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Research Article

Hepatoprotection and Phytochemistry of the Vietnamese Herbs *Cleome chelidonii* and *Cleome viscosa* Stems

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The study aims to determine the hepatoprotective effect of *n*-hexane, ethyl acetate, and methanol extracts of the leaves and stems of two *Cleome* species against carbon tetrachloride- (CCl_4-) induced liver toxicity both *in vitro* using human hepatoma (HepG2) cells and *in vivo* in rats as well as the hepatoprotective property of all isolated compounds on HepG2. After 72 h of treatment, at the concentrations of 25, 50, and 100 μg/mL, the methanol of *C. chelidonii* stems (CCSM) ranged from 18.6% to 20.8%, whereas the methanol of *C. chelidonii* stems (CVSM) increased from 12.3% to 17.2% cell viability. The results show that CCSM and CVSM significantly expressed *in vitro* hepaprotective activity on HepG2. Therefore, the animals were daily treated with these extracts at the doses of 15, 30, and 45 mg/kg body weight for 5 days, and CCl_4 was injected (2 ml/kg body weight, i.p.) on the 2nd and 3rd days. Levels of aspartate aminotransferase (ALT) and alanine aminotransferase (AST) in the blood were measured and compared to the silymarin control. The treatments with CCSM and CVSM (30, and 45 mg/kg) possessed significant hepatoprotection and were comparable with the activity of silymarin. Further, phytochemical studies of these ones were conducted and led to the identification of eight flavonoids: visconoside A (1), visconoside B (2), quercetin 3-*O*-β-D-glucopyranoside 7-*O*-α-L-rhamnopyranoside (3), kaempferol 3-*O*-β-D-glucopyranoside 7-*O*-α-L-rhamnopyranoside (4), cleomeside A (5), cleomeside B (6), cleomeside C (7), and quercetin-3-*O*-[β-D-glucopyranosyl-(1⟶2)]-α-L-rhamnopyranoside 7-*O*-α-L-rhamnopyranoside (8). Two major flavonoids (1 and 4) displayed significant hepatoprotective property (at the concentration of 100 μM, the prevention percentage values were 66.5% and 74.2%, respectively, compared to the quercetin control, with value of 80.3%).

1. Introduction

The genus *Cleome* belonging to the Cleomaceae family comprises about 170 species[1]. Five species were found in Vietnam [2]. In the traditional Vietnamese medicine, *C. chelidonii* is used for treatment of fever, flu, headache, cough, snake bite, and nephritis, whereas *C. viscosa* is used to treat diarrhea, fever, inflammation, liver diseases, bronchitis, skin diseases, and malarial fever [2]. Pharmacological investigations proved that *C. chelidonii* possessed antipyretic [3], antihyperglycemic [4], and anthelmintic [5] properties, while *C. viscosa* expressed anticonvulsant [6], antitumor [7], cytotoxic [8–12], antiangiogenic [12], antimalarial [13], larvicidal [14], antiallergic, diuretic [15], analgesic, antipyretic [16], α-glucosidase, and α-amylase inhibitory [17] activities. Additionally, both species exhibited antimicrobial [9–21], antinociceptive [3, 10, 22, 23], anti-inflammatory [3, 21, 23, 24], and antioxidant activities [5, 8, 9, 12, 14, 25–27].

In vivo study on rats against CCl_4-induced liver injury indicated that hydroalcohol, methanol, ethyl acetate, and hexane extracts of *C. chelidonii* root revealed hepatoprotective activity [28]. Another study on rats against paracetamol- and ethanol-induced liver toxicity also confirmed that a methanol extract of *C. chelidonii*'s whole plant displayed hepatoprotective property [24]. *In vivo* study on ethanol extract of *C. viscosa*'s whole plant against CCl_4-induced hepatotoxicity [29], as well as methanol extract against streptozotocin- (STZ-) induced diabetic rats [30], *C. viscosa* leaves against thioacetamide-induced hepatotoxicity [31, 32], and *C. viscosa* seeds against paracetamol-induced hepatotoxicity [33, 34] also showed that *C. viscosa* possessed a hepatoprotective effect on rat models.

So far, there has been no report on the hepatoprotection and phytochemical constituents of the *C. chelidonii* and *C. viscosa* stems. Continuing our study on bioactive composition of traditional Vietnamese medicines [35] and the *Cleome* genus [36–39], this paper detailed the evaluation of the hepatoprotective effect of different extracts (*n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) extracts) from the stems of two *Cleome* species against CCl_4-induced liver intoxication in both *in vitro* and *in vivo* assays. All compounds isolated from the most active extracts were also measured for the hepatoprotective activity using *in vitro* assay.

2. Materials and Methods

2.1. Plant Materials. *C. chelidonii* and *C. viscosa* stems were collected in Ben Cat, Binh Duong province, Vietnam, in May 2015 and certificated by Professor Vo Van Chi. The voucher specimens (No. VH/MINH-1012 and No. VH/MINH-0515, respectively) were deposited in the Institute of Chemical Technology, Vietnam Academy of Science and Technology.

2.2. Extraction. Dried stems powders of *C. chelidonii* (8 kg) and *C. viscosa* (7 kg) were extracted with 96% EtOH for three times (3 × 30 L, total amount 90 L) at room temperature. The supernatants were filtered, and the solvents were removed under vacuum to obtain crude extracts CCS (970 g) and CVS (770 g), respectively. Those extracts were subjected to solid-phase separation and successively fractionated into *n*-hexane, EtOAc, and MeOH extracts, respectively, to afford six extracts: CCSH (155 g), CCSE (355 g), CCSM (420 g), CVSH (130 g), CVSE (310 g), and CVSM (330 g). Similar protocols were used for powdered leaves of *C. chelidonii* (5 kg) and *C. viscosa* (7.5 kg), resulting in six extracts: CCLH (120 g), CCLE (228 g), CCLM (170 g), CVLH (150 g), CVLE (260 g), and CVLM (400 g). All extracts were stored at 4°C for further studies.

2.3. Chemicals and Reagents. Eagle's Minimum Essential Medium (EMEM), fetal calf serum (FCS), and trypsin-EDTA were purchased from Gibco, USA; L-glutamine, penicillin, streptomycin, phosphate buffer, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin, and carbon tetrachloride (CCl_4) were from Sigma-Aldrich, USA; dimethyl sulfoxide (DMSO) and isopropanol were from Merck, Germany. All chemicals met cell culture standards.

2.4. Cell Culture. HepG2 cells (the American Type Culture Collection, Manassas, Rockville) were seeded and cultured in EMEM containing 10% FCS (v/v), 2 mM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 5% CO_2 at 37°C to attain confluency.

2.5. Animals. Swiss albino mice weighing 26–30 g were purchased from Pasteur Institute in Ho Chi Minh City, Vietnam Ministry of Health. The mice were housed in standard cages (48 cm × 35 cm × 22 cm) at room temperature and provided with pelleted food and water.

2.6. Evaluation of the In Vitro and In Vivo Hepatoprotective Activity

2.6.1. Cell Viability. HepG2 cells were harvested and seeded in 96-well plates at 4.0×10 cells/cm^2. Then, cells were treated with EMEM containing 2 mM CCl_4 and compounds 1–8 alone or combined at different concentrations. Cell viability was measured as mitochondrial succinate dehydrogenase activity, a marker of viable cells using MTT test. Doxorubicin was used as positive control for cytotoxicity. The assay was performed using the MTT test, as previously described [17, 18]. Doxorubicin was used as a positive control for cytotoxicity.

For the cytotoxicity, the percentage of control (%) was calculated = OD_{570} sample/OD_{570} control × 100% measured at the different concentrations (25, 50, and 100 μg/mL) by MTT assay.

2.6.2. Study on Hepatic Protective Effect in Mice Acute Liver Injury Induced by CCl_4. Mice were divided into six groups with six animals in each group.

Group I (normal control) received distilled water with 0.3% sodium carboxymethylcellulose (CMC-Na) (1 mL/kg body weight, p.o.) for 5 days and olive oil (1 mL/kg body weight, i.p.) on days 2 and 3.

Group II (CCl_4-intoxicated) received 0.3% CMC-Na (1 mL/kg body weight, p.o.) for 5 days and CCl_4–olive oil (1 : 1, 2 mL/kg body weight, i.p.) on days 2 and 3.

Group III (positive group) was treated daily with the positive silymarin drug (100 mg/kg body weight, p.o.) for 5 days and CCl_4–olive oil (1 : 1, 2 mL/kg body weight, i.p.) on days 2 and 3, 30 min after silymarin administration.

Test groups (IV–VI) were administered orally with 100, 200, and 400 mg/kg TFs, respectively, for 5 days. The three test groups received CCl_4–olive oil (1 : 1, 2 mL/kg, i.p.) on days 2 and 3, 30 min after TFs administration.

The mice were killed after the 24 h treatment. Blood was collected via heart puncture and serum was separated for examination of various biochemical parameters. The liver was carefully dissected and cleaned of extraneous tissue. A portion of the liver tissue was immediately transferred into 10% formalin for histopathologic investigation. Levels of biochemical parameters ALT and AST were measured and compared with silymarin control [15].

2.7. General Experimental Procedures for Isolation and Structural Identification. Column chromatography was carried out using Merck Silica gel normal-phase (230–240 mesh) and reversed-phase C_{18} (Merck). Analytical TLC was carried out in silica gel plates (Merck DC-Alufolien 60 F_{254}). Compounds were visualized by spraying with 10% H_2SO_4 in EtOH and heating for 3–5 min.

The high-resolution electrospray ionization mass spectra (HR-ESI-MS) were acquired on a Bruker MicrOTOF-QII spectrometer. The ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), DEPT, COSY, HSQC, and HMBC spectra were recorded on a Bruker AM500 FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard.

2.8. Isolation of Pure Compounds. CVSM extract (330 g) was subjected to silica gel column chromatography and eluted with gradient solvent systems of chloroform and methanol (95 : 5 ⟶ 5 : 95, v/v) to collect six fractions: M1 (20 g), M2 (32 g), M3 (90 g), M4 (80 g), M5 (47 g), and M6 (62 g). The fraction M4 (80 g) was chromatographed on silica gel and eluted with $CHCl_3$–MeOH (6 : 1⟶3 : 1, v/v) to give four subfractions (M4.1–M4.4). The subfraction M4.2 (18 g) was separated and further purified by RP-18 with MeOH–H_2O (4 : 1, v/v) to deliver compounds 1 (150 mg), 3 (2 g), and 4 (250 mg). The fraction M5 (5 g) was applied on a silica gel chromatographic column and eluted with $CHCl_3$–MeOH (2 : 1, v/v) to yield compound 2 (50 mg).

Similarly, CCSM extract (420 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (95 : 5–5 : 95, v/v) to get seven fractions: M1 (15 g), M2 (25 g), M3 (20 g), M4 (18 g), M5 (15 g), M6 (11 g), and M7 (52 g). The fraction M3 (20 g) was eluted with $CHCl_3$–MeOH–H_2O (5 : 1 : 0.1, v/v/v) by silica gel column chromatography to receive compound 8 (30 mg). The fraction M4 (18 mg) was loaded on silica gel column chromatography using $CHCl_3$–MeOH–H_2O (4 : 1 : 0.1, v/v/v) and furnished compound 5 (70 mg). The fraction M5 (15 g) was separated on a silica gel column with $CHCl_3$–MeOH–H_2O (3 : 1 : 0.1, v/v/v) to obtain compound 6 (45 mg). The fraction M6 (11 g) was eluted with $CHCl_3$–MeOH–H_2O (2 : 1 : 0.1, v/v/v) on a silica gel column chromatography to yield compound 7 (18 mg).

3. Results and Discussion

3.1. Protective Activity of Extracts against CCl_4-Induced Hepatoxicity in HepG2 Cells. The *in vitro* cytotoxic and hepatoprotective effects of extracts were shown in Tables 1 and 2.

The ethyl acetate extract of *C. chelidonii* stems mostly increased cell viability. Particularly, after 24 h of treatment, it increased 25% and 26% at the concentrations of 50 and 100 μg/mL, respectively; after 48 h of treatment, at 50 μg/mL, it increased 25% and 50%, respectively; after 72 h of treatment, at 100 μg/mL, it increased 26% and 60%, respectively.

The *n*-hexane and methanol extracts of *C. chelidonii* stems, after 72 h of treatment, at 100 μg/mL, approximately increased 21.4%, while the *n*-hexane and methanol extracts of *C. chelidonii* leaves, after 72 h of treatment at 100 μg/mL, increased 26.9% and 30%, respectively. Meanwhile, the ethyl acetate and methanol extracts of *C. viscosa* leaves and the *n*-hexane extract of *C. viscosa* stems, after 72 h treatment, at 100 μg/mL, increased from 20% to 30% cell viability.

The results show that the methanol extracts of the stems of *C. chelidonii* and *C. viscosa* significantly revealed *in vitro* hepatoprotective activity. Thus, these ones were further examined for *in vivo* hepatoprotection against CCl_4-induced liver toxicity in mice.

3.2. Hepatic Protective Effect of Extracts against CCl4-induced Liver Injury in Mice. The *in vivo* hepatoprotective effects of methanolic extracts of the stems of *C. chelidonii* and *C. viscosa* (Table 3 and Figure 1) were tested against CCl_4-induced toxicity of liver in mice.

At the doses of 30 mg/kg and 45 mg/kg, the methanol extracts of the stems of *C. chelidonii* and *C. viscosa* (CCSM and CVSM) significantly decreased ALT and AST concentrations in comparison with untreated extracts and the hepatic protection of these extracts was comparable to that of silymarin.

This result warranted the CCSM and CVSM extracts to be further investigated on phytochemical components.

3.3. Phytochemical Components of the Most Hepatic Protective Effect Extracts. The most *in vitro* and *in vivo* liver protection extracts of two species stems (CCSM and CVSM) were subjected to silica gel normal-phase and reversed-phase RP-18 chromatography to give eight known flavonoids (**1**–**8**) whose structures were confirmed by HR-ESI-MS, NMR experiments, and comparisons with the published data: visconoside A (1), visconoside B (2) [20], quercetin 3-*O*-β-D-glucopyranoside 7-*O*-α-L-rhamnopyranoside (3), kaempferol 3-*O*-β-D-glucopyranoside 7-*O*-α-L-rhamnopyranoside (4) [18], cleomeside A (5), cleomeside B (6) [19],

Table 1: The cytotoxicity using HepG2 of extracts of *Cleome chelidonii*.

	$OD_{570} \pm SEM$						Percentage of control (%)		
Concentration (μg/mL)	CCSH	Control	CCSE	Control	CCSM	Control	CCSH	CCSE	CCSM
After 24 h of treatment									
100	0.281 ± 0.010	0.243 ± 0.002	0.269 ± 0.002	0.213 ± 0.003	0.293 ± 0.009	0.313 ± 0.012	108.3	126.4	93.7
50	0.281 ± 0.008	0.260 ± 0.006	0.266 ± 0.004	0.216 ± 0.007	0.300 ± 0.010		107.0	123.6	95.9
25	0.270 ± 0.007	0.263 ± 0.008	0.250 ± 0.005	0.231 ± 0.007	0.281 ± 0.010		102.7	108.1	90.0
DOX 10	0.149 ± 0.005		0.149 ± 0.005		0.164 ± 0.006		56.7	59.3	52.5
After 48 h of treatment									
100	0.402 ± 0.010	0.282 ± 0.005	0.420 ± 0.003	0.265 ± 0.009	0.313 ± 0.007	0.326 ± 0.005	117.8	158.4	95.9
50	0.367 ± 0.004	0.341 ± 0.011	0.396 ± 0.006	0.319 ± 0.004	0.323 ± 0.010		97.1	124.3	98.9
25	0.367 ± 0.005	0.378 ± 0.011	0.360 ± 0.007	0.359 ± 0.005	0.314 ± 0.007		97.2	100.3	96.3
DOX 10	0.113 ± 0.004		0.112 ± 0.003		0.091 ± 0.002		29.9	31.2	27.9
After 72 h of treatment									
100	0.429 ± 0.012	0.315 ± 0.010	0.441 ± 0.014	0.289 ± 0.012	0.403 ± 0.014	0.339 ± 0.022	121.4	152.2	118.6
50	0.394 ± 0.016	0.353 ± 0.009	0.420 ± 0.012	0.329 ± 0.009	0.410 ± 0.017		98.7	127.6	120.8
25	0.388 ± 0.011	0.399 ± 0.006	0.441 ± 0.017	0.393 ± 0.006	0.406 ± 0.010		97.3	112.3	119.5
DOX 10	0.071 ± 0.002		0.076 ± 0.001		0.068 ± 0.001		17.8	19.3	20.0

Table 2: The cytotoxicity using HepG2 of extracts of *Cleome viscosa*.

	$OD_{570} \pm SEM$						Percentage of control (%)		
Concentration (μg/mL)	CVSH	Control	CVSE	Control	CVSM	Control	CVSH	CVSE	CVSM
After 24 h of treatment									
100	0.232 ± 0.014	0.234 ± 0.004	0.274 ± 0.009	0.244 ± 0.009	0.267 ± 0.008	0.230 ± 0.007	92.9	112.2	116.3
50	0.254 ± 0.008	0.250 ± 0.008	0.256 ± 0.007	0.242 ± 0.005	0.265 ± 0.011		98.0	105.7	115.5
25	0.260 ± 0.004	0.259 ± 0.012	0.258 ± 0.010	0.266 ± 0.004	0.250 ± 0.013		100.3	96.7	108.7
DOX 10	0.157 ± 0.004		0.186 ± 0.006		0.164 ± 0.006		60.5	69.8	51.7
After 48 h of treatment									
100	0.314 ± 0.009	0.268 ± 0.008	0.274 ± 0.009	0.271 ± 0.012	0.322 ± 0.008	0.312 ± 0.007	97.2	101.4	103.4
50	0.308 ± 0.011	0.306 ± 0.011	0.304 ± 0.009	0.291 ± 0.008	0.311 ± 0.002		85.4	104.6	99.8
25	0.329 ± 0.008	0.365 ± 0.003	0.297 ± 0.008	0.332 ± 0.004	0.311 ± 0.005		91.0	89.4	99.8
DOX 10	0.129 ± 0.003		0.130 ± 0.006		0.114 ± 0.003		35.7	39.3	36.4
After 72 h of treatment									
100	0.405 ± 0.014	0.299 ± 0.019	0.279 ± 0.013	0.303 ± 0.014	0.365 ± 0.007	0.325 ± 0.008	121.7	92.2	112.3
50	0.368 ± 0.005	0.333 ± 0.011	0.374 ± 0.028	0.345 ± 0.012	0.372 ± 0.013		97.2	108.3	114.5
25	0.368 ± 0.015	0.378 ± 0.008	0.356 ± 0.020	0.390 ± 0.017	0.381 ± 0.014		97.3	91.4	117.2
DOX 10	0.073 ± 0.002		0.075 ± 0.001		0.066 ± 0.001		19.2	19.3	20.3

Table 3: The hepatoprotection of the methanol extracts of the stems of *C. viscosa* and *C. chelidonii* against CCl_4-induced toxicity of liver in mice.

Group	CVSM extract		CCSM extract	
	ALT	AST	ALT	AST
I (normal control)	22.50 ± 7.42	21.75 ± 6.34	30.50 ± 10.15	72.75 ± 13.60
II (CCl_4-intoxicated)	188.75 ± 81.81	233.00 ± 71.36	508.50 ± 113.09	470.50 ± 112.34
III (positive group)	29.00 ± 11.40	23.50 ± 9.15	26.75 ± 19.52	23.50 ± 12.34
IV (test group, 15 mg/kg)	66.25 ± 72.91	54.50 ± 46.92	41.75 ± 23.37	38.75 ± 2.06
V (test group, 30 mg/kg)	53.00 ± 25.22	58.00 ± 32.79	37.00 ± 34.30	24.50 ± 16.26
VI (test group, 45 mg/kg)	83.75 ± 72.54	78.00 ± 59.70	20.25 ± 9.07	21.00 ± 9.13

cleomeside C (7), and quercetin-3-*O*-[β-D-glucopyranosyl-(1⟶2)]-α-L-rhamnopyranoside 7-*O*-α-L-rhamnopyranoside (8) [17] (Figure 2).

The phytochemical study confirmed that flavonoids are the main components of two species, which might be representative of their hepatoprotective effect. Therefore, the hepatoprotections of flavonoids (1–8) were screened using HepG2 cell line.

3.4. Cytotoxicity and Hepatoprotective Activity of Purified Compounds. The cytotoxicity (Table 4) and hepatoprotection

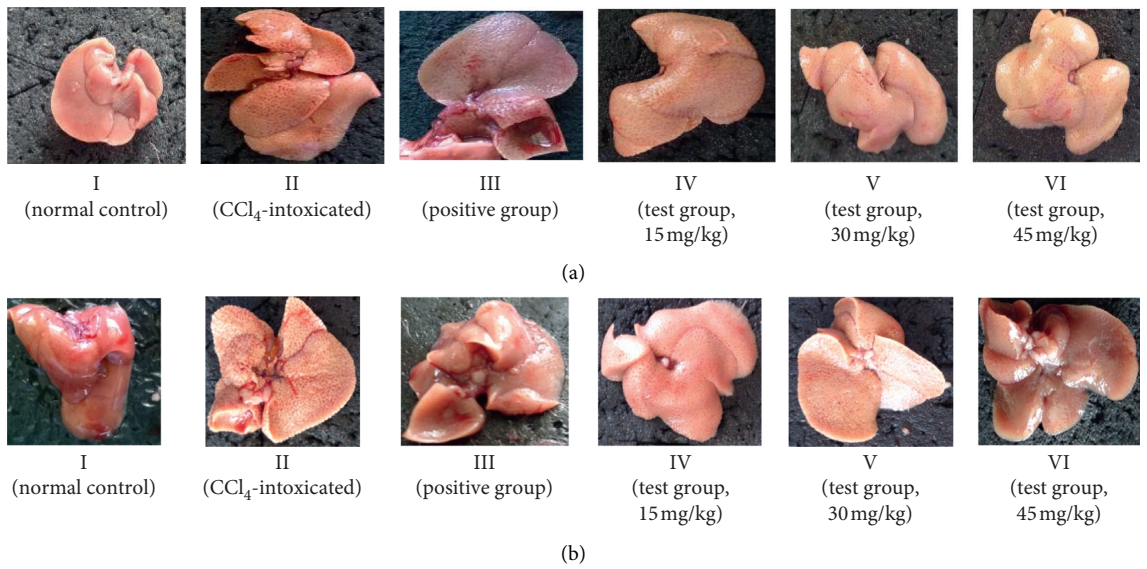
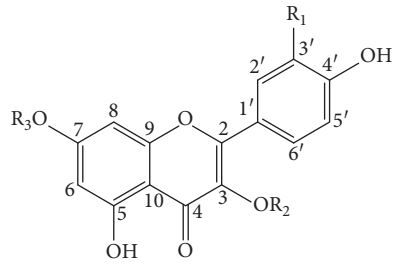


Figure 1: Photographs of livers from normal and treated mice. (a) The methanol extract of the stems of *C. viscosa* (CVSM). (b) The methanol extract of the stems of *C. chelidonii* (CCSM).



Compound	R_1	R_2	R_3	R_4
1	OH	OH	Glc-(1→3)-4-OAc-Rha	Rha
2	OH	OH	[Sinapinoyl-(1→6)]-Glc-(1→3)-4-OAc-Rha	Rha
3	OH	OH	Glc	Rha
4	H	OH	Glc	Rha
5	OH	OH	Glc-(1→2)-Rha	4-OAc-Rha
6	OH	OH	[Coumaroyl-(1→6)]-Glc-(1→2)-Rha	4-OAc-Rha
7	H	OH	[(6-Feruloyl)-(1→2)-Glc; (6-Coumaroyl)-(1→3)-Glc]-Rha	Rha
8	OH	OH	Glc-(1→2)-Rha	Rha

Figure 2: Structure of isolated flavonoids 1–8.

Table 4: The cytotoxicity using HepG2 of isolated compounds 1–8.

Sample	Concentration (μM)	$OD_{570} \pm SEM$	Control	Percentage of control (%)
Quercetin	10	0.183 ± 0.004	0.191 ± 0.003	95.8
	100	0.151 ± 0.007	0.161 ± 0.003	94.1
1	50	0.169 ± 0.008	0.177 ± 0.010	95.3
	25	0.157 ± 0.003	0.194 ± 0.010	81.0
	100	0.175 ± 0.004	0.161 ± 0.003	109.1
2	50	0.167 ± 0.005	0.177 ± 0.010	94.1
	25	0.188 ± 0.005	0.194 ± 0.010	97.0
	100	0.187 ± 0.012	0.161 ± 0.003	116.4
3	50	0.174 ± 0.006	0.177 ± 0.010	98.1
	25	0.141 ± 0.004	0.194 ± 0.010	72.6

Table 4: Continued.

Sample	Concentration (μM)	$OD_{570} \pm SEM$	Control	Percentage of control (%)
4	100	0.167 ± 0.012	0.161 ± 0.003	103.6
	50	0.166 ± 0.008	0.177 ± 0.010	93.8
	25	0.164 ± 0.006	0.194 ± 0.010	84.8
5	100	0.169 ± 0.172	0.161 ± 0.003	68.2
	50	0.147 ± 0.139	0.177 ± 0.010	78.6
	25	0.140 ± 0.132	0.194 ± 0.010	68.2
6	100	0.165 ± 0.010	0.161 ± 0.003	102.7
	50	0.162 ± 0.008	0.177 ± 0.010	91.6
	25	0.152 ± 0.008	0.194 ± 0.010	78.3
7	100	0.143 ± 0.006	0.161 ± 0.003	89.0
	50	0.153 ± 0.018	0.177 ± 0.010	86.3
	25	0.406 ± 0.018	0.194 ± 0.010	209.8
8	100	0.188 ± 0.010	0.161 ± 0.003	116.7
	50	0.140 ± 0.003	0.177 ± 0.010	78.8
	25	0.149 ± 0.009	0.194 ± 0.010	77.0

Table 5: The hepatoprotective activities using HepG2 of all isolated compounds 1–8 at a concentration of 100 μM.

Sample	OD_{570} nm ± SEM		Prevention percentage (%)
	CCl_4 2 mM (−)	CCl_4 2 mM (+)	
Control	0.191 ± 0.003	0.157 ± 0.010	—
Control DMSO 1%	0.161 ± 0.003	0.119 ± 0.004	
1	0.151 ± 0.007	0.147 ± 0.004	66.5
2	0.175 ± 0.004	0.146 ± 0.006	64.1
3	0.187 ± 0.012	0.106 ± 0.005	−33.5
4	0.167 ± 0.012	0.150 ± 0.006	74.2
5	0.172 ± 0.009	0.125 ± 0.005	13.3
6	0.165 ± 0.010	0.133 ± 0.003	32.3
7	0.143 ± 0.006	0.105 ± 0.008	−34.7
8	0.188 ± 0.010	0.125 ± 0.009	14.1
Quercetin 10 μM	0.183 ± 0.004	0.184 ± 0.004	80.3

(Table 5) using HepG2 cell line of all separated compounds 1–8 were measured by MTT assay.

At tested concentrations, samples did not show cytotoxicity, except compounds 3, 5, 6, and 8 at 25 μM (cell viability decreased, ranging from 25.0% to 30.0%).

At the concentration of 100 μM, compounds 1 and 4 significantly showed hepatoprotective effect (with prevention percentages of 66.5% and 74.2%, respectively), whereas compounds 5 and 8 disclosed weaker activity (with prevention percentages of 32.3% and 34.3%, respectively, compared to that of 80.3% of quercetin positive control).

The hepatoprotective effects of compounds 1 and 4 were tested for the first time.

4. Conclusions

In vitro and *in vivo* hepatoprotections using HepG2 and in mice of *C. chelidonii* and *C. viscosa* stems and their phytochemical constituents were investigated for the first time. The phytochemical study evidenced that flavonoids are the main compounds of two species. Furthermore, the hepatoprotections of visconoside A (1) and kaempferol 3-*O*-β-D-glucopyranoside 7-*O*-α-L-rhamnopyranoside (4) were identified for the first time. However, further clinical examinations are required to determine the molecular mechanisms of hepatoprotection as well as qualitative and quantitative identification of main biological flavonoid markers (1, 4, and 6) from these species.

The present study suggests that *C. chelidonii* and *C. viscosa* plants are good sources of natural hepatoprotective agents and contribute to understanding the biological activities of *Cleome* species in traditional Vietnamese medicine.

Data Availability

All the data related to the study are available from the corresponding author and can be provided upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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