BIODIVERSITAS
Volume 24, Number 7, July 2023
Pages: 3665-3672

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240703

Chemical constituents and antioxidant activity of Britton's wild petunia (*Ruellia brittoniana*) flower

GRACIA LASMA ROHANA SIANTURI[1], ELYNA WAHYU TRISNAWATI[1], MAMORU KOKETSU[2], VENTY SURYANTI[1,♥]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel/fax.: +62-271-669376, ♥email: venty@mipa.uns.ac.id
[2]Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University. 1-1 Yanagido, Gifu 501-1193, Japan

Manuscript received: 20 April 2023. Revision accepted: 3 July 2023.

Abstract. *Sianturi GLR, Trisnawati EW, Koketsu M, Suryanti V. 2023. Chemical constituents and antioxidant activity of Britton's wild petunia (*Ruellia brittoniana*) flower. Biodiversitas 24: 3665-3672.* Britton's wild petunia or *kencana ungu* (*Ruellia brittoniana)*, which has purple flowers, is widely used as a decorative plant. Isolation and structure determination of the antioxidant compound of *R. brittoniana* flower were carried out. Extraction was carried out by maceration, and compound isolation was conducted by column chromatography. Structure elucidation was accomplished by FTIR, NMR, and MS Spectrometry. Apigenin (4',5,7-trihydroxyflavone) was isolated from the B1 fraction using chloroform: methanol (10: 1) eluent. Fraction A1, obtained from elution by chloroform: methanol (10: 0), consisted of 24 compounds. Two compounds were identified as 1-hexadecanol and 1-phenyl ethanone, which have antioxidant properties of fraction A1. Fraction B2 was eluted by chloroform: methanol (10:1), consisting of 20 compounds. A compound was identified as 2-methoxy phenol, contributing to the antioxidant properties of fraction B2. The total phenolic content of *R. brittoniana* flower extract was 1.033 mg GAE/g, and its total anthocyanin content was 16.97%. Fractions A1 and B2 possessed strong antioxidant activities due to their phenolic compounds. Apigenin has three -OH groups in the 4', 5, and 7 positions; only the -OH group at the 4' position contributes to its antioxidant activity. Therefore, apigenin has moderate antioxidant activity by DPPH assay. The modification structure of apigenin needs further study to enhance its antioxidant activity. This research shows that *R. brittoniana* flower can be a source of apigenin, also known to have anticancer properties.

Keywords: Antioxidant activity, *kencana ungu*, *Ruellia brittoniana*, total anthocyanins content, total phenolics content

INTRODUCTION

Acanthaceae is a dicotyledonous flowering plant with over 250 genera and 2500 species (Matos et al. 2022). Some are epiphytes, while others are herbs, shrubs, or twining vines. The Acanthaceae family is known for its diverse range of tropical and subtropical habitats. Several species are found in temperate areas (Khan et al. 2017). Several species are widespread in Indonesia, Malaysia, Africa, Brazil, and Central America (Afzal et al. 2015; Khan et al. 2017; Matos et al. 2022). Some of these are plants with medicinal uses. The Acanthaceae family has been extensively utilized in Indonesian traditional medicine. The genus has long been recommended to treat diseases such as diabetes, high blood pressure, dermatitis, asthma, fever, and bronchitis. It contains numerous secondary metabolites with many therapeutic utilities, such as flavonoids, alkaloids, terpenoids, tannins, phenols, saponins, and quinones (Khan et al. 2017). Further study is required to identify the active compounds in this genus responsible for their bioactivities to introduce them to the commercial health market and offer the community their potential benefits.

Ruellia is a genus in the Acanthaceae family as a perennial and decorative plant. Many species of this genus contain essential constituents such as flavonoids, alkaloids, triterpenoids, and glycosides. *Ruellia* plant extracts exhibit antioxidant, anti-hypertensive, analgesic, anti-inflammatory, antidiabetic, and antipyretic properties (Khan et al. 2017). *Ruellia* species are traditionally used to treat influenza, fever, and inflammation. The leaves of the *R. prostrata* plant have been widely used in treating rheumatism, eczema, and other skin diseases. *Ruellia asperula* is commonly used to treat bronchitis, asthma, influenza, fever, and inflammation of the uterus. *Ruellia hygrophila* has antispasmodic analgesic activity (Afzal et al. 2015). *Ruellia tuberosa* leaves are regularly used to treat gonorrhea, ear disease, skin diseases and to heal burns. The dried roots of the *R. tuberosa* were used to treat eye and bladder diseases (Chothani et al. 2010). *R. tuberosa* has antimicrobial activity, antioxidant activity, anticancer and anti-inflammatory (Chothani et al. 2010; Arirudran et al. 2011).

Britton's wild petunia (*Ruellia brittoniana)*, also known as *kencana ungu* (Indonesian), is a herbaceous ornamental perennial herb with a flowering period from April to November. It greatly tolerates dry and harsh conditions (Figure 1) (Khan et al. 2017). The purple blossom of *R. brittoniana* contains anthocyanins (Le et al. 2019). Anthocyanins are natural water-soluble flavonoid pigments abundantly found in the vacuoles of plants' stems, flowers, fruits, and leaves (Tan et al. 2022). It has various colors of purple, red, violet, pink, and blue (Mohammed and Khan 2022). Anthocyanin has antioxidant activity due to

phenolate groups in its structure (Suryanti et al. 2020). *R. brittoniana* shows various biological activities such as anticancer, anti-microbes, anti-inflammatory, and antioxidant activities (Afzal et al. 2015; Ahmad 2017). *R. brittoniana* contains 5,2,3-trihydroxy 7-O-glucoflavone; 5,7,4-trimethoxy 3-O-Rhamnoflavone; and 2,2,4,6 -tetrahydroxy-calcone (Khan et al. 2017). Several flavonoids, such as 2-O-α-D-galactopyranosil glycerol hexaacetate; 5,2′,3′-trihydroxy-7-O-glucoflavone; 5,7,4′- trimethoxy-3-O-ramnopyranoside; and 2,2′,4′,6′-tetrahydroxy-chalcone, have been isolated from the whole plant of *R. brittoniana* (Elgindi et al. 2015).

Antioxidant compounds are natural or synthetic compounds that inhibit or delay oxidation at relatively low concentrations. Antioxidants interact with free radicals before damaging host cells (Abeyrathne et al. 2022). Antioxidant compounds in plants are β-carotene, ascorbic acid, alkaloids, saponins and tannins, and phenolics, such as flavonoids, cinnamic acid derivates, coumarin, and tocopherols (Suryanti et al. 2016; Suryanti et al. 2021; Suryanti et al. 2022). Phenolic compounds have a wide range of structural, functional, and biological properties (Abeyrathne et al. 2022). Phenolate compounds can scavenge free radical compounds and inhibit singlet oxygen due to electron-donating groups such as the hydroxyl group. Modification structure of secondary metabolites is often carried out to enhance their antioxidant activity (Suryanti et al. 2018). Studies have analyzed the phytochemical, antioxidant properties, and anticancer activity of the *R. brittoniana* flower (Tejaputri et al. 2019; Tejaputri et al. 2020). Meanwhile, isolation and structure identification of the *R. brittoniana* flower has never been carried out. This paper discusses the isolation and structure determination of antioxidant active compounds of the *R. brittoniana* flower.

MATERIALS AND METHODS

Study area

R. brittoniana flowers were collected from the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia. This study was conducted at the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia, and the Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, Japan.

Extraction of *R. brittoniana* flowers

The *R. brittoniana* flowers were macerated using methanol and evaporated to obtain methanol extract. The methanol extract was dissolved in aquadest (250 mL) and transferred into a separating funnel. Hexane (250 mL) was added to the solution to obtain the hexane and water fractions. The water fraction was then re-fractioned using ethyl acetate. The ethyl acetate fraction was evaporated to obtain ethyl acetate extract, and further purification of ethyl acetate extract was conducted by column chromatography.

Figure 1. *Ruellia brittoniana* flower

Column chromatography of ethyl acetate extract

The Friko 24/40 column was filled with 350 g of silica gel slurry. Sea sand (15 g) was placed on top of the silica gel. Ethyl acetate extract (8 g) was put on the packed column. Gradient polarity of solvent was introduced using methanol, chloroform, and acetone with the composition as follows, methanol: chloroform (10: 1), (5: 1), (5: 2) and acetone (10:0). Eluted fractions were analyzed by thin layer chromatography (TLC). The eluted fractions with similar R_f values were collected and evaporated. NMR Spectroscopy analyzed the single compound, while GC-MS Spectroscopy analyzed the fractions.

Total phenolics content (TPC) determination

TPC of flower extract was determined by the Folin-Ciocalteu method (Tian et al. 2021). One mL of methanol extract (10 ppm) in aquadest was put into a glass vial. Folin-Ciocalteu reagent (0.5 mL) was added and stirred for 1 min. 7.5% of Na_2CO_3 solution was added and stirred again for 1 min. The mixture was incubated at the maximum time, and the UV-Vis spectrophotometer measured its absorbance. The same procedure was used to examine gallic acid at concentrations of 2, 4, 6, 8, and 10 ppm for standard curves of total phenolics content determination. Total phenolic was calculated using equation (1), where C is the concentration of gallic acid established from the calibration curve (mg/mL), V is the volume of extract (mL), and m is the weight of the plant extract (g). TPC was expressed as mg Gallic Acid Equivalent (GAE)/g sample.

$$TPC = \frac{C \times V}{m} \quad (1)$$

Total anthocyanins content (TAC) determination

The pH differential method was used to determine the TAC of flower extract (Jaafar et al. 2020). TAC was expressed as cyanidin-3-glucoside. Flower extract (0.05 g) was added with 4 mL of KCl buffer solution pH = 1, and the mixture was then incubated for 2 h, followed by centrifugation at 150 rpm for 1 min. The supernatant was measured with a UV-Vis spectrophotometer at 520 and 700 nm. The same procedures were conducted for CH_3COONa buffer solution pH = 4.5. Absorbance data was introduced into equation (2), where A_{520} is the absorbance at 520 nm, and A_{700} is the absorbance at 700 nm.

$$A = (A_{520}-A_{700})_{pH1} - (A_{520}-A_{700})_{pH4.5} \quad (2)$$

TAC value was determined by equation (3), where A is the absorbance from eq. (2), Ɛ is 30175 M, l is the path length (cm), Mw is the molecular weight of cyanidin-3-glucoside (611 g/mol), DF is the dilution factors, V is the volume (mL), W is the weight of samples (g).

$$TAC = \frac{A}{\varepsilon \times l} \times Mw \times DF \times \frac{V}{W} \times 100\ \% \quad (3)$$

Antioxidant activities assay

Antioxidant activity of the sample was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Sethi et al. 2020). The sample was diluted with methanol to obtain 5, 10, 15, 20, and 25 ppm concentrations. Each sample was added 1 mL of DPPH 0.2 mM solution and then incubated for 30 mins before being measured with the UV-Vis spectrophotometer at the maximum wavelength determined by measuring DPPH 100 ppm solution at 800-400 nm. The same procedure was conducted for Vitamin E as a positive control. Antioxidant activities were determined by measuring % inhibition using equation (4), where An is the negative control's absorbance, and As is the samples' absorbance. The IC_{50} was calculated by interpolation and plotting the correlation between each concentration and its scavenging percentage. The effective concentration of each extract, which scavenges 50% of DPPH radicals, was used to measure the antioxidant activity, which was expressed as the IC_{50} value.

$$\% \text{ inhibition} = \frac{(An-As)}{An} \times 100\% \quad (4)$$

Data analysis

The single spot eluted fraction on the TLC plate was recognized as a pure compound. FTIR, NMR, and MS Spectrometry were used to identify the single spot. MS analysis was conducted using electron impact ionization and 50 scans. The multiple spots eluted fractions were subjected to GC-MS analysis with column type Rtx 5 MS 30 m in length with helium gas as the carrier. The oven temperature was 70°C, and the injection temperature was 300°C with split-less mode injection at 13.7 kPa. Total phenolics, total anthocyanins, and antioxidant activity of samples were determined.

RESULTS AND DISCUSSION

Chemical compounds determination

The Folin-Ciocalteu technique was used to determine the total phenolic content (TPC) of ethyl acetate extract (Çayan et al. 2022). The TPC of ethyl acetate extract was 3.84 mg GAE/g. Phenolic compounds possess a chemical structure comprising an aromatic ring with one or more hydroxyl substituents. The main groups of phenolic compounds are flavonoids, phenolic acids, tannins, stilbenes, and lignans (Machmudah et al. 2017). Phenolic content is responsible for bioactivity; therefore, this extract was expected to exhibit good antioxidant activity. Ethyl acetate extract of *R. brittoniana* flower was originally purple colored and turned into light pink or pink when reacted with CH_3COONa and KCl buffer solution.

There are three types of anthocyanin structures: cyanidin, pelargonidin, and delphinidin for dark red/pink, bright red/orange, and blue/violet/purple, respectively (Inggrid et al. 2016). These types of anthocyanin structures can be distinguished from the number of hydroxyl groups attached to the parent compound. The number of -OH groups in anthocyanins indicates their potential as antioxidants (Le et al. 2019). The distinctive purple color of the *R. brittoniana* flower indicates the presence of anthocyanin pigments. The total anthocyanins content (TAC) of ethyl acetate extract was 16.97%. The purple color of the *R. brittoniana* flower indicates that the extracted anthocyanins were delphinidin. It is the most polar anthocyanin, with three phenolic hydroxy groups in the B ring in the molecular structure. The ortho-dihydroxyphenyl structure at the B-ring appears essential for its bioactivity properties. Delphinidin has an inhibitory effect on the growth of human vulva carcinoma cell line A431 in vitro, uterine carcinoma (HeLa S3 cells), and colon adenocarcinoma cells (CaCo-2 cells) (Jing and Giusti 2010).

Column chromatographic of ethyl acetate extracts resulted in 8 fractions, i.e., fractions A1, A2, B1, B2, C1, C2, C3, and D1. The weight of the B1 fraction was 0.05 g of yellow powders and showed a single spot-on TLC, and FT-IR, NMR, and MS Spectrophotometry determined its structure. The weight of the green powder of the A1 and B2 fractions were 0.12 and 0.03 g, respectively. TLC plates of both fractions showed many spots on TLC; therefore, they were subjected to GC-MS analysis. Since other fractions were obtained very small amounts, no further analyses were subjected to GC-MS analysis.

FT-IR spectrum of B1 fraction showed the broad peak of the hydroxyl group at 3500-3300 cm^{-1} (Figure 2). Peaks of aromatic were found at 3030-3150 cm^{-1} for CH (stretching), 1466 cm^{-1} for C=C (stretching) and 1605 cm^{-1} for C=C (bending). The carbonyl group peak was found at 1735 $cm^{-1,}$ and the C-O group of ester peak was found at 1297 cm^{-1} (Peng et al. 2016; Shoubaky et al. 2016).

The B1 fraction was dissolved in DMSO-d_6 solvent for ^1H and ^{13}C NMR Spectroscopy analyses (Table 1 and 2). ^1H NMR of B1 fraction showed 8 peaks with 10 protons in total. The peak of the -OH group as a chelate was found at 12.96 ppm. Two other broad and low-intensity peaks of the -OH group were found at 10.79 and 10.3 ppm. Aromatic peaks of -CH were found at 6.1-8.5 ppm. Three multiplet peaks were found at 7.2-7.5 ppm, which were characteristics of meta disubstituted aromatic. Two doublet peaks were found at 6.48 and 6.16 ppm, characteristic of para disubstituted aromatic (Peng et al. 2016). ^{13}C NMR of the B1 fraction showed 13 peaks with 15 protons in total. A characteristic peak of the carbonyl group of esters was found at 182.28 ppm. Characteristic peaks of quaternary carbon and C-O groups were found at 164.70, 164.31, 162.00, 161.74, and 157.86 ppm. Two peaks at 129.14 and 116.55 ppm showed the integration of two carbon atoms.

Three aromatic CH group peaks were found at 104.33, 100.02, and 94.61 ppm (Peng et al. 2016).

Two-dimensional NMR, including COSY, HMQC, and HMBC, were performed to determine the correlation between ^{1}H NMR and ^{1}H NMR, ^{1}H NMR, and ^{13}C NMR (Table 3). B1 fraction was identified as Apigenin or 4',5,7-trihydroxyflavone (Figure 3). Two-dimensional NMR correlation of apigenin is shown in Figure 4. MS analysis of the B1 fraction was performed using EI ionization (Figure 5), which showed base peak of apigenin in the area of m/z 270.0366 (Shoubaky et al. 2016; Dou et al. 2020). The MS data confirmed that apigenin was the isolated compound. Apigenin is a bioflavonoid compound (specifically a flavone) that is found in a wide variety of plants and herbs. Surprisingly, apigenin has never been isolated from any genus of the Acanthaceae family. Apigenin from *Tripleurospermum disciforme* has been isolated with a similar extraction process and similar characteristics of NMR data (Tofighi et al. 2015). This result indicated that *R. brittoniana* flower could be a source of apigenin. Apigenin-rich foods are chamomile, celery, parsley, vine spinach, artichokes, and oregano (Shankar et al. 2017).

GC-MS chromatogram of A1 fraction showed 24 peaks (Figure 6), and two compounds were identified as 1-hexadecanol and 1-phenyl ethenone or acetophenone (Figure 7). The 1-hexadecanol has a hydroxyl group and the 1-phenyl ethenone has a conjugate double bond that contributes to antioxidant activities (Chen et al. 2020; Santos and Silva 2020). The 1-hexadecanol had 0.41% of the total percent area with a retention time (RT) of 50.142 mins. The 1-phenyl ethenone had 0.45% of the total percent area with an RT of 12.849 mins.

GC-MS analysis of the B2 fraction showed 20 peaks (Figure 8). One compound was successfully identified as 2-methoxy phenol, which had a conjugate double bond and a hydroxyl group (Figure 9). The 2-methoxy phenol had 2.62% of percent total area with RT of 13.501 mins.

Antioxidant activity

The antioxidant activity (IC_{50}) of samples was measured by the DPPH method (Table 4). Antioxidants scavenge DPPH radicals by donating hydrogen atoms resulting in a yellow-colored non-radical state (Akter et al. 2019). A decrease in absorbance and color change of the sample solution from purple to yellow confirmed that the sample has antioxidant activity. All samples showed very strong antioxidant activity except for apigenin which showed moderate antioxidant activity. Ethyl acetate had the highest antioxidant activity, followed by B2 and A1 fractions. Ethyl acetate extract, fractions A1 and B2 of *R. brittoniana* flower extract, possessed very strong antioxidant activities due to the synergic work of compounds in enhancing their antioxidant activity.

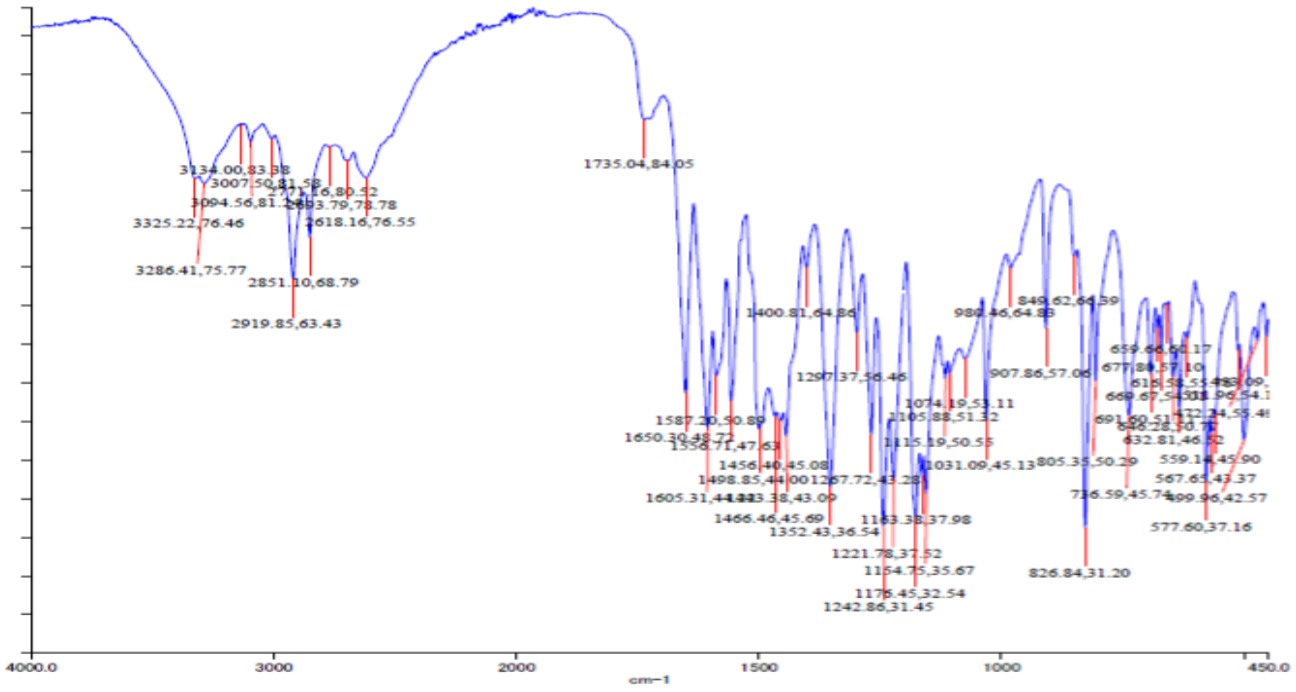

Figure 2. FTIR spectrum of B1 fraction

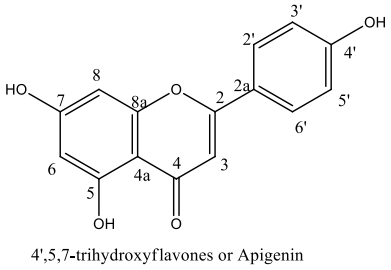

Figure 3. Structure of Apigenin

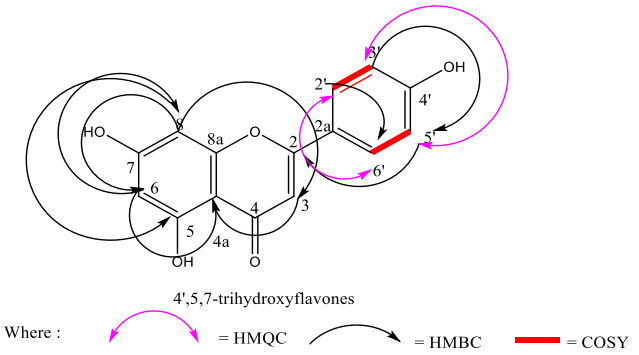

Figure 4. The two-dimensional NMR correlation of apigenin

TPC and TAC of ethyl acetate extract showed that the extract possessed phenolic and anthocyanin compounds, which play significant roles in antioxidant activities (Sethi et al. 2020). Ethyl acetate extract contains many active compounds contributing to the high antioxidant activity value. The A1 and B2 fraction contained 24 and 20 compounds, respectively, contributing to their antioxidant activities. Antioxidant activity was enhanced with the presence of functional groups, such as -OH, heterocyclic, conjugated double bond, and electron donating group, which attached in the ortho or para position toward phenolic compounds (Lee et al. 2015; Akter et al. 2019). Functional groups of compounds that enhanced antioxidant activities were found in both fractions. The 1-hexadecanol has a hydroxyl group, and the 1-phenyl ethenone has a conjugate double bond contributing to antioxidant activities (Chen et al. 2020; Santos and Silva 2020). The 2-methoxy phenol has a conjugate double bond and a hydroxyl group.

Table 1. ^{1}H NMR data of B1 fraction

δ (ppm)	Multiplicity, integration, position, and J coupling (Hz)	Proton type
12.96	OH5	Chelator OH group
10.79	OH4’	OH
10.3	OH7	OH
7.91	d, 2H, H3' and H5‘, J = 8 Hz	Aromatic CH
6.91	d, 2H, H2' and H6‘, J = 8 Hz	Aromatic CH
6.74	s, 1H, H3	Aromatic CH
6.48	d, 1H, H6, J = 2 Hz	Aromatic CH
6.16	d, 1H, H8, J = 2 Hz	Aromatic CH

Table 2. ^{13}C NMR data of B1 fraction

δ (ppm)	Multiplicity, integration, position	Carbon type
182.28	s, C4	Carbonyl
164.7	s, C7	C-O
164.31	s, C2	Aromatic C
162	s, C5	C-O
161.74	s, C8a	Aromatic C
157.86	s, C4'	C-O
129.14	s, 2C, C3' and C5'	Aromatic CH
121.72	s, C2a	Aromatic C
116.55	s, 2C, C2' and C6'	Aromatic CH
104.33	s, C3	Aromatic CH
103.36	s, C4a	Aromatic C
100.02	s, C8	Aromatic CH
94.61	s, C6	Aromatic CH

Table 3. Table of correlation between ^{1}H NMR and ^{1}H NMR, ^{1}H NMR, and ^{13}C NMR based on COSY, HMQC, and HMBC NMR

COSY NMR		HMQC NMR		HMBC NMR	
δ H (ppm)	δ H (ppm)	δ H (ppm)	δ C (ppm)	δ H (ppm)	δ C (ppm)
7.87	6.9	7.91	129.14	7.91	129.14
		6.91	116.55		164.31
		6.74	104.33	6.91	116.55
		6.48	94.61	6.74	103.36
		6.16	100.02	6.48	100.02
				6.16	103.36
					94.61
					104.33
					162.00

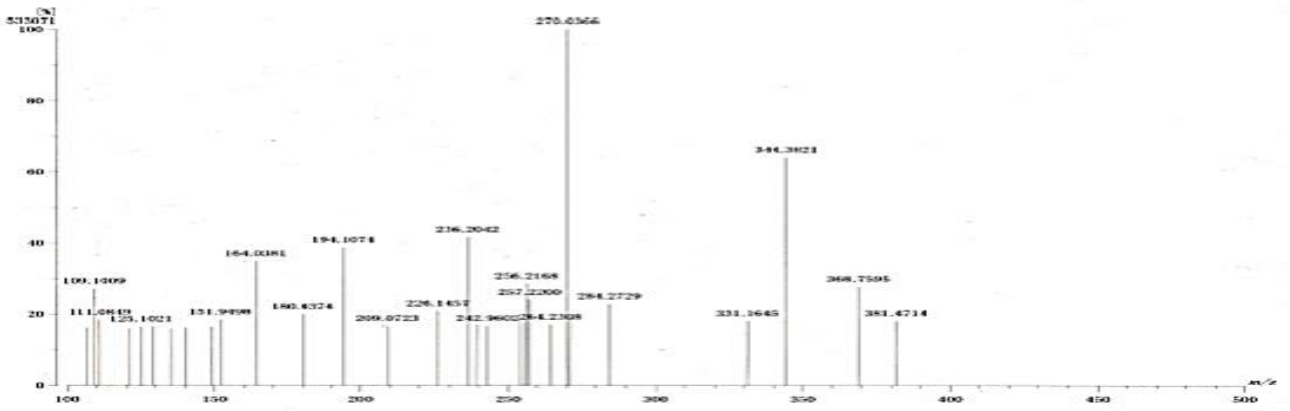

Figure 5. MS spectrum of B1 fraction

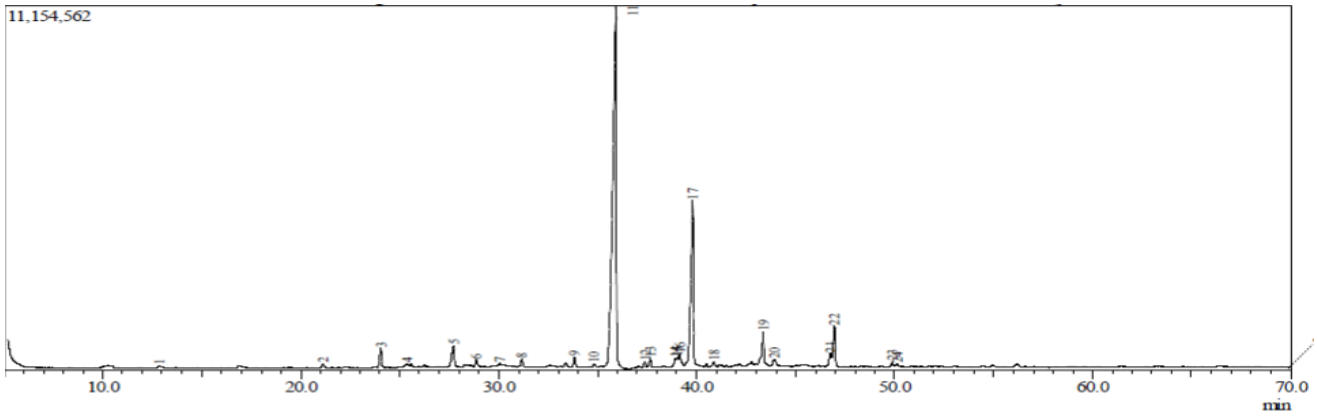

Figure 6. GC-MS chromatogram of A1 fraction

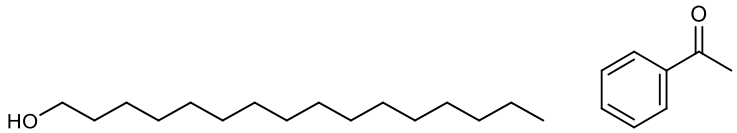

Figure 7. Structure of 1-hexadecanol (left) and 1-phenyl ethenone (right)

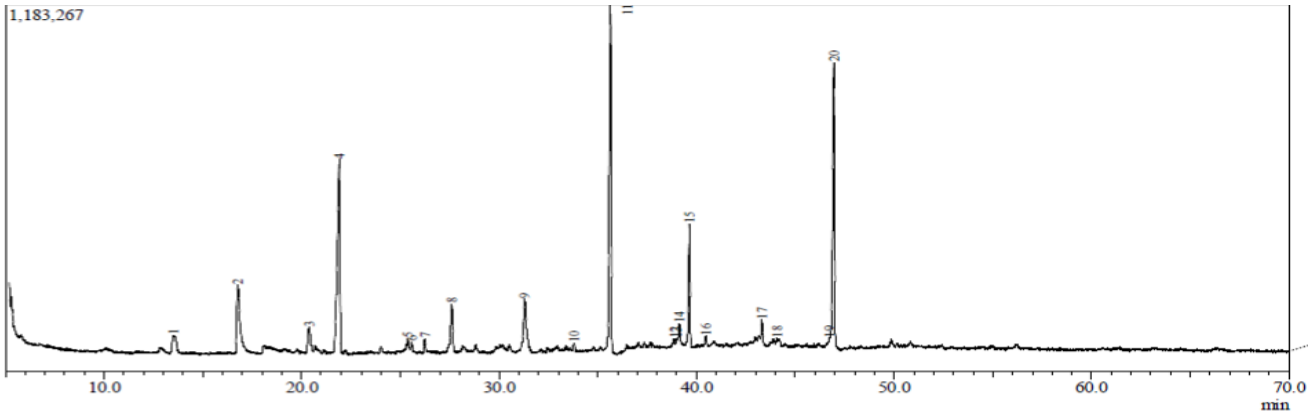

Figure 8. GC-MS chromatogram of B2 fraction

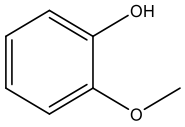

Figure 9. Structure of 2-methoxy phenol

Table 5. IC_{50} value of antioxidant activities of samples and positive control

Sample	IC_{50} (ppm)	Category
Ethyl acetate extract	16.62	Very strong
A1 fraction	22.28	Very strong
B2 fraction	20.12	Very strong
Apigenin	109.24	Moderate
Vitamin E	11.50	Very strong

Apigenin possessed moderate antioxidant activity with an IC_{50} value is 109.24 ppm. A previous study by Seyoum et al. (2006) showed that apigenin also had moderate antioxidant activity. The -OH groups of 3' and 4' positions in the B ring of flavonoids enhance its antioxidant activity. Apigenin has three -OH groups in the 4', 5, and 7 positions. However, only the -OH group at the 4' position contributes to apigenin antioxidant activity. The bond dissociation enthalpy of C4'-OH is most negligible than C7-OH and C5-OH; hence the hydrogen atom transfer activity of C4'-OH was strongest in scavenging free radicals DPPH. The -OH groups at positions 5 and 7 do not support much antioxidant activity because they form intramolecular hydrogen bonds with adjacent carbonyl (Chen et al. 2022). Therefore, apigenin only possessed moderate antioxidant activity despite three -OH groups in its structure. Apigenin is a type of flavonoid with good medicinal properties. It is abundant in various fruits, vegetables, and medicinal

plants. Apigenin has been utilized as a dietary supplement due to its anticancer properties and anti-inflammatory and antioxidation activities. Apigenin exhibits anticancer activity with low cytotoxicity and no mutagenic activity in numerous human cancer cells, such as breast cancer, prostate cancer, and colon carcinoma (Liu et al. 2013). Modifications of apigenin have been made as the lead compound in anticancer drugs. Structural-activity relationship (SAR) studies indicate that the A and C rings of Apigenin are suitable for various modifications (Chen et al. 2023).

In conclusion, isolated compound from *R. brittoniana* flower (B1 fraction) was identified as apigenin (4',5,7-trihydroxyflavone). Apigenin has never been isolated from any genus of the Acanthaceae family. Fraction A1 consisted of 24 compounds, with two compounds identified as 1-hexadecanol and 1-phenyl ethanone. Fraction B2 consisted of 20 compounds, and a compound was identified as 2-methoxy phenol. The flower of *R. brittoniana* extract had total phenolic content of 1.033 mg GAE/g and total anthocyanin content of 16.97%. Ethyl acetate extract possessed strong antioxidant activity, with an IC_{50} value of 16.62 ppm. Fraction A1 and B2 possessed strong antioxidant activity, with IC_{50} values were 22.28 and 20.12 ppm, respectively; meanwhile, apigenin possessed moderate antioxidant activity, with an IC_{50} value of 109.24 ppm.

ACKNOWLEDGEMENTS

The author would like to thank Universitas Sebelas Maret, Surakarta, Indonesia for financial support through research number 228/UN27.22/PT.01.03/2023.

REFERENCES

Abeyrathne ED, Nam K, Huang X, Ahn DU. 2022. Plant- and animal-based antioxidant's structure, efficacy, mechanisms and applications: A review. Antioxidants 11 (5): 1025. DOI: 10.3390/antiox11051025.

Afzal K, Uzair M, Chaudhary BA, Ahmad A, Afzal S, Saadulah M. 2015. Genus *Ruellia* pharmacological, phytochemical importance in ethnopharmacology. Acta Pol Pharm Res 72 (5): 821-827.

Ahmad AR, Elya B, Mun'im A. 2017. Antioxidant activity and isolation of xanthine oxidase inhibitor from *Ruellia tuberosa* L. leaves. Pharmacogn J 9 (5): 607-610. DOI: 10.5530/pj.2017.5.96.

Akter J, Hossain MA, Takara K, Islam MZ, Hou DX. 2019. Antioxidant activity of different species and varieties of turmeric (*Curcuma* spp.): Isolation of active compounds. Comp Biochem Physiol Part C Toxicol Pharmacol 215: 9-17. DOI: 10.1016/j.cbpc.2018.09.002.

Çayan F, Tel-Çayan G, Deveci E, Duru ME, Öztürk M. 2022. Isolation and identification of compounds from truffle *Reddellomyces westraliensis* and their antioxidant, cytotoxic and enzyme inhibitory activities. Process Biochem 121: 553-562. DOI: 10.1016/j.procbio.2022.08.001.

Chen J, Yang J, Ma L, Li J, Shahzad N, Kim CK. 2020. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. Sci Rep 10 (1): 2611. DOI: 10.1038/s41598-020-59451-z.

Chen B, Su J, Hu Y, Liu S, Ouyang X, Cai R, You X, Li X. 2023. Antioxidant mechanisms and products of four 4′,5,7-trihydroxyflavonoids with different structural types. RSC Med Chem 14 (1): 173-182. DOI: 10.1039/D2MD00333C.

Dou X, Zhou Z, Ren R, Xu M. 2020. Apigenin, flavonoid component isolated from *Gentiana veitchiorum* flower suppresses the oxidative stress through LDLR-LCAT signaling pathway. Biomed Pharmacother 128: 110298. DOI: 10.1016/j.biopha.2020.110298.

Elgindi MR, Hagag EG, Mohamed SE. 2015. Phytochemical and biological studies of *Ruellia brittoniana*. Res J Pharm Biol Chem Sci 6 (2): 926-933.

Inggrid HM, Jaka, Santoso H. 2016. Natural red dyes extraction on roselle petals. IOP Conf Ser: Mater Sci Eng 162: 012029. DOI: 10.1088/1757-899X/162/1/012029.

Jaafar NF, Ramli ME, Salleh RM. 2020. Optimum extraction condition of *Clitorea ternatea* flower on antioxidant activities, total phenolic, total flavonoid and total anthocyanin contents. Trop Life Sci Res 31 (2): 1-17. DOI: 10.21315/tlsr2020.31.2.1.

Jing P, Giusti MM. 2010. Contribution of Berry Anthocyanins to Their Chemopreventive Properties. In: Stroner GD, Seeram NP (eds). Berries and Cancer Prevention. Springer, New York.

Khan I, Jan SA, Shinwari ZK, Ali M, Khan Y, Kumar T. 2017. Ethnobotany and medicinal uses of folklore medicinal plants belonging to family Acanthaceae: An updated review. MOJ Biol Med 1 (2): 34-38. DOI: 10.15406/mojbm.2017.01.00009.

Liu R, Zhang H, Yuan M, Zhou J, Tu Q, Liu JJ, Wang J. 2013. Synthesis and biological evaluation of apigenin derivatives as antibacterial and antiproliferative agents. Molecules 18 (9): 11496-11511. DOI: 10.3390/molecules180911496.

Le XT, Huynh MT, Pham TN, Than VT, Toan TQ, Bach LG, Trung NQ. 2019. Optimization of total anthocyanin content, stability and antioxidant evaluation of the anthocyanin extract from Vietnamese *Carissa carandas* L. fruits. Processes 7: 468. DOI: 10.3390/pr7070468.

Lee CY, Nanah CN, Held RA, Clark AR, Huynh UG, Maraskine MC, Uzarski RL, McCracken J, Sharma A. 2015. Effect of electron donating groups on polyphenol-based antioxidant dendrimers. Biochimie 111: 125-134. DOI: 10.1016/j.biochi.2015.02.001.

Machmudah S, Wahyudiono, Kanda H, Goto M. 2017. Hydrolysis of Biopolymers in Near-Critical and Subcritical Water. In: González HD, Muñoz JG (eds). Water Extraction of Bioactive Compounds. Elsevier. DOI: 10.1016/B978-0-12-809380-1.00003-6.

Matos P, Batista MT, Figueirinha A. 2022. A review of the ethnomedicinal uses, chemistry, and pharmacological properties of the genus *Acanthus* (Acanthaceae). J Ethnopharmol 293: 115271. DOI: 10.1016/j.jep.2022.115271.

Mohammed HA, Khan RA. 2022. Anthocyanins: Traditional uses, structural and functional variations, approaches to increase yields and product's quality, hepatoprotection, liver longevity, and commercial products. Intl J Mol Sci 23 (4): 2149. DOI: 10.3390/ijms23042149.

Peng H, Zhang X, Xu J. 2016. Apigenin-7-O-β-d-glycoside isolation from the highly copper-tolerant plant *Elsholtzia splendens*. J Zhejiang Univ Sci B 17 (6): 447-454. DOI: 10.1631/jzus.B1500242.

Shankar E, Goel A, Gupta K, Gupta S. 2017. Plant flavone apigenin: An emerging anticancer agent. Curr Pharmacol Rep 3 (6): 423-446. DOI: 10.1007/s40495-017-0113-2.

Santos CMM, Silva AMS. 2020. The antioxidant activity of Prenylflavonoids. Molecules 25: 696. DOI: 10.3390/molecules25030696.

Sethi S, Joshi A, Arora B, Bhowmik A, Sharma RR, Kumar P. 2020. Significance of FRAP, DPPH, and CUPRAC assays for antioxidant activity determination in apple fruit extracts. Eur Food Res Technol 246 (3): 591-598. DOI: 10.1007/s00217-020-03432-z.

Seyoum A, Asres K, El-Fiky FK. 2006. Structure-radical scavenging activity relationships of flavonoids. Phytochemistry 67 (18): 2058-2070. DOI: 10.1016/j.phytochem.2006.07.002.

Shoubaky GAEl, Abdel-Daim MM, Mansour MH, Salem EA. 2016. Isolation and identification of a flavone apigenin from marine red alga *Acanthophora spicifera* with antinociceptive and anti-inflammatory activities. J Exp Neurosci 10: JEN.S25096. DOI: 10.4137/JEN.S25096.

Suryanti V, Marliyana SD, Putri HE. 2016. Effect of germination on antioxidant activity, total phenolics, β-carotene, ascorbic acid and β-tocopherol contents of lead tree sprouts (*Leucaena leucocephala* (LMK.) de Wit). Intl Food Res J 23 (1): 167-172.

Suryanti V, Wibowo FR, Khotijah S, Andalucki N. 2018. Antioxidant activities of cinnamaldehyde derivatives. IOP Conf Ser: Mater Sci Eng 333: 012077 DOI: 10.1088/1757-899X/333/1/012077.

Suryanti V, Kusumaningsih T, Marliyana SD, Setyono HA, Trisnawati EW. 2020. Identification of active compounds and antioxidant activity of teak (*Tectona grandis*) leaves. Biodiversitas 21 (3): 941-947. DOI: 10.13057/biodiv/d210313.

Suryanti V, Marliyana SD, Rohana GL, Trisnawati EW, Widiyanti. 2021. Bioactive compound contents and antioxidant activity of fermented lead tree (*Leucaena Leucocephala* (Lmk.) De Wit) seeds. Molekul 16(3): 192-199. DOI: 10.20884/1.jm.2021.16.3.756

Suryanti V, Sariwati A, Sari F, Handayani DS, Risqi HD. 2022. Metabolite bioactive contents of *Parkia timoriana* (DC) Merr seed extracts in different solvent polarities. Hayati 29 (5): 681-694 DOI: 10.4308/hjb.29.5.681-694.

Tan J, Han Y, Han B, Qi X, Cai X, Ge S, Xue H. 2022. Extraction and purification of anthocyanins: A review. J Agric Food Res 8: 100306. DOI: 10.1016/j.jafr.2022.100306.

Tejaputri NA, Arsianti A, Qorina F, Fithrotunnisa Q. 2019. Phytochemical analysis and antioxidant properties by DPPH radical scavenger activity of *Ruellia brittoniana* flower. Intl J Appl Pharm 11 (6): 24-28.

Tejaputri NA, Arsianti A, Qorina F, Fithrotunnisa Q, Azizah NN, Putrianingsih R. 2020. Anticancer activity of *Ruellia britoniana* flower on cervical HeLa cancer cells. Pharmacogn J 12 (1): 29-34. DOI: 10.5530/pj.2020.12.6.

Tian W, Chen G, Gui Y, Zhang G, Li Y. 2021. Rapid quantification of total phenolics and ferulic acid in whole wheat using UV-Vis spectrophotometry. Food Control 123: 107691. DOI: 10.1016/j.foodcont.2020.107691.

Tofighi Z, Molazen M, Doostdar B, Taban P, Shahverdi AR, Samadi N, Yassa N. 2015. Antimicrobial activities of three medicinal plants and investigation of flavonoids of *Tripleurospermum disciforme*. Iran J Pharm Res 14 (1): 225-231.