Proximate analysis, phytochemical screening, and bioactivities evaluation of *Cissus rotundifolia* (Forssk.) Vahl. (Fam. Vitaceae) and *Sansevieria cylindrica* Bojer ex Hook. (Fam. Dracaenaceae) growing in Egypt

Ataa A. Said^a, Elsayed Ali Aboutabl^c, Sally A. El Awdan^b, Mona A. Raslan^a

Departments of ^aPharmacognosy, ^bPharmacology, National Research Centre, Dokki, Giza, ^oPharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Correspondence to Mona A. Raslan, PhD, Pharmacognosy Department, National Research Centre, Dokki, 12622 Giza, Egypt Tel: +20 1143036424; fax: +20 233 370 931; e-mail: azzam_mona@yahoo.com

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Objectives

This work aimed to carry out a proximate analysis, phytochemical screening, and bioactivities evaluation of the nonflowering aerial parts of each of *Cissus rotundifolia* (Forssk.) Vahl. (Fam. Vitaceae) and *S. cylindrica* Bojer ex Hook. (Fam. Dracaenaceae) growing in Egypt.

Materials and methods

The total phenolic contents (TPC) and total flavonoid contents were estimated spectrophotometrically. The oral acute toxicity of each of the two plant extracts was evaluated. Analgesic activities of both plants were evaluated using hot plate and writhing test using indomethacin as a reference drug. Anti-inflammatory activities were evaluated using carrageenan-induced rat-paw edema. Antiulcerative activities were measured using indomethacin-induced ulcer in pyloric-ligated rats. Thioacetamide-induced acute hepatic encephalopathy was used to evaluate the hepatoprotective effects of the plants.

Results and conclusion

Proximate analysis of air-dried nonflowering aerial parts of each of C. rotundifolia and S. cylindrica yielded a moisture content of 7.95 and 7.52%, total ash of 8.35 and 9.83%, watersoluble ash of 3.96 and 2.94%, acid-insoluble ash of 2.14 and 3.42%, crude fiber content of 8.52 and 13.43%, and methanolic extractive value of 41 and 35%, respectively. Phytochemical analysis of C. rotundifolia extract indicated the presence of triterpenes, carbohydrates, and/or glycosides, tannins, flavonoids, coumarins, and saponins, whereas S. cylindrica extract showed the presence of sterols, carbohydrates, and/or glycosides, flavonoids, coumarins, and saponins. Total phenolic content of C. rotundifolia and S. cylindrica were 3.18 and 4.82 mg (GAE)/g, respectively, whereas total flavonoid contents of C. rotundifolia and S. cylindrica were 1.35 and 4.13 mg (QE)/g plant dry weight, respectively. Both extracts showed no toxicity up to 1 g/kg body weight. Both methanolic extracts showed significant central and peripheral analgesic effects. C. rotundifolia extract showed percentages of edema inhibition higher than or equal to the reference drug indomethacin in carrageenan-induced paw edema assay, whereas S. cylindrica extract showed percentages of edema inhibition less than that of the reference drug indomethacin. In the ulcer model induced by indomethacin, the groups pretreated with both plants received significant protection against ulcer formation. In the thioacetamide-induced acute hepatopathic encephalopathy model, rats treated with S. extract showed significant improvement in their plasma AST and ALT, whereas rats treated with C. rotundifolia extract showed increased levels of their ALT and AST.

Keywords:

analgesic, anti-inflammatory, antiulcerative, *Cissus rotundifolia*, flavonoids, hepatoprotective, phenolics, preliminary phytochemical screening, proximate analysis, *Sansevieria cylindrica*

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Introduction

Cissus rotundifolia (Forssk.) Vahl. (Fam. Vitaceae) and *Sansevieria cylindrica* Bojer ex Hook. (Fam. Dracaenaceae) are native to the subtropical regions of the African continent, and cultivated in Egypt for ornamental purposes. Both plants are reputed to have beneficial effects in the traditional system of medicine. *Cissus rotundifolia* (Forssk.) Vahl. is a climber known as Arabian Wax *Cissus* or Peruvian grape ivy. It was reported to be used in Yemeni ethnomedicine to treat fever and digestive ailments [1]. Certain *Cissus* spp. were reported

to contain sterols, triterpenoids [2], phenolics [3], flavonoids, stilbene derivatives, iridoids [4], and coumarin glycosides [5]. Different therapeutic effects were attributed to certain *Cissus* spp. [6]. *C. rotundifolia* leaves showed antioxidant [7] and antibacterial activities [1].

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On the other hand, *S. cylindrica* Bojer ex Hook., xerophytic perennial herb known as the Cylindrical Snake Plant, African Spear or Spear *Sansevieria*. Several steroidal sapogenins and saponins were isolated from certain *Sansevieria* spp. [8–11]. A new steroidal saponin isolated from the leaves of *S. cylindrica* showed inhibition of the capillary permeability activity [12].

The main aim of the present study was to carry out a proximate analysis, phytochemical screening, and bioactivities evaluation of the two plants.

Materials and methods Plant material

The nonflowering aerial parts (stems and leaves) of each of *C. rotundifolia* (Fam. Vitaceae) and *S. cylindrica* (Fam. Dracaenaceae) were collected from Orman botanical garden, Giza, Egypt, in June 2009, kindly authenticated by Dr Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC), and confirmed by Dr Abd El-Magali, Flora and Phytotaxonomy Research – Horticultural Research Institute, Agricultural Research Centre, Ministry of Agriculture. Voucher specimens are deposited in the Herbarium, Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Preparation of the methanolic extracts

The air-dried powdered nonflowering aerial parts (1 kg) of each plant were extracted by maceration with 70% aqueous methanol until exhaustion. The solvent was evaporated to dryness under reduced pressure to yield the crude extract of each plant.

Preparation of successive extracts

The powder of each plant (100 g) was extracted successively with petroleum ether, chloroform, ethyl acetate, and 70% methanol in a Soxhlet apparatus. Each extract was evaporated to dryness under reduced pressure. The solvent-free residue in each case was weighed.

Proximate analysis

Percentages of moisture content, total ash, watersoluble ash, acid-insoluble ash, and crude fiber values were assessed according to the official methods [13].

Preliminary phytochemical screening

All the extracts were screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests [14,15].

Total phenolic assay

The total phenolic content was determined applying the Folin–Ciocalteu colorimetric method using gallic acid as a standard [16] and expressed as milligrams of gallic acid equivalents (GAE)/g of the dry plant material.

Total flavonoid assay

Total flavonoid content (TFC) was measured using an aluminum chloride colorimetric assay [17]. A calibration curve was established using quercetin as a standard. TFC was expressed as mg quercetin equivalent (QE)/g of the dry plant material.

Bioactivity studies

Experimental animals

Adult male albino Sprague–Dawley rats weighing 130–150 g and adult male Swiss albino mice weighing 20–25 g were obtained from the animal house colony in the National Research Centre (Giza, Egypt). They were housed in groups in properly numbered stainless-steel cages, maintained on a 12 h light and dark cycle at $28 \pm 2^{\circ}$ C in a well-ventilated animal house under natural conditions, and given pelleted food and drinking water *ad libitum*.

Acute oral toxicity study

Seventy albino rats were grouped randomly into one control group and six treated groups, each including ten animals. Animals were orally dosed 0.25, 0.5, and 1 g/kg body weight of the tested extracts following the method of Lorke [18]. Control animals received the vehicle and were kept under the same conditions without any treatment. Animals were observed for 24 h for signs of toxicity or death.

Analgesic activity

Hot-plate test

For 3 consecutive days preceding the experiment, mice were adapted on the hot plate by placing them on a plate maintained at room temperature for 15 min each day. Each animal was then placed gently onto a $52^{\circ}C$ ($\pm 0.1^{\circ}C$) hot plate to perform the test. Six groups (six mice each) were administered vehicle and/or the tested extract of each plant (100 and 200 mg/kg body weight of extract) and the last group received indomethacin (10 mg/kg body weight). All drugs were injected subcutaneously 30 min before placing the animal on the hot plate. Latency to show nociceptive responses, such as licking paws or jumping off the hot plate, was determined 30, 60, and 120 min after administration of the test substances or saline [19].

Writhing test

Six separate groups (six mice each) were administered the vehicle and/or the tested extract (100 and 200 mg/kg body weight) subcutaneously 30 min before the intraperitoneal injection of 0.6% acetic acid in distilled water (10 ml/kg). The stretching reaction was observed [20]. Each mouse was then placed in an individual clear plastic observational chamber. The number of writhes (muscular contractions) was counted for 30 min immediately after the acetic acid injection and expressed as writhing numbers [21].

Anti-inflammatory activity

The carrageenan-induced rat-paw edema

Paw edema was induced by a subplantar injection of 100 μ l of 1% sterile carrageenan in saline into the right hind paw [22]. Thirty-six adult male albino rats, divided into six groups, each of six animals, were orally treated with 100 or 200 mg/kg body weight of 70% methanolic extract of *C. rotundifolia* and *S. cylindrica* nonflowering aerial parts, indomethacin (10 mg/kg body weight) (positive control), and saline (negative control). The rats received vehicle or extracts 1 h before carrageenan injection. The hind paw volume was measured immediately before carrageenan injection and at selected times thereafter with the water displacement method [23] using 7410, plethysmometer (Ugo Basile, Comerio, Italy).

Antiulcerative activity

Indomethacin-induced ulcer in pyloric-ligated rats

Pyloric-ligated rats were treated according to the method described by Shay and colleagues [24,25]. Rats were starved for 18 h, but allowed free access to drinking water. A midline ventral incision starting from the xiphoid cartilage downwards was made to expose the stomach and the duodenum. The pylorus was ligated and the abdominal wall was sutured. The tested extracts were administered immediately after pyloric ligation, followed by oral administration of indomethacin (30 mg/kg body weight). Four hours later, animals were killed by cervical dislocation, the abdominal cavity was opened, and a ligature was placed at the esophagocardiac junction and the stomach was removed. The mucosa was examined [26] and the total lesion number was counted and the severity of lesions was calculated on the basis of the following score: $0 = \text{no ulcer}, 1 = \text{lesion} \le 1 \text{ mm}, 2 = \text{lesion of size } 1-2$ mm, 3 = 1 lesion of size 2-3 mm, 4 = 1 lesion of size 3-4mm, and 5 = lesion of size more than 4 mm.

Evaluation of hepatoprotective activity

Thioacetamide-induced acute hepatic encephalopathy Forty-two rats were used to carry out the study and they were divided into seven groups (six animals each) as follows. Group 1 (control group): rats received saline (2 ml/kg body weight) intraperitoneally once, and were then administered saline and corn oil orally at a dose of 5 ml/kg body weight daily for 3 days.

Group 2 [hepatotoxic thioacetamide (TAA) group]: the rats received TAA (300 mg/kg body weight) intraperitoneally once [27].

Groups 3, 4, 5, and 6 (treated groups): rats received TAA (300 mg/kg body weight) once intraperitoneally on the first day and were then orally administered a 70% aqueous methanolic extract of *C. rotundifolia* or *S. cylindrica* nonflowering aerial parts at two dose levels (100 or 200 mg/kg body weight) daily for 3 days.

Group 7 (silymarin and TAA): the rats received TAA (300 mg/kg body weight) once intraperitoneally on the first day and were then administered silymarin (100 mg/kg body weight) daily for 3 days.

The animals received dextrose water and ringer lactate solutions (10 mg/kg/day, intraperitoneally) to prevent renal failure, hypoglycemia, and electrolyte imbalance until the end of the experiment. All rats were killed under anesthesia 24 h after the last treatment and overnight fasting. Blood samples (~4 ml) were taken from each rat from retro-orbital veins, using a sterile syringe, after they were killed at the end of the experiments. These samples were kept at room temperature for 30 min and centrifuged at 3000 rpm for 10 min. Serum samples were stored in a freezer (-20°C) for use in biochemical analyses.

Hepatic biochemical parameters in serum

Serum aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to the method of Reitman and Frankel [28] using ELISA kits supplied by Bio-diagnostic Company (Cairo, Egypt).

Statistical analysis

Values were expressed as means ± SE. Data of carrageenan-induced rat-paw edema, the hot-plate test, and the writhing test were analyzed using repeated-measures two-way analysis of variance, followed by the Tukey HSD test for multiple comparisons. Results of ulcer numbers and severity were analyzed using the Kruskal–Wallis nonparametric test, followed by Dunn's multiple-comparisons test. Hepatic biochemical parameters were analyzed using one-way analysis of variance or the corresponding nonparametric (Kruskal–Wallis) test, as required. A probability level of less than 0.05 was considered significant in all types of statistical tests.

Results and discussion Proximate analysis

Percentages of moisture content, total ash, watersoluble ash, acid-insoluble ash, and crude fiber of each of the two plants were estimated and the results are presented in Table 1. Successive extracts of each of the two plants are shown (Tables 2 and 3) as percentage of yield, physical characteristics, and phytoconstituents.

Phytochemical screening

Phytochemical analysis of 70% methanolic extract of *C. rotundifolia* nonflowering aerial parts indicated the presence of triterpenes, carbohydrates, and/ or glycosides, tannins, flavonoids, coumarins, and saponins, whereas the 70% methanolic extract of *S. cylindrica* nonflowering aerial parts contains sterols, carbohydrates, and/or glycosides, flavonoids, coumarins, and saponins (Tables 2 and 3).

Total phenolic and flavonoids content

Total phenolic contents of *C. rotundifolia* and *S. cylindrica* nonflowering aerial parts estimated using the Folin-Ciocalteu method were 3.18 and 4.82 mg (GAE)/g, respectively, whereas the TFC were 1.35 and 4.13 mg (QE)/g plant dry weight, respectively.

Table 1 Pharmacopoeial constants of Cissus rotundifolia and Sansevieria cylindrica nonflowering aerial parts

Pharmacopoeial	Cissus	Sansevieria	
constants (%)	rotundifolia	cylindrica	
Moisture	7.95	7.52	
Ash	8.35	9.83	
Water-soluble ash	3.96	2.94	
Acid-insoluble ash	2.14	3.42	
Crude fiber	8.52	13.43	

Acute oral toxicity

At all of the doses tested, none of the extracts produced any mortality up to 24 h observation. Accordingly, the doses used for pharmacological studies were (100 and 200 mg/kg body weight) of each of the two extracts. These doses represent 1/10 and 1/5 of the maximum soluble dose of the aqueous extract that induced no mortalities in mice.

Analgesic activity

Hot-plate test

The results presented in Table 4 indicate that the mean reaction time on the hot plate was significantly delayed after the administration of the tested extracts compared with indomethacin as a positive control, indicating the significant central analgesic effects of the tested extracts.

Writhing test

The results presented in Table 5 indicate that acetic acid-induced writhing was significantly reduced in mice receiving tested extracts at the two dose levels, compared with indomethacin as a positive control, confirming the peripheral analgesic effect.

Anti-inflammatory activity

Carrageenan-induced rat-paw edema

Pretreatment with *C. rotundifolia* and *S. cylindrica* extracts significantly decreased carrageenan-induced edema (Table 6). The highest anti-inflammatory potency at 4 h was shown by *C. rotundifolia*. Both dose levels showed percentages of edema inhibition higher than or equal to the reference drug indomethacin. However, *S. cylindrica*, at both doses, showed lower percentages of edema inhibition than the reference drug indomethacin.

Table 2 Percentages, physical characteristics, and constituents of successive extracts of Cissus rotundifolia nonflowering	J
aerial parts	

Characteristic	Successive extractives				
	Petroleum ether	Ether	Chloroform	Methanol (70%)	
Percentage (w/w)	1.8	0.5	6	41	
Physical characteristics					
Color	Dark green	Dark green	Dark green	Dark green	
Condition	Gummy	Gummy	Gummy	Gummy	
Constituents					
Sterols and/or triterpenes	+	+	+	+	
Carbohydrates and/or glycosides	_	_	_	+	
Anthraquinones	—	—	_	—	
Flavonoids	_	_	_	++	
Coumarins	_	+	+	+	
Alkaloids and/or nitrogenous compounds	_	_	_	_	
Tannins	—	_	_	+	
Saponins	_	_	_	++	

+, present; -, absent; ++, appreciably present.

Table 3 Percentages, physical characteristics, and constituents of successive extracts of Sansevieria cylindrica nonflowering	j
aerial parts	

Characteristic	Successive extractives				
	Petroleum ether	Ether	Chloroform	Methanol (70%)	
Percentage (w/w)	2.1	1.2	3	35	
Physical characteristics					
Color	Dark green	Dark green	Dark green	Dark brown	
Condition	Gummy	Gummy	Gummy	Solid	
Constituents					
Sterols and/or triterpenes	+	+	+	++	
Carbohydrates and/or glycosides	—	—	—	+	
Anthraquinones	—	—	—	—	
Flavonoids	_	—	—	++	
Coumarins	—	—	—	+	
Alkaloids and/or nitrogenous compounds	_	_	—	—	
Tannins	—	—	—	—	
Saponins		_	_	++	

+, present; -, absent; ++, appreciably present.

Table 4 Central analgesic effect of 70% methanolic extracts of nonflowering aerial parts of *Cissus rotundifolia* and *Sansevieria cylindrica* as shown by the hot-plate test

Groups	Reaction time (s)				
	0 min	30 min	60 min	90 min	
Control	3.84 ± 0.32	3.40 ± 0.51	3.70 ± 0.27	3.68 ± 0.23	
Indomethacin (10 mg/kg body weight)	3.94 ± 0.32	$6.36 \pm 0.40^{*}$	8.16 ± 0.46*	10.30 ± 0.39*	
Cissus rotundifolia (100 mg/kg body weight)	3.82 ± 0.49	$6.24 \pm 0.63^{*}$	$7.64 \pm 0.47^*$	9.86 ± 0.50*	
Cissus rotundifolia (200 mg/kg body weight)	4.16 ± 0.37	6.26 ± 0.06	$7.94 \pm 0.32^*$	10.56 ± 0.69*	
Sansevieria cylindrical (100 mg/kg body weight)	3.60 ± 0.29	5.58 ± 0.46	7.10 ± 0.47*	8.54 ± 0.53*	
Sansevieria cylindrica (200 mg/kg body weight)	3.94 ± 0.90	5.70 ± 0.32	8.20 ± 0.58*	9.20 ± 0.39*	

Data are presented as mean \pm SE, *P < 0.05.

Table 5 Antinoeiceptive effect of 70% methanolic extracts of nonflowering aerial parts of *Cissus rotundifolia* and *Sansevieria cylindrica* as shown by the acetic acid-induced writhing test

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Treatment	Number of contractions ($X \pm SE$)	% of change
Control	31.40 ± 2.74	
Indomethacin	17.60 ± 1.21*	43.9
(10 mg/kg body weight)		
Cissus rotundifolia	19.22 ± 0.86*	38.9
(100 mg/kg body weight)		
Cissus rotundifolia	18.25 ± 0.58*	42
(200 mg/kg body weight)		
Sansevieria cylindrica	18.81 ± 1.15*	40.1
(100 mg/kg body weight)		
Sansevieria cylindrica	$12.23 \pm 0.86^*$	61.1
(200 mg/kg body weight)		

The data represent the mean \pm SEM (n = 6-10), n = number of animals in each group, *Statistically significant from the control group: P < 0.05.

Antiulcerative activity

The number of lesions in the untreated ulcer group was 4.03 ± 0.79 , with an ulcer severity value of 9.5 ± 1.78 . The groups pretreated with both methanolic extracts had no lesions, indicating a significant protection against ulcer. The number of lesions present on the gastric mucosa is indicative of the gastric damage [29]. The absence of lesions in the groups pretreated with plant extract, compared with the ranitidine pretreated group, can possibly be attributed to inhibition of gastric acid secretion, an important factor in ulcer, or inhibition of lipid peroxidation because of their antioxidant activities. Ranitidine, an antisecretory drug, has often been reported to possess antioxidant and immunosuppressive actions, which might be responsible for its antiulcerogenic activity [30].

Evaluation of hepatoprotective activity

In the TAA group, serum levels of AST and ALT were significantly higher than those of the healthy controls (P < 0.0001), indicating liver injury (Table 7). Rats treated with silymarin (100 mg/kg body weight) showed a significant reduction in their plasma AST and ALT by 66.48 and 41.59%, respectively, compared with the TAA control group, indicating the hepatoprotective effect. Rats treated with the 70% aqueous methanolic extract of nonflowering aerial parts of *C. rotundifolia* at doses of 100 and 200 mg/kg body weight showed elevated levels of their ALT and AST, indicating that *C. rotundifolia* does not exert any hepatoprotective effect. However, rats treated with 70% aqueous methanolic extract of *S. cylindrica* nonflowering aerial parts at a dose of 100 mg/kg

Table 6 Anti-inflammatory effect of 70% methanolic extracts of nonflowering aerial parts of *Cissus rotundifolia* and *Sansevieria cylindrica* at doses of 100 and 200 mg/kg body weight on carrageenan-induced rat-paw edema

Groups	Paw edema volume (ml) (% inhibition)				
	0 h	After 1 h (%)	2 h (%)	3 h (%)	4 h (%)
Control	0.28 ± 0.01	0.43 ± 0.02	0.46 ± 0.02	0.50 ± 0.001	0.51 ± 0.02
Indomethacin 10 mg/kg body weight	0.28 ± 0.01	0.33 ± 0.01* 23.26	0.33 ± 0.001* 28.26	6 0.33 ± 0.01* 34.00	0.33 ± 0.01* 35.29
Cissus rotundifolia (100 mg/kg body weight)	0.27 ± 0.01	0.33 ± 0.02* 23.26	0.32 ± 0.02* 30.43	3 0.34 ± 0.02* 32.00	0.34 ± 0.01* 33.33
Cissus rotundifolia (200 mg/kg body weight)	0.28 ± 0.01	$0.34 \pm 0.02^{*} 20.93$	0.31 ± 0.02* 32.61	$0.29 \pm 0.02^{*}$ 42.00	0.27 ± 0.01* 47.06
Sansevieria cylindrica (100 mg/kg body weight)	0.28 ± 0.01	0.37 ± 0.01* 13.95	0.38 ± 0.01* 17.39	$0.38 \pm 0.03^{*}$ 24.00	0.38 ± 0.02* 25.49
Sansevieria cylindrica (200 mg/kg body weight)	0.29 ± 0.01	$0.33 \pm 0.02^{*} 23.26$	0.35 ± 0.01* 23.91	$0.36 \pm 0.01^{*}$ 28.00	$0.37 \pm 0.01^* 27.45$

The data represent the mean \pm SEM (*n* = 6), Values represent the mean \pm SE of six animals for each groups, **P* < 0.05: statistically significant from control (two-way ANOVA, followed by the Bonferroni test).

Table 7 Effect of 70% aqueous methanolic extract of nonflowering aerial parts of *Cissus rotundifolia* and *Sansevieria cylindrica* in thioacetamide-induced liver injury

Group	AST (U/I)% reduction relative to the TAA group (%)	ALT (U/I)% reduction relative to the TAA group (%)	
Control	97.00 ± 18.71	43.57 ± 14.53	
Thioacetamide (TAA)	355.53 ± 50.32	144.90 ± 26.80	
Cissus rotundifolia (100 mg/kg body weight)	379.00 ± 27.11	189.90 ± 75.80	
Cissus rotundifolia (200 mg/kg body weight)	309.20 ± 43.83	196.90 ± 62.25	
Sansevieria cylindrica (100 mg/kg body weight)	209.00 ± 39.9041.21	72.94 ± 72.9049.66	
Sansevieria cylindrica (200 mg/kg body weight)	381.83 ± 50.02	104.97 ± 19.3127.56	
Silymarin (100 mg/kg body weight)	119.17 ± 31.3166.48	84.63 ± 14.6641.59	

Data are expressed as mean ± SD (minimum–maximum), ALT, alanine aminotransferase; AST, aspartate aminotransferase, *P*: significance versus control <0.0001.

body weight showed a significant reduction in their plasma AST and ALT by 41 and 49.66%, respectively. Rats treated with a dose of 200 mg/kg body weight of *S. cylindrica* extract showed a significant reduction only in their plasma ALT by 27.56%, indicating its hepatoprotective effect.

Conclusion

The different parameters developed in this study will enable phytochemical identification and standardization of both plants. The results of the preliminary phytochemical screening and bioactivities evaluation indicate that *C. rotundifolia* (Forssk.) Vahl. and *S. cylindrica* Bojer ex Hook can be potential sources of phytomedicines with analgesic, antiinflammatory, and antiulcerative activities. Moreover, *S. cylindrica* is a potential source of a hepatoprotective phytomedicine.

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Conflicts of interest

There are no conflicts of interest.

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