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# Nephroprotective activity of *Vetiveria zizanioides* (L.) Nash supplement in doxorubicin-induced nephrotoxicity model of Wistar rats

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## Abstract

The nephroprotective effect of standardized aqueous root extract of *Vetiveria zizanioides* (L.) Nash (Family: Poaceae) was investigated in doxorubicin-induced (20 mg/kg, ip) experimental nephrotoxicity model of Wistar rats. The freeze-dried aqueous refluxed (4 hr) root extract of *V. zizanioides* (25, 50; equivalent human therapeutic dose and 100 mg/kg) was administered separately to nephrotoxic Wistar rats ($n = 6$/group). Supplement of *V. zizanioides* resulted a dose-dependent reduction in raised serum creatinine, $\beta_2$-microglobulin, and blood urea nitrogen and a subsequent increase in serum total protein and albumin in nephrotoxic rats ($p < .05$). An attenuation of the doxorubicin-induced features of renal parenchymal injury was observed on H- and E-stained sections of the kidney tissues. Nootkatone, dehydroaromadendrene, isokhusenic acid, α-vetivone, and isolongifolene were identified in the methanol extract of *V. zizanioides* based on the GC-MS chromatogram analysis. The findings revealed that the supplement of standardized aqueous root extract of *V. zizanioides* had a significant dose-dependent nephroprotective activity against doxorubicin-induced experimental nephrotoxicity.

## Practical applications

*Vetiveria zizanioides* is a medicinal plant with a variety of therapeutic applications in kidney-related diseases. Apparently, it is used as a food ingredient due to its fresh and elegant scent and potential bioactivities. The aqueous root extract of *V. zizanioides* exerted relatively high antioxidant potential in vitro, substantiating the health effects of the plant pertaining to kidney diseases as a potential source of dietary antioxidant. The administration of the plant extract resulted in significant nephroprotection against doxorubicin-induced experimental nephrotoxicity revealing the significance of *V. zizanioides* as a promising dietary supplement in the management of kidney disease.

KEYWORDS

chemical standardization, doxorubicin, nephroprotective activity, nephrotoxicity, *Vetiveria zizanioides*

  

## 1 | INTRODUCTION

Medicinal plants are considered as vital sources of potentially useful bioactive compounds that could be useful in the development of dietary supplements to combat a variety of diseases. *Vetiveria zizanioides* (L.) Nash (Family: Poaceae), commonly known as Savandara, is one such medicinal plant, with various dietary and therapeutic uses. The plant is cultivated in a number of South Asian countries for the production of vetiver oil, which is a commercially and medicinally valued essential oil, distilled from the roots of the plant. Vetiver oil is often used as a flavoring agent, preservative, and an additive due to its fresh and elegant scent. It raises a high price in the traditional Chinese markets as a safe and valuable natural food additive (Chou et al., 2016). The flavor of the vetiver grass is similar to that of the lemongrass and citronella due to its botanical relationship. Hence, the edible oil is used as a flavoring agent for different food items as syrups, ice cream, and beverages despite of its use as an additive and a preservative (Chou et al., 2016; Mahmoudi et al., 2019; Raman et al., 2018). Moreover, application of the vetiver oil as a food flavor has been increased due to its excellent blending potential with many other frequently used essential oils including cedar wood, ginger, jasmine, lemongrass, sandalwood, and vanilla. (Chou et al., 2016). The root extract of *V. zizanioides* is used for the preparation of popular beverage; sherbet, which is known to have a cooling effect on brain. It is a refreshing drink in summer and serves as an additive to milk, kulfi, ice-cream, and food salads (Moon et al., 2020).

A common cause of acute kidney injury is administration of certain drugs. In fact, a wide range of kidney impairments, ranging from acute kidney injury to chronic kidney disease and subsequent end-stage renal disease is mediated via oxidative stress (Al-Okbi et al., 2014). Oxidative damage mediated through the generation of reactive oxygen species has been identified as a major pathway of causing and amplifying nephrotoxicity by the anticancer drug, doxorubicin (Principal et al., 2010). Biotransformation of doxorubicin to its toxic metabolite; semiquinone radical which has a short half-life, initiates a cascade of reactions producing reactive oxygen species in aerobic conditions (Ajith et al., 2008; Benzer et al., 2018; Heravi et al., 2018). The increased production of reactive oxygen species and reduced activity of antioxidant enzymes may lead to a state of oxidative stress resulting in tissue injury which links with protein oxidation, DNA damage, and membrane lipid peroxidation in kidney tissues (Ayla et al., 2011; El-Sheikh et al., 2012).

Antioxidants with the potential of scavenging reactive oxygen species are promising sources for combatting associated free radical pathologies in oxidative stress-related diseases (Marcadenti & Assis -Coelho, 2015). Apparently, in the current practice of modern allopathic medicine, there is no successful medication available for improving kidney function in kidney diseases, rather than addressing the comorbidities. Indeed, therapeutic approaches are limited to retardation of the disease progression by normalization of blood pressure, plasma glucose level, cardiovascular problems etc. In this sense, the development of dietary supplements targeting the treatment of kidney impairment or kidney disease which can be used as an adorn therapy is highly recommended. Indeed, the use of exogenous antioxidants, present in certain dietary ingredients would be beneficial as dietary supplements in the management of kidney disorders (Hosohata, 2016; Yoo et al., 2010).

A number of published reports has stated the potential antioxidant activity of *V. zizanioides* in vitro, and the high levels of phenolic content in the root extract, particularly flavonoids are accounted for the free radical scavenging and antioxidant potential of *V. zizanioides* (Luqman et al., 2009; Muthukrishnan & Manogaran, 2018). The potential correlation between the anti-inflammatory and antioxidant activities of vetiver oil has been further specified in a study by Chou and the coworkers through decreasing lipopolysaccharide-induced superoxide anion production and malondialdehyde levels (Chou et al., 2012). Apart from its dietary uses, *V. zizanioides* is a main ingredient of "Rath pith kethakee oil," "Maha narayana kalkaya," "Saraswathee churnaya" etc. used in Sri Lankan traditional medicine (Pharmacopoeia, 1985). The stem decoction is used for urinary tract infections due to its diuretic effects. The use of Vetiver roots in the management of multiple aspects of kidney diseases including dysuria, urethritis, burning urethra, and kidney stones is documented in Ayurveda texts (Pharmacopoeia, 1985; Jayaweera, 1982; Krishnaveni & Ampritha, 2016). Based on the therapeutic applications in clinical practice, and the reported antioxidant activity of *V. zizanioides*, we hypothesized that the extract may exert beneficial effects as a dietary supplement in diverse pathophysiological conditions of the kidney. Hence, herein we aimed to investigate the nephroprotective effects of *V. zizanioides* using doxorubicin-induced experimental nephrotoxicity in rats with an intension of developing a commercially viable dietary supplement particularly to be used as an adorn therapy for patients with kidney disease.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals

Analar grade chemicals, reagents, and solvents were used as received from chemical suppliers without any purification.

### 2.2 | Plant material

Roots of *V. zizanioides* were collected from a natural habitat in the Southern province (6°04′05″N, 80°13′35″E), Sri Lanka in October, 2016. The plant was authenticated by the curator of National Herbarium, Royal Botanical Gardens, Kandy, Sri Lanka. A detailed specimen (PG/2016/55/06) was reposited at the Biochemistry Research Laboratory, Faculty of Medicine, University of Ruhuna, Sri Lanka (Figure 1).

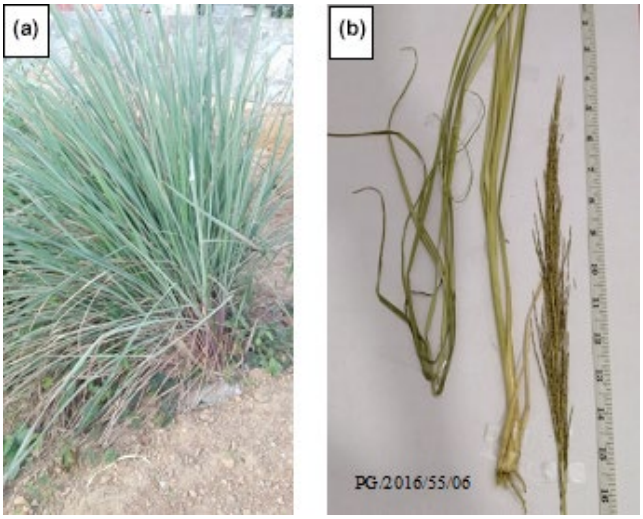


**FIGURE 1** *Vetiveria zizanioides*: (a) in the natural habitat and (b) herbarium sheet with flowers and leaves

## 2.3 | Plant extraction

Fresh roots of *V. zizanioides* were washed thoroughly with tap water and dried in a hot air oven at 40°C. The powdered plant material (12 g) was dissolved in distilled water (240 ml) and refluxed for 4 hr. The mixture was strained though cheesecloth and Whatman grade 1 filter paper and the resulting plant extract with an initial concentration of 50 mg/ml was used in the in vitro assays. The extract was freeze dried at −40°C and the resultant lyophilized powder (percentage yield 4.28%) was reconstituted in distilled water to obtain desired concentrations for the evaluation of nephroprotective activity in vivo.

## 2.4 | Standardization of the roots of *Vetiveria zizanioides*

### 2.4.1 | Physicochemical analysis

Physicochemical analysis was performed according to the procedures mentioned in the WHO guidelines (WHO, 2011). Accordingly, ash values, extractive values, and the moisture content were determined in the powdered plant material.

### 2.4.2 | Determination of microbial contamination and heavy metal analysis

Determination of microbial contamination was carried out as per the Sri Lanka Standards (SLS 1982, 1992). Heavy metal analysis was carried out by Inductively Coupled Plasma Mass Spectrometry (Agilent 7900 ICP-MS) according to the standard protocols (SLS 1973; AOAC, 2000). The limits of quantification were 0.2, 5.0, 0.3, and 10.0 ppm, respectively, for the selected heavy metals; mercury, arsenic, cadmium, and lead according to the WHO guidelines (WHO, 2011).

### 2.4.3 | Thin layer chromatography fingerprint

Dichloromethane extract of the powdered plant material was used for the development of the Thin layer chromatography (TLC) profile. A mixture of cyclohexane, dichloromethane, ethyl acetate, and methanol were used in a ratio of 4:1:0.5:0.4 as the mobile phase.

### 2.4.4 | Gas chromatography–mass spectrometry analysis

The methanol extract of *V. zizanioides* was analyzed using an Agilent 6,890 series gas chromatograph equipped with an HP-5 MS (5% phenyl methyl siloxane) capillary column (30 m × 0.25 mm i.d, film thickness; 0.25 μm) interfaced to an Agilent 5,973 N series mass-selective detector. The oven temperature was initially held at 35°C for 5 min and then increased at a rate of 5°C/min to 280°C with 1 min hold time. The injector temperature was 250°C. Helium was used as the carrier gas at a flow rate 0.9 ml/min. The MS source and MS quadrupole temperatures were set at 230 and 150°C, respectively. Parameters were scanned at 15 (amu)–550 (amu). Library search for the identification of compounds was performed in the Wiley W9N08 and NIST databases.

### 2.4.5 | Preliminary phytochemical analysis

The aqueous refluxed extract of *V. zizanioides* was subjected to preliminary phytochemical analysis for the detection of phenolic compounds, tannins, flavonoids, alkaloids, terpenoids, steroid glycosides, and saponins using standard procedures as described by Farnsworth (1996).

## 2.5 | In- vitro total antioxidant activity

The total antioxidant potential of the freeze dried aqueous root extract of *V. zizanioides* was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Williams et al. 1995), ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1999), and thiobarbituric acid (TBA) assay (Ottolenghi, 1959). The scavenging potential was given in terms of $IC_{50}$ in DPPH assay. The in vitro antioxidant potential was determined in terms of the concentration of the ascorbic acid standard (1,000 μM) in FRAP assay. The values were expressed as ascorbic acid equivalents (μg AAE)/gram of dry weight (g) based on the standard curve of L- ascorbic acid ($y = 0.0002x + 0.0633$) in TBA method.

### 2.6 | Total polyphenol and flavonoid content

The Folin Ciocalteu reagent method was followed for the quantification of polyphenols in the aqueous refluxed *V. zizanioides* root extract (Singleton et al., 1999). The total polyphenol content was measured as gallic acid equivalents (μg GAE)/gram of dry weight (g) based on the standard curve of gallic acid ($y = 0.01017x + 0.004810$, $R^2 = 0.99$). The total flavonoid content was quantified using the regression equation of quercetin under the same experimental conditions ($y = 0.0074x - 0.0182$, $R^2 = 0.99$) by aluminum chloride method and the values were given as quercetin equivalents (μg QE)/gram of dried weight (Chang et al., 2002).

### 2.7 | Evaluation of nephroprotective activity against doxorubicin-induced nephrotoxicity in Wistar rats

#### 2.7.1 | Experimental animals

Adult male rats of Wistar strain, weighing 300 ± 25 g was purchased from the Medical Research Institute, Sri Lanka. The animals were maintained under standard environmental conditions (28°C, 12-hr light/12-hr dark) in the animal house at the Faculty of Medicine, University of Ruhuna, Sri Lanka. The Wistar rats were fed with a pelleted rodent diet and allowed free access to water. Ethical clearance was granted from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka (Reference No. 14.12.2015:3.1). Experiments were carried out according to the principle of 3R's within an ethical framework. The potential pain, suffering or distress to the experimental animals was minimized through improved animal husbandry and housing, careful handling, and employing appropriate anesthetics. Personal Protective Equipment (PPE) including double nitrile gloves, cytotoxic safety goggles, lab coat, and facial masks were used during experimental induction of nephrotoxicity with the cytotoxic drug, doxorubicin (DOX).

#### 2.7.2 | Experimental design

The experimental protocol as reported by our research group in Amarasiri et al. (2020) was followed for the evaluation of protective effects of the aqueous root extract of *V. zizanioides* against doxorubicin-induced experimental nephrotoxicity. Nephrotoxicity was induced with a single intraperitoneal dose of doxorubicin (United Biotech) at 20 mg/kg in experimental rats of all groups except in the healthy control group. Experimental Wistar rats were grouped ($n = 6$ per group), 24 hr following the induction of nephrotoxicity. The treatment regimens were continued as a single dose on a daily basis for three consecutive days.

Group 1: Healthy control.
Group 2: DOX-induced nephrotoxic control.
Group 3: DOX +V. *zizanioides* (25 mg/kg, orally).
Group 4: DOX +V. *zizanioides* (50 mg/kg, orally).
Group 5: DOX +V. *zizanioides* (100 mg/kg, orally).
Group 6: DOX +fosinopril (0.09 mg/kg, orally).

Animals were housed individually in metabolic cages and 24-hr urine samples were collected following the last dose of plant extracts or standard drug (fosinopril). Blood samples were collected from the sacrificed animals by cardiac puncture and serum was separated. Kidney tissues were removed and dipped in 10% of formalin for fixation.

#### 2.7.3 | Biochemical parameters of kidney function

The creatinine concentration in serum (Bartels et al., 1972), blood urea nitrogen (BUN) (Sampson et al., 1980), total protein concentration in serum (Weichselbaum, 1946), albumin concentration in serum (Bartholomew & Delaney, 1964), and total protein concentration in urine (Watanabe et al., 1986) were estimated using spectrophotometric assay kits (UV-1800, SHIMADZU). Serum $\beta_2$-microglobulin and cystatin C concentration were determined by enzyme-linked immunosorbent assay method (BIO TEK).

#### 2.7.4 | Assessment of histopathology

The formalin-fixed, bisected kidneys were embedded in paraffin after being dehydrated in a series of ethanol and subsequently cleared with xylene. Kidney sections with five-micrometer thickness were prepared from the paraffin blocks and stained with hematoxylin and eosin for the examination under light microscope (Olympus CX 21). A blinded analysis of histopathological features was carried out using previously reported score system by two independent investigators (Amarasiri et al., 2020). A value of "1" was assigned for the presence of the selected features in each field and a "0" value was assigned in the absence of damage. The mean score for each section (in 10 high power fields) was calculated and scores for each experimental rat and experimental groups were calculated.

### 2.8 | Statistical analysis

Statistical analysis was performed using SPSS software version, 22.0. The in vitro experiments were done in triplicates and results are mentioned as mean ± *SEM*. Group means were compared by one-way analysis of variance; least significant difference post hoc test in in vivo experiments. Semiquantitative data of histopathological examination was analyzed using Kruskal-Wallis test. The values of $p < .05$ was considered significant. Triplicates of each sample were used for statistical analysis in the in vitro study.

## 3 | RESULTS

### 3.1 | Chemical standardization of *Vetiveria zizanioides*

The total ash value, acid insoluble ash value, and water-soluble ash value were found to be 30.2 ± 0.9, 26.2 ± 0.9, and 0.8 ± 0.1 (% w/w),

respectively, in the root powder of *V. zizanioides*. The moisture content was 7.5 ± 0.4 (% w/w). The extractive values were found to be 0.8 ± 0.0, 0.3 ± 0.0, 1.4 ± 0.0, and 0.6 ± 0.0 (% w/w), respectively, for the cold water, cold ethanol, hot water, and hot ethanol extracts.

Scientific evaluation of the content of heavy metals in the root powder of *V. zizanioides* revealed that the said metals were present within the standard safe range. Further, the investigations carried out for the detection of *Escherichia coli*, *Staphylococcus aureus*, coliforms, yeast, and mould in the roots of *V. zizanioides* disclosed absence of microbial contamination.

The TLC fingerprint of the dichloromethane extract of *V. zizanioides* roots is shown in Figure 2. The dichloromethane extract exhibited 10 spots in the TLC fingerprint at $R_f$ 0.13, 0.23, 0.32, 0.43, 0.47, 0.49, 0.56, 0.62, 0.71, and 0.94.

The identified compounds from the gas chromatography–mass spectrometry (GC-MS) chromatogram of the methanol extract of *V. zizanioides* with the retention time and their relative percentage values are shown in Figure 3 and Table 1, respectively. The molecular structures of the secondary metabolites including; nootkatone, dehydroaromadendrene, isokhusenic acid, α-vetivone, and isolongifolene were identified (Figure 4).

Preliminary phytochemical analysis revealed the presence of steroid glycosides, flavonoids, saponins, tannins, phenolic compounds, terpenoids, alkaloids, and coumarins in the aqueous root extract of *V. zizanioides*.

## 3.2 | In vitro total antioxidant activity, total polyphenol content, and total flavonoid content

The resulted $IC_{50}$ value by DPPH assay (67.33 ± 1.85 μg/ml) of the aqueous root extract of *V. zizanioides* was relatively high in this study (Figure 5). The $IC_{50}$ value of the reference compound, L- ascorbic acid was 10.67 ± 0.02 μg/ml. The total antioxidant activity by FRAP and TBA methods were 8.10 ± 0.22 μM and 24.54 ± 0.57 μg AAE/g of dry weight of the plant, respectively, for the aqueous root extract of *V. zizanioides* in this study. The polyphenol and flavonoid content of the aqueous root extract of *V. zizanioides* were 1.78 ± 0.03 μg GAE/g of dry weight and 0.55 ± 0.01 μg QE/g of dry weight, respectively.

## 3.3 | Nephroprotective activity of *Vetiveria zizanioides* in nephrotoxic rats

### 3.3.1 | Biochemical parameters of kidney function

The findings on biochemical parameters of kidney function are shown in Table 2. A significant increase in the concentrations of serum creatinine (67%) and BUN (43%) were observed in the nephrotoxic control rats with respect to the normal healthy untreated rats ($p < .05$). Treatment with the aqueous root extract of *V. zizanioides* at 25, 50, and 100 mg/kg doses markedly improved the kidney functions as manifested by a reduction in creatinine and BUN by 12%, 27%, 47%, and 10%, 20%, 13%, respectively, with respect to nephrotoxic untreated control group. Significant fall in serum creatinine was observed only with the 50 and 100 mg/kg doses of *V. zizanioides* ($p < .05$). However, the changes in BUN were not significant in all three doses of *V. zizanioides* ($p > .05$). A significant elevation of urine total protein over 70% and a decline in serum total protein concentration (43%) and albumin (54%) was found in the nephrotoxic control Wistar rats with respect to the healthy rats. The plant extract at the selected doses were able to reduce proteinuria (24%, 54%, 60%) and increase the serum concentration of total protein (12%, 23%, 36%) and albumin (4%, 14%, 21%) significantly in dose-dependent manner ($p < .05$). Treatment with the aqueous root extract of *V. zizanioides* resulted in dose-dependent reduction of both $\beta_2$-microglobulin and cystatin-C with significant changes in $\beta_2$-microglobulin in nephrotoxic rats treated with *V. zizanioides* ($p < .05$).

The standard drug, fosinopril also showed comparable results alleviating doxorubicin-induced nephrotoxic changes. A fall

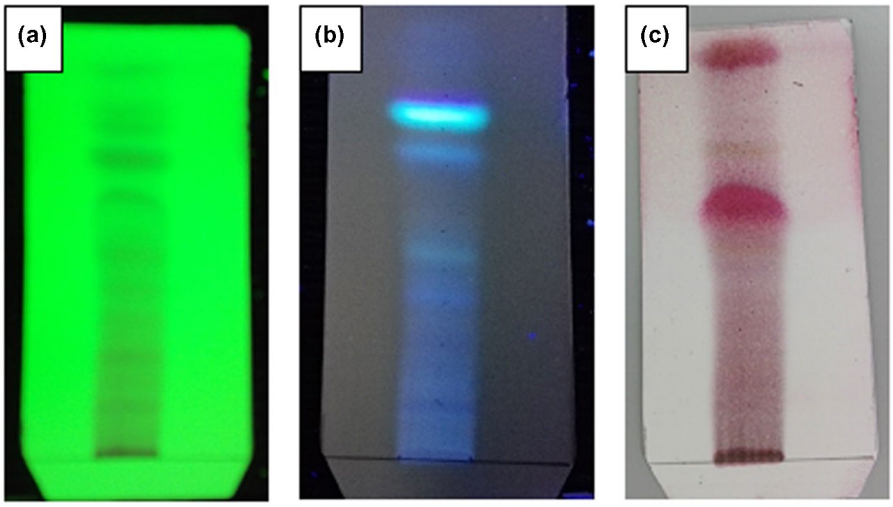


**FIGURE 2** Thin Layer Chromatography fingerprint for the dichloromethane root extract of *Vetiveria zizanioides* at UV 254 nm (a), UV 366 nm (b), and after spraying vanillin sulfuric acid (c)

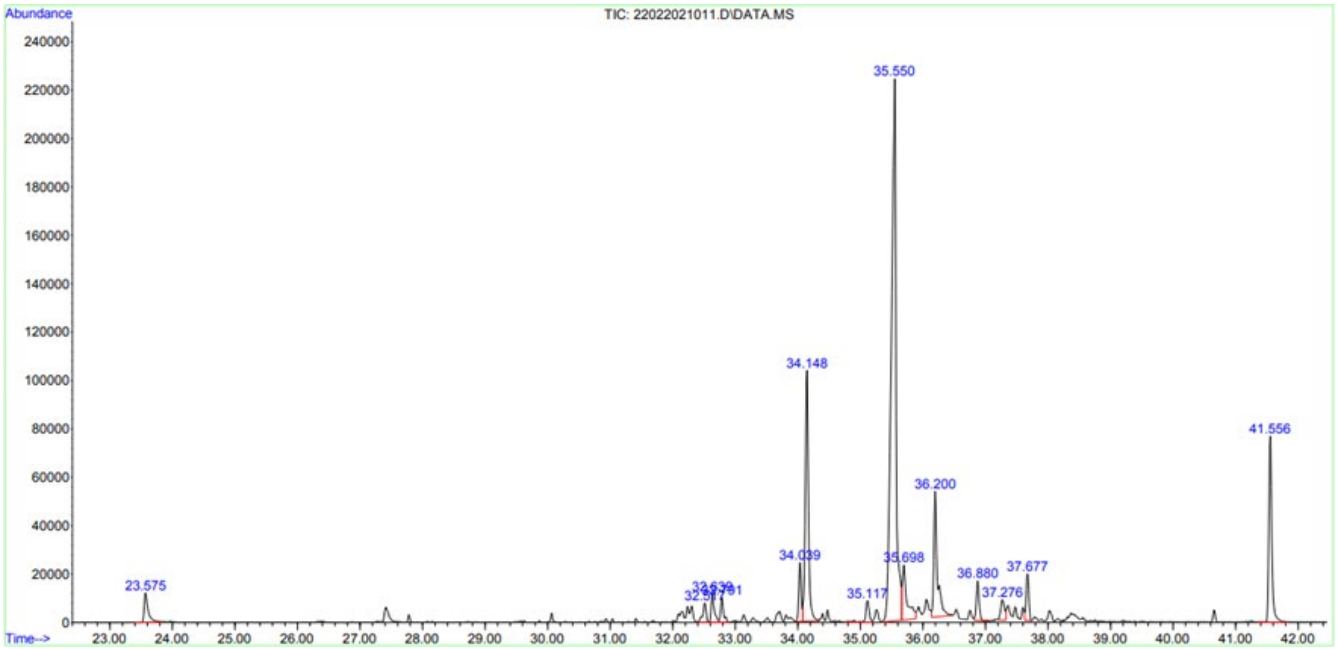


**FIGURE 3** The GC-MS chromatogram of the methanol extract of *Vetiveria zizanioides*

**TABLE 1** The chemical constituents identified from the methanol extract of *Vetiveria zizanioides* with their retention time and the relative percentage values

| Name of the compound | Retention time | Relative percentage (%) |
|---|---|---|
| Nootkatone | 32.517 | 1.17 |
| Dehydroaromadendrene | 34.148 | 14.42 |
| Isokhusenic acid | 35.550 | 46.87 |
| α-Vetivone | 36.200 | 8.083 |
| Isolongifolene | 37.276 | 1.352 |

in creatinine (33%) and BUN (39%) values were observed in the fosinopril-treated rats with respect to nephrotoxic untreated control animals ($p < .05$). Similarly, a remarkable reduction in proteinuria was observed following the treatment with the standard drug even exceeding the level of normal controls. The serum total protein (19%) and albumin (24%) in fosinopril-treated rats were increased and comparable to the findings in the therapeutic (50 mg/kg) and in the selected highest dose (100 mg/kg) of *V. zizanioides*, respectively. However, the selected doses of *V. zizanioides* were more potent in decreasing the elevated levels of $\beta_2$-microglobulin with respect to fosinopril ($p < .05$). Yet, the percentage reduction in cystatin-C by fosinopril (31%) was higher than the three selected doses of *V. zizanioides* (20%, 26%, 28%).

### 3.3.2 | Assessment of histopathology

The results of semiquantitative analysis of kidney injury are shown in Table 3. Normal renal histology was observed in healthy control animals (Figure 6a), whereas early histological features of acute tubular necrosis were shown in the nephrotoxic control group (Figure 6b). These findings are confirmed by the reported highest (46) and lowest (26) mean histological scores by the nephrotoxic and healthy control groups, respectively. A dose-dependent attenuation of the doxorubicin-induced features of renal parenchymal injury was observed following treatment with the three selected doses of *V. zizanioides* (Figure 6d–f) as depicted by the decreased mean histological scores (40, 37, 36). The degree of nuclear pyknosis and loss of brush border in the tubular epithelium was also significantly decreased with the increased dose of *V. zizanioides*. However, a significant improvement in the mean histological scores could be observed only with the 50 and 100 mg/kg doses.

## 4 | DISCUSSION

*V. zizanioides* is a medicinal plant with therapeutic efficacy for combatting a variety of diseases. However, scarce evidence is available on scientific scrutinization of the plant as a dietary supplement in kidney-related diseases. Therefore, this is the first attempt undertaken for scrutinizing the nephroprotective potential of the aqueous root extract of *V. zizanioides* in an animal model of nephrotoxicity.

Scientific evaluation of the potential bioactivities of herbal medicines is greatly hampered by the associated quality control issues such as widespread adulteration, deterioration, and variation in composition of raw plant material, affecting the validity of the test results (Che et al., 2013; Griffiths et al., 2009; Kumari & Kotecha, 2016). Hence, standardization of raw plant materials has become a fundamental requirement in ensuring the safety of the herbal medicines. Accordingly, determination of physicochemical

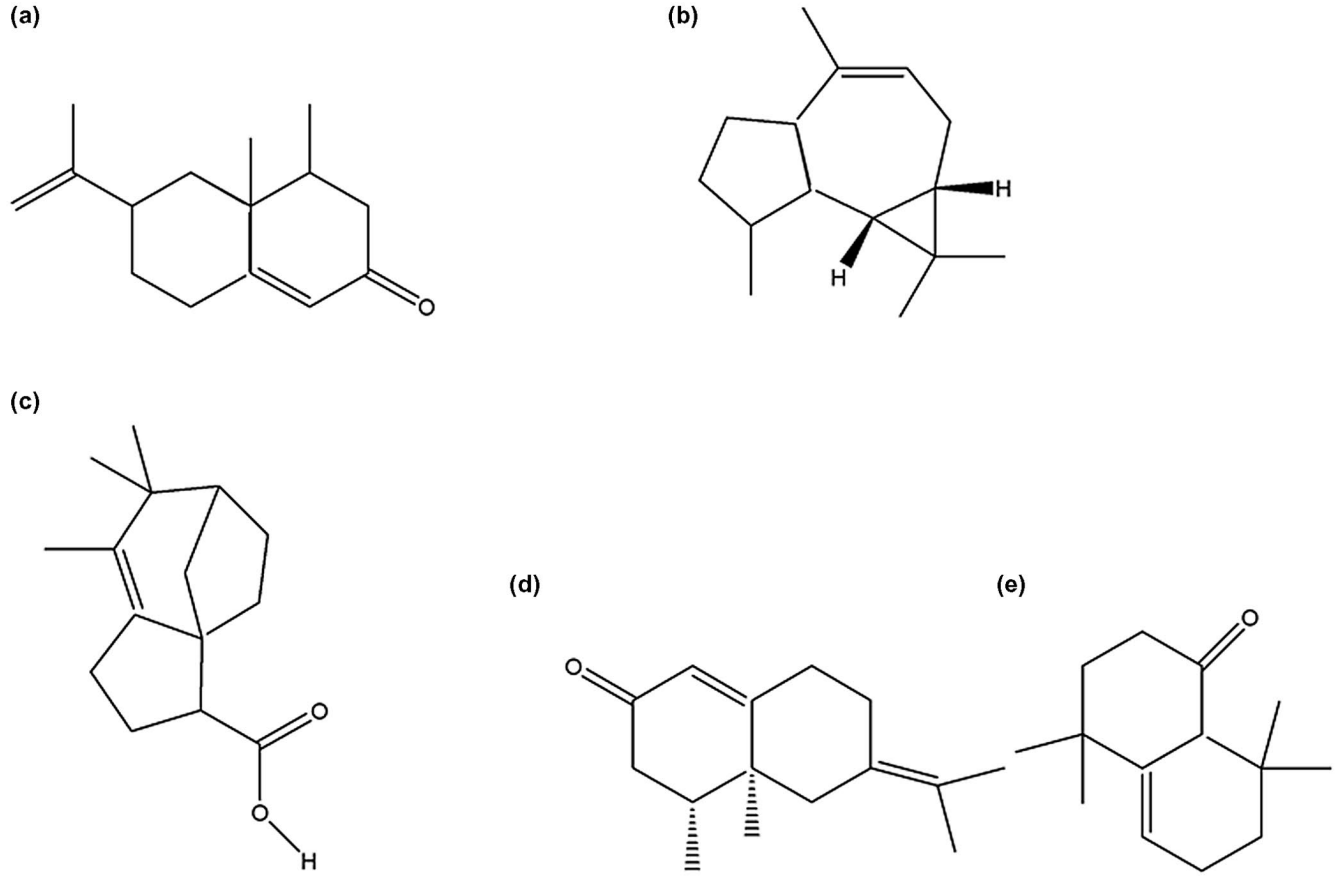


**FIGURE 4** Molecular structures of substances identified in *Vetiveria zizanioides* (L.) Nash: nootkatone (a), dehydroaromadendrene (b), isokhusenic acid (c), α-vetivone (d), and isolongifolene (e)

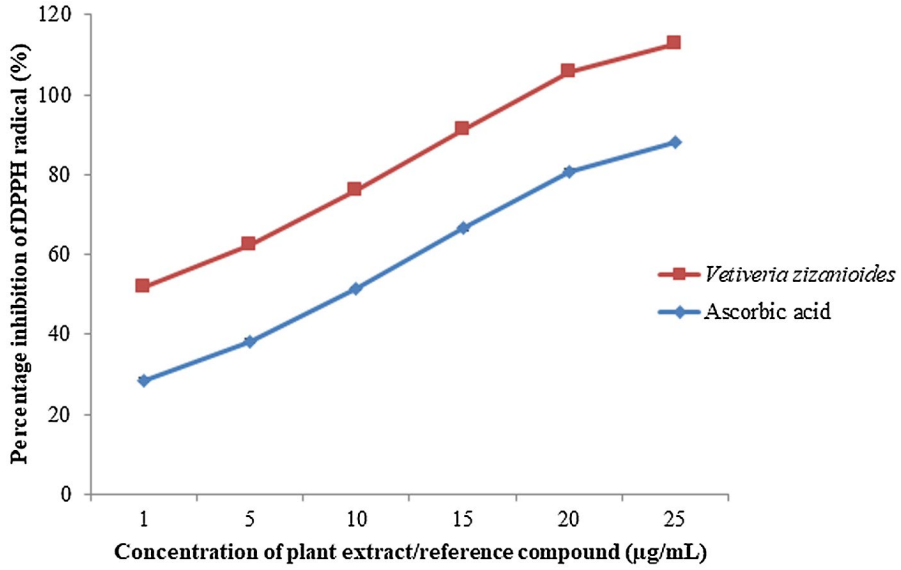


**FIGURE 5** DPPH radical scavenging activity of the aqueous root extract of *Vetiveria zizanioides*. Each data point is expressed as mean ± *SEM*

parameters, qualitative analysis of phytoconstituents, determination of microbial limits, heavy metal analysis, and chromatographic profiling was carried out on the root powder of *V. zizanioides* in this study.

The root powder of *V. zizanioides* demonstrated relatively higher total and acid insoluble ash values in this study, suggesting potential contamination of the plant material from siliceous materials like earth and sand. However, similar variation in total (10.6%, 6.33%) and acid

**TABLE 2** Effect of *Vetiveria zizanioides* on biochemical parameters of renal function

| Treatment | BUN (mmol/L) | Serum creatinine (μmol/L) | Serum total protein (g/dl) | Serum albumin (g/dl) | Serum $\beta_2$-MG (μg/ml) | Serum cystatin-C | Urine total protein (g/dl) |
|---|---|---|---|---|---|---|---|
| Healthy control | 5.32 ± 0.38$^{**}$ | 30.94 ± 4.05$^{***}$ | 66.42 ± 1.50$^{***}$ | 35.17 ± 0.42$^{***}$ | 3.74 ± 0.29$^{***}$ | 17.99 ± 1.52$^{*}$ | 81.28 ± 8.17$^{***}$ |
| DOX induced nephrotoxic control | 9.28 ± 1.26 | 92.93 ± 13.38 | 46.31 ± 1.08 | 22.88 ± 0.48 | 12.81 ± 2.12 | 28.11 ± 2.04 | 316.18 ± 20.99 |
| DOX +*V. zizanioides* 25 mg/kg | 8.33 ± 0.94 | 81.92 ± 7.24 | 51.67 ± 5.44 | 23.78 ± 1.59 | 7.22 ± 0.22$^{**}$ | 22.50 ± 4.15 | 240.00 ± 36.23$^{*}$ |
| DOX +*V. zizanioides* 50 mg/kg | 7.98 ± 0.90 | 67.41 ± 1.11$^{*}$ | 56.77 ± 2.19$^{**}$ | 26.17 ± 0.22$^{*}$ | 4.90 ± 1.03$^{*}$ | 20.87 ± 2.57 | 144.95 ± 13.89$^{***}$ |
| DOX +*V. zizanioides* 100 mg/kg | 8.03 ± 1.05 | 49.28 ± 6.17$^{***}$ | 63.20 ± 1.17$^{***}$ | 27.73 ± 0.43$^{**}$ | 4.38 ± 1.04$^{*}$ | 20.15 ± 0.36 | 125.63 ± 34.26$^{***}$ |
| DOX +fosinopril 0.09 mg/kg | 5.64 ± 0.21$^{**}$ | 61.88 ± 2.53$^{**}$ | 55.04 ± 0.77$^{**}$ | 28.27 ± 0.47$^{***}$ | 11.54 ± 3.73 | 19.38 ± 2.70$^{*}$ | 48.32 ± 5.93$^{***}$ |

*Note*: The biochemical parameters are expressed as mean ± *SEM* ($n = 6$/group). Statistical significance with respect to doxorubicin (DOX)-induced nephrotoxic control group at: $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$.

Abbreviations: BUN, blood urea nitrogen, $\beta_2$-MG, $\beta_2$-microglobulin.

insoluble ash (9.1%, 4.83%) values were noticed in studies done by Issaravanich et al. (2008) and Mishra et al. (2014), respectively. The reported water-soluble ash content (0.66%) by Mishra et al. (2014) was much similar to our findings. These findings may validate the results of this study excluding the risk for potential contamination. A possible explanation for the discrepancy of results between the studies might be due to the differences in the sources and the origin of the plant materials.

The highest extractive value was found in the hot water extract of *V. zizanioides*. This finding favors the use of the hot water extract in the assessment of therapeutic efficacy of the selected medicinal plant, simulating the conventional medical practice of using the plant as decoctions. Comparable variations of ethanol soluble (4.9 ± 3.2) and water soluble (48.0 ± 13.3) extractive values were observed in Issaravanich et al. (2008) even though the values were relatively low in this study.

Medicinal plants grown in a natural environment are often vulnerable to contamination with heavy metals and potential pathogenic microorganisms from soil, irrigation, and postharvest water. The consumption of such herbs might result in adverse health consequences leading to poisoning (Azi et al., 2018). The present findings revealed that the said metals were present within the standard safe range in the selected plant material. Moreover, the absence of microbial contamination, further safeguard the human consumption of the plant in therapeutic and food applications.

The findings on preliminary phytochemical analysis are comparable with the previous literature (Muthukrishnan & Manogaran, 2018; Ratha et al., 2012). Phenolic compounds are potent antioxidants with the ability of preventing harmful physiological effects in human body. Besides the antioxidant properties, phenolic compounds from roots of *V. zizanioides* have been reported for antimicrobial and anticancer activities. The major phenolic compounds identified from the roots of *V. zizanioides* including ferulic acid and vanillin are widely known as a food preservative and a flavoring agent, respectively (Moon et al., 2020). Further, alkaloids from *V. zizanioides* have shown effective antimicrobial and antifungal activities (Chou et al., 2016). These findings substantiate the value of *V. zizanioides* in medicinal and food applications.

The findings on TLC and GC-MS profiles of *V. zizanioides* roots would be further beneficial as quality control parameters for differentiation of the plant from its adulterants and substitutes. Moreover, these findings would facilitate as quality control measures in future applications of developing a commercially viable dietary supplement. The identified secondary metabolites by GC-MS profiling; nootkatone, dehydroaromadendrene, isokhusenic acid, α-vetivone, and isolongifolene corroborate the findings on *V. zizanioides* by Mao et al. (2006), David et al. (2019), and Nigam and Komae (1967).

Most of the kidney-related adverse health consequences are mediated via oxidative stress (Al-Okbi et al., 2014). The array of reactions which provokes the generation of highly reactive free radicals is assumed to contribute to lipid peroxidation, protein degradation, and DNA damage during the events of oxidative stress (Meo and Venditti 2020). This can be either delayed or even halted

**TABLE 3** Effect of *Vetiveria zizanioides* on kidney histopathology

| Treatment group | Score value | | | | | | Mean histological score |
|---|---|---|---|---|---|---|---|
| | Loss of brush border | Congestion in the glomerulus | Hemorrhage | Cytoplasmic vacuolization | Pyknosis | Cast formation | |
| Healthy untreated animals | 7** | 3** | 8** | 8** | 1** | 0 | 26*** |
| DOX induced nephrotoxic control | 10 | 9 | 10 | 10 | 7 | 0 | 46 |
| DOX +V. *zizanioides* 25 mg/kg | 7* | 10 | 10 | 10 | 3 | 1 | 40 |
| DOX +V. *zizanioides* 50 mg/kg | 6** | 9 | 10 | 9 | 1** | 0 | 37* |
| DOX +V. *zizanioides* 100 mg/kg | 5*** | 9 | 10 | 10 | 2* | 0 | 36** |
| DOX +fosinopril 0.09 mg/kg | 9 | 7 | 9* | 10 | 0*** | 0 | 35** |

*Note:* Scores for the individual features observed were given; 10 as the highest score value in each feature observed. Mean score for each group is given ($n = 6$/group). Statistical significance with respect to doxorubicin (DOX)-induced nephrotoxic control group at: $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$.

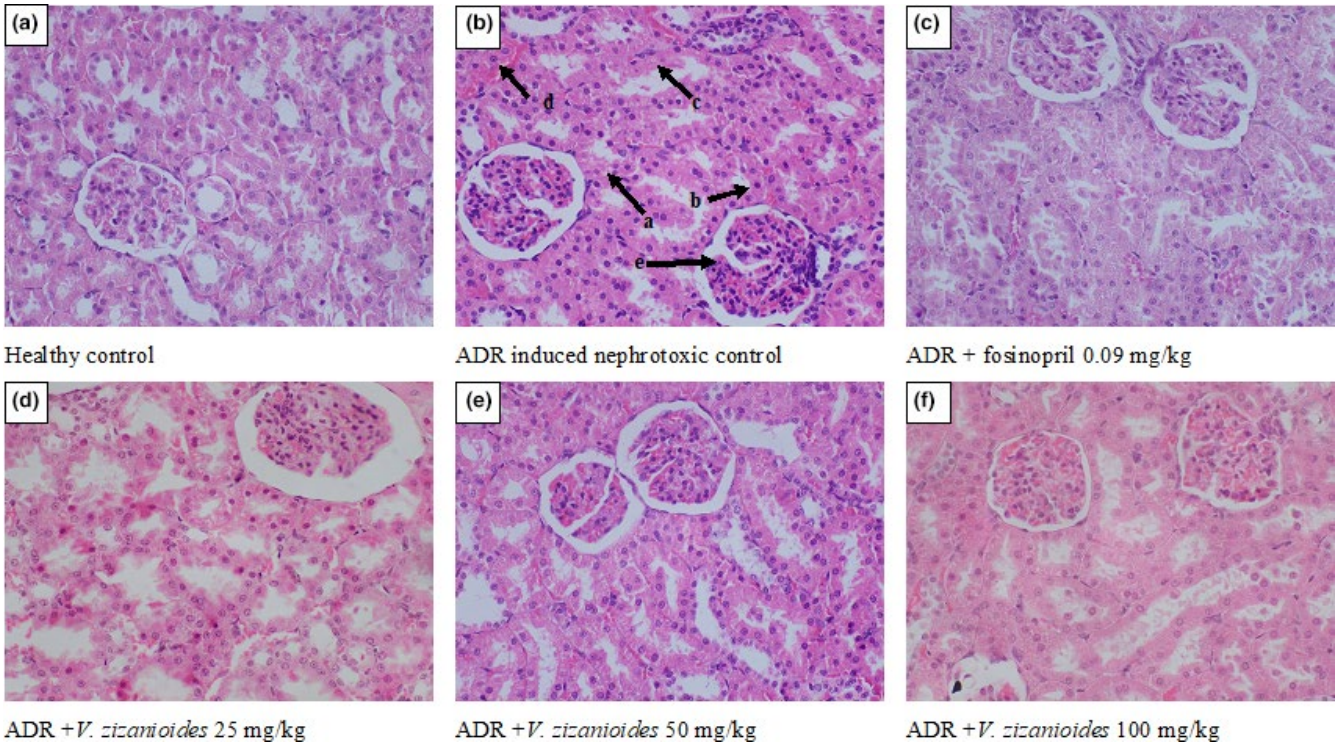


**FIGURE 6** Photomicrographs H- and E-stained kidney tissues of doxorubicin (DOX) nephrotoxic untreated animals upon the treatment of *Vetiveria zizanioides* root extract (x400). Normal control group (a), doxorubicin (DOX)-induced nephrotoxic untreated Wistar rats (b), fosinopril; standard drug-treated group (c), animals upon the treatment of *Vetiveria zizanioides* root extract at 25, 50, and 100 mg/kg doses (d–f). The histological features considered to signify acute tubular injury in Figure 5 (b) are; (a) cytoplasmic vacuolization, (b) nuclear pyknosis, (c) loss of brush border in the epithelium of the tubule, (d) intertubular hemorrhages and (e) glomerular congestion

by protecting the cells from damaging free radicals. Generally, endogenous antioxidants are produced by the body to defend against free radicals; however, it might be insufficient if the free radical damage is severe. The use of dietary antioxidants plays a crucial role in this context preventing cellular damage and repairing biological molecules to a certain extent (Marcadenti & Assis -Coelho, 2015; Yoo et al., 2010). Hence, estimation of total antioxidant activity of the aqueous root extract of *V. zizanioides* was carried out in this study, in order to assess the potential benefits of the plant in the management oxidative stress-related problems in kidney disease.

The aqueous root extract of *V. zizanioides* exerted relatively a low $IC_{50}$ value for DPPH radical scavenging assay, suggesting relatively a higher antioxidant potential in vitro. The $IC_{50}$ value of the reference compound, L- ascorbic acid is comparable with previously published reports, further validating the findings of this study (Shekhar & Anju, 2014). However, the resulted FRAP value for the aqueous root extract of *V. zizanioides* is relatively lower than the FRAP value

reported by Devprakash and Subburaju (2011) (51.47 ± 0.43 mg GAE/g). The differences in the origin of the plant as well as the use of different reference compounds might give rise to these deviations in results (Devprakash & Subburaju, 2011). Interestingly, the present finding on antioxidant activity by TBA method was in line with the report by Attanayake et al. (2016) on *V. zizanoides* of Sri Lankan origin. These findings substantiate the health effects of *V. zizanioides* pertaining to kidney diseases as a potential dietary source of free radical scavenger. Based on the literature, β-vetinene, β-vetinone, and α-vetinone isolated from *V. zizanioides* are the major contributors to the antioxidant activity of the plant (Kim et al., 2005; Luqman et al., 2009). In addition, the identified sesquiterpenes; nootkatone and isolongifolene have been reported for antioxidant activity in vivo and in vitro, respectively (Nemmar et al., 2018; Rangasamy & Namasivayam, 2014). Hence, the presence of α-vetivone, nootkatone, and isolongifolene in the GC-MS fingerprint of the methanol root extract of *V. zizanioides* in this study further substantiates the reported higher antioxidant potential of the plant. These findings emphasize the importance of *V. zizanioides* as a potent source of exogenous antioxidants in the management of kidney diseases.

Polyphenols contribute directly to the antioxidant activity of medicinal plants (Abuajah et al. 2014; Subhadradevi et al., 2010). The quantification of polyphenols in the aqueous root extract of *V. zizanioides* by Folin Ciocalteu method substantiated the results of Devprakash and Subburaju (2011), where the fresh aqueous root extracts of *V. zizanioides* showed a polyphenol content of 15.43 ± 0.31 mg tannic acid equivalents per 100 g dry weight of sample. Flavonoids are the largest group of phenolic compounds and represent the most abundant species of phenolic compounds found in the human diet (Krzyzanowska et al., 2010). The findings on total flavonoid content of *V. zizanioides* in this study corroborated the findings of Devprakash and Subburaju (2011) (0.94 ± 0.21%w/w of quercetin), however, slightly deviated from the findings of Attanayake et al. (2016) (2.6 ± 0.2 μg QE/g of dry weight). The differences might be due to the variations in assay conditions and the seasonal variations in the plant even though both were collected from Sri Lanka. The reported high flavonoid content might contribute directly to the antioxidant activity of *V. zizanioides* in neutralizing the free radicals.

Supplement of dietary antioxidants has been identified as an effective therapeutic approach in reducing oxidative stress caused by the doxorubicin-induced nephrotoxicity. A number of studies were reported on investigation of nephroprotective activity of dietary antioxidants such as curcumin, resveratrol, quercetin, and proanthocyanidins in doxorubicin-induced experimental nephrotoxicity model (Benzer et al., 2018; El-Sayed et al. 2017; Eugenio-Perez et al., 2016; Heeba and Mahmoud 2014; Shahbazi et al. 2020). Hence, in this study, the potential protective effect of *V. zizanioides* root extract was investigated against the experimental model of doxorubicin-induced nephrotoxicity.

The significant changes observed in the biochemical parameters, markers of kidney function, and histopathology of kidney tissues in the nephrotoxic control rats compared to the untreated normal control group, substantiate the potential nephrotoxicity induced by doxorubicin in this study. Post-treatment with the standardized aqueous root extract of *V. zizanioides* provided a significant protection toward kidney dysfunction in the studied model of nephrotoxicity as reflected by biochemical and histopathological assessments. Treatment of the nephrotoxic rats with an equivalent therapeutic dose and the selected highest dose of *V. zizanioides* resulted in similar or better improvement in most of the biochemical parameters and markers of kidney function and the kidney histopathology compared to the experimental rats treated with the standard drug. Interestingly, the three selected doses of *V. zizanioides* significantly reduced the serum concentration of $\beta_2$-microglobulin compared to the standard drug, fosinopril. $\beta_2$-microglobulin is an early biomarker of glomerular damage, which facilitates the identification of kidney injury at the early stage of the disease. Hence, significant dose-dependent reduction in $\beta_2$-microglobulin following the treatment with the three selected doses of *V. zizanioides* indicates the effective management of the kidney patients at early stages of the disease. However, a remarkable reduction in proteinuria, even exceeding the level of normal controls was noted following the treatment with the therapeutic dose of the standard drug. The inhibition of renin-angiotensin system by fosinopril causing hypoalbuminuria might lead to this reduction in urinary albumin excretion ameliorating proteinuria (Xiao et al., 2013).

However, present results demonstrated that the standardized aqueous root extract of *V. zizanioides* has a significant dose-dependent nephroprotective effect against doxorubicin-induced experimental nephrotoxicity based on the biochemical and histopathological assessments. The nephroprotective activity of *V. zizanioides* might be via reduction of the oxidative stress due to the presence of antioxidative secondary metabolites, particularly α-vetivone, nootkatone, and isolongifolene. Additionally, the anti-inflammatory potential of the plant as stated in the recent literature might account for the nephroprotective effects of the plant via suppression of the inflammatory response induced by doxorubicin (Chou et al., 2012; Heravi et al., 2018; Rahul et al., 2013). The anti-inflammatory potential of nootkatone, might play a significant role in attenuating cellular inflammatory cascades resulted from doxorubicin (Nemmar et al., 2018). The subsequent activation of apoptotic pathways in doxorubicin-induced oxidative stress also plays a critical role in amplifying the cellular toxic effects to a significant extent (Ibrahim et al., 2020). The potential inhibition of apoptosis by nootkatone through inhibition of NF-κB activation as reported by Nemmar et al. (2018) might be beneficial in amelioration of doxorubicin-induced apoptosis and down regulation of inflammatory responses in nephrotoxic animals. However, further investigations on underlying cellular anti-inflammatory mechanisms of aqueous root extract of *V. zizanioides* in nephrotoxic experimental animals are underway in our laboratory.

Isokhusenic acid, a compound identified in GC-MS analysis further substantiates the use of *V. zizanioides* in food applications in addition to its contribution to nephroprotective activity (Dwivedi et al., 2013). The unique flavor and the reported bioactivities of the plant fortify its use as a novel dietary supplement targeting the

management of kidney disease (Mahmoudi et al., 2019). In fact, the dietary supplement of *V. zizanioides* might be beneficial in enhancing the quality of life of kidney patients by preserving kidney functions and preventing/delaying the progression to an end-stage kidney disease (Ash et al., 2014; Cosola et al., 2018). However, the possible role of gut microbiota in the pathogenesis of the kidney disease has been reported (Eshghjoo et al., 2021). Even though, diet is considered a major factor driving the composition and metabolism of the gut microbiota, the effect of dietary supplements on gut microbiota and its mechanistic links to bioactivities are largely undefined (Eshghjoo et al., 2021). Hence, the effect of *V. zizanioides* as a dietary supplement on microbial enzyme expression in the microbiota is also warranted in future.

## 5 | CONCLUSIONS

The aqueous root extract of *V. zizanioides* possessed relatively high in vitro antioxidant activity emphasizing the potential use of the plant as a dietary antioxidant. The in vivo experiments revealed the significant dose-dependent protective effects of the aqueous root extract of *V. zizanioides* against doxorubicin-induced experimental nephrotoxicity. A better protection could be observed at the doses of 50 and 100 mg/kg of *V. zizanioides* as signified by the improved biochemical parameters, markers of kidney function, and histological scores. The antioxidant potential of the plant generated through identified secondary metabolites might account for the nephroprotective activity in vivo. Based on the present findings, we conclude that, administration of the aqueous root extract of *V. zizanioides* would be a promising dietary supplement for combatting the free radical pathologies associated with the kidney diseases.

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### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

### AUTHOR CONTRIBUTIONS

**Sachinthi Sandaruwani Amarasiri:** Data curation; Formal analysis; Investigation; Methodology; Validation; Writing-original draft. **Anoja Priyadarshani Attanayake:** Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. **Liyanage Dona Ashanti Menuka Arawwawala:** Formal analysis; Methodology; Supervision; Writing-review & editing. **Kamani Jayatila Ayoma Perera Wijewardana Jayatilaka:** Conceptualization; Supervision; Writing-review & editing. **Lakmini Kumari Boralugoda Mudduwa:** Conceptualization; Methodology; Supervision; Writing-review & editing.

### ETHICS APPROVAL STATEMENT

Ethical clearance was granted from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka (Reference No. 14.12.2015:3.1).

### DATA AVAILABILITY STATEMENT

The mean grouped data supporting the findings of this study are included within the article and the raw data is available upon request.

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