± 306.6
2	1594.1 ± 327.7	2085 ± 190	2403.7 ± 371.9
3	2115.9 ± 416.4	2648.9 ± 226.6	3206.4 ± 347.8
4	2507.7 ± 499.6	3253.9 ± 214.4	4095.8 ± 365.7*
5	3006.5 ± 530.6	3607.9 ± 255.6	4756.3 ± 374.3*
6	3262.4 ± 621.3	4217.9 ± 218.1	5299.3 ± 500.4*
7	3324.6 ± 653.8	4537.6 ± 291.4	5918.2 ± 594.6*
K^+ excreted (mmol/100 g body weight)			
1	840.8 ± 157.1	860.4 ± 92	1066.2 ± 169.3
2	993.4 ± 278.8	1444.8 ± 140	1948.9 ± 169.3*
3	1409.6 ± 352.6	1794.5 ± 165.4	2541.7 ± 200.3*
4	1680.5 ± 450.9	2077.8 ± 191.3	3252.4 ± 253.5*
5	2007.7 ± 457.8	2327.9 ± 214.5	3864.4 ± 335.1*
6	2116.6 ± 498.9	2611.1 ± 207.5	4287.1 ± 459.5*
7	2224.8 ± 588.3	2768.4 ± 207.9	4681.1 ± 408.7*

Mean ± S.E.M. $n = 9$. Na^+: Sodium; K^+: potassium.
* $p < 0.05$ vs. control.

3.5. Hyperuricemic effect of potassium oxonate

Potassium oxonate (250 mg/kg, po) increased the uric acid level in rats. A sharp rise in serum urate level was noticed 3 h after induction. Although the urate level fell down, the hyperuricemic effect of potassium oxonate prolonged for the next few hours as shown in Fig. 2.

3.6. Hypouricemic effect of allopurinol and methanol:water extract of Orthosiphon stamineus

As shown in Table 3, allopurinol effectively decreased the uric acid level after administration and wash persisted throughout the experiment ($p < 0.05$ and 0.01). Methanol:water extract at 2, 1 and 0.5 g/kg only decreased the serum urate level in hyperuricemic rats after 6 h from the extract administration as shown in Table 3. Meanwhile, at a dose of 0.25 g/kg, a slight decrease in uric acid level was observed but it was not statistically significant. The hyperuricemic effect of uricase inhibitor, potassium oxonate, declined at 8 h.

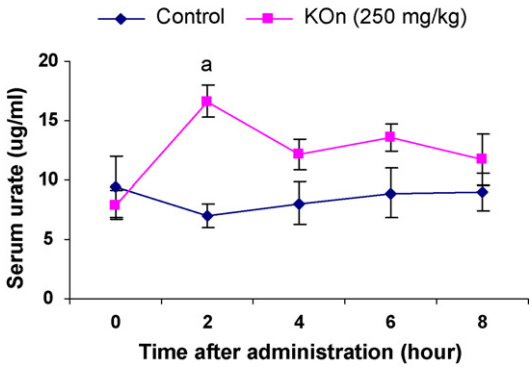

Fig. 2. Hyperuricemic effect of potassium oxonate (KOn) at 250 mg/kg i.p. on rats. Mean ± S.E.M., $n = 6$, (a) $p < 0.05$ vs. KOn.

4. Discussion

Orthosiphon stamineus Benth. (Labiateae) has widely been used in Southeast Asia for the treatment of kidney disorders, gallstone, gout and rheumatism. The popularity of its use in Malaysia for treating stone disease and gout increased its potential for being developed into a modern herbal product. Diuretics have been introduced and medically been used as prophylactic agents for urolithiasis (urinary stone), due to their key role in regulating kidney function and alleviating the urinary risk factors for stone formation. Biochemical investigations of serum and urine compositions in stone formers showed metabolic abnormalities, including hyperuricemia and hyperuricosuria, which have been found to play a role in disease development (Hesse et al., 1997). Therefore, inhibition of the excess production of uric acid, one of the metabolic abnormalities, has been introduced as an effective treatment in uric acid-related diseases; kidney stone and gout. This study has shown the diuretic and hypouricemic effects of methanol extracts of *Orthosiphon stamineus* using animal models.

Results showed that single doses of 2 g/kg of MeOH and MeOH:water extracts of *Orthosiphon stamineus* were unable to cause a significant increase in urinary output within 24 h in treated rats though a significant increase in electrolytes excretion was detected. The excretion of sodium and potassium at an early time (4 h after administration) was more pronounced and predominant with MeOH:water extract and comparable to the synthetic diuretic; hydrochlorothiazide, but with a lower profile. This similarity in the natriuretic effect of MeOH:water extract with the standard diuretic hydrochlorothiazide may reflect the tendency of *Orthosiphon stamineus* to have a diuretic property similar to that of hydrochlorothiazide upon prolonged administration.

Table 3
Effect of allopurinol (50 mg/kg, p.o.) and methanol:water (MeOH) (1:1) extract of *Orthosiphon stamineus* at 2, 1, 0.5 and 0.25 g/kg on serum urate level in hyperuricemic rats at 0, 2, 4, 6 and 8 h

Treatment	Time after administration (h)				
	0	2	4	6	8
KOn (250 mg/kg)	7.9 ± 1.2	16.6 ± 1.3	12.1 ± 1.3	13.6 ± 1.1	11.7 ± 2.2
KOn + allopurinol (50 mg/kg)	9.1 ± 1.0	9.9 ± 2.2*	3.2 ± 0.2*	2.9 ± 0.5*	4.2 ± 0.9*
KOn + MeOH:water 1:1 (2 g/kg)	9.5 ± 1.1	13.9 ± 1.6	9.2 ± 1.9	7.4 ± 0.7*	9.8 ± 1.4
KOn + MeOH:water 1:1 (1 g/kg)	9.4 ± 1.5	13.4 ± 0.9	11.4 ± 2.1	7.6 ± 1.1*	9.5 ± 2.1
KOn + MeOH:water 1:1 (0.5 g/kg)	9.9 ± 1.9	17.8 ± 1.9	11.5 ± 1.7	7.9 ± 0.8*	9.7 ± 1.8
KOn + MeOH:water 1:1 (0.25 g/kg)	8.3 ± 0.9	20.3 ± 2	14.6 ± 3.8	9.5 ± 1.9	10.8 ± 1.8

Mean ± S.E.M., $n = 6$. KOn: Potassium oxonate.
* $p < 0.05$ vs. KOn.

The absence of a significant diuresis activity in herbal extracts, including *Orthosiphon* species, upon single doses while these effects appeared upon prolonged administration was noticed and explained (Englert and Harnischfeger, 1992; Haloui et al., 2000; Jouad et al., 2001; Galati et al., 2002). These findings have inspired the present study to find out whether repeated administrations of MeOH extracts to rats over a period of seven days have any effect on diuresis parameters.

The results clearly showed that repeated oral administration of a MeOH:water extract at a dose of 0.5 g/kg caused a remarkable time-dependent increase in urinary output and electrolytes excretions. In contrast, MeOH extract has shown a delay effect. The prominent effect of MeOH:water extract on urinary output and electrolytes excretion over MeOH extract demonstrate a relation between these prominent effects and the presence of more polar components. The contribution and responsibility of aqueous extracts, aqueous (caffeic acid derivatives; RA) and semi-polar components (flavonoids; SIN, EUP, TMF and tetra-*O*-methylscutellarein) to diuretic properties have been reported (Bombardelli et al., 1972; Schut and Zwaring, 1993; Shetty, 1997; Haloui et al., 2000; Jouad et al., 2001; Galati et al., 2002; Kreydiyyeh and Usta, 2002; Makino et al., 2002; Olah et al., 2003). According to our chromatographic analysis, flavonoids and RA were found in MeOH:water extract. Therefore, it is worth noting that both flavonoids and caffeic acid derivatives, such as RA may account for the prominent effect of the extract over the diuresis parameters and these component(s) may act synergistically (Dat et al., 1992; Olah et al., 2003). Therefore, conducting further experiment to determine the chronic diuretic effect of *Orthosiphon stamineus* comparing to a standard diuretics, such as hydrochlorothiazide or furosemide, is highly recommended.

The presence of potassium in urinary output and also in *Orthosiphon stamineus* leaves, as our results showed, may have an explanation. Though it may be early to discuss, it should be noted that the remarkable increase in potassium excretion upon repeated administration of MeOH:water extract suggests a correlation between its potassium content and the pronounced effect on the diuresis parameters (De Ribeiro et al., 1988). It has been reported that inhibition of sodium reabsorption in the more proximal segments will cause an increase in distal delivery and increases potassium secretion into the tubular lumen in a flow-dependent manner (Shinkawa et al., 1993). More investigations are desirable to identify clearly whether or not potassium may play a role in some of the biological properties of *Orthosiphon stamineus*.

It should be mentioned that the increase in the urinary excretion volume (diuresis) will facilitate the removal of small crystals and reduce the chance of these crystals to grow or aggregate which has been explained for certain herbal exhibiting anti-lithiatic activity (Selvam et al., 2001; Al-Ali et al., 2003; Afzal et al., 2004). On the other hand, reducing uric acid production, a crystallization salt which may facilitate the heterogeneous nucleation of the calcium oxalate stone, will not only exert anti-gout effect but also will contribute to the anti-lithiatic property and other uric acid-related diseases (Spector, 1977; Finlayson and Reid, 1978; Kong et al., 2002; Ishibuchi et al., 2001). Therefore, we have used hyperuricemic rats as a model to investigate the ability of *Orthosiphon stamineus* to reduce uric acid production. Our results showed that MeOH:water extract reduced the serum urate level after 6 h of treatment. The xanthine oxidase inhibitor, allopurinol, has been used in our study as standard to compare its activity with *Orthosiphon stamineus* extract. Methanol:water extract showed a marked decrease in uric acid formation as late as 6 h comparing to the more effective allopurinol which may indicate a level of similarity between *Orthosiphon stamineus* and the standard been used.

High levels of flavonoids, triterpenoids and caffeic acid derivatives, such as eupatorin, sinensetin, rutin, TMF, betulinic acid, caffeic acid and rosmarinic acid have been reported in *Orthosiphon stamineus* and their anti-oxidant properties have also been documented (Akowuah et al., 2004; Amzad and Zhari, 2003). Therefore, it is believed that the presence of these components in MeOH:water extract may contribute to its hypouricemic activity via their anti-oxidant properties. Yet, studying the effect of MeOH:water extract and its constituents on xanthine oxidase enzyme becomes essential to proof this assumption.

5. Conclusion

In conclusion, this study showed the high tendency of *Orthosiphon stamineus* towards diuretic property and provided an evidence for hypouricemic activity of *Orthosiphon stamineus* MeOH:water extract using animal models. It appears that the more polar MeOH:water extract, the more prominent is the diuretic activity in MeOH extract. More than one mechanism can possibly be involved and more than one component might be responsible for the activity whereby these component(s) may act synergistically (Capasso et al., 2000).

This study also showed the effectiveness of MeOH:water extract of *Orthosiphon stamineus* in reducing uric acid level in serum. The presence of flavonoids, triterpenes and caffeic acid derivatives in *Orthosiphon stamineus* is responsible for its activities. This may explain some of the reasons behind its anti-lithiatic property.

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References

Afzal, M., Khan, N.A., Ghufran, A., Iqbal, A., Inamuddin, M., 2004. Diuretic and nephroprotective effect of Jawarish *Zarooni Sada*—a polyherbal unani formulation. Journal of Ethnopharmacology 91, 219–223.

Akowuah, G.A., Zhari, I., Norhayati, I., Sadikun, A., Khamsah, S.M., 2004. Sinensetin, eupatorin, 3′-hydroxy-5,6,7,4′-tetramethoxyflavone and rosmarinic acid contents and antioxidant effect of *Orthosiphon stamineus* from Malaysia. Food Chemistry 87, 559–566.

Al-Ali, M., Wahbi, S., Twaij, H., Al Badr, A, 2003. *Tribulus terrestris*: preliminary study of its diuretic and contractile effects and comparison with *Zea mays*. Journal of Ethnopharmacology 85, 257–260.

Amzad, H.M., Zhari, I., 2003. Hydroxy betulinic acid from the leaves of *Orthosiphon stamineus*. Asian Coordinating Group for Chemistry 16, 69–73.

Awale, S., Tezuka, Y., Banskota, A.H., Kouda, K., Tun, K.M., Kadota, S., 2001. Five novel highly oxygenated diterpenes of *Orthosiphon stamineus* from Myanmar. Journal of Natural Products 64, 592–596.

Awale, S., Tezuka, Y., Banskota, A.H., Shimoji, S., Taira, K., Kadota, S., 2002. Norstaminane- and isopimarane-type diterpenes of *Orthosiphon stamineus* from Okinawa. Tetrahedron 58, 5503–5512.

Awale, S., Tezuka, Y., Banskota, A.H., Kadota, S., 2003. Siphonols A–E: novel nitric oxide inhibitors from *Orthosiphon stamineus* of Indonesia. Bioorganic and Medicinal Chemistry Letters 13, 31–35.

Bames, J., Anderson, L.A., Phillipson, J.D., 2002. Herbal Medicines. Pharmaceutical Press, London, p. 313.

Blumenthal, M., Busse, W.R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C.W., Rister, R.S., 1998. The Complete German Commission E Monographs; Therapeutic Guide to Herbal Medicine. Integrative Medicine Communications, Boston, p. 155.

Bombardelli, E-Bonati, A., Gabetta, B., Mustich, G., 1972. Flavonoid constituents of *Orthosiphon stamineus* Benth. Fitoterapia 43, 35–40.

Capasso, R., Izzo, A., Pinto, L., Bifulco, T., Vitobello, C., Mascolo, N., 2000. Phytotherapy and quality of herbal medicines. Fitoterapia 71, S58–S65.

Cohanim, M., Yendt, E.R., 1980. Reduction of urine oxalate during long-term thiazide therapy in patients with calcium urolithiasis. Investigative Urology 18, 170–173.

Dat, D.D., Ham, N.N., Khae, D.H., Lam, N.T., Son, T., Dau, N.V., Grabe, M., Johansson, R., Lindgren, G., Stjernström, N.E., 1992. Studies on the individual and

combined diuretic effects of four Vietnamese traditional herbal remedies (*Zea mays*, *Imperata cylindrical*, *Plantago major* and *Orthosiphon stamineus*). Journal of Ethnopharmacology 36, 225–231.
De Ribeiro, R.A., De Barros, F., De Melo, M.M.R.F., Muniz, C., Chieia, S., Wanderley, M., Das, G., Gomes, C., Trolin, G., 1988. Acute diuretic effects in conscious rats produced by some medicinal plants used in the state of Sao Paulo, Brasil. Journal of Ethnopharmacology 24, 19–29.
Dharmaraj, S., Hossain, M.A., Zhari, S., Harn, G.L., Ismail, Z., 2006. The use of principal component analysis and self-organizing map to monitor inhibition of calcium oxalate crystal growth by *Orthosiphon stamineus* extract. Chemometrics and Intelligent Laboratory Systems 81, 21–28.
Englert, J., Harnischfeger, G., 1992. Diuretic action of aqueous *Orthosiphon* extract in rats. Planta Medica 58, 237–238.
Finlayson, B., Reid, F., 1978. The effect of allopurinol on calcium oxalate (whewellite) precipitation. Investigative Urology 156, 489–492.
Galati, E.M., Tripodo, M.M., Trovato, A., Miceli, N., Monoforte, M.T., 2002. Biological effect of *Opuntia ficus* (L). Mill. (Cactaceae) waste matter. Note I: diuretic activity. Journal of Ethnopharmacology 79, 17–21.
Haloui, M., Louedec, L., Michel, J.-B., Lyoussi, B., 2000. Experimental diuretic effects of *Rosmarinus officinalis* and *Centaurium erythraea*. Journal of Ethnopharmacology 71, 465–472.
Hesse, A., Tiselius, H.-G., Jahnen, A., 1997. Urinary stones: Diagnosis, Treatment and Prevention of Recurrence. KargerBasel, Switzerland.
Hollman, P.C., Katan, M.B., 1999. Dietary flavonoids: intake, health effects and bioavailability. Food Chemistry and Toxicology 37, 937–942.
Hossain, M.A., Salehuddin, S.M., Ismail, Z., 2006. Rosmarinic acid and methyl rosmarinate from *Orthosiphon stamineus* Benth. Journal of Bangladesh Academy of Sciences 30, 167–172.
Hossain, M.A., Ismail, Z., Rahman, A., Kang, S.C., 2008. Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth. Industrial Crops and Products 27, 328–334.
Ishibuchi, S., Morimoto, H., Oe, T., Ikebe, T., Inoue, H., Fukunari, A., Kamezawa, M., Yamada, I., Naka, Y., 2001. Synthesis and structure-activity relationships of 1-phenylpyrazoles as xanthine oxidase inhibitors. Bioorganic and Medicinal Chemistry Letters 11, 882–897.
Jouad, H., Lacaille-Dubois, M.A., Eddouks, M., 2001. Chronic diuretic effect of the water extract of *Spergularia purpurea* in normal rats. Journal of Ethnopharmacology 75, 219–223.
Kong, L.D., Cai, Y., Huang, W.W., Christopher, H.K., Cheng, Tan, R.X., 2002. Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. Journal of Ethnopharmacology 73, 199–207.
Kreydiyyeh, S.I., Usta, J., 2002. Diuretic effect and mechanism of action of parsley. Journal of Ethnopharmacology 79, 353–357.
Makino, T., Ono, T., Liu, N., Nakamura, T., Muso, E., Honda, G., 2002. Suppressive effects of rosmarinic acid on mesangioproliferative glomerulonephritis in rats. Nephron 92, 898–904.
Masuda, T., Masuda, K., Shiragami, S., Jitoe, A., Nakatani, N., 1992. Orthosiphol A and B, novel diterpenoid inhibitors of TPA (12-O-tetradecanoylphorobol-13-acetate)-induced inflammation, from *Orthosiphon stamineus*. Tetrahedron 48, 6787–6792.
Olah, N.K., Radu, L., Mogoan, C., Hanganu, D., Gocan, S., 2003. Phytochemical and pharmacological studies on *Orthosiphon stamineus* Benth. (Lamiaceae) hydroalcoholic extracts. Journal of Pharmacy and Biomedical Analysis 33, 117–123.
Osada, Y., Tsuchimoto, M., Fukushima, H., Takahashi, K., Kondo, S., Hasaichi, M., Komoriya, K., 1993. Hypouricemic effect of the novel xanthine oxidase inhibitor, TEI-6720, in rodents. European Journal of Pharmacology 241, 183–188.
Resnick, M.I., Persky, L., 1995. Summary of the national institutes of arthritis, diabetes, digestive and kidney diseases conference on urolithiasis: state of the art and future research needs. Journal of Urology 153, 4–9.
Schut, G.A., Zwaring, J.H., 1993. Pharmacological investigation of some lipophilic flavonoids from *O. aristatus*. Fitoterapia 2, 99–102.
Selvam, R., 2002. Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. Urological Research 30, 35–47.
Selvam, R., Kalaiselvi, P., Govindaraj, A., Bala Murugan, V., Kumar, S.S., 2001. Effect of *Alanata* leaf extract and *Vediuppu chunnam* on the urinary risk factors of calcium oxalate urolithiasis during experimental Hyperoxaluria. Pharmacological Research 43, 89–93.
Shekarriz, B., Stoller, M.L., 2002. Uric acid nephrolithiasis: current concepts and controversies. The Journal of Urology 168, 1307–1314.
Shetty, K., 1997. Biotechnology to harness the benefits of dietary phenolics; focus on Lamiaceae. Asia Pacific Journal of Clinical Nutrition 6, 162–171.
Shinkawa, T., Yamasaki, F., Notsu, T., Nakakuki, M., Nishijima, K., Yoshitomi, K., Imai, M., 1993. Loop and distal actions of a novel diuretic, M17055. European Journal of Pharmacology 238, 317–325.
Spector, T., 1977. Inhibition of urate production by allopurinol. Biochemical Pharmacology 26, 355–358.
Sriplang, K., Adisakwattana, S., Rungsipipat, A., Yibchok-anun, S., 2007. Effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 09, 510–514.
Sumaryona, W., Proksch, P., Hartmann, T., Nimtz, M., Wray, V., 1991. Induction of rosmarinic acid accumulation in cell suspension cultures of *Orthosiphon aristatus* after treatment with yeast extract. Phytochemistry 30, 3267–3271.
Tezuka, Y., Stampoulis, P., Banskota, A.H., Awale, S., Tran, K.Q., Saiki, I., Kadota, S., 2000. Constituents of the Vietnamese Medicinal Plant *Orthosiphon stamineus*. Chemical and Pharmaceutical Bulletin 48, 1711–1719.