Research Article

Diuretic Activity of Roots from *Carica papaya* L. and *Ananas comosus* L.

Y.Adam[ab], A.A. Nasaruddin[c], A. Zuraini[a], A.K. Arifah[d], M.S. Omar Fauzee[e] Z.A. Zakaria[a], M.N. Somchit[af*]

[a] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia.
[b] Sports Academy, University Putra Malaysia, Serdang, Selangor, Malaysia.
[c] Faculty of Medicine, Cyberjaya University College of Medical Sciences, Cyberjaya, Malaysia.
[d] Faculty of Veterinary Medicine, University Putra Malaysia, Serdang, Selangor, Malaysia.
[e] Northen University of Malaysia, Sintok Kedah, Malaysia.
[f] Institute of Halal Product Research, University Putra Malaysia, Serdang, Selangor, Malaysia.
*Corresponding author's E-mail: nhareet@medic.upm.edu.my

Accepted on: 28-08-2013; **Finalized on:** 31-10-2013.

ABSTRACT

Water extracts of *Carica papaya* and *Ananas comosus* extracts were administered orally at doses of 5 and 10 mg/kg to Spraque-Dawley rats. Two other groups were given commercial diuretics, furosemide and hydrochlorthiazide at 10 mg/kg. Urine volume, urine pH, urine density and urine electrolytes were determined every hour for 4 hours. Blood was taken to determine the serum level of glucose, albumin, blood urea nitrogen (BUN) and creatinine. Both, *C. papaya* and *A. comosus* extracts exhibited moderate to potent diuretic activity. C. papaya extract increased the 4-hour urine volume when administered at both 5 and 10 mg/kg. *A. comosus* extracts increased urine volume only at 10 mg/kg dose. Na^+ and Cl^- elimination was unchanged for the whole period of study. However, the 4-hour urinary excretions of K^+ were significantly increased for both plant extracts. Both plant extracts induced increment of serum BUN and creatinine level significantly when compared to controls ($p<0.05$) but these levels were still within the normal range. Blood glucose level and albumin were not significantly for both plant extract. In conclusion, both plants investigated had diuretic activity and roots of *C. papaya* activity were statistically similar potency to the activity of furosemide and hydrochlorothiazide. However, care must be taken when using these herbs as increased urinary K^+ being excreted and marginal increase of serum BUN and creatinine were detected.

Keywords: Word diuretic, electrolytes, *Carica papaya*, *Ananas comosus*.

INTRODUCTION

Medicinal plants are used for many various diseases. Plants commonly used for traditional treatment of renal diseases have been reported to show significant diuretic activity[1]. Herbal plants may also be a useful tool in the treatment of hypertension[2]. These previous studies emphasized the application of plants as a diuretic agent in treatment of dysuria and hypertension. Currently, commercial synthetic diuretic drugs are prescribed in the treatment hypertension, congestive heart failure and other cardiovascular diseases[3].

Carica papaya or 'betik' in Malay is a pantropical, small, soft-wooded, usually unbranched tree, 7 to 8 meter tall, lactiferous, fast growing and short lived. Large glabrous palmitifid and palmatinerved leaves (30-60 cm) and flowers are aromatic. Fruits are large ovoid and smooth, green while young and yellow-greenish or orange when ripe[4]. *Ananas comosus* or 'nanas' in Malay is perennial, stout herbaceous, sometimes spinescent succulent and up to 1 m tall. It producing numerous, elongated, finely toothed, long, sword-like, arranged in a tight spiral around a short stem, edges very sharply dentate to nearly entire, often variegated, or red or brown streaked. Stems are erect and long. Flowers are purplish-blue, timorous, and progressive toward apex of stem, with oldest flowers at base of inflorescence. A fruit is cone-like, spirally gathered into a large ovoid fleshy, edible mass crowned by persistent bracts[4].

Even though these plants have been used in traditional medicine for centuries, the mechanism of actions is yet to be discovered. This present study was aimed to evaluate the diuretic activity of the aqueous extract of the *Carica papaya* and *Ananas comosus* and elucidate the possible mechanism of action of diuretic activity of plant extracts.

MATERIALS AND METHODS

Plants materials and Preparation of the extracts

The plants were collected from Sepang District, Selangor, Malaysia and were verified. Samples were deposited in the Phytomedicinal Herbarium Institute of Bioscience, Universiti Putra Malaysia. The plants were prepared using the method describes previously by Sripanidkulchai *et al.*,[5] and Zakaria *et al.*,[6] with slight modification. Briefly, the freshly collected roots were washed and weighed. After that the parts were cut in small and dried at 50 to 60°C for 5 days. The dried parts were weighed and then grinded to obtain the powder form of plants. The dried powders of plants were soaked in 2 liter of distilled water for 2 hours at room temperature. Then, the mixture boiled until the volume was decreased to one-third of the initial volume. After boiling, the mixture was cooled and kept at 60 to 80°C for an hour. The mixture was filtered using Whatman filter paper No.1 and the filtrate collected. The filtrate was then freezing dried and final

extract was stored at minus 20°C prior to use (yield: *C.papaya* 14.8% w/w and *A.comosus* 16% w/w). The extracts were reconstituted with distilled water prior to diuretic assessment in different concentration of the treatment.

Animals and Experimental Design

Twenty-one adult male Spraque-Dawley rats (180-200 g) were housed in cages of three. They were first acclimatized to the laboratory environment for 7 days prior to experiment. They were maintained under standard and uniform laboratory condition with free access to rat pellet diet and water *ad libitium*. All animals were fasted overnight prior to experiment. All experiments are approved by the University Animal care and use committee (ACUC).

Diuretic assessment

Method of Sripanidkulchai *et al.*,[5] and Adam *et al.*,[7] were used for the determination of diuresis. Extracts and drugs were administered orally. Animals were given bicarbonate saline solution (40 ml/kg) 30 minutes before the experiment. They were divided into 7 groups and placed in an individual metabolic cage. Negative control (Group 1) was given 1 ml distilled water orally. The positive control groups (Group 2 and 3) were given furosemide and hydrochlorothiazide (Sigma Chemicals, US) at 10 mg/kg in distilled water respectively. Treatment groups (Groups 4-7) were given plants extracts at a dose of 5 and 10 mg/kg for each rat. Animals were then put into metabolic cages individually. Water and feed were available to the animals. Urine sample were collected and measured hourly for 4 hours. Urine density and urine pH were also measured hourly. The content of urinary electrolytes that included sodium (Na^+), potassium (K^+) and chloride (Cl^-) was determined by using ion selective electrodes (ISE) automatic analyzer (Radiometer EML 100, Copenhagen).

Blood serum analysis

Rats were anesthetized using pentobarbitone (50 mg/kg ip) and intracardiac blood samples (3 to 5 ml) were taken[8]. Rats eventually sacrificed by cervical dislocation. Blood samples were centrifuged at 4000 rpm for 10 minutes. The serum was separated and frozen at -20°C for analysis of glucose, albumin, blood urea nitrogen (BUN) and creatinine level. These tests were performed with an automatic chemical analyzer (Hitachi Roche 902, Japan)[9].

Statistical Analysis

The results are presented as mean ± standard error of mean (S.E.M). All assessment data were analyzed using One-way analysis of variance (ANOVA) and result will be considered significant if ($p<0.05$). Post hoc test that used is Duncan Test that can be determined significant statistically difference between the group of experiment[10].

RESULTS AND DISCUSSION

Urine Volume

Table 1 shows volume of all treatment groups. The extract of *C. papaya* (10 mg/kg) provided maximum excreted volume which was similar to that of positive control; furosemide and hydrochlorothiazide. On the other hand, the extract from *A. comosus* showed less potent diuretic action.

Urinary electrolytes

The amount of urinary sodium, potassium and chloride were measured every hour from the collected urine, as shown in Table 2. The positive control groups; furosemide and hydrochlorothiazide, excretion of sodium and chloride were markedly increased every hour throughout the experiment compared to negative control. *C. papaya* (10 mg/kg) and *A. comosus* (5mg/kg) were slightly increased urinary sodium excretion compared to the negative control. In the other hand, *A. comosus* (10mg/kg) had the similar pattern of sodium excretion to the negative control group. The extract from *C. papaya* (5 mg/kg) produced reduced amount of the urine sodium compared to the negative control.

Both plant extracts showed markedly increased amount of the urine potassium every hour throughout the experiment compared to the negative control. Total urinary potassium for both plants aqueous extracts are significant compared to the negative control. *C. papaya* aqueous extracts revealed dose-dependently effects; however, *A. comosus* activity was dose-independently, lower dose (5 mg/kg) of *A. comosus* more potent compared to the high dose (10 mg/kg). Among the two plant extracts tested, a higher amount of the urinary potassium excretion was observed in the group that treated with *A. comosus* at dose 5 mg/kg during the third hour.

Both plant extracts showed moderate increased amount of the urine chloride every hour throughout the experiment compared to the negative control; but the difference was not statistically significant. The urinary chloride excretion profiles of both plant extracts were similar.

Blood serum analysis

The effects on glucose, albumin, blood urea nitrogen (BUN) and creatinine concentration in rat's blood serum treated with aqueous extracts of plants are summarized in Table 3. The concentration of blood serum glucose, albumin, BUN and creatinine were measured after the diuretic assessment. The result showed that both plants extract slightly increased the concentration of glucose. *C. papaya* extract at dose 5 mg/kg revealed decreased glucose concentration. However, the change was not statistically significant relative to negative control.

Both plant extracts were markedly increased the albumin, BUN and creatinine concentration, statistically significant relative to negative control. The action of *A. comosus* was

dose-independent to albumin and creatinine concentration. Otherwise was dose-dependent to BUN concentration. *C. papaya* was dose-independents to albumin, BUN and creatinine concentration.

This study revealed that the aqueous extracts from the roots of *C. papaya and A. comosus* have a demonstrable diuretic activity. Furosemide caused the expected increase in the urine volume, renal excretion of Na^+ and K^+, whereas had relatively no effect on Cl^- loss. Furosemide acts by inhibiting electrolyte reabsorption in the thick, ascending limp of the loop of Henle[11,12]. On the other hand, hydrochlorothiazide enhanced urine output, Na^+ and Cl^- loss significantly. Hydrochlorothiazide inhibits sodium and chloride reabsorption in the distal tubules[5,7,13]. The diuretic actions of these both extracts were less potent than that of positive control; furosemide and hydrochlorothiazide, except to *C. papaya* extract at dose 10 mg/kg. *C. papaya* extract at dose 5 mg/kg caused a significant increase of urinary output. The increment of urine volume is qualitatively similar potency to hydrochlorothiazide and furosemide[14] and the elevated of potassium ions is more potent than the positive control. The effect of diuretic activity of *C. papaya* extract was dose-independent, whereas *A. comosus* extract is dose-dependent.

Table 1: Effects of plant extracts on urine volume

Treatment	Dose (mg/kg)	Urine volume (4 hr in ml/kg body weight)	Diuretics action[1]
Distilled water (control)	-	1.073 ± 0.54[a]	1
Forusemide	10	25.071 ± 4.79[c]	23.43
Hydrochlorothizide	10	23.163 ± 0.61[c]	21.64
C.papaya	5	11.718 ± 2.17[b]	10.95
C.papaya	10	23.774 ± 2.50[c]	22.21
A.comosus	5	7.313 ± 2.16[b]	6.83
A.comosus	10	12.092 ± 3.99[b]	11.3

Each value represents the mean ± S.E.M; [1] Diuretics action = (urinary excretion of treated group (4 hr)) / (Urinary excretion of control group (4 h))
[a-c] Mean with different superscripts significantly at (P< 0.05) All assessment data were analyzed using One-way analysis of variance (ANOVA) and Duncan Test is used to determine differences between the treatment means.

Table 2: Total excretions over a period of 4 hour of urinary electrolytes

Treatment	Dose (mg/kg)	Na^+ (mol)				K^+ (mol)				Cl^- (mol)			
		Hr 1	Hr 2	Hr 3	Hr 4	Hr 1	Hr 2	Hr 3	Hr 4	Hr 1	Hr 2	Hr 3	Hr 4
Distilled water	-	ND	17± 0.1	20.5± 1.22	ND	ND	67.3 ±0.11	80.1 ±10.86	ND	ND	17 ± 0.32	31± 6.53	ND
Forusemide	10	139.67 ± 11.9	140 ±3.21	117.33 ± 2.73	157.5 ± 8.57	142.3 ±21.47	77.3 ±28.82	47.95 ±11.23	24.87 ±3.74	188 ± 8.98	163 ± 33.4	133.5 ± 4.1	135.1 ± 4.5
Hydrochlorothizide	10	159 ± 28.36	227± 43.92	175.67 ±24.88	174.5 ±15.11	207.47 ±45.76	47.03 ±10.23	38.8 ±4.16	85.6 ± 7.92	142.3 ±16.4	115.6± 11.3	110± 15.3	205± 22.86
C. papaya	5	13 ± 1.63	6 ± 0.82	9.33 ±3.38	10.33 ±3.18	246.5 ±10.12	99.65 ±2.16	202.47 ±100.6	190.9 ±76.47	38 ± 2.45	14.5 ± 0.41	24± 8.0	36± 14.84
C. papaya	10	30.6 ± 11.2	37.67 ±13.9	15± 0.817	17 ± 3.27	382.3 ±21.7	404.43 ±18.24	186.245 ±131.58	302.1 ±16.25	31.3 ± 2.9	35 ± 7.57	20.5 ±5.3	19.67± 3.84
A. comosus	5	37 ± 0.21	28 ± 2.89	28 ±0.1	22.5± 0.41	122 ± 12.5	359.43 ± 54.05	527.9 ±8.0	431.2 ±4.31	28.5 ± 4.5	30.67 ±3.67	54± 13.8	40 ± 15.51
A. comosus	10	23 ± 0.13	16.5 ±4.49	17.33 ±9.35	25.0± 1.34	131.2 ±7.34	59.45 ±36.78	340.43 ±139.2	420.3 ±32.2	20 ± 1.32	28.0 ±1.63	46± 20.8	38 ± 5.0

Each value represents the mean ± S.E.M: ND-Not determined (No urine)

Table 3: Mean concentration of glucose, albumin, Blood Urea Nitrogen (BUN) and creatinine of rat's blood serum.

Treatment	Dose (mg/kg)	Mean Glucose Conc. (mmol/L)	Mean Albumin Conc. (g/dL)	Mean BUN Conc. (mg/dL)	Mean Creatinine Conc. (mg/dL)
Distilled water	-	5.26 ± 0.524	24.0 ± 3.279[a]	3.86 ± 0.374[a]	0.59 ± 0.079[a]
Forusemide	10	5.78 ± 0.246	28.43 ± 2.563[a]	5.08 ± 0.421[a]	0.748 ± 0.059[a]
Hydrochlorothizide	10	4.13 ± 0.722	16.1 ± 2.933[a]	3.18 ± 0.709[a]	0.43 ± 0.090[a]
C.papaya	5	4.32 ± 0.233	34.23 ± 1.047[b]	8.87 ± 0.524[b]	0.842 ± 0.031[b]
C.papaya	10	6.42 ± 0.826	32.72 ± 2.299[b]	5.8 ± 0.277[a]	0.792 ± 0.041[b]
A.comosus	5	6.03 ± 0.850	35.28 ± 1.164[b]	11.65 ± 0.542[b]	0.87 ± 0.053[b]
A.comosus	10	5.23 ± 0.067	29.23 ± 0.983[a]	21.67 ± 0.261[b]	0.767 ± 0.015[b]

Each value represents the mean ± S.E.M; [a-b] mean with different superscript differ significantly at (P<0.05); All assessment data were analyzed using One-way analysis of variance (ANOVA) and Duncan Test is used to determine differences between the treatment means.

The mechanism of action by which diuresis was induced by the plant extracts was also investigated by comparing with standard reference drugs, furosemide (a high-ceiling loop diuretic) and Hydrochlorothiazide (a carbonic anhydrase inhibitor)[12,15]. In general, diuretics act as by increasing the quantity of the sodium ion in the urine, the increased salt excretion being accompanied by an increased water excretion to maintain osmotic balance. Diuretics actions through three ways; by inhibit the reabsorbtion of sodium ions by a direct action on the kidney tubules, inhibit tubular reabsorbtion of sodium ions by an indirect mechanism, and increase the filtered load of sodium ions in the glomeruli. In other words, diuretics will give net effect to increase urine volume due to sodium and water excretion[7]. The observed decrease of urine density could be explained by a marked increase in urinary flow, which was more important than the urinary electrolytes excretion. The pH alteration that presented involves an accounting of the carbonic (HCO^{-3}). The carbonic acid system is the most important buffering system of the plasma.

Hydrochlorothiazide blocks Cl^- reabsorption, creating intraluminal negative charge that impedes Na+ reabsorption resulting in increased K^+ losses because of increase Na^+ delivery with increased aldosterone. Basically, Hydrochlorothiazide inhibits sodium and chloride reabsorption in the kidney distal tubules and produces a corresponding increase in sodium, water and potassium excretion. Hydrochlorothiazide produces alkaline urine containing increased Na^+, K^+, HCO^-_3 and Cl^{-13}. Furosemide acts by inhibition of the sodium pump in all part of the nephron and to a lesser extent by inhibition of carbonic anhydrase. They promote the excretion of a higher percentage of filtered salt. Urine that excreted is alkaline and contains excess Na^+, K^+ and HCO^-_3[16]. It is possible that *C. papaya* and *A. comosus* extracts exerted its diuretic activity by inhibiting tubular reabsorption of water and accompanying anions, as such action has been hypothesized for some plants[14,17,18].

Mechanism that presented by *C. papaya and A. comosus* extracts maybe similar. Our result presented *C. papaya* and *A. comosus* extracts significantly increased ($P<0.05$) potassium ions. *C. papaya and A. comosus* extracts that studied do not give significant alteration of urinary levels of sodium. Mechanism of action of extracts is not totally analogous to hydrochlorothiazide and furosemide. The diuretic action observed may depend on stimulation of the urinary tract and is linked to the activation of neurohumoral mechanism, mediators of stimuli acting on glomerules, tone acid on the pyelo-uretral peristaltis[19]. The diuretic activity of *C. papaya* may associate with the high salt or electrolyte content of its extract. These effects might be due to the influence that the electrolytes, present in considerable quantities on the plant, exert on renal epithelium[19]. However, this activity appeared to not correlate well with the maximum urine volume and the amount of electrolytes excreted during the first hour of urine collection[5,14].

The plant extracts exerted its diuretic activity by inhibiting tubular reabsorption of water and accompanying anions[17,18]. The other possibility for the observed diuretic properties could be due to direct action of K^+ content of *C. papaya* extracts caused by highest potassium ions contents[14,20]. Our results indicated that the existing diuretic activity of both plants seems thus to be mediated through a change in potassium transport. Plant extracts may be inhibiting potassium absorption or stimulating potassium secretion, or both, leading, in either case, to more potassium retention in the lumen of the kidney tubules and osmotic water flow[21].

The mechanism of action of the plant extracts on the urine and electrolyte output may not totally be identical. This could be due to the different active components (or mechanisms) extracted. The data presented in this study indicate that plant extracts contained compound(s) that mediated diuretic effects by increasing the rate of urine output as well as electrolyte excretion[11].

In the present study, the diuretic effect observed does not exclude the possibility that changes in the diuresis may occur as a consequence of the presence of polar drug or active compounds[22], for examples flavonoid glycosides[23-25], saponosids[25] and ascorbic acid[25-27]. These natural compounds might be acting synergetically or individually promoting an initial vasodilatation[28]. It is also possible that plant extracts might manifest cumulative effect of several substances in the extract and/or due to secondary active metabolite[29]. Other monovalent and bivalent cations are present in this plant and might have a diuretic activity synergetically with K^+ [19,30].

Results from the presents study demonstrate variation changes of concentration of the parameter in the blood serum analysis. There are significant change occurred in the blood chemistry parameters, including creatinine, blood urea nitrogen (BUN), and albumin of treated group. Glucose level was slightly increases but not significantly for both plants extracts. Even though, this finding is not enough to categorize as diabetic. The mechanism of action of the extract is unknown in this case due to the increasing of glucose level is not correlated well with dose of extract.

CONCLUSION

As a conclusion, *A. comosus,* and *C. papaya* plant extracts exhibited diuretic properties. The finding results show that *A. comosus and C. papaya* are paralleled and totally agree with the ethnomedical information for those plants that used for dysuria treatment. However, both extracts also increased serum level of albumin, BUN and creatinine indicating potential nephrotoxic effects. Therefore, care should be taken when patients ingesting these herbs.

Acknowledgements: This work was financially supported by the IRPA grant from Ministry of Science Technology and Innovations, Malaysia and University Putra Malaysia.

REFERENCES

1. Caceres, A.,Giron, L.M., and Martinez, A.M. Diuretic activity of plants used for the treatment of urinary ailments in Guatemala. Journal of Ethanopharmacology, 19, 1987, 233-245.
2. Maghrani M., Zeggwagh N.-A., Haloui M., Eddouks M., Acute diuretic effect of aqueous extract of *Retama raetam* in normal rats. Journal of Ethnopharmacology, 99, 2005, 31–35.
3. Henry, R., Clinical Trials in Hypertension, 1st edition, Marcel Dekker Inc, New York. 2001, 11-26.
4. Wiart, C. Medicinal plant of Southeast Asia, Pelanduk Publication Sdn.Bhd., Selangor. 2000.
5. Sripanidkulchai B, Wongpanich V, Laupattarakasem P, Suwansaksari J, Diuretic effects of selected Thai indigenous medicinal plants in rats. Journal of Ethnopharmacology, 75(2-3), 2001, 185-190.
6. Zakaria, Z.A , Mat Jais, A.M., Goh, Y.M., Sulaiman, M.R., Somchit, M.N. Amino acid and fatty acid composition of an aqueous extract of *Channa striatus* (Haruan) that exhibits antinociceptive activity. Clinical and Experimental Pharmacology and Physiology, 34(3), 2007, 198-204
7. Adam Y., Somchit M.N., Sulaiman M.R., Nasaruddin A.A., Zuraini A., Bustamam A.A., Zakaria Z.A. Diuretic properties of *Orthosiphon stamineus* Benth. Journal of Ethnopharmacology, 124(1), 2009, 154-158.
8. Somchit, N., Hassim, S.M., Samsudin, S.H. Itraconazole- and fluconazole-induced toxicity in rat hepatocytes: A comparative *in vitro* study. Human and Experimental Toxicology, 21(1), 2002, 43-48.
9. Hashemi, S.R., Zulkifli, I., Bejo, M.H., Farida, A., Somchit, M.N. Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. International Journal of Pharmacology, 4(5), 2008, 352-360.
10. Zakaria, Z.A., Safarul, M., Valsala, R., Sulaiman, M.R., Fatimah, C.A., Somchit, M.N., Mat Jais, A.M. The influences of temperature and naloxone on the antinociceptive activity of *Corchorus olitorius* L. in mice. Naunyn-Schmiedeberg's Archives of Pharmacology, 372(1), 2005, 55-62.
11. Johnson, Patricia B., Abdurahman, Ezzeldin M., Tiam, Emmanuel A., Ibrahim Abdu-Aguye, Hussaini, Isa M., *Euphorbia hirta* leaf extracts increase urine output and electrolytes in rats. Journal of Ethnopharmacology, 65, 1999, 63–69.
12. Shinkawa, T., Yamaski, F., Notsu, T., Nakakuki, M., Nishijima, K., Yoshitomi, K., Imai, M., Loop and distal actions of a novel diuretics, M17055. European Journal of Pharmacology, 238 (2–3), 1993, 317–325.
13. Lakshman, K., Henry, J.A. Handbook of Drug Interaction, 1st edition, Arnold Publisher, London. 1998, 177-183.
14. Jouad, H., Lacaille-Dubois, M.A., Eddouks, M., Chronic diuretic effect of the water extract of *Spergularia purpurea* in normal rats. Journal of Ethnopharmacology, 75, 2001, 219–223.
15. Brechure, W.F., Stager, J.M., Lukaski, H.C. Body water and electrolyte responses to acetazolamide in humans. Journal Applied Physiology, 69(4), 1990, 1397–1401.
16. Goth, A., Medical Pharmacology, 5th edition. The C.V Mosby Company, Maruzen Company, Limited. 1970, 387-402.
17. Pantoja, C.V., Chiang, L.C.H., Norris, B.C., Concha, J.B., Diuretic, natriuretic and hypotensive effects produced by *Allium sativum* (Garlic) in anesthetized dogs. Journal of Ethnopharmacology, 31, 1991, 325–331.
18. Bevevino, L.H., Mello Aires, M., Effect of crude extract of roots of *Bredemeyera floribunda* Willd II. Effect on glomerular filtration rate and renal tubular function of rats. Journal of Ethnopharmacology, 43, 1994, 203–207.
19. Galati, E.M., Tripodo, M.M., Trovato, A., Miceli, N. and Monforte, M.T., Biological effect of *Opuntica ficus indica* (L.) Mill. (Cactaceae) waste matter note I: diuretic activity. Journal of Ethnopharmacology, 79, 2002, 17-21.
20. Nilveses, N., Wammanachinda, W., Wanverakul, B., Pidech, P., Diuretic effect of *Pluchea indica*. Thai Journal of Pharmacology, 11, 1989, 1–7.
21. Kreydiyyeh, S.I., and Usta, J. Diuretic effect and mechanism of action of parsley. Journal of Ethnopharmacology, 79, 2002, 353–357.
22. Szentmiha´lyi, K., Kre´ry, A., Then, M., Lakatos, B., Sa´ndor, Z., Vinkler, P. Potassium/sodium ratio for the characterization of medicinal plants extracts with diuretic activity. Phytotherapy Research, 12, 1998, 163–166.
23. Alard, D., Wray, V., Grotjahn, L., Reznik, H., Strack, D. N-neobetanin: isolation and identification from *Beta_ulgaris*. Phytochemistry, 10, 1985, 2383–2385.
24. Forni, E., Polesello, A., Montefiori, D., Maestrelli, A. High performance liquid chromatographic analysis of the pigments of blood-red prickly pear (*Opuntia ficus indica*). Journal of Chromatography, 19, 1992, 719–720.
25. Haloui, M., Louedec, L., Jean-Baptiste Michel, JB., Lyoussi, B. Experimental diuretic effects of *Rosmarinus officinalis* and *Centaurium erythraea*. Journal of Ethnopharmacology, 71, 2000, 465–472.
26. Kuti, J.O. Growth and compositional changes during the development of prickly pear fruit. Journal of Horticulture Science, 67, 1992, 861–868.
27. Teles, F.F.F., Price, R.L., Whiting, F.M., Reid, B.L. Circadian variation of non-volatile organic acids in the prickly pear (*Opuntia ficus indica* L.). Revista Ceres, 41, 1994, 614–622.
28. Stanic, G., Samarˇzija, I., Diuretic activity of *Satureja Montana* subsp. Montana extracts and oil in rats. Phytotherapy Research, 7, 1993, 363–366.
29. Tanira, M.O.M., Ageel, A.M., Al-Said, M.S. A study on some Saudi medicinal plants used as diuretics in traditional medicine. Fitoterapia, 5, 1988, 443–447.
30. Kanias, G.D., Loukis, A., Philianos, S.M. Trace element pharmacognostical study on diuretic drugs by neutron activation analysis. Journal of Radioanalytical Chemistry, 54, 1979, 103–112.

Source of Support: Nil, **Conflict of Interest:** None.