SURGICAL CHEMICAL COMPOSITION FOLLOWED ANTIOXIDANT EFFECTS OF *CAYRATIA TRIFOLIA*

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ABSTRACT:

Cayratia trifolia is a wild vines that grow a lot in different ecosystems in the Mekong Delta provinces in particular and in Vietnam in general. This is a rich source of raw materials, easy to find, but so far, the research works in the country and in the world on this plant are still limited. Therefore, the study was carried out to study the antioxidant-oriented chemical composition of the whole and fractional extracts (*n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, water) from roots, stems, leaves and fruits of *C. trifolia* by DPPH (1,1-diphenyl-2-picrylhydrazyl) test with vitamin C as reference. The results of the study identified high antioxidant activity of ethyl acetate of *C. trifolia* root with the strongest antioxidant effect, with IC_{50} = 51.27 µg/mL, followed by high ethyl acetate of *C. trifolia* stem, with IC_{50} = 119.62 µg/mL, followed by high ethyl acetate of *C. trifolia* leaves, with IC_{50} = 159.92 µg/mL and finally high ethyl acetate of *C. trifolia* fruit, with IC_{50} = 268.11 µg/mL. Simultaneously, from the ethyl acetate fraction of *C. trifolia* roots, we isolated and recognized the structure of 3 compounds: 5-hydroxymethylfurfural (1), liquigitigenin (2), kaempferol-3-*O*-rhamnoside (3) . Among them, compounds (1) and (2) were found for the first time in *C. trifolia* roots compared with previous studies.

Keywords: *Antioxidant, Cayratia trifolia (L.) Domino, compound, DPPH.*

INTRODUCTION

The excessive formation of free radicals beyond the neutralizing capacity of the body's antioxidant defense system leads to oxidative stress and is a major cause of many chronic and degenerative diseases in humans (Liguori *et al*., 2018). Natural antioxidants play an important role in promoting health, preventing disease and supporting disease treatment for humans, gaining increasing attention because they have no or fewer side effects than natural antioxidants. synthetic exogenous antioxidants (Fernandes *et al.*, 2016). Among them, medicinal plants rich in polyphenols and flavonoids are recognized to have strong antioxidant effects (Liguori *et al*., 2018), (Fernandes *et al.*, 2016). Can Tho city, Vietnam has a rich source of medicinal herbs, with suitable environmental conditions for the development of many types of medicinal herbs, including *Cayratia trifolia*.

Cayratia trifolia belongs to the family vitaceae, this is a wild plant, often grows widely in Vietnam in general and the Mekong Delta in particular, does not have high economic value, but has the potential to become a potential medicinal plant potential because people have used as well as some studies on *C. trifolia's* antioxidant, antibacterial, cancer prevention, ... on the total extracts of *C. trifolia*. Previous studies showed that *C. trifolia* contains two main compounds, alkaloids and flavonoids (Perumall *et al*., 2012). The whole plant *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, delphinidin. This plant also contains kaempferol, myricetin, quercetin, triterpenes and epiftriedelanol (Kumar *et al*., 2011). This is a very favorable condition for the development of the field of production of functional foods from natural sources of medicinal herbs, making an important contribution to the cause of people's health care, in addition to being input materials. for other industries such as cosmetics, food processing… To provide scientific data for further studies on medicinal properties and active ingredients of *C. trifolia* and promote the value of herbal species. Therefore, the research topic is carried out on different segments and parts used in order to create natural products for the protection of public health.

MATERIALS AND METHODS

Materials

Cayratia trifolia (L.) Domin was collected at coordinates 10.00789^{o} B, 105,72298^{o} 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 identified by observing plant morphology, microbiological investigation and comparison with botanical taxonomic documents. Medicinal materials studied are mainly roots, stems, leaves and fruits of *C. trifolia*. The stem, leaves and roots of *C. trifolia* are dried in the shade and finely ground. *C. trifolia* fruit material is collected, pest removed, washed and dried.

Methods

Preliminary analysis of phytochemical composition

Follow the modified Ciulei method (Ciulei, 1982) Extract the test sample with three solvents of increasing polarity (diethyl ether, ethanol, water) to obtain a diethyl ether extract containing groups of less polar substances. Alcohol and aqueous extracts contain more polar groups of substances. Confirm the presence of groups of compounds in the extracts by coloration or precipitation reactions. Carry out hydrolysis by heating the extracts with 10% HCl acid to investigate the aglycon fraction.

High preparation of total ethanol and high fractions

From root, stem and leaf powder of *C. trifolia* extracted by reflux method with ethanol 96% obtained ethanol extract. The evaporator rotates under reduced pressure at 40 °C to obtain a total elevation. Take a little of the total extract to test the antioxidant activity, the rest proceed to mix a sufficient amount of water to obtain the liquid form, and the highly diluted form is shaken with a liquid-liquid distribution in turn with solvents of different concentrations. Increasing polarization n-hexane, dichloromethane, ethyl acetate, n-butanol obtained the respective solutions, evaporating and recovering the solvent under reduced pressure to the respective highs. These extracts were used to test the antioxidant effects.

Investigation of total and fractional high antioxidant activity

Prepare a 0.6 mM DPPH solution in methanol by dissolving 5.915 mg of DPPH with an adequate amount of methanol, then transfer to a volumetric flask and add 25 mL of methanol. After mixing, use immediately, store in colored glass bottles. Investigation of DPPH free radical scavenging activity of total extracts from samples of medicinal materials. The extracts were dissolved with methanol to obtain an initial concentration of 1 mg/mL for the dried medicinal plant. If it is difficult to dissolve, you can use DMSO to help dissolve. The positive control used was vitamin C. Prepare a solution of DPPH with a concentration of 1 mM in methanol. This solution is not stable to light, only prepared before use. Test solution: Take a sample for the phase in MeOH according to table 1:

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

Shake the tubes well for 15s, stabilize at room temperature for 30 min, and photometrically at λ = 517 nm.

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

$$HTCO\ (\%) = [(OD_{control} - OD_{test})/ OD_{control}\ ] \times 100$$

Test results are expressed as the mean of three different independent measurements. From HTCO (%) and sample concentration, a standard curve was constructed. Based on the standard curve, calculate IC_{50} (capability of catching 50% of the DPPH of the sample) by replacing y = 50 into the logarithmic linear regression equation of the form y = aln(x) + b. The lower IC_{50} value corresponds to a higher HTCO and vice versa (Chanda and Dave, 2009; Huang *et al*., 2005). Select the part used and the extract with the strongest antioxidant effect for isolation and purification.

Methods of isolation and structure determination

The extract with the highest antioxidant activity was carried out by column chromatography (silica gel, Sephadex LH-20), fractional crystallization, repeated filtration and washing of the precipitate with different solvents to obtain pure compounds. Determine the structures of the isolated compounds by means of UV, MS, NMR spectroscopy and compare with reference materials.

RESULTS AND DISCUSSION

Preliminary analysis results of chemical composition

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. Among them, flavonoids are the most reactive components of *C. trifolia* species.

Investigation results of antioxidant activity by DPPH *in vitro* test

Investigation of antioxidant activity of extracts 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 high at a concentration of 1 mg/mL. Calculate the average antioxidant activity (%) of each high. Then, select the high with the highest antioxidant activity (%), proceed to build a logarithmic curve and calculate IC_{50} at 5 different concentrations. Compare the results with the IC_{50} of the sample with the vitamin C control.

Stem of C. trifolia

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*-hexan extract 1000 μg/mL	0.053	92.44
5	*n*-hexan 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

No	Ingredient name	Abs medium	anti-oxidation (%)
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

Based on the results, 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 anti-inflammatory activity. Oxidation > 50%, should continue to investigate antioxidant activity to find IC_{50}.

Leaves of C. trifolia

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*-hexan extract 1000 μg/mL	0.794	11.48
5	*n*-hexan 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

No	Ingredient name	Abs medium	anti-oxidation (%)
13	Water extract 2000 μg/mL	1.113	-24.08

Based on the results, 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}.

Roots of C. trifolia

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*-hexan extract 1000 μg/mL	0.056	92.74
5	*n*-hexan 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

Based on the results, all the extracts had antioxidant activity > 50%, it is advisable to continue investigating the antioxidant activity to look for IC_{50}.

Fruit of C. trifolia

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*-hexan extract 1000 μg/mL	0.348	61.04
5	*n*-hexan 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

Based on the results, 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 extract 1000 µg/mL (79.07%), EA extract 2000 µg/mL (92.39%), *n*-butanol extract 1000 µg/mL (63.28%) has antioxidant activity > 50%, should continue to investigate antioxidant activity to find IC_{50}.

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:

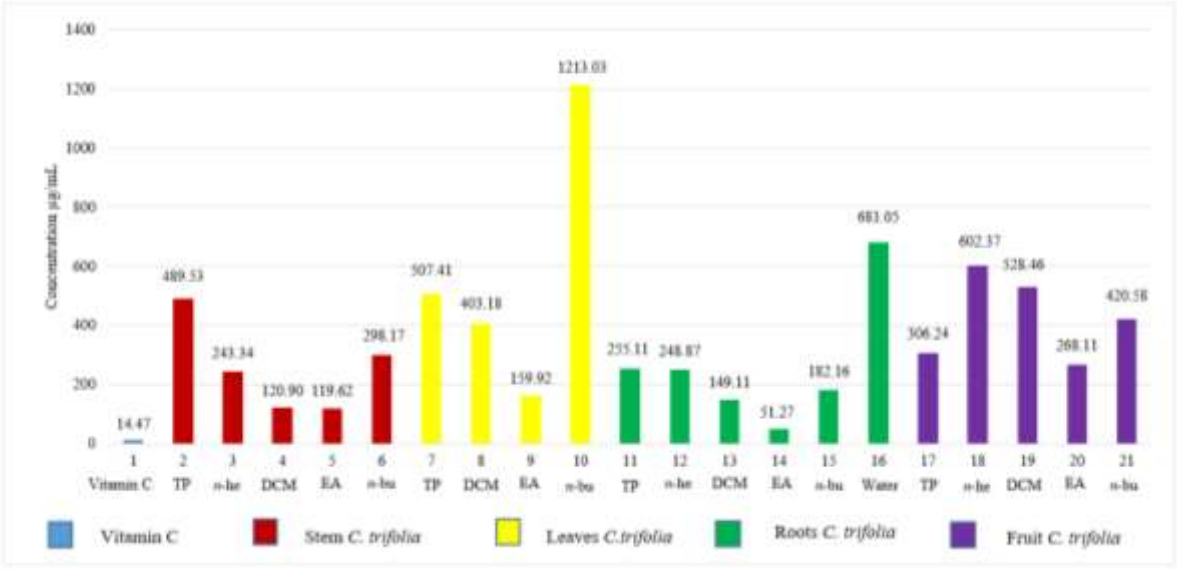

Figure 1. Graph showing antioxidant activity of tested extracts by division *C. trifolia*

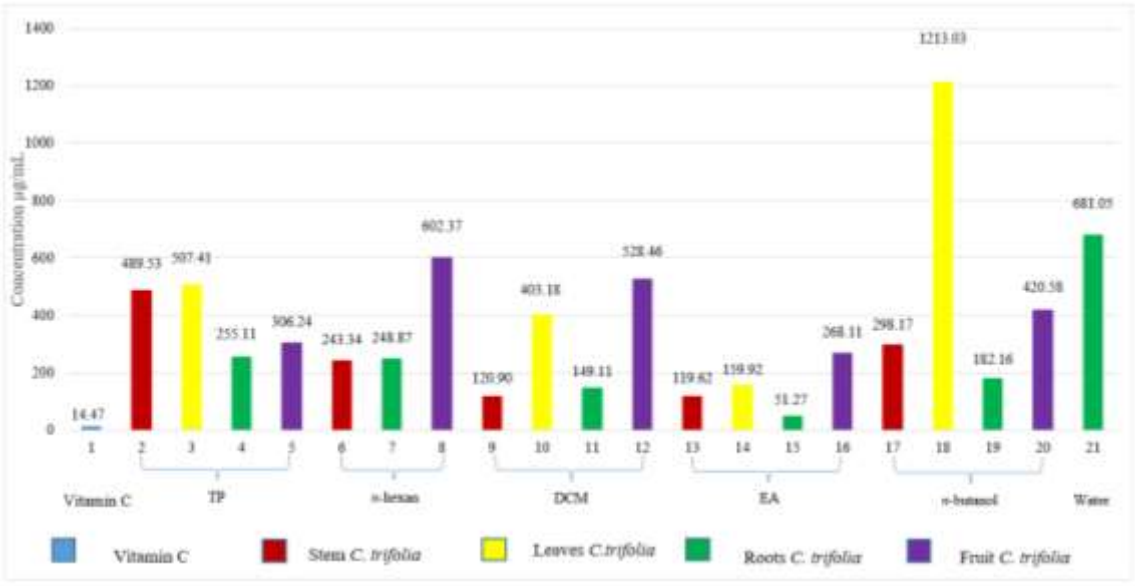

Figure 2. Graph showing the antioxidant activity of the tested extracts by fractions between *C. trifolia* parts

The results of antioxidant activity screening showed that high ethyl acetate of roots *C. trifolia* had the strongest antioxidant effect, with IC_{50} = 51.27 µg/mL, followed by high 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 2014, Kavi Gour and Vidya Patni studied the antioxidant capacity of methanol, ethanol, petroleum ether extracts by DPPH free radical scavenging method with IC_{50} values of 43,396 ± 0.52 respectively; 52.38 ± 0.36; 116.82 ± 0.12 µg/mL compared with the IC_{50} of the vitamin C positive control was 57 ± 0.03 µg/mL; The results showed that the extracts of *C. trifolia* in methanol solvent had higher antioxidant capacity than the extracts of ethanol, petroleum ether solvents. In 2007, Homhua *et al*. investigated the antioxidant capacity of ethyl acetate and high methanol extracts of *C. trifolia* by DPPH free radical scavenging method with IC_{50} results of 10.24 µg/mL and 11.36 µg/mL respectively compared to that of *C. trifolia*. Trolox positive control was 3.2

µg/mL. The experimental results of the study showed that the high ethyl acetate of roots *C. trifolia* had a high antioxidant effect when compared with ethanol extract and petroleum extract, with IC_{50} = 51.27 µg/mL. *C. trifolia* is a wild plant species that often grows widely in Vietnam in general and the Mekong Delta in particular, with no high economic value, but has the potential to become potential medicinal plants for humans. People have used as well as some studies on *C. trifolia* with antioxidant, antibacterial, cancer prevention, etc. on the total extracts of *C. trifolia*. Some foreign journals also reported that *C. trifolia* has a high antioxidant capacity, possibly due to the presence of two main compounds, alkaloids and flavonoids (Perumall et al, 2012).

Results of isolation and structure determination

The study selected the ethyl acetate fraction of roots *C. trifolia* with the strongest 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 - CHCl3 (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. Continue to purify this fraction by column chromatography on RP18 with solvent system MeOH - H_2O (30:70) to obtain compound 2 (20 mg). The EA8 fraction (3190 mg) was further investigated to isolate compounds. Perform Sephadex LH-20 gel chromatography with MeOH solvent for the EA8 fraction (3190 mg) to obtain 4 EA8.1-EA8.4 fractions. Carry out normal phase silica gel column chromatography EA8.3 (190 mg) with solvent $CHCl_3$-EtOAc-Me_2CO-H_2O (7:10:10:0.05, v/v) to obtain 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 that compound 1 had an absorption maximum at 281 nm. ^{1}H-NMR spectra for tip 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 (Nguyen *et al.*, 2011), compound 1 was identified as 5-hydroxymethylfurfural, this compound has been reported to be present in a number of other medicinal herbs and can be found in other medicinal plants. In terms of antioxidant activity, it also has potential for practical applications in the fields of food, cosmetics and pharmaceuticals (Zhao *et al*., 2013), (Li *et al*., 2009).) has the following structural formula:

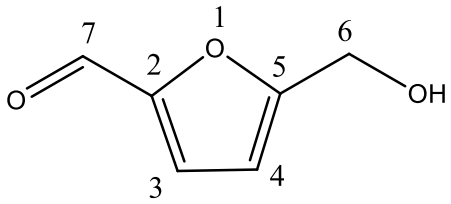

Figure 3. Structural formula of 5-hydroxymethylfurfural

Compound 2: Light yellow powder, easily soluble in acetone. HR-ESI-MS spectrum for molecular ion tip [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, ^{13}C-NMR spectral data, combined with HMBC, HSQC spectra and compared with the literature (Nguyen *et al*., 2021), there are similarities. Therefore, the structure of compound 2 identified as liquiritigenin has the following structural formula:

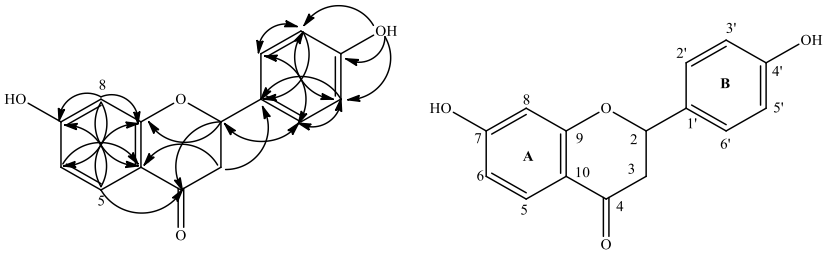

Figure 4. Structure of liquiritigenin

Compound 3: Compound 3 was obtained as a pale yellow powder, easily soluble in acetone. The HR-ESI-MS spectrum gives the molecular ion tip [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). 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, HSQC spectra and comparison with the literature (Do *et al*., 2021), there are similarities. Therefore, compound 3 was identified as kaempferol-3-O-rhamnoside. This is a flavonoid compound that has been shown to have antioxidant activity (Akter *et al*., 2022) with the following structure:

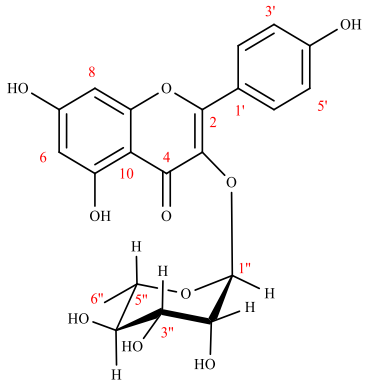

Figure 5. Structure of kaempferol-3-*O*-rhamnosid

CONCLUSION

Regarding the antioxidant activity of total and fractionated extracts, ethyl acetate extract had the highest antioxidant activity. In which high ethyl acetate of roots *C. trifolia* had the strongest antioxidant effect, with IC_{50} = 51.27 µg/mL, followed by high ethyl acetate of stem *C. trifolia*, with IC_{50} = 119.62 µg/mL, followed by high ethyl acetate of leaves *C. trifolia*, with IC_{50} = 159.92 µg/mL and finally high ethyl acetate of fruit *C. trifolia*, with IC_{50} = 268.11 µg/mL. From the ethyl acetate fraction of roots *C. trifolia* grown in Can Tho with the strongest antioxidant effect (IC_{50} = 51.27 µg/mL) carried out column chromatography, we isolated and identified the structure 3 compounds are: 5-hydroxymethylfurfural (1), liquiritigenin (2), kaempferol-3-O-rhamnoside (3). In which compounds (1) and (2) were found for the first time in roots *C. trifolia* compared with previous domestic and foreign studies.

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