Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 155-161
Received: 15-05-2018
Accepted: 20-06-2018

AMSS Amarasiri
Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka

AP Attanayake
Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

KAPW Jayatilaka
Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

LKB Mudduwa
Department of Pathology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

Correspondence
AMSS Amarasiri
Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka

Acute nephroprotective and antioxidant activities of aqueous leaf extract of *Plectranthus amboinicus* (Roxb.) grown in Sri Lanka

AMSS Amarasiri, AP Attanayake, KAPW Jayatilaka and LKB Mudduwa

Abstract
Plectranthus amboinicus (Roxb.) is a medicinal plant, widely used in the management of various chronic diseases in Sri Lankan traditional medicine including kidney diseases. The aim of the present study was to investigate nephroprotective and total antioxidant activities of the aqueous leaf extract of *P. amboinicus* (Lamiaceae). The nephroprotective activity was evaluated in Adriamycin induced nephrotoxic Wistar rats while the total antioxidant activity was determined using three standard *in vitro* assays. Blood and urine were used for the estimation of selected renal parameters. H and E stained sections of the kidney tissues were used for the assessment of treatment related histological changes in the kidney tissues in order to prove the nephroprotective activity of *P. amboinicus* extract. The results revealed that the aqueous leaf extract of *P. amboinicus* possesses significant nephroprotective and antioxidant activities which scrutinize the therapeutic use of the plant in the management of kidney diseases.

Keywords: antioxidant activity, biochemical assessment, histopathological assessment, nephroprotective activity, *Plectranthus amboinicus*

1. Introduction
Plectranthus amboinicus (Roxb.), known as "Country Borage" in English (Sinhala; Kapparawalliya), is a medicinal plant of family Lamiaceae [1, 2]. It is a perennial herb which grows up to 30- 90 cm in height with oregano like flavour and odour [3, 4]. The plant has highly aromatic, fleshy leaves, 2.5- 5 cm in length with short soft erect hairs [5]. *P. amboinicus* is a medicinal plant with a wide geographical distribution [6]. It is a native species from Asia and distributed in Africa, America, Australia etc [7, 8, 9]. It is mostly grown throughout India and Sri Lanka [1].

P. amboinicus is a well-known medicinal plant used in the management of several ailments in traditional Ayurvedic and Chinese folk medicine [10]. It is commonly used for the treatment of digestive, skin, kidney and lung diseases [6, 7, 10, 11]. *P. amboinicus* leaves are specifically used for whooping cough in children. It is used in the treatment of laryngitis, bronchitis, pneumonia and tuberculosis due to its bronco-dilator properties [1, 6]. In addition, *P. amboinicus* leaves have been used as a traditional food in Indonesia. It is used as a substitute for oregano as well as a usual ingredient in soup prepared to stimulate lactation [9].

The phytochemical studies revealed the presence of essential oils, flavonoids, terpens, cinnamic derivatives, phenolics and esters in the leaves of *P. amboinicus* [5, 7, 11]. A wide range of bioactivities of the leaf extract of *P. amboinicus* as antibacterial, antifungal, antiviral, insecticidal, antiinflammatory, antimalarial, antileptospiral, antilithiatic, antiepileptic, antitumorogenic, antimutagenic etc. have been reported to date [6, 9, 11, 12].

Even though *P. amboinicus* is widely being used in the management of kidney diseases in Sri Lankan traditional medicine, it has not been screened for nephroprotective activity yet. Moreover, the chemical constituents in the plant can vary depending on geographical location, climate and different stages of collection of plant material [5]. Therefore, it is worth scrutinizing the *P. amboinicus* extract of Sri Lankan origin for its respective bioactivities specifically for nephroprotective potential *in vivo.* Hence, the aim of the present study was to evaluate the acute nephroprotective activity of the aqueous leaf extract of *P. amboinicus* grown in Sri Lanka prior to the investigation of nephroprotective mechanisms *in vivo*. In addition, the total antioxidant activity, total polyphenol and flavonoid contents of the aqueous leaf extract were determined using standard *in vitro* protocols.

Fig 1: Photographs of *Plectranthus amboinicus* plant (aerial part) and leaves grown in Sri Lanka

2. Material and Methods

2.1 Chemicals and Reagents

Aluminium chloride ($AlCl_3$), L-ascorbic acid, 95% ethanol, 2,2-diphenyl-1-picryldydrazyl (DPPH), ferric chloride, Folin-Ciaocalteu reagent, gallic acid, potassium acetate, sodium carbonate (Na_2CO_3), 2-Thiobarbituric acid (TBA), trichloro acetic acid (TCA) and 2,4,6,-tripyridyl-S-triazine (TPTZ) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Adriamycin (Doxutec) and fosinopril sodium were purchased from United Biotech, India and Sigma-Aldrich Company (St. Louis, MO, USA) respectively. All the other general chemicals and solvents that were not mentioned above were of analytical grade and used without any purification.

UV-1800, SHIMADZU recording double beam spectrophotometer (USA) and the BIO TEK microplate reader (USA) were used for biochemical analysis.

2.2 Plant material

Leaves of *P. amboinicus* were collected in October, 2016 from the natural habitat of Southern province, Sri Lanka. The botanical identity of the plant was determined by the description given by Jayaweera [1]. A voucher specimen was deposited at the Research Laboratory, Department of Biochemistry, Faculty of Medicine, University of Ruhuna.

2.3 Preparation of the aqueous plant extract

The plant parts (leaves) were washed under running tap water to remove surface pollutants, cut into small pieces and dried at 40℃ to a constant weight. The powdered leaves (12.00 g in 240 mL of distilled water) were subjected to the preparation of crude aqueous refluxed (4 hour) extract. The extract was filtered, concentrated and freeze dried to obtain the lyophilised powder of the aqueous extract with a yield of 33.92% (w/w, with reference to dry material). The lyophilised powder was then dissolved in distilled water for the preparation of equivalent human therapeutic dose in rats (400 mg/kg body wt.) for the *in vivo* study. The equivalent human therapeutic dose was calculated by extrapolating to rats considering the therapeutic dose used by the Ayurvedic practitioners in the management of kidney diseases.

Aqueous plant extract (2.50 g of powdered plant material in 60 mL of distilled water), refluxed for four hours, filtered and adjusted to an initial concentration of 50 mg/mL (0.05 g/mL) was used for *in vitro* studies.

2.4 *In vivo* study

2.4.1 Experimental Animals

Healthy male rats of Wistar strain (200±25g, 10-12 weeks of age), purchased from the Medical Research Institute, Colombo, Sri Lanka were used in the experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka. The animals were maintained on a standard laboratory diet of pellets (MRI rat formulae) and water *ad libitum*. The rats were allowed to acclimatize for a period of seven days under standard environmental conditions before the commencement of experiments. Ethical clearance was obtained from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka (14.12.2015:3.1).

2.4.2 Determination of acute nephroprotective activity of *P. amboinicus* extract

The nephroprotective activity was determined using the lyophilised powder of the aqueous refluxed leaf extract of *P. amboinicus* (400 mg/kg) against adriamycin (ADR) induced nephrotoxicity in adult Wistar rats.

Induction of nephrotoxicity in wistar rats

A single dose of ADR (20 mg/kg body wt., ip) was used for the induction of nephrotoxicity to Wistar rats.

Study design

Wistar rats were randomly divided into four groups each with six rats.

- Group I (normal control): Administered with equivalent volume of normal saline (0.9% NaCl).
- Group II (nephrotoxic control): Administered with equivalent volume of normal saline (0.9% NaCl).
- Group III (test): Administered orally with the lyophilised powder of the aqueous extract of *P. amboinicus* (400 mg/ kg body wt.; equivalent human therapeutic dose).
- Group IV (positive control): Administered orally with fosinopril sodium (0.09 mg/ kg body wt.).

The treatments were continued for three days. Rats of each group were individually housed in metabolic cages at the end of the period of intervention and 24 h urine samples were collected. Animals were anaesthetised using diethyl ether and sacrificed on the 5th day of the study. Blood was collected by cardiac puncture and left at room temperature (27^{0}C) for one hour for coagulation and centrifuged at 3000 rpm for 15 min for the separation of serum. Serum and urine samples were stored at –70℃ to be used in biochemical assays. Kidney tissues were excised immediately and transferred into 10% formalin for the preparation of haematoxylin and eosin (H and E) stained sections. H and E stained sections of the kidney tissues were used for the assessment of treatment related histological changes.

Assessment of biochemical parameters

Serum concentrations of creatinine (Biorex, UK), total protein, albumin and urine total protein (Stanbio, USA) were measured with commercially available test kits for the assessment of renal functions. The concentration of β2 - microglobulin in serum was estimated by enzyme-linked immunosorbent assay (ELISA) method (DRG Instruments GmbH, Germany).

Assessment of histopathology on H and E stained sections of the kidney tissues

H and E stained sections of the kidney tissues were examined for histological changes by two independent investigators of the study including a consultant histopathologist. Tubular vacuolisation, loss of brush border, pyknosis, intertubular haemorrhage, glomerular congestion and formation of hyaline casts were examined as selected histopathological features.

In vitro study

2.5 Determination of total antioxidant capacity *in vitro*

Total antioxidant activity of the aqueous refluxed plant extract of *P. amboinicus* was determined by 2, 2'-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant potential (FRAP) assay and thiobarbituric acid (TBA) method.

DPPH radical scavenging activity was performed using the reported method by Brand Williams *et al*. [13]. L-ascorbic acid was used as the reference compound. The decrease in absorbance values of DPPH on addition of test samples/ reference compound in relation to the control (DPPH solution alone) was used to calculate the antioxidant activity, in terms of IC_{50} (concentration of the extract /reference compound required to inhibit DPPH radical formation by 50%).

The ferric reducing antioxidant power (FRAP) assay was performed according to the method described by Benzie and Strain (1999) with slight modifications [14]. L-ascorbic acid (1000 µM) was used as the reference compound and the FRAP value of the plant extract was calculated.

TBA method, described by Ottolenghi (1959) was followed for the determination of total antioxidant activity [15]. Quantification was done with respect to the standard curve of L- ascorbic acid in the range 0-500 µg/mL (y = 0.0002x + 0.0633) in TBA assay. The results were expressed in the terms of ascorbic acid equivalents/ g dry weight of plant material.

Moreover, total polyphenol content of the *P. amboinicus* leaf extract was estimated using Folin Ciocalteu method following the procedure of Singleton *et al* (1999) [16]. The gallic acid solution prepared freshly in a graded series of concentrations (0 - 50 µg/mL) was used to establish the standard curve (y=0.01017x + 0.004810). Total polyphenol content was expressed in terms of gallic acid equivalent (µg)/g of dry weight.

The aluminum chloride method was adapted for the determination of total flavonoid content of the *P. amboinicus* leaf extract. The quantification of flavonoids was determined as quercetin equivalent from the calibration curve of quercetin standard solutions in range of 0-50 µg (y= 0.0074x- 0.0182). The results were expressed as quercetin equivalent (µg)/g of the dry weight [17, 18].

2.6 Statistical analysis

Data were statistically analyzed using Minitab statistical package. Quantitative data were expressed as mean ± SD. Analysis of variance (ANOVA) was followed by Dunnets' comparison test for the determination of level of significance in different groups in the *in vivo* study. The values of $p < 0.05$ were considered statistically significant. All the analyses were performed in triplicates in *in vitro* experiments.

3. Results and Discussion

In the present study, we evaluated the nephroprotective potential of the *P. amboinicus* leaf extract used in traditional Ayurvedic medicine on ADR induced acute kidney injury in a rat model. Further, the antioxidant activity was determined using standard *in vitro* protocols.

Among the various solvents used in the extraction of plant materials, the aqueous extract is preferred as it is the least toxic solvent with high solubility of considerable number of phytochemicals. Further, the aqueous extracts have been highly recommended for the development of neutraceuticals with strong antioxidant potentials targeting the dietary management of chronic diseases. It is further supported with the fact that traditional Ayurvedic practitioners primarily have used the aqueous form of herbal preparations in their treatments since ancient times [19]. Accordingly, the aqueous leaf extract of *P. amboinicus* was selected to determine the nephroprotective activity *in vivo* and antioxidant activity *in vitro*.

Acute nephroprotective activity of *P. amboinicus*: *in vivo* study

Acute kidney injury (AKI) is a reversible condition in which there is a sudden loss of kidney functions [20]. It is a state of losing the ability to eliminate excess fluids and waste materials from blood. Oxidative stress and inflammatory response play major roles in the progression of AKI [21]. In recent years, several studies provided *in vitro* and *in vivo* data confirming the nephroprotective activity of several medicinal plants used in the management of AKI [21, 22].

The acute nephroprotective activity of the *P. amboinicus* extract was evaluated in Adriamycin induced nephrotoxic rats. Adriamycin is an anthracycline drug which is widely used for the induction of nephrotoxicity in animal models [23]. ADR induced nephrotoxicity involves increased production of free radicals leading to oxidative stress [24, 25]. In addition, ADR exerts direct toxic damage to the glomerular and tubular structure leading to acute tubular necrosis and glomerular congestion, resulting in renal dysfunction [25]. Therefore, ADR is a well-known inducer of renal injury in rodents and the model mimics most of the features of human renal disease [23, 26].

Assessment of biochemical parameters

The concentration of serum creatinine is critically important in assessing renal functions. It is the most reliable first line biochemical investigation of glomerular function. Elevated levels of serum creatinine indicate impaired renal functions [27, 28]. Accordingly, elevated levels of serum creatinine (61%) were observed in ADR induced nephrotoxic control rats in the present study. Administration of the aqueous leaf extract of *P. ambonicus* (400 mg/kg body wt.) was able to significantly limit the elevation of serum creatinine concentration compared to the nephrotoxic control group rats ($p<0.05$).

Fosinopril was used as the standard drug in the present study. It is an angiotensin-converting enzyme inhibitor, with antihypertensive effects [26, 29]. It is commonly used to treat chronic kidney disease due to its long term nephroprotective effects [29]. Even though the mechanism of action is not related to AKI, fosinopril was used as the standard drug during the present study as it has been widely used as the standard drug in screening of nephroprotective activity of medicinal plants in number of studies [26, 30, 31].

Administration of the fosinopril (0.09 mg/kg body wt.) was also effective in reducing the elevation of creatinine concentration by 42% compared to the nephrotoxic control group during the study ($p<0.05$). Interestingly, there was no significant difference between the serum creatinine levels of rats treated with the plant extract and fosinopril ($p>0.05$). Thus, the nephroprotective activity of the plant extract was comparable to the effect of fosinopril.

β_2 -microglobulin is considered as a promising marker used to assess glomerular and tubular functions of the kidney. Estimation of serum levels of β_2 -microglobulin is a reliable method, which can be used as an ideal screening tool [32]. A 50% increase in the concentration of β_2 -microglobulin was observed in nephrotoxic control group rats compared to the healthy control group ($p<0.05$). As shown in Table 1, the

elevation of serum concentration of β_2 -microglobulin was decreased in both groups of rats treated with *P. ambonicus* (66%) and fosinopril (11%) compared to the nephrotoxic control group. However, the reduction in β2 -macroglobulin in ADR rats was not statistically significant in fosinopril treated rats ($p>0.05$). The above results revealed that, *P. ambonicus* is more effective in reducing the elevated level of β_2 –microglobulin compared to the standard drug.

Serum total protein and albumin are two additional markers of kidney function that are being commonly done in studies related to kidney diseases [26, 31]. As shown in Table 1, the administration of the plant extract significantly reduced the decrease in serum concentration of albumin (14%) and total protein (25%) compared to the ADR induced nephrotoxic rats ($p<0.05$). Similarly, a decrease in reduction of levels of serum albumin (16%) and total protein (12%) were observed in the fosinopril treated group ($p<0.05$).

In a normal healthy individual, almost all the protein filtered by the glomeruli are reabsorbed and subsequently catabolized in the proximal tubules [28]. Therefore, detection of protein in urine is an important marker of diagnosis and prognosis of renal diseases in an acute stage. Proteinuria is one of the earliest sign of renal damage. Hence, several studies have focused on the improvement of proteinuria under several therapies [26, 30, 31].

Urinary protein loss in 24hr urine samples were significantly decreased in rats treated with both fosinopril (86%) and *P. ambonicus* (56%) during the present study ($p<0.01$). However, as shown in Fig. 2, fosinopril, which was used as the standard drug, counteracts urinary protein loss more effectively than the plant extract. The reason for the significant reduction of urine protein loss in fosinopril treated rats compared to the other groups of rats may due to the antiproteinuric effect of fosinopril result from the reduction of glomerular capillary pressure [29, 33].

Results of all biochemical parameters substantiate the fact that, ADR causes a significant nephrotoxicity in rats. These findings were able to prove the use of ADR as an inducer of renal injury in rodents, as recognized in previous studies [23, 26]. However, the results of all the selected biochemical parameters revealed that *P. amboinicus* possesses significant nephroprotective activity against ADR induced nephrotoxicity.

Assessment of histopathology

Induction of nephrotoxicity caused significant changes in the morphology of kidney tissues in nephrotoxic control group (Fig. 3b), compared to the normal control group (Fig. 3a). The early features of acute tubular necrosis were observed with loss of brush border, cytoplasmic vacuolisation and pyknosis in renal tubular epithelial cells of ADR induced nephrotoxic rats. In addition, haemorrhages, glomerular congestion and hyaline casts were observed in the kidney tissues of ADR induced control group. These pathological changes were attenuated in the rats treated with the plant extract (Fig. 3c). These findings coincide with the observations of Palani *et al.* on acetaminophen-induced nephrotoxic rats treated with ethanol extract of *P. amboinicus* [22]. A similar attenuation of morphological destruction was observed in the H and E stained sections of rats treated with fosinopril (Fig. 3d).

A number of studies have provided scientific data confirming the nephroprotective activity of medicinal plants used in the management of renal diseases. *Hygrophila spinosa* (Acanthaceae), *Aerva javanica* (Anemarrhenaceae), *Euterpe oleracea* (Arecaceae), *Momordica dioica* (Cucurbitaceae), *Casuarina equisetifolia* (Casuarinaceae), *Drynaria fortune* (Polypodiaceae) are few of those medicinal plants with proven nephroprotective activity [21]. Suppression of oxidative stress by antioxidant defense mechanism has been suggested as one of the nephroprotective mechanisms in most of the experimental studies [21, 22, 26, 34]. Therefore, it would be worth evaluating the antioxidant activity of the medicinal plants in parallel to the investigation of nephroprotectivity *in vivo*.

Table 1: Effect of aqueous leaf extract of *P. ambonicus* on selected kidney parameters in ADR induced rats

Group	Creatinine (mg/dL)	β_2 -microglobulin (µg/ mL)	Total protein (g/dL)	Albumin (g/dL)
Group I	0.45± 0.10	0.11±0.01	6.06±0.28	3.34±0.11
Group II	1.14±0.18	0.22±0.04	4.72±0.12	2.32±0.04
Group III	0.70±0.03*	0.08±0.00*	5.89±0.13**	2.63±0.05**
Group IV	0.66±0.03*	0.19±0.07	5.29±0.12*	2.69±0.08**

Data are expressed as mean ± SD (n = 6). *p <0.05, **p <0.01 compared to adriamycin induced nephrotoxic control group.

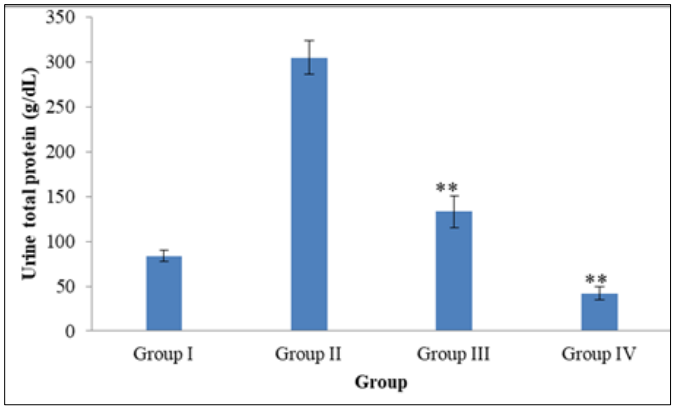

Fig 2: Effect of aqueous leaf extract of *P. amboinicus* on urine total protein level of rats. Data are expressed as mean ± SD (n = 6). *p<0.05, **p<0.01 compared to adriamycin induced nephrotoxic control group

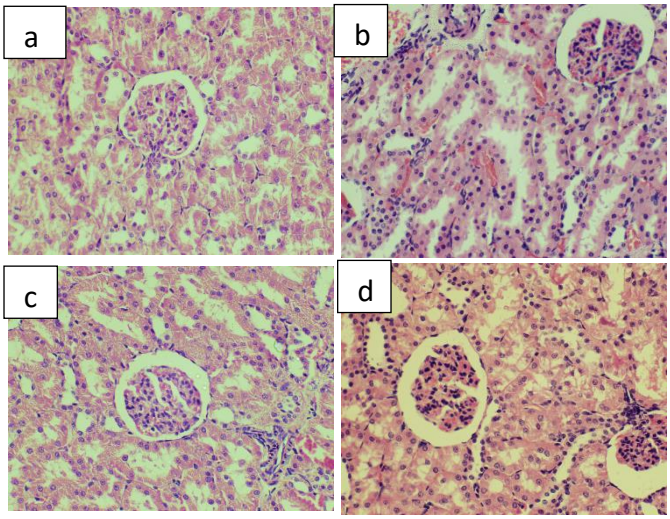

Fig 3: Photomicrographs of H and E stained kidney sections by light microscopy

(a) Healthy control group (Group I), (b) Adriamycin induced nephrotoxic control group with cytoplasmic vacuolisation, pyknosis, haemorrhages, glomerular congestion and destructed epithelial cells (Group II), (c) Group of rats treated with the lyophilised powder of the aqueous extract of *P. amboinicus* showing early features of acute tubular necrosis to a lesser degree (Group III) and (d) Group of rats treated with

fosinopril sodium showing similar attenuation of morphological destruction (Group IV) (×400).

***In- vitro* total antioxidant activity**

DPPH, FRAP and TBA assays were selected in the present investigation to determine the total antioxidant activity. These methods have been used to evaluate the antioxidant potential of pure chemicals, fruit juices and medicinal plant extracts in a number of scientific reports [35]. Generally a single assay is not sufficient to determine the antioxidant activity of natural products as crude extracts. Therefore, in practice a number of *in vitro* assays are being used for the evaluation of antioxidant activity of plant extracts [36, 37]. However, these assays may differ from each other in terms of reaction mechanisms, reaction conditions as well as from the way of expression of results. The results may vary even in the same method according to the solvent, time of reaction, pH and selected reference compound employed etc. Therefore, it is recommended to use at least three methods to determine the total antioxidant potential of plant materials [38].

The DPPH radical scavenging assay has been widely used in the determination of antioxidant activity of different plant extracts [39]. It is the simplest and widely used method in screening of total antioxidant activity of herbal extracts. In this assay, the purple chromogenic radicals, DPPH are reduced to the corresponding pale yellow hydrazine with the presence of antioxidants in the plant extracts. The reaction involves a decrease in absorbance of DPPH at 517 nm, which is proportional to the free radical scavenging potential of the plant extract. The activity is expressed as inhibitory concentration IC_{50}; the concentration of antioxidant required to decrease the percentage of inhibition by 50% of the initial DPPH concentration. A low IC_{50} value represents high level of antioxidant activity and *vice versa* [38].The percentage inhibition of DPPH radical in an aqueous leaf extract of *P. amboinicus* was relatively high with an IC_{50} value of 67.81±1.94 µg/mL in the present study. The results of the present study shows a higher antioxidant activity of the aqueous leaf extract compared to the findings of Ramos *et al* on hydroalcoholic leaf extract of *P. amboinicus* [40]. The reference compound L- ascorbic acid showed an IC_{50} value of 10.67±0.02 µg/mL. Accordingly, the IC_{50} value of L-ascorbic acid obtained in the present investigation is comparable with the value in the study reported by Shekhar *et al* [41].

Ferric reducing antioxidant power (FRAP) assay is a quick and simple method for the determination of total antioxidant activity *in vitro*. It is a highly sensitive antioxidant assay with excellent reproducibility and linearity [39]. In the assay, the antioxidant activity is determined on the basis of the potential of antioxidants in plant extracts to reduce ferric (III) ions in the form of ferrus 2, 4, 6-tripyridyl-s-triazine (TPTZ) [42]. The FRAP value of *P. amboinicus* aqueous leaf extract was 17.96±0.15 in the present study.

During the process of lipid oxidation, carbonyl compounds are formed from the decomposed peroxides. Thiobarbituric acid assay method is used in the determination of total antioxidant activity based on the measurement of those carbonyl compounds [19]. Total antioxidant activity of *P. amboinicus* by the TBA method was 30.84±0.42 in terms ascorbic acid equivalents (µg)/g dry weight of plant material in the present study.

Phytochemicals as polyphenols and flavonoids are generally found to be attributed for the nephroprotective activity *in vivo* [21, 34]. Therefore, herbal compounds with hydroxyl groups, such as phenols and flavonoids have become most effective bioactive compounds in therapeutic applications [21]. Hence, total polyphenol and flavonoid contents of the aqueous leaf extract of *P. amboinicus* were determined using the Folin Ciocalteu method and the aluminum chloride colorimetric method respectively in the present investigation. The total polyphenol content was 5.68±0.04 gallic acid equivalents (µg) per dry weight (g) of plant material as determined by the regression equation of calibration curve ($y=0.0102x+0.0048$, $R^2=0.99$). According to the calibration curve of quercetin as the reference compound ($y=0.0074x-0.0182$, $R^2=0.99$), total flavonoid content was 1.8±0.05 quercetin equivalent (µg)/ g of dry plant material.

Present study reveals that the aqueous leaf extract of *P. amboinicus* possesses relatively high antioxidant activity and free radical scavenging activity *in vitro*. Phenolic substances are considered as important group of natural antioxidants widely distributed in medicinal plant extracts [35]. Hence, the polyphenols present in the plant extract might be attributed to the total antioxidant activity of *P. amboinicus.*

Antioxidants are the chemicals which counteract the deleterious effects of free radicals at the cellular level. Excessive formation of free radicals or diminished levels of free radical scavengers cause oxidative stress [43]. ADR, the drug which was used to induce nephrotoxicity in animal model, increases the production of free radicals leading to oxidative stress [24, 25]. Therefore, the protective effects of *P. amboinicus* as evidenced by the results of renal biochemical parameters might be due to the antioxidant potential of the plant extract which counteract against the oxidative stress.

Conclusions

The results of the present study revealed that the aqueous leaf extract of *P. amboinicus* possesses significant nephroprotective activity against adriamycin induced acute nephrotoxicity. The improved kidney functions were supported with the results of selected renal parameters and histological changes observed in H and E stained sections of the kidney tissues of Wistar rats. Moreover, the aqueous extract showed relatively high antioxidant activity *in vitro* based on standard antioxidant assays. Further investigations are in progress to investigate the long term nephroprotective mechanism(s) of the aqueous leaf extract of *P. amboinicus* in the same animal model.

Acknowledgment

Authors are thankful to Mr. G.H.J.M. Priyashantha and Mr. O.M.P. Chaminda, Faculty of Medicine, University of Ruhuna for their technical assistance provided in animal experiments and obtaining photomicrographs. National Science Foundation research grant (RG/2016/HS -03) and University Grants Commissions special allocation for strengthening research grant (RU/PG- R/16/14) are gratefully acknowledged for the financial support provided.

References

1. Jayaweera DMA. Medicinal Plants (Indigenous and Exotic) Used in Ceylon- Part III. National Science Council of Sri Lanka, Colombo 1982, 59-60.
2. Patel R, Mahobia NK, Gendle R, Kaushik B, Singh SK. Diuretic activity of leaves of *Plectranthus amboinicus* (Lour) Spreng in male albino rats. Phcog Res 2010; 2:86-8.
3. Fu L, Fu Z. *Plectranthus amboinicus* leaf extract–assisted biosynthesis of ZnO nanoparticles and their

photocatalytic activity. Ceramics International 2015; 41(2):2492-2496.
4. Manjamalai A, Alexander T, Grace VMB. Bioactive evaluation of the essential oil of *Plectranthus amboinicus* by GC-MS analysis and its role as a drug for microbial infections and inflammation. Int J Pharm Pharm Sci. 2012; 4(3):205-211.
5. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: Botanical, phytochemical, pharmacological and nutritional significance. Molecules 2016; 21:369-395.
6. Costa JGM, Pereira CKB, Rodrigues FFG, Lima SG. Chemical composition, antibacterial and fungicidal activities of leaf oil of *Plectranthus amboinicus* (Lour.) Spreng. J Oil Res. 2010; 22(2):183-185.
7. Gurgel AP, Da Silva JG, Grangeiro AR, Oliveira DC, Lima CM, Da Silva AC, *et al*. *In vivo* study of the anti-inflammatory and antitumor activities of leaves from *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae). J Ethnopharmacol. 2009; 125(2):361-363.
8. Rice LJ, Brits GJ, Potgieter CJ, Van Staden J. *Plectranthus*: A plant for the future?. S Afr J Bot. 2011; 77(4):947-959.
9. El-hawary SS, El-sofany RH, Abdel-Monem AR, Ashour RS, Sleem AA. Seasonal variation in the composition of *Plectranthus amboinicus* (Lour.) Spreng essential oil and its biological activities. American Journal of Essential Oils and Natural Products. 2013; 1(2):11-18.
10. Manjamalai A, Narala Y, Haridas A, Grace VMB. Antifungal, antiinflammatory and GC-MS analysis of methanolic extract of *Plectranthus amboinicus* leaf. Int J Curr Pharm Res. 2011; 3(2):129136.
11. Pillai PG, Suresh P, Aggarwal G, Doshi G, Bhatia V. Pharmacognostical standardization and toxicity profile of the methanolic leaf extract of *Plectranthus amboinicus (Lour) Spreng.* J Appl Pharm Sci. 2011; 1(2):75-81.
12. Bhatt P, Negi PS. Antioxidant and antibacterial activities in the leaf extracts of Indian borage (*Plectranthus amboinicus*). Food Nutr Sci 2012; 3:146-152.
13. Brands Williams W, Cuveilier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995; 28:25-30.
14. Benzie IFF, Strain JJ. Ferric Reducing Antioxidant Power assay: Direct measure of total antioxidant activity of biological fluids and modified version of simultaneous measurement of total *antioxidant* power and ascorbic acid concentration. Methods Enzymol. 1999; 299:15-27.
15. Ottolenghi A. Interaction of ascorbic acid and mitochondria lipids. Arch Biochem Biophys. 1959; 79:355-365.
16. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999; 299:152-178.
17. Koksal E, Gulcin L. Antioxidant Activity of Cauliflower (*Brassica oleracea* L.). Turk J Agric For. 2008; 32:65-78.
18. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10:178-182.
19. Zahin M, Aqil F, Ahmad I. The *in vitro* antioxidant activity and total phenolic content of four indian medicinal plants. Int J Pharm Pharm Sci. 2009; 1:88-95.
20. Konda VR, Arunachalam R, Eerike M, Rao R, radhakrishnan AK, Raghurman LP, *et al*. Nephroprotective effect of ethanolic extract of *Azima tetracantha* root in glycerol induced acute renal failure in Wistar Albino rats. J Tradit Complement Med. 2016; 6(4):347-354.
21. Boozari M, Hosseinzadeh H. Natural medicines for acute renal failure: A review. Phytother Res. 2017; 1-12.
22. Palani S, Raja S, Naresh R, Kumar BS. Evaluation of nephroprotective, diuretic, and antioxidant activities of *Plectranthus amboinicus* on acetaminophen-induced nephrotoxic rats. Toxicol Mech Methods 2010; 20(4):213-221.
23. Lee VW, Harris DC. Adriamycin nephropathy: A model of focal segmental glomerulosclerosis. Nephrology 2011; 16:30-38.
24. Gumaa SA, Hassan EM, Khalifa DM. Hepatoprotective effect of aqueous extracts of some medicinal plant mixtures on CCl4-induced liver toxicity. Int J Pharm Biol Sci. 2017; 12(1):43-52.
25. Szalay CI, Erdelyi K, Kokeny G, Lajtar E, Godó M, *et al*. Oxidative/nitrative stress and inflammation drive progression of doxorubicin-induced renal fibrosis in rats as revealed by comparing a normal and a fibrosisresistant rat strain. Plos One 2015; 10(6):e0127090.
26. Qi Y, Xiao H, Xu C, Tian X, Wu H, Shen W. Cyprinuscarpio decoction improves nutritionand immunity and reduces proteinuria through nephrin and CD2AP expressions in rats with Adriamycin-induced nephropathy. Evid.-Based Complementary Altern Med. 2012; 237482.
27. Mayne PD. Clinical Chemistry in Diagnosis and Treatment. Ed 6, Arnold, London, 1994, 17-20.
28. Marshall WJ, Bangert SK. Clinical chemistry. Ed 5, Elsevier Limited, Philadelphia, 2007, 65-68.
29. Navis G, Faber HJ, de Zeeuw D, de Jong PE. ACE inhibitors and the kidney. A risk-benefit assessment. Drug Saf, 1996; 15(3):200-211.
30. Song LQ, Si-Ming Y, Xiao-Peng M, Li-Xia J. The protective effects of *Cordyceps sinensis* extract on extracellular matrix accumulation of glomerular sclerosis in rats. Afr J Pharm Pharmacol. 2010; 4(7):471-478.
31. Zhao J, Lin X, Xiao X, Yang J, Liu H, Yi W, *et al*. Inhibition of adriamycin-induced nephropathy in rats by herbs based kangshenoral solution. Int J Clin Exp Med. 2015; 8(12):22469-22476.
32. Argyropoulos CP, Chen SS, Ng YH, Roumelioti ME, Shaffi K, Singh PP, *et al*. Rediscovering beta-2 microglobulin as a biomarker across the spectrum of kidney diseases. Front Med, 2017; 4:73.
33. Harty J. Prevention and Management of Acute Kidney Injury. Ulster Med J. 2014; 83(3):149-157.
34. Ajith TA, Aswathy MS, Hema U. Protective effect of Zingiber officinale roscoe against anticancer drug doxorubicin-induced acute nephrotoxicity. Food Chem Toxicol, 2008; 46(9):3178-3181.
35. Chan S, Li S, Kwok C, Benzie I, Szeto Y, Guo DJ, *et al*. Antioxidant activity of Chinese medicinal herbs. Pharm Biol, 2008; 46:587-595.
36. Alam MDN, Bristi NJ, Rafiquzzaman MD. Review on *in vivo* and *in vitro* methods of evaluation of antioxidant activity. Saudi Pharm J. 2013; 21:143-152.
37. Attanayake AP, Jayatilaka KAPW, Malkanthi BMAS. Total flavonoid content, total antioxidant activities and phytochemical constituents of selected medicinal plant extracts used for oxidative stress related chronic diseases in Sri Lanka. J Med Plants Stud. 2016; 4(6):26-29.

38. Boligon AA, Machado MM, Athayde ML. Technical evaluation of antioxidant activity. J Med Chem. 2014; 4:517-522.
39. Shi F, Jia X, Zhao C, Chen Yan. Antioxidant activities of various extracts from *Artemisisa selengensis* turcz (LuHao). Molecules, 2010; 15:4934-4946.
40. Ramos A, Visozo A, Piloto J, Garcıa A, Rodrıguez CA, Rivero R. Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants. J Ethnopharmacol. 2003; 87:241-246.
41. Shekhar TC, Anju G. Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. leaves. A J Ethno. 2014; 1:244-249.
42. Devprakash, Sreenivasan KK, Subburaju T. Comparative antioxidant studies of ethanol extract and fresh aqueous extract of *Vetiveria zizanioides*. Int J Pharm Pharm Sci. 2011; 3:325-331.
43. Veeru P, Kishor MP, Meenakshi M. Screening of medicinal plant extracts for antioxidant activity. J Med Plants Res. 2009; 3(8):608-612.