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Original Research Article

Antioxidant Activity of Compounds of Fractions from *Cayratia trifolia* Species

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ABSTRACT

Cayratia trifolia (Vitaceae, commonly known as three-leaf cayratia), a wild vine abundant in the Mekong Delta, Vietnam, is traditionally used in folk medicine but underexplored scientifically. This study investigated the antioxidant-oriented chemical composition of whole and fractional extracts (n-hexane, dichloromethane, ethyl acetate, n-butanol, water) from roots, stems, leaves, and fruits of *C. trifolia* using the DPPH assay with vitamin C as a reference. The ethyl acetate fraction of *C. trifolia* roots exhibited the most potent antioxidant activity (IC_{50} = 51.27 µg/mL), followed by stems (IC_{50} = 119.62 µg/mL), leaves (IC_{50} = 159.92 µg/mL), and fruits (IC_{50} = 268.11 µg/mL). From the ethyl acetate fraction of roots, three compounds were isolated and identified as 5-hydroxymethylfurfural, liquiritigenin, and kaempferol-3-O-rhamnoside, with the first two reported in *C. trifolia* roots for the first time.

Keywords: Antioxidant, *Cayratia trifolia* (L.) Domino, Phenolics, 1,1-diphenyl-2-picrylhydrazyl.

Introduction

The excessive formation of free radicals beyond the neutralizing capacity of the body's antioxidant defense system leads to oxidative stress. It is a significant cause of many chronic and degenerative diseases in humans.[1] Natural antioxidants are essential in promoting health, preventing disease, and treating human disease. Natural antioxidants are increasingly valued for their minimal or no side effects compared to synthetic exogenous antioxidants.[2] Among them, medicinal plants rich in polyphenols and flavonoids are recognized to have potent antioxidant effects.[1,2] Can Tho City, Vietnam, has a rich source of medicinal herbs, with suitable environmental conditions for developing many medicinal herbs, including *Cayratia trifolia*. Can Tho City, located in the Mekong Delta of Vietnam, is known for its rich biodiversity and favorable tropical climate, supporting various medicinal plants, including *Cayratia trifolia*.

Cayratia trifolia belongs to the family Vitaceae. This wild plant often grows widely in Vietnam and the Mekong Delta. It does not have high economic value, but it has the potential to become a potential medicinal plant because people have harnessed its antioxidant, antibacterial, and cancer-prevention properties of the total extracts of *C. trifolia*. Previous research identified two main compounds in C. trifolia: alkaloids and flavonoids.[3] The whole plant of *C. trifolia* has been studied to contain yellow waxes, steroids/terpenoids, flavonoids, and tannins. Leaves contain stilbenes (piceid, resveratrol, viniferin, ampelopsin). Stems, leaves, and roots are believed to contain hydrocyanic acid and delphinidin. This plant also contains kaempferol, myricetin, quercetin, triterpenes and epiftriedelanol.[4] This is a favorable condition for the development of functional foods from natural sources of medicinal herbs, making an essential contribution to the cause of health care and being input materials for other industries such as cosmetics and food processing.

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This study aimed to evaluate the antioxidant activity of *C. trifolia* extracts and isolate bioactive compounds from the most active fraction, provide scientific data for further studies on the medicinal properties and active ingredients of *C. trifolia*, and promote the value of herbal species. The study investigated different parts of the plant to create natural products that could be used for human health protection.

Materials and Methods

Collection and identification of plant material

Cayratia trifolia was collected in February 2024 at coordinates 10.00789º B, 105,72298º E, An Binh ward, Ninh Kieu district, Can Tho City and identified by Dr. Duong Thieu Van, Nursing Pharmaceutical Sciences, Tay Do University. Materials were determined by observing plant morphology, microbiological investigation, and comparison with botanical taxonomic documents in the Dictionary of Vietnamese Medicinal Plants by Vo.[5] Plant parts studied include roots, stems, leaves, and fruits of C. trifolia. The plant materials were shade-dried at room temperature (25-30 °C) for 7–10 days until constant weight, then ground into a fine powder (particle size < 1 mm) using a laboratory grinder. *C. trifolia* fruit material was also collected, pest removed, washed, and dried.

Preliminary analysis of phytochemical composition

The modified method of Ciulei was used in this study.[6] The test sample was extracted with three solvents of increasing polarity (diethyl ether, ethanol 96%, water) to obtain a diethyl ether extract containing groups of less polar substances. Alcohol and aqueous extracts contain more polar groups of substances. The presence of different classes of compounds in the extracts was confirmed by coloration or precipitation reactions. The extract was hydrolyzed by heating with 10% HCl to investigate the aglycon fraction.

Extraction and fractionation of extracts

The root, stem, and leaf powder of *C. trifolia* were extracted by reflux with ethanol 96% (Merck, 96%) to obtain ethanol extract using a rotary evaporator under reduced pressure at 40 °C. An Aliquot of the total extract was used to investigate the antioxidant activity. The crude extract was mixed with sufficient water to obtain the liquid form, and the mixture was shaken to effect a liquid-liquid partition of mixtures using solvents of different increasing polarities: n-hexane, dichloromethane, ethyl acetate, and n-butanol, respectively. The collected fractions were evaporated under reduced pressure to obtain

the respective fractions. These extracts were used to test for antioxidant activity.

Determination of Antioxidant activity of crude extract and fractions

A 0.6 mM DPPH (Sigma-Aldrich, 97% purity, 0.6 mM) solution was prepared in methanol by dissolving 5.915 mg of DPPH in an adequate amount of methanol, then transferred into a volumetric flask and 25 mL of methanol was added. After mixing, the mixture was vortexed immediately and incubated in dark-colored glass bottles. The DPPH free radical scavenging activity of total extracts from samples of *C. trifolia* was evaluated using a spectrophotometer (Shimadzu UV-1800, Japan) at λ = 517 nm. The extracts were dissolved with methanol to obtain an initial 1 mg/mL concentration for the dried crude extracts. Vitamin C (Sigma-Aldrich, 97% purity) was used as the positive control (Table 1).

The HTCO (Antioxidant activity) free radical scavenging activity (%) was calculated by the formula:

$$\text{HTCO}(\%) = \left[\frac{(ODcontrol - ODtest)}{ODcontrol}\right] X\ 100$$

Test results are expressed as the mean of three different independent measurements. A standard curve was constructed based on HTCO (%) and sample concentration. From the standard curve, the IC_{50} (capability of catching 50% of the DPPH of the sample) was calculated using the logarithmic linear regression equation of the form y = ln(x) + b. The lower IC_{50} value corresponds to a higher HTCO and vice versa.[7,8] The fraction with the highest activity was selected for further phytochemical screening (isolation).

Isolation and structure determination

The extract with the highest antioxidant activity was loaded into a chromatographic column (silica gel, Sephadex LH-20) and eluted using different solvents systems, and their combination ($CHCl_3$-MeOH, 100:0 - 0:100), (PE - $CHCl_3$, 3:7 - 2:8), ($CHCl_3$ – MeOH, 100:0 - 80:20), (EtOAC - MeOH - H_2O, 100:5:13 - 100:17:13), and (MeOH - H_2O, 30:70) (RP18 silica gel). The eluates were repeatedly filtered, washed, and recrystallized to give the pure compounds. The structures were determined through UV, MS, and NMR spectroscopy and compared with reference materials.

Results and Discussions

Analytical results showed that *C. trifolia* extracts gave positive reactions to the following groups of compounds: Carotenoids, essential oils, free triterpenoids, polyphenols, tannins, hydrolyzed triterpenoids, saponins, flavonoids, organic acids, and polyuronides. Preliminary phytochemical analysis revealed flavonoids and polyphenols in all parts, while roots showed the highest flavonoid content, followed by leaves. Saponins were detected in stems and roots but absent in fruits. Among them, flavonoids were the most reactive components of *C. trifolia* species.

The antioxidant activity of extracts was investigated by measuring UV-Vis spectral absorbance. To confirm the antioxidant capacity of the stem, leaf, root, and fruit fractions of C. trifolia, the antioxidant activity was evaluated based on the IC_{50} value. Survey 6 was extracted at a concentration of 1 mg/mL. The average antioxidant activity (%) of each fraction was calculated. The highest antioxidant activity (%) was used to determine the logarithmic curve and compute the IC_{50} at 5 concentrations. The results were compared with the IC_{50} value of vitamin C control.

Based on the results, different extracts exhibited antioxidant activity: ethanol 96% extract 1000 µg/mL (69.04%), ethanol 96% extract 2000 µg/mL (95.01%), *n*-hexane extract 1000 µg/mL (92.44%), *n*-hexane extract 2000 µg/mL (92.58%), DCM extract 1000 µg/ml (94.72%), DCM extract 2000 µg/mL (93.44%), EA extract 1000 µg/mL (93.58%), EA extract 2000 µg/mL (91.87%), n-butanol extract 1000 µg/mL (89.87%), *n*-butanol extract 2000 µg/mL (90.87%) had antioxidant activity. If the antioxidant activity is > 50%, further investigation is conducted to find the IC_{50} (Table 2).

Results of the leaf extract *C. trifolia* showed ethanol 96% extract 1000 µg/mL (74.80%), ethanol 96% extract 2000 µg/mL (82.83%), DCM extract 1000 µg/ml (77.03%), DCM extract 2000 µg/mL (91.3%), EA extract 1000 µg/mL (90.41%), *n*-butanol extract 2000 µg/mL (78.15%) with antioxidant activity > 50%, should continue investigating antioxidant activity to find IC_{50} (Table 3). The results from the antioxidant activity of the roots showed that all the extracts had antioxidant activity > 50%; hence, they were further evaluated for IC_{50} (Table 4). Also the fruits showed ethanol 96% extract 1000 µg/mL (88.02%), ethanol 96% extract 2000 µg/mL (97.87%), *n*-hexane extract 1000 µg/mL (61.04%), *n*-hexane extract 2000 µg/mL (91.49%), DCM extract 2000 µg/mL (73.36%), EA extracts 1000 µg/mL (79.07%), EA extract 2000 µg/mL (92.39%), *n*-butanol extract 1000 µg/mL (63.28%) antioxidant activity > 50%, and was further investigated to determine its IC_{50} value (Table 5). From the logarithmic equations of the form y = aln(x) + b in the diagram above. Substituting y = 50 we get the IC_{50} (µg/mL) result as follows (Figure 1), (Figure 2).

The results of antioxidant activity screening showed that ethyl acetate extract of the roots *C. trifolia* had the most potent antioxidant effect, with IC_{50} = 51.27 µg/mL, followed by extract ethyl acetate of stem C. trifolia, IC_{50} = 119.62 µg/mL. However, the activity of both compounds was still lower than that of the positive control vitamin C, IC_{50} = 14.47 µg/mL. In 2007, Homhua et al.[9] investigated the antioxidant capacity of ethyl acetate and methanol extracts of *C. trifolia* by DPPH free radical scavenging method with IC_{50} values of 10.24 µg/mL and 11.36 µg/mL respectively compared to that of C. trifolia. Vitamin C positive control was 3.2 µg/mL. The study's experimental results showed that the ethyl acetate extract of roots of *C. trifolia* had a high antioxidant effect when compared with ethanol extract and petroleum extract, with IC_{50} = 51.27 µg/mL.

The study selected the ethyl acetate fraction of roots *C. trifolia* with the most potent antioxidant effect (IC_{50} = 51.27 µg/mL) and conducted column chromatography on the ethyl acetate fraction (EA, 15 g) with the solution system $CHCl_3$-MeOH lysates (100:0 - 0:100) yielded 10 fractions of EA1 (150 mg), EA2 (510 mg), EA3 (920 mg), EA4 (340 mg), EA5 (800 mg), EA6 (630 mg), EA7 (1250 mg), EA8 (3190 mg), EA9 (320 mg), EA10 (90 mg). Column chromatography of EA2 fractionation (510 mg) on silica gel with the solvent system is PE - EA (8:2 - 5:5) to obtain the main fraction EA2.2. Further purification of this fraction by column chromatography on silica gel with solvent system PE - $CHCl_3$ (3:7 - 2:8) obtained compound 1 (25 mg). Column chromatography of EA6 fractions (630 mg) on silica gel several times with the solvent system $CHCl_3$ - MeOH (100:0 - 80:20) obtained the main fraction EA6.4. Further purification of this fraction by column chromatography on silica gel with solvent system EtOAC - MeOH - H_2O (100:5:13 - 100:17:13) obtained the main fraction EA6.4.2. Continued purification of this fraction by column chromatography on RP18 with solvent system MeOH - H_2O (30:70) led to compound 2 (20 mg). The EA8 fraction (3190 mg) was further investigated to isolate compounds. Chromatography of EA8 on Sephadex LH-20 gel with MeOH as a solvent obtained 4 fractions (EA8.1-EA8.4.). A normal phase silica gel column chromatography of EA8.3 (190 mg) with solvent $CHCl_3$-EtOAc-Me_2CO-H_2O (7:10:10:0.05, v/v) led to the isolation of compound 3 (32 mg).

Compound 1: Amorphous, dark yellow, soluble in chloroform. On a silica gel 60 F_{254} plate, compound 1 turned off under a 254 nm UV lamp, showing color with 10% H_2SO_4 reagent in alcohol. UV (MeOH) spectra showed compound 1 had a maximum absorption of 281 nm. ^{1}H-NMR spectra for peak resonances: Two protons of two olefin carbons coupled at 6.51 ppm (d; J = 3.5 Hz; H-3) and 7.21 ppm (d; J = 3.5 Hz; H-4). So compound 1 is most likely a derivative of the furan ring. There is also a singlet signal of a proton belonging to an aldehyde group (-CHO) at 9.58 ppm (s; H-7) and an oxymethylene group (-CH_2OH) at 4.71 ppm (s; H-6). The ^{13}C-NMR spectrum gives signals of 6 carbons, including signals of 2 quaternary carbons of the furan ring at 152.60 ppm (C-2) and 160.70 ppm (C-5). The two methyl carbons of the furan ring are at 122.67 ppm (C-3) and 110.09 ppm (C-4). One carbon of the -CHO group at 177.77 ppm (C-7). A methylene carbon of the -CH_2OH group at 57.80 ppm (C-6). From the above data and compared with references,[10] compound 1 was identified as 5-hydroxymethylfurfural. This compound has been reported to be present in several other

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medicinal herbs and can be found in other medicinal plants. Regarding antioxidant activity, it also has the potential for practical applications in food, cosmetics, and pharmaceuticals[11,12], which have the following structural formula (Figure 3).

Table 1: DPPH test response

Tube	Test solution (mL)	MeOH solution (mL)	DPPH solution (mL)
White	0	4	0
Control	0	3.5	0.5
Test	0.5	3	0.5

Table 2: Investigating the antioxidant capacity of stem *C. trifolia* extract at concentrations of 2000 µg/mL and 1000 µg/mL

No	Ingredient name	Abs medium	anti-oxidation (%)
1	DPPH	0.701	
2	Ethanol 96% extract 1000 μg/mL	0.271	69.04
3	Ethanol 96% extract 2000 μg/mL	0.035	95.01
4	n-hexane extract 1000 μg/mL	0.053	92.44
5	n-hexane extract 2000 μg/mL	0.052	92.58
6	DCM extract 1000 μg/mL	0.037	94.72
7	DCM extract 2000 μg/mL	0.046	93.44
8	EA extract 1000 μg/mL	0.045	93.58
9	EA extract 2000 μg/mL	0.057	91.87
10	n-butanol extract 1000 μg/mL	0.071	89.87
11	n-butanol extract 2000 μg/mL	0.064	90.87
12	Water extract 1000 μg/mL	0.590	15.83
13	Water extract 2000 μg/mL	0.864	-23.25

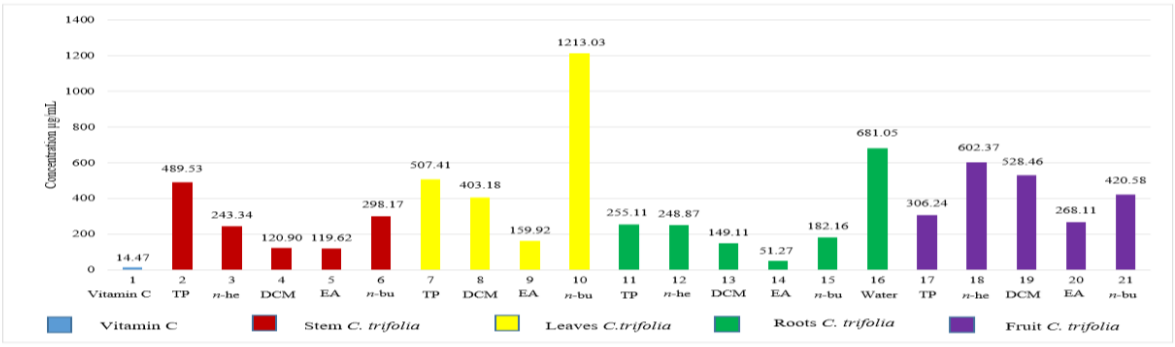

Figure 1: Graph showing antioxidant activity of tested extracts by division C. trifolia

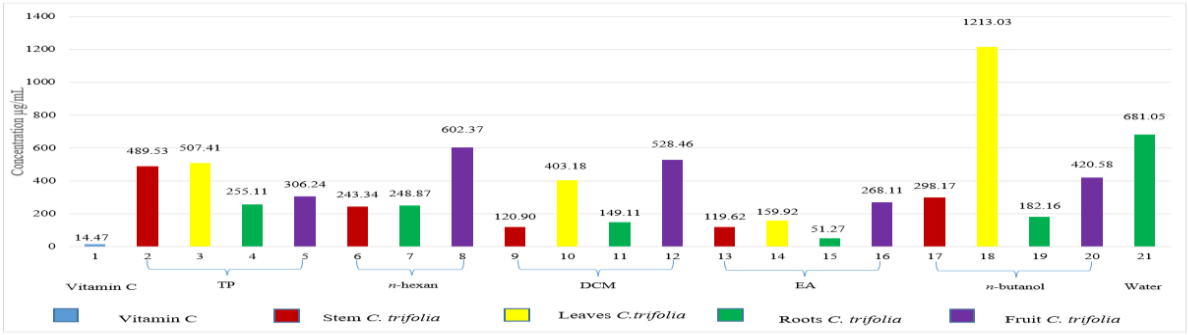

Figure 2: Graph showing the antioxidant activity of the tested extracts by fractions between *C. trifolia* parts

Compound 2: Light yellow powder, easily soluble in acetone. HR-ESI-MS spectrum for molecular ion peak [M-H]- at m/z 255.0661 (theoretical 255.0657), allowing the molecular formula to be determined as $C_{15}H_{12}O_4$. The ^{1}H-NMR spectrum of compound 2 in the low-field region shows the presence of seven aromatic ring proton signals, including the proton of the aromatic ring A corresponding to an aromatic ring with substituents at positions 1, 3, 4. δ_H 6.41 (1H, d, J = 2.5 Hz, H-8), δ_H 7.71 (1H, d, J = 8.5 Hz, H-5) and δ_H 6.56 (1H, dd), J =

8.5; 2.0 Hz, H-6)] and 4 pairwise symmetry protons of the B aromatic ring [δ_H 7.39 (2H, d, J = 8.5, H-2' and H) -6') and δ_H 6.89 (2H, d, J = 8.5 Hz, H-3' and H-5')]. In the high-field region, a resonance signal of a methylene group [δ_H 2.66 (1H, dd, J = 16.5; 3.0 Hz, H-3a) and δ_H 3.06 (m, H-3b) appears in the high-field region.)] and an oxymethin group at δ_H 5.44 (1H, dd, J = 13.0; 3.0 Hz, H-2). The ^{13}C-NMR spectrum combined with the HSQC spectrum shows that compound 2 has the presence of 15 carbon signals including 1 carbonyl carbon (δ_C 190.9), 12 aromatic ring carbons (δ_C 128.3; 110.2; 164.4; 102.8; 65.6; 114.4; 130.7; 128.0; 115.5; 158.1; 115.5; 128.0), 1 carbon methylene (δ_C 43.4), and 1 carbon methyl (δ_C 80.0). Based on the characteristic spectral signals, proton δ_H 5.44 (C-2) and 2 protons δ_H 2.66 and δ_H 3.00 (C-3) and 1 carbonyl signal at δ_C 190.9 (C-) 4) can predict compound 2 with a flavan framework. HMBC spectra show that compound 2 has a proton correlation at δ_H 7.71 (d, 8.5, H-5) and δ_H 6.41 (d, 2.5, H-8) with a quaternary carbon at δ_C 164.4 (C-7), which helps to identify the -OH substituent identified at the C-7 position. Proton HMBC correlation at δ_H 7.39 (H-2') and δ_H 6.89 (H-6') with the same δ_C 80.0 (C-2) helps determine the alignment of the B nucleus and the C nucleus at C-2. From the analysis of HR-ESI-MS, ^{1}H- C-NMR, and ^{13}C-NMR spectral data, combined with HMBC and HSQC spectra compared with the literature,[10] shows similarities. Therefore, the structure of compound 2 was identified as liquiritigenin and has the following structural formula (Figure 4).

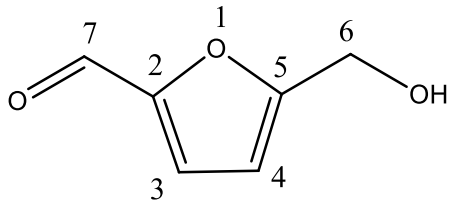

Figure 3: The structural formula of 5-hydroxymethylfurfural

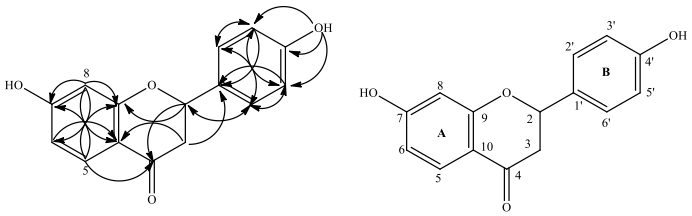

Figure 4: Structure of liquiritigenin

Table 3: Investigating the antioxidant capacity of leaves *C. trifolia* extract at concentrations of 2000 µg/mL and 1000 µg/Ml

No	Ingredient name	Abs medium	anti-oxidation (%)
1	DPPH	0.897	
2	Ethanol 96% extract 1000 μg/mL	0.226	74.80
3	Ethanol 96% extract 2000 μg/mL	0.154	82.83
4	n-hexane extract 1000 μg/mL	0.794	11.48
5	n-hexane extract 2000 μg/mL	1.026	-14.38
6	DCM extract 1000 μg/mL	0.206	77.03
7	DCM extract 2000 μg/mL	0.078	91.30
8	EA extract 1000 μg/mL	0.086	90.41
9	EA extract 2000 μg/mL	1.108	-23.52
10	n-butanol extract 1000 μg/mL	0.629	29.88
11	n-butanol extract 2000 μg/mL	0.196	78.15
12	Water extract 1000 μg/mL	1.354	-50.95
13	Water extract 2000 μg/mL	1.113	-24.08

Table 4: Investigating the antioxidant capacity of roots *C. trifolia* at concentrations of 2000 µg/mL and 1000 µg/mL

No	Ingredient name	Abs medium	anti-oxidation (%)
1	DPPH	0.771	
2	Ethanol 96% extract 1000 μg/mL	0.145	81.19
3	Ethanol 96% extract 2000 μg/mL	0.065	91.57
4	n-hexane extract 1000 μg/mL	0.056	92.74
5	n-hexane extract 2000 μg/mL	0.073	90.53
6	DCM extract 1000 μg/mL	0.077	90.01
7	DCM extract 2000 μg/mL	0.139	81.32
8	EA extract 1000 μg/mL	0.105	86.38
9	EA extract 2000 μg/mL	0.072	90.66
10	n-butanol extract 1000 μg/mL	0.061	92.09
11	n-butanol extract 2000 μg/mL	0.081	89.49
12	Water extract 1000 μg/mL	0.059	92.35
13	Water extract 2000 μg/mL	0.066	91.44

Compound 3: Compound 3 was obtained as a pale yellow powder, easily soluble in acetone. The HR-ESI-MS spectrum gives the molecular ion peak $[M+Na]^+$ at m/z 455.0930 (theoretical 455.0954), allowing the molecular formula to be determined as $C_{21}H_{20}O_{10}$. ^{1}H-NMR spectrum of compound 3 at the weak magnetic field region, showing the presence of 1 -OH group at δ_H 12.71 (s, 1H) and 2 aromatic proton signals at δ_H 6.48 (d, 1H), J = 2.0 Hz, H-8), δ_H 6.27 (d, 1H, J = 2.0 Hz, H-6) are meta-paired to characterize the aromatic nucleus A of the flavonoid framework. The ^{1}H–NMR spectrum also shows two aromatic proton signals at δ_H 7.86 (d, 2H, J = 8.5 Hz), δ_H 7.02 (d, 2H, J = 9.0 Hz), demonstrating the presence of an aromatic nucleus with 2 substituents at position 1,4 (nucleus B). The ^{1}H-NMR spectrum also shows signals of 5 oxymethin groups at δ_H 5.54 (brs, 1H), δ_H 4.23 (brs, 1H), δ_H 3.70 (dd, 1H, J = 9.0, 2.5 Hz), δ_H 3.33 (m, 1H), δ_H 3.30 (m, 1H) and 1 double-ended methyl group at δ_H 0.90 (d, 3H, J = 5.5 Hz), confirmed presence of sugar molecule α-L-rhamnopyranosyl in the structure of compound 3. ^{13}C-NMR spectrum combined with HSQC spectrum helps identify 21 carbon signals of compound 3, which includes 15 carbon signals of flavonoids (including 1 carbonyl group at δ_C 179.3; there are 6 sp^2 carbon signals linked directly with the oxygen atom at δ_C 165.0; 163.2; 160.9; 158.5; 158.0 and 135.6; there are 5 aromatic carbon signals at δ_C 131.7; 116.3; 105.8; 99.8; 94.3) and 6 signals of sugar molecule α-L-rhamnopyranosyl (5 methine signals at δ_C 102.8; 73.0; 72.2; 71.5; 71.4 and 1 signal of the methyl group at δ_C 17.7). A survey of HMBC spectral data confirmed that compound 3 is a flavonoid glycoside. Besides, the correlation between the proton anomer signal at δ_H 5.54 (H-1") and the carbon signal at δ_C 135.6 (C-3) demonstrates the association of the sugar molecule at C-3. of the aglycon fraction. From the analysis of HR-ESI-MS, ^{1}H-NMR, ^{13}C-NMR spectral data, combined with HMBC and HSQC spectra and comparison with the literature,[13] there are similarities. Therefore, compound 3 was identified as kaempferol-3-O-rhamnoside (Figure 5). This flavonoid compound has been shown to have antioxidant activity[14-16] with the following structure (Figure 5). The high antioxidant activity of the ethyl acetate fraction of roots (IC^{50} = 51.27 µg/mL) may likely be due to flavonoids like liquiritigenin and kaempferol-3-O-rhamnoside, which possess phenolic hydroxyl groups that scavenge free radicals. Kaempferol-3-O-rhamnoside, a glycosylated flavonoid, has been reported to exhibit strong DPPH scavenging activity due to its catechol structure.[14] Similarly, 5-hydroxymethylfurfural, a furan derivative, contributes to antioxidant capacity through its aldehyde group, as noted in. [11]

Table 5: Investigating the antioxidant capacity of fruit *C. trifolia* at concentrations of 2000 µg/mL and 1000 µg/mL

No	Ingredient name	Abs medium	anti-oxidation (%)
1	DPPH	0.893	
2	Ethanol 96% extract 1000 μg/mL	0.107	88.02
3	Ethanol 96% extract 2000 μg/mL	0.019	97.87
4	n-hexane extract 1000 μg/mL	0.348	61.04
5	n-hexane extract 2000 μg/mL	0.076	91.49
6	DCM extract 1000 μg/mL	0.485	45.71
7	DCM extract 2000 μg/mL	0.238	73.36
8	EA extract 1000 μg/mL	0.187	79.07
9	EA extract 2000 μg/mL	0.068	92.39
10	n-butanol extract 1000 μg/mL	0.328	63.28
11	n-butanol extract 2000 μg/mL	0.483	45.93
12	Water extract 1000 μg/mL	0.668	22.53
13	Water extract 2000 μg/mL	0.506	43.36

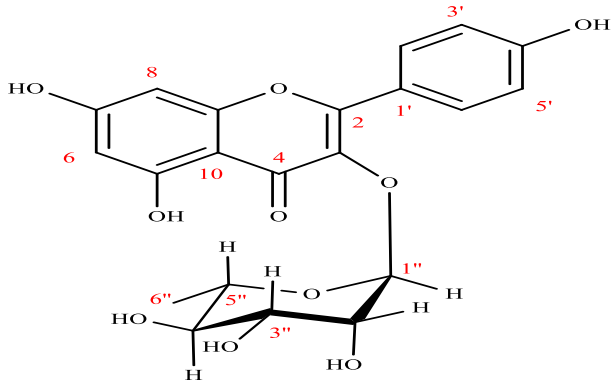

Figure 5: Structure of kaempferol-3-O-rhamnoside

Conclusion

This study demonstrated that *Cayratia trifolia*, particularly the ethyl acetate fraction of the root, exhibits potent antioxidant activity attributed to flavonoids and other phenolics. Three compounds—5-hydroxymethylfurfural, liquiritigenin, and kaempferol-3-O-rhamnoside—were isolated, with the first two identified in *C. trifolia* roots for the first time. Preliminary phytochemical analysis confirmed the presence of flavonoids, tannins, and saponins, supporting the plant's potential as a source of natural antioxidants for functional foods, cosmetics, and pharmaceuticals. This study shows that *Cayratia trifolia* has the potential to become a medicinal herb with antioxidant properties. *Cayratia trifolia* also contains two main compounds: alkaloids and flavonoids. In addition, *Cayratia trifolia* could serve as an essential raw material for other industries, such as cosmetics and food processing. However, further investigations of the different parts of the plant are needed for the possible development of a potent health-promoting supplement.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018; 13:757–772.
2. Fernandes RPP, Trindade MA, Tonin FG, Lima CGD, Pugine SMP, Munekata PES, Lorenzo JM, De Melo MP. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidants in lamb burgers. J Food Sci Technol. 2016; 53:451–460.
3. Perumal PC, Sophia D, Raj CA, Ragavendran P, Starlin T, Gopalakrishnan VK. In vitro antioxidant activities and HPTLC analysis of ethanolic extract of *Cayratia trifolia* (L.). Asian Pac J Trop Dis. 2012; 2:S952–S956.
4. Kumar D, Kumar S, Gupta J, Arya R, Gupta A. A review on chemical and biological properties of *Cayratia trifolia* Linn. (Vitaceae). Pharmacogn Rev. 2011; 5(10):184–188.
5. Vo VC. Dictionary of Vietnamese medicinal plants. Vietnam: Hanoi Medical Publishing House. 2021.
6. Ciulei I. Methodology for analysis of vegetable drugs. Practical manual on the industrial utilisation of medicinal and aromatic plants. Bucharest, Romania. 1982; 1–62.
7. Chanda S, Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afr J Microbiol Res. 2009; 3(13):981–996.
8. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005; 53(6):1841–1856.
9. Homhua S, Tongngok P, Bonjim J. Evaluation of biological activities of crude extracts from Cratoxylum formosum (Jack.) Dyer. and *Cayratia trifolia* (L.) Domin young shoots. J Ubon Rajathanee Uni. 2007; 9:54–60.
10. Nguyen TVA, Le Hong L, Nguyen TMH, Vu TT, Bui TT. Compounds isolated from the ethyl acetate fraction of *Canna edulis* Ker Gawl rhizomes. VNU J Sci Med Pharm Sci. 2021; 37(2):18–23.
11. Zhao L, Chen J, Su J, Li L, Hu S, Li B, Zhang X, Xu Z, Chen T. In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. J Agric Food Chem. 2013; 61(44):10604–10611.
12. Li CY, Xu HX, Han QB, Wu TS. Quality assessment of *Radix Codonopsis* by quantitative nuclear magnetic resonance. J Chromatogr A. 2009; 1216(11):2124–2129.
13. Do VM, Tran CL, Duong TH, Phan HVT, Nguyen HH, Nguyen TP, Sichaem J. Chemical constituents of the leaves of *Polyscias fruticosa*. Chem Nat Compd. 2021; 57(6):1125–1127.
14. Akter M, Parvin MS, Hasan MM, Rahman MAA, Islam ME. Anti-tumor and antioxidant activity of kaempferol-3-O-alpha-L-rhamnoside (Afzelin) isolated from *Pithecellobium dulce* leaves. BMC Complement Med Ther. 2022; 22(1):169.
15. Chantan W, Kanchanarach W, Noppawan P, Khunwong C, Senakun C, Appamaraka S, Cumrae N, Udchachone S, Siriamornpun S, Promprom W. Total Phenolics, Flavonoids Contents and Antioxidant Activity in Different Flavor Plants in Northeast (Isaan) Thailand: Enhancing Commercial Value. Trop J Nat Prod Res. 2023; 7(11): 5115-5122.
16. Ali MA, Shallan M, Meshrf WA, Marrez DA. Phenolic constituents, antioxidant, and antimicrobial activities of *globe artichoke* (*Cynara scolymus* L.) aqueous extracts. Trop J Nat Prod Res. 2021; 5(11): 1986-1994.