Journal of Ethnopharmacology 299 (2022) 115510

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Costus spiralis extract restores kidney function in cisplatin-induced nephrotoxicity model: Ethnopharmacological use, chemical and toxicological investigation

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ARTICLE INFO

Keywords:
Cisplatin
Costus spiralis
Flavonoids
Vicenin II
Schaftoside
Nephrotoxicity

ABSTRACT

Ethnopharmacological relevance: Costus spiralis (Jacq.). Roscoe (Costaceae) is traditionally used in Brazil for the treatment of kidney diseases such as pyelonephritis, urethra inflammation, kidney stones, and inflammatory conditions. There are reports of its use by Brazilian Indians since the 17th century when it was known as "pacocatinga." Currently, the use of the *Costus* species in Brazil is widespread, which was evidenced by the inclusion of the genus in the Brazilian National List of Medicinal Plants of Interest to the Unified Health System (RENISUS).

Aim of the study: This study aimed to confirm the ethnopharmacological use of *Costus spiralis* in the treatment of kidney diseases, toxicity study using animal models, and the phytochemistry of the species.

Materials and methods: The chemical profile of *Costus spiralis* leaves extract (CSLE) was obtained for the hydroethanolic extract by ultra-performance liquid chromatography coupled to a mass spectrometer and ultraviolet detector with diode array (UPLC-UV/DAD-ESI-MS). The acute oral toxicity of the extract was predicted using the neutral red uptake cytotoxicity assay. Wistar rats were used in a model *in vivo* for confirmation of acute oral toxicity (2000 mg/kg p.o. for 14 days.) and determination of the effect on a cisplatin-induced nephrotoxicity model.

Results: The analysis by UPLC-UV/DAD-ESI-MS showed that the chemical composition of the extract is mostly diglycosylated flavones of apigenin. In the extract were identified the flavones vicenin II and schaftoside. The quantification of total flavonoids by spectrometry showed 0.880%. CSLE proved to be safe for acute oral administration (2000 mg/kg) with an IC_{50} value of 222.9 μg/mL and predicted oral toxic dose of 523.82 μg/mL in a neutral red uptake cytotoxicity assay. The absence of death allows the classification of the extract in class 5 according to OECD 423 guidelines and therefore it can be considered as a high acute safety product, which is highly relevant, considering the wide popular use of the species. In the cisplatin-induced nephrotoxicity model, *C. spiralis* extract (5, 15, and 30 mg/kg) significantly improved renal function, reversing almost completely the effects on plasma creatinine levels and creatinine clearance ($p < 0.001$).

Conclusions: This study demonstrates that oral administration of *Costus spiralis* extract leaves is safe and effective in restoring the renal function in rats in a cisplatin-induced nephrotoxicity. It is suggested that the observed

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https://doi.org/10.1016/j.jep.2022.115510
Received 28 January 2022; Received in revised form 21 June 2022; Accepted 22 June 2022
Available online 27 June 2022

activity is related to the flavonoids present. This hypothesis should be confirmed, and the participation of other secondary metabolites should be investigated in the future.

List of abbreviations

CEUA	Ethics Committee on the Use of Animals (CEUA/UFMG)
CSLE	*Costus spiralis* leaves extract
DAD	Diode Array Detector
DMEM	Eagle's Minimal Essential Medium
ESI	Electrospray Ionization
I.P	intraperitoneal
HPLC	High Performance liquid chromatography
OECD	Organization for Economic Co-operation and Development
RENISUS	Brazilian National List of Medicinal Plants of Interest to the Unified Health System
SFB	Fetal Bovine Serum
SISGEN	National System of Genetic Heritage and Associated Traditional Knowledge Management
UPLC	Ultra Performance liquid chromatography
UPLC-ESI-MS/UV/DAD	Ultra-Performance liquid chromatography coupled to electrospray ionization mass spectrometry and ultraviolet detector with diode array
UV/DAD	Ultraviolet Detector with Diode Array

1. Introduction

Costus spiralis (Jacq.). Roscoe (Costaceae) is a medicinal plant widely used in Brazil, with reports from European naturalists on its use by Brazilian indigenous people in the 17 th century (DATAPLAMT, 2019). Ethnopharmacological studies and historical records report the use of the roots, rhizomes, stems, and leaves for different conditions, mostly related to the genitourinary tract. The juice of the stem and the tea from fresh leaves are used for gonorrhea, syphilis, nephritis, and bladder diseases. There are also reports of topical use of the decoct from the leaves and stems to relieve vaginal irritation and leucorrhea (Duarte et al., 2017; DATAPLAMT, 2019).

Costus piralis is indicated in folk Brazilian medicine for the treatment of nephritis, inflammation of the urethra, bladder infections, kidney stones, renal failure, and atherosclerosis (Duarte et al., 2017). The genus *Costus* was even included in the National List of Medicinal Plants of Interest to Unified Health System (RENISUS) in Brazil, justified by the wide ethnopharmacological use of these species in Brazil, as well as their potential for the development of herbal medicines (Almeida et al., 2014; Brazil, 2009).

Thus, investigation of popular medicinal use of the *Costus* species, as well as their chemical constituents and toxicity, may provide new therapeutic options for the treatment of kidney diseases (Burgos-Calderón et al., 2021; Joyce et al., 2017). Kidney diseases are a global public health problem and are the leading cause of death and disability worldwide. In addition, the high costs and complications of hospitalization and renal replacement therapy also justify the investigation of new and multiple therapeutic approaches, especially for prevention or management at the early stages of these diseases.

In this context, this study aims to assist the safe and efficient use of *C. spiralis*, to support its ethnopharmacological use, and to contribute to the chemical characterization of the species, identifying the major substances present in the species and conducting a preliminary analysis of the safety of its use. Taking into account the reasoning, the objective of this study was to perform a chemical analysis of the *C. spiralis* extract using ultra-performance liquid chromatography coupled to ultraviolet detector with diode array and electrospray ionization mass spectrometry (UPLC-UV/DAD-ESI-MS) and spectrophotometric methods, and to evaluate the acute oral toxicity and the effect of *Costus spiralis* (Jacq.) Roscoe leaves extract on a model of cisplatin-induced nephrotoxicity in rats.

2. Materials and Methods

2.1. Chemicals/reagents

HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany) and formic acid 85% was acquired from CRQ Produtos Químicos (São Paulo, Brazil). Reference standards of vicenin (94.12%) and quercetin (99.50%) were purchased from Sigma-Aldrich/Merck (Darmstadt, Germany) and the schaftoside (98.98%) from Aktin Chemicals (Chengdun, China). Ultra-pure water was obtained through the Milli-Q-Plus system (Millipore, USA). Cisplatin was purchased from Sigma-Aldrich/Merck (Darmstadt, Germany).

2.2. Plant material

Leaves of *Costus spiralis* (Jacq.) Roscoe, Costaceae, were collected at Botanical Garden of Fundação de Parques Municipais e Zoobotânica, Belo Horizonte, Minas Gerais, Brazil (19°51′26,4‘‘ S; 44°0′24,8″ W) in March of 2015. The cultivated specimens were previously identified, and a voucher specimen was deposited in the institution's herbarium (CV JBFZB1874 BHZB, Belo Horizonte, Brazil). The registration was carried out in the National System of Genetic Heritage and Associated Traditional Knowledge Management (SisGen) (code A61CD8C).

2.3. Extraction

The fresh leaves of C. *spiralis* were dried in a ventilated oven (T < 40 °C). Dried leaves were ground in tamis (840 μm), and the obtained powder (5.0 g) was extracted by maceration with 100 mL of mixture Ethanol: water (80:20) assisted by ultrasound for 20 min. This procedure was repeated three times. After extraction, solvents were evaporated under reduced pressure and water was lyophilized. The powder extracts (CSLE) were stored at −20 °C until analysis. The procedure was repeated until 5.0 g extract was obtained. The average extractive yield was 12.30 ± 0.77%.

2.4. UPLC-UV/DAD-ESI-MS analysis

2.4.1. Sample solution

CSLE was accurately weighed (5.0 mg) and solubilized in 1.0 mL of a mixture of ultrapure water and HPLC grade methanol (7:3), sonicated in an ultrasound bath for 20 min, and centrifuged (9184 g) for 10 min. The supernatant was filtered through a Millipore membrane (0.22 μm).

2.4.2. Standard solutions

Vicenina II and schaftoside reference standard were accurately weighed (1.0 mg), separately, and solubilized in 2.0 mL of a mixture of ultrapure water and HPLC grade methanol (7:3), sonicated in an ultrasound bath for 20 min, and centrifuged (9184×*g*) for 10 min. The supernatant was filtered through a Millipore membrane (0.22 μm).

2.4.3. Sample solution contaminated with vicenin II and schaftoside standards

To perform the co-injection experiment, the sample solution (1.0 mL)

was mixed with 40 μL of vicenin II standard solution and 60 μL of schaftoside standard solution and 1,0 mL of a mixture of ultrapure water and HPLC grade methanol (7:3).

2.4.4. Analyses by UPLC-UV/DAD-ESI-MS

The sample (item 2.4.1) and standard solutions (item 2.4.2) were analyzed by UPLC-UV/DAD-ESI-MS in an ACQUITY Ultra Performance LC system (Waters, USA) linked to an ACQUITY TQ detector and ACQUITY UV/DAD detector (Waters MS Technologies, UK), equipped with Z-spray electrospray ionization (ESI) in both negative and positive mode. For this, the extracts were solubilized in a mixture of methanol and water (3:7) (1.0 mL) HPLC grade, sonicated in an ultrasound bath for 20 min, and centrifuged (625 *g*) for 10 min. The supernatant was filtered through a Millipore membrane (0.2 μm). Standard solutions of schaftoside (30 μg/mL) and vicentin II (18 μg/mL) were prepared similarly. Chemical profiles were obtained using a mobile phase of 0.1% HCOOH water (A) and 0.1% HCOOH methanol (B) with a flow rate of 0.25 mL/min in an ACQUITY UPLC® BEH C-18 column (1.7 μm, 100 mm × 2.1 mm i. d.; ACQUITY, Ireland) at 30 °C. The following gradient elution scheme was used: 10–25% B at 0–1 min, 25–35% B at 1–18 min, 35–95% B at 18.0–18.1 min, 95–95% B at 18.1–25 min, 95–10% B at 25.0–26.0 min, and −26%–30% B at 36–30 min. The MS operating parameters were as follows: scan mode negative and positive within the mass range of *m/z* 100–1000, cone voltage of 5 kV, and a capillary voltage and temperature of 3.5 kV and 400 °C respectively. The nebulizer gas was nitrogen and the source temperature was 120 °C. The system was coupled with MassLynx software (version 4.1, Waters, USA) to control the instruments, as well as for data acquisition and processing.

2.4.5. Co-injection experiment by UPLC-UV/DAD

To perform the co-injection experiment the sample (item 2.4.1), standard solutions (item 2.4.2), and sample solution contaminated with vicenin II and schaftoside standards (item 2.4.3) were analyzed by ACQUITY® UPLC-UV/DAD (Waters MS Technologies, UK). The chromatographic conditions were the same as those employed for UPLC-UV/DAD-ESI-MS analysis, except the final gradient step was changed to shorten the chromatographic run time. The mobile phase used consisted of 0.1% HCOOH water (A), 0.1% HCOOH methanol (B) and acetonitrile The following gradient elution scheme used was: A–B–C (90:10:0, v/v) at 0 min, A–B–C (75:25:0, v/v) at 0,0–1.0 min, A–B–C (65:35:0,v/v) at 1–18 min, A–B–C (5:0:95 v/v) at 18.1–20 min for column cleaning, and finally A–B–C (90:10:95 v/v) at 20–25 for stabilization of the initial condition.

2.5. Determination of total flavonoid content

The total flavonoid content was determined using a method developed and validated by Garcia (2011). The analyses were performed in triplicate.

2.5.1. Sample solution

CSLE (0.250 g) was refluxed in methanol (12 mL) for 30 min. The extract obtained, cooled to room temperature, was filtered. The process was repeated two times. The filtrate was collected in a 25 mL volumetric flask and the volume was completed with methanol. An aliquot of the resulting solution (15 mL) was partitioned between liquid-liquid with water (9 mL) and chloroform (6 mL). The hydromethanolic phase was diluted to 25 mL using methanol. To an aliquot (5 mL) of this solution was transferred to a 25 mL volumetric flask and added 0.6 mL glacial acetic acid, 10 mL pyridine: water (2: 8, v/v) solution, and 2.5 mL of 12% methanolic aluminum chloride hexahydrate solution, completing the volume with water. After 30 min the absorbance of solution was measured at λ 420 nm. Results were expressed in g of quercetin per 100 g of sample (% w/w).

2.5.2. Standard solution

Quercetin standard were accurately weighed (3.0 mg), and quantitatively transferred to a 25 mL volumetric flask using methanol to dissolve and fill the volume. An aliquot of the resulting solution (15 mL) was diluted to 25 mL using methanol. To an aliquot (5 mL) of this solution was transferred to a 25 mL volumetric flask and added 0.6 mL glacial acetic acid, 10 mL pyridine: water (2: 8, v/v) solution, and 2.5 mL of 12% methanolic aluminum chloride hexahydrate solution, completing the volume with water. After 30 min the absorbance of solution was measured at λ 420 nm.

2.5.3. Calculation of the percentage of total flavonoids

The total flavonoid content was calculated according to the following equation and results were expressed in g of quercetin per 100 g of sample (% w/w).

$$\%\ of\ total\ flavonoids = \frac{Abs\ (samples\ solution)\ \times\ quercetin\ mass\ (3.0\ mg)\ x\ 0.995\ \times\ 100}{Abs\ (standard\ solution)\ x\ CSLE\ mass\ (250.0\ mg)}$$

2.6. Prediction of acute oral toxicity

The neutral red uptake cytotoxicity assay was carried out according to the guidelines of the Organization for Economic Co-operation and Development (OECD) number 129 (OECD, 2010). Fibroblasts from Balb/c mice 3T3-A3 were seeded (3×10^3 cells/well) into 96-well plates for 24 h at 37 °C and exposed to sample solutions for 48 h. After the exposure time, 250 μL/well of neutral red dye (25 μg/mL) in Eagle's minimal essential medium (DMEM) 5% Fetal Bovine Serum (SFB) was added, and the plates were incubated again for 3 h. Absorbance was measured at λ 540 nm in a microplate reader. Cell viability was expressed as a percentage of untreated controls and this value was used to determine the IC_{50} value using the GraphPad Prism 7.0 software. To predict the toxic dose, the following equation was used:

$$\text{Toxic dose} = \mathbf{10}^{(0{,}372\ \mathbf{x}\ \log\ \mathbf{IC_{50}}\ (\mu g/mL)\ +\ 2{,}024)}$$

2.7. Animals

Wistar rats of either sex, 6–8 weeks old and weighing 180–230 g, were obtained from the animal facility of the Faculty of Pharmacy, UFMG, Brazil. Animals were housed in standard cages or metabolic cages, according to the experiment performed, at a room temperature 22 °C (±3 °C) and 50–60% relative humidity under 12 h light-dark cycles. Water and feed were provided ad libitum. The experimental protocols (nº 201/2016 and nº 129/2019) were approved by the Ethics Committee on the Use of Animals (CEUA/UFMG).

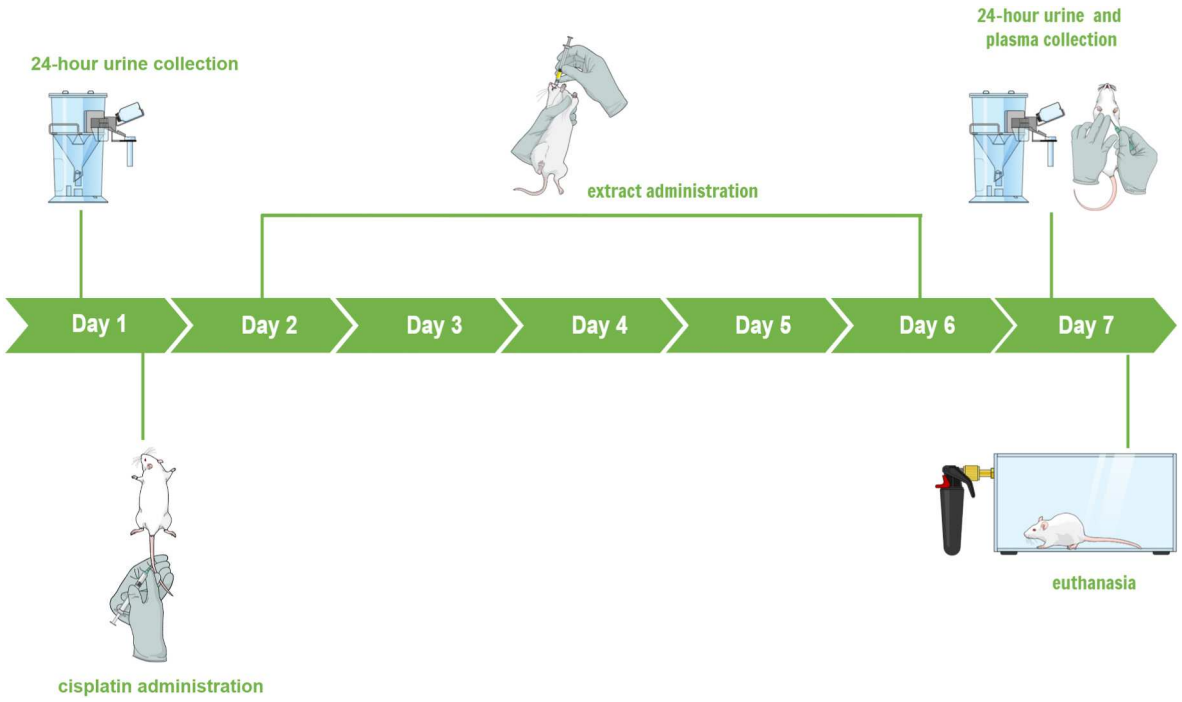

Fig. 1. Treatment scheme for animals subjected to the experimental model of cisplatin-induced nephrotoxicity. Animals treated with hydroethanolic extract (20:80) of *Costus spiralis* in a single dose of intraperitoneal cisplatin (7.5 mg/kg) on the first experimental day, and on the following five days the extract was administered by gavage at doses of 5, 15, and 30 mg/kg. On the seventh experimental day, euthanasia was performed using a CO_2 chamber after plasma was collected by intracardiac puncture. For groups I, II, and III at a dose of 5 mg/kg dose, the animals' 24-h urine was collected on the first and seventh experimental days using metabolic cages.

2.8. Acute toxicity

To ensure the safety of extract, an oral acute toxicity assay was performed at dose 2000 mg/kg using a female rat model (n = 6), following Guideline 423 of the OECD (OECD, 2001). For this, approximately 2.5 g CSLE were dispersed in 12 mL of saline solution with assisted ultrasound. A group of rats (n = 3) that did not receive treatment was used as a control group. The general behavior of the rats was monitored individually after administration of CSLE dispersion (approximately 2.0 mL) at intervals of 30 min, 1 h, 2 h, and then every 2 h until completing 8 h post treatment. Subsequently, the animals were observed daily for a total of 14 days for signs of toxicity, stress, pain, suffering, and behavioral changes (aggression, unusual vocalization, agitation, sedation and somnolence, convulsions, tremors, ataxia, catatonia, paralysis, fasciculation, prostration, and unusual locomotion, and asphyxia). The average consumption of food and water and the average weight of each group were also recorded daily. All animals were euthanized after 14 days and selected vital organs were excised, weighed, and macroscopically examined.

2.9. Cisplatin-induced nephrotoxicity

The animals were randomLy assigned into five groups (n = 6) and conditioned in common cages for 4 days. Group I (control) received saline solution orally for 5 d. Group II (cisplatin-treated) received a single dose of cisplatin intraperitoneally (7.5 mg/kg i. p.) on the first day. Groups III, IV, and V (extract treatment) received CSLE solution at doses 5, 15, and 30 mg/kg orally, respectively. For this, solutions of

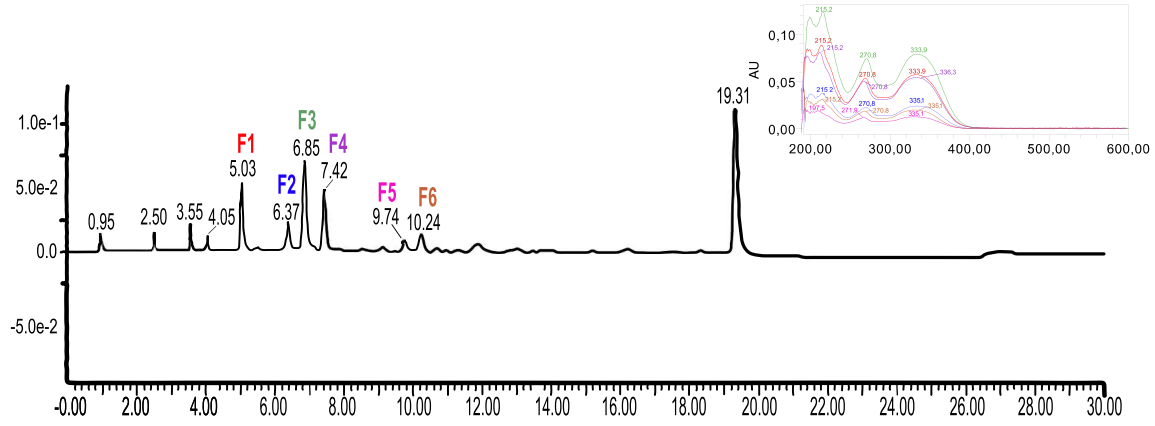

Fig. 2. UPLC chromatographic profile of *Costus spiralis* leaves extract and UV spectra of the identified flavonoids. The chromatographic profile obtained by UPLC-UV/DAD-ESI-MS indicated the presence of 6 majority peaks (F1–F6) with UV spectrum and fragmentation pattern characteristic of flavones. Chromatographic conditions: see Materials and Methods, item UPLC-UV/DAD-ESI-MS analysis. (Group I =control, Group II =single dose of cisplatin 7.5 mg/kg i. p., Group III =5 mg/kg, Group IV =15 mg/kg Group V =30 mg/kg.).

Table 1
Peaks assignments and UPLC-UV/DAD-ESI-MS fragmentation data obtained for the constituents of *Costus spiralis* leaves extract and vicenin II and schaftoside reference standards. Chromatographic conditions: Acquity UPLC® BEH C18 column, at 30 °C, λ 335 nm, flow rate 0.25 µl/min, scan mode negative within the mass range of m/z 100–1000, cone voltage of 5 kV, capillary voltage of 3.5 kV, nebulizer gas nitrogen, capillary temperature of 400 °C.

Peak	Retention time (min)	λ max (nm)	Ions ESI$^-$(m/z)	MM (g/mol)	Molecular Formula	Sugested Compound
F1	5.03	270,87; 333,87	593 [M-H], 503, 473,383,353	594	$C_{27}H_{30}O_{15}$	Vicenin II
F2	6.37	270,87; 333,87	563 [M-H], 503, 473, 443, 383, 353	564	$C_{26}H_{28}O_{14}$	C-glycosylated apigenin flavone
F3	6.85	270,87; 333,87	563 [M-H], 503, 473, 443, 383,353	564	$C_{26}H_{28}O_{14}$	C-glycosylated apigenin flavone
F4	7.42	270,87; 333,87	563 [M-H], 563,473,443,383,353	564	$C_{26}H_{28}O_{14}$	Schaftoside
F5	9.74	271,87; 332,87	563 [M-H]; 503, 473, 443,383,353	564	$C_{26}H_{28}O_{14}$	C-glycosylated apigenin flavone
F6	10.24	269,87; 332,87	563 [M-H],545, 473,443,383,353	564	$C_{26}H_{28}O_{14}$	C-glycosylated apigenin flavone
Vicenin II standard	5.02	270,87,333,87	593 [M-H], 503, 473,383,353	594	$C_{27}H_{30}O_{15}$	–
Schaftoside standard	7,37	269,87; 333,87	563 [M-H], 563,473,443,383,353	594	$C_{26}H_{28}O_{14}$	–

CSLE in saline were prepared at concentrations of 0.5 mg/mL, 1.5 mg/mL and 3.0 mg/mL, and administered for animals in the groups III, IV and V, respectively, in a volume of about 2 mL, adjusted according to the weight of each rat. CSLE was administered from the second to the sixth day and the animals received a single dose of cisplatin (7.5 mg/kg) intraperitoneally on the first day. On the seventh day, animals were anesthetized intraperitoneally and submitted to intracardiac puncture for blood collection. Afterwards, the animals were euthanized in a carbon dioxide chamber. The treatment scheme is shown in Fig. 1. Animals in groups I and II and those receiving the 5 mg/kg dose of the extract were kept in metabolic cages during the 24 h preceding the beginning and end of the experimental period (day 0 and day 6, respectively) for urine collection and single determination of mass and hydric and food consumption. After euthanasia, the kidneys were removed and weighed. The right kidney was stored in 4% formaldehyde until histological analysis.

2.10. Biochemical assays

The measurements of serum and urinary creatinine were performed using a colorimetric method, based on the reaction of creatinine with the alkaline picrate solution (Labtest, Brazil). The determination of uric acid and plasma urea was performed by colorimetric enzymatic test using the commercial kit from Bioclin (Brazil). The proteinuria was quantified by a colorimetric method, based on the reaction of proteins present in the sample with pyrrogallol red in an acid medium (Proteinuria kit, Gold Analisa, Brazil; Proteinuria kit, Bioclin, Brazil).

2.11. Histopathological examination

Longitudinal cuts were made to the right kidney of the animals. After fixation in buffered formaldehyde for 24 h, the kidneys were preserved in 70% alcohol. After inclusion in paraffin, the tissues were sliced in 4 µm sections and stained using the techniques of hematoxylin-eosin and Masson's trichrome. The slides were analyzed using conventional microscopy with the aid of a digital camera to record images.

2.12. Statistical analysis

Statistical analyses were performed using the Windows version of GraphPad Prism 7.0 software (San Diego, CA, USA). Data were checked for normality and the variation between groups was evaluated by multiple t tests using Holm-Sidak method or one-way-analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison post-test according to the evaluated results. Differences between groups with $p < 0.05$ were considered statistically significant.

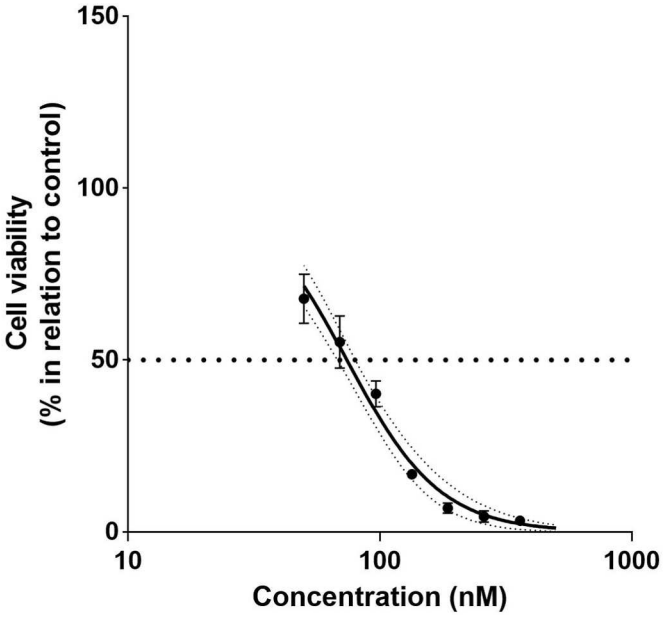

Fig. 3. Cell viability of the 3T3-A31 fibroblast lineage against *Costus spiralis* extract. Data expressed as the mean ± SD. The IC_{50} value calculated was 73.92 µg/mL.

3. Results and discussion

3.1. Chemical composition of Costus spiralis leaves extract

The chemical profiling of CSLE was performed by UPLC-UV/DAD-ESI-MS. The chromatographic profile obtained are shown in Fig. 2. Most peaks (F1–F6) were compatible with apigenin C-glycosylated flavones of apigenin considering combined interpretation of retention times, UV spectra, and fragmentation patterns obtained by UPLC-UV/DAD-ESI-MS/MS and comparisons with previously reported for these substances (Abad-García et al., 2012, 2008; Argentieri et al., 2015; Barreca et al., 2016, 2013; Ferreres et al., 2011, 2003; Kite et al., 2006; Mabry, Tom J.; Markham, K. R; Thomas, 1970). F1 and F4 were identified respectively as vicenin II and schaftoside by comparison of retention times, UV spectra, and fragmentation patterns obtained by UPLC-UV/DAD-ESI-MS/MS of these peaks with reference standards. Co-injection experiments also confirmed the identity of the two substances by obtaining unique and pure peaks for F1 and F4 in sample contaminated with vicenin II and schaftoside reference standards. Assignments of F1–F6 peaks and reference standards are shown in Table 1,

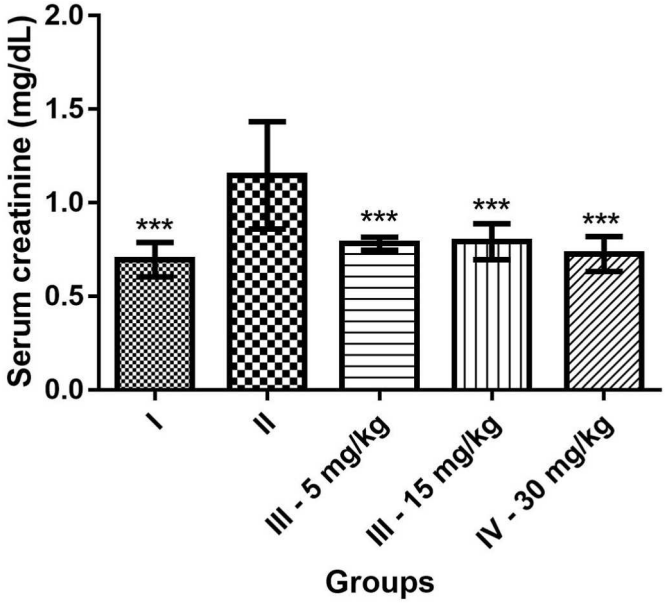

Fig. 4. Effect of the *Costus spiralis* leaves extract (CSLE) on the serum creatinine. The animals that received oral administration of CSLE showed a reduction in plasma creatinine concentrations to the level of the control group at all doses. Data expressed as the mean ± SD. Group I = control, Group II = single dose of cisplatin 7.5 mg/kg i. p., Group III = 5 mg/kg, Group IV = 15 mg/kg Group V = 30 mg/kg. ANOVA One-way followed by Newman-Keuls multiple comparison post-test (n = 6). ***$p < 0.001$ in relation to group II.

as well as in the Supplementary Material, where the results of the co-injection experiment are also presented. Previous studies have already identified principally the presence of flavonoids in the species, including the glycosylated flavones schaftoside and isoschaftoside corroborating the results obtained in this work (de Oliveira et al., 2018). Vicenin II was identified for the first time in the specie. Considering the identification of flavonoids as a major class of the extract, the determination of total flavonoids calculated as quercetin was performed, obtaining a value of 0.880% ± 0.006. It is possible to suggest that biosynthetic mechanisms may favor the occurrence of flavonoids vicenin II, schaftoside and its isomers in the same plant, since reports of the presence of these flavonoids in other plant species are common in the literature (Argentieri et al., 2015; Ferreres et al., 2003; Picariello et al., 2017; Raffaelli et al., 1997; Yuan et al., 2019).

3.2. Prediction of acute oral toxicity

The neutral red uptake assay is recommended by OECD 423 to predict the oral toxic dose (OECD, 2001). The results obtained are shown in Fig. 3, and the IC_{50} value calculated for *Costus spiralis* extract was 73.92 μg/mL (Fig. 3). From these results, the predicted oral toxic dose for the extracts was 523.82 μg/mL, suggesting that acute administration of the extract is relatively safe and indicating that evaluation of acute toxicity *in vivo* should be initiated at the highest dose recommended by the OECD (2000 mg/kg).

3.3. Acute toxicity

As recommended by the OECD, the evaluation was carried out in two steps. First, 3 animals per group were evaluated and, in the absence of death, the exposure was repeated with another 3 rats, totaling 6 animals. Parallel physiological and behavioral parameters were observed, such as changes in food and water consumption, weight loss, presence of secretion in the eyes and nose, changes in coat and body posture, aggressiveness, vocalization, resistance to handling, poor hygiene, feces

Table 2
Effect of *Costus spiralis leaves* extract on change in body weight and balance between experimental days 1 and 11.

Groups	Change in body weight (g)	Change in urine volume (mL)	Change in hydric balance
Group I	(+) 48.1 ± 8.6a	(+) 3.5 ± 1.5a	(−) 2.5 ± 4.3a
Group II	(+) 23.3 ± 17.9a	(+) 3.4 ± 3.6a	(−) 2.5 ± 2.9a
Group III – 5 mg/kg	(+) 13.6 ± 7.1b	(+) 14.5 ± 9.4b	(−) 4.3 ± 14.3a

Group I: control group; Group II: cisplatin group; Group III – 5 mg/kg: treatment group at dose 5 mg/kg; data expressed as the mean ± SEM. ANOVA One-way followed by Newman-Keuls multiple comparison post-test (n = 6). Different superscript letters on the same line indicate statistically significant differences ($p < 0.05$).

altered in volume, consistency, and color (Andrade et al., 2002). No significant change in weight and percentage of kidney and liver weight relative to body weight was observed in animals that received the extract compared to the control group (n = 3). The present study was the first of its kind to investigate the acute toxicity of *Costus spiralis* extract. The absence of death allows the classification of the extract in class 5 according to the OECD 423 (OECD, 2001) and therefore it can be considered a high acute safety product, which is highly relevant, considering the wide popular use of the species in treatment of kidney affections.

3.4. Pharmacological analyses

The experimental model of cisplatin-induced acute nephrotoxicity is associated with development of acute renal injury in rats, characterized by tubular damage with changes in urine concentration capacity and reduced creatinine clearance (Ali and Al Moundhri, 2006; Singh et al., 2012). Creatinine dosage is a common parameter for assessing renal function in experimental models of cisplatin-induced nephrotoxicity. Increased plasma concentrations of this metabolite have been systematically observed in this model (Ezz-Din et al., 2011; Nasr and Saleh, 2014; Perše and Veceric-Haler, 2018; Sahu et al., 2013; Sherif, 2015). In the present study, there was a significant increase of creatinine in plasma in the group II animals compared to the control group and groups treated with the extract. Administration of *Costus spiralis* leaves extract significantly decreased plasma creatinine concentration compared to group II in the 3 evaluated doses (5, 15, and 30 mg/kg). Considering the increase in plasma creatinine level in group I relative to group II as the maximum degree of damage caused by the drug, the groups treated with the extract had an average recovery above 80% for all doses tested. The groups treated with the extract did not show plasma creatinine values significantly different from the control group, indicating that there was recovery of renal function to a near baseline condition (Fig. 4).

The observed activity may be related to the significant presence of flavonoids in *Costus spiralis*, since this class of natural products is widely known to possess antioxidant and anti-inflammatory activities, although confirmation of this hypothesis by metabolomics and pharmacokinetic studies, for example, is necessary. Many studies have evaluated the potential use of flavonoids as therapeutic agents to reduce the nephrotoxic side effects of cisplatin. Several flavonoids, including apigenin and aglycone of the flavones identified in the present study, attenuated cisplatin-induced nephrotoxicity in *in vivo* models (Athira et al., 2016; Badary et al., 2005; He et al., 2016; Loreto et al., 2004; Sahu et al., 2013). Additionally, apigenin reduced cisplatin-induced nephrotoxicity in human renal proximal tubular epithelial cells (Ju et al., 2015).

Another possible mechanism of action may be related to the faster elimination of cisplatin from renal tubules. The high concentrations of the drug in the kidneys are one of the most important factors for the observed nephrotoxicity (Peres and da Cunha, 2013). It has been shown that the nephrotoxicity of cisplatin is partially reduced in the clinic using

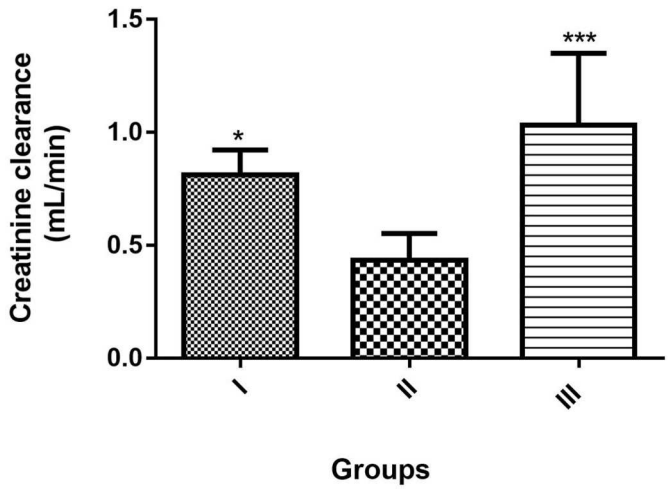

Fig. 5. Effect of the *Costus spiralis* leaves extract on the serum creatinine. The animals in the control group and group III, which received the extract at a dose of 5 mg/kg, had significantly higher creatinine clearance values than group II. Data expressed as the mean ± SD. ANOVA One-way followed by Newman-Keuls multiple comparison post-test (n = 6). *$p < 0.05$; ***$p < 0.001$ in relation to group II.

diuretics and hydration, which reduces the concentration of cisplatin and the contact time of the drug with the tubular epithelium (Ali and Al Mounchri, 2006; Ouchi et al., 2014). *Costus spiralis* is traditionally used in Brazil as a diuretic (Duarte et al., 2017), so this use may be a hypothesis to be considered.

To evaluate the effect of the extract on urinary excretion, 24-h urine samples were collected from the animals that received the 5 mg/kg dose of the extract, as well as from the rats in groups I and II. It was observed that, at the end of the experimental period, the animals treated with extract presented higher urinary excretion than the other groups, and, at the same time, ingested a greater amount of water, which generated compensation when the water balance was rated, demonstrating that this may be a possible mechanism for the protection of renal function observed for the extract (Table 2).

The urine collection also allowed the determination of creatinine clearance. The reduction in clearance of this metabolite has been demonstrated in previous studies (Badary et al., 2005; Perše and Veceric-Haler, 2018). The data obtained were consistent with the results observed for plasma creatinine clearance. The animals treated with the extract at dose 5 mg/kg showed a creatinine clearance higher than that observed for group II, confirming its effect on increasing renal function (Fig. 5).

Histological analyses were performed considering three qualitative levels of tubular alterations in mild (1–30%), moderate (30–60%), and severe (above 60%). The results obtained showed desquamation of the vascular epithelium and mild acute tubular necrosis (1–30%), with focal tubular nuclear atypia in the group that did not receive IP injection of cisplatin, while the control group did not show significant changes in the tubular/interstitial compartment. The animals treated with the extract (group III) showed histological results similar to group II (Supplementary Material – Fig. 7). Positive results for tubular changes were mild (1–30%) in all slides, so it was not possible to determine whether there was a difference between treated animals that showed changes and the positive control animals.

Thus, the results demonstrate the activity of *Costus spiralis* extract in recovering renal function in the experimental model evaluated. It can be suggested that flavonoids present in the *Costus spiralis* extract could act synergistically restoring renal function.

4. Conclusions

Chemical profiling of the extract by UPLC-UV/DAD-ESI-MS allowed the demonstration of the major presence of apigenin glycosides, having been identified the schaftoside and vicenin II flavones, the latter for the first time for the species. Costus spiralis hydroethanolic extract proved to be safe for acute oral administration and was evaluated in a cisplatin-induced acute nephrotoxicity model, being able to recover renal function in the experimental model, *in vivo*, probably by the presence of flavonoids. This pharmacological potential of the Costus spiralis extract justifies its ethnobotanical uses, based on the combination of different mechanisms discussed.

Funding sources

Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG- 02002312), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 303757/2019-1/307494/2015-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – 88882.380764/2019–01).

CRediT authorship contribution statement

Juliana Mendes Amorim: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Larissa Camila Ribeiro de Souza:** Methodology, Investigation, Formal analysis, Writing – review & editing. **Rebecca Almeida Lemos de Souza:** Methodology, Investigation. **Roberta da Silva Filha:** Methodology, Investigation. **Juliana de Oliveira Silva:** Methodology, Investigation. **Stanley de Almeida Araújo:** Investigation, Formal analysis. **Carlos Alberto Tagliti:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Ana Cristina Simões e Silva:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Rachel Oliveira Castilho:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and PRPq of Universidade Federal de Minas Gerais for funding and support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jep.2022.115510.

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