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Original Research Article

Antioxidant Activity of Red Lip (*Syzygium myrtifolium* Walp.) Leaves Fractions and their Secondary Metabolites

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ABSTRACT

Antioxidant compounds can prevent or delay certain types of cell damage caused by oxidation processes involving oxidants. One of the sources of antioxidant compounds is the red lip plant (*Syzygium myrtifolium* Walp.). *S. myrtifolium* is a medicinal plant that has traditionally been used for the treatment of hypertension, facilitating labor, and alleviating abdominal pain. This research was conducted to evaluate the antioxidant activity of fractions derived from *S. myrtifolium* leaves and to identify the chemical profile of the antioxidant-active fractions. Fractionation of *S. myrtifolium* leaves was carried out through successive maceration in graded solvents. Each fraction was tested for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Quercetin exhibits very strong antioxidant activity and is used as a standard for comparison. The chemical components with strong antioxidant activity were further separated by gravity column chromatography into several subfractions. The chemical profiles of the active subfractions were identified by using 1D Nuclear Magnetic Resonance (NMR) Spectroscopy and Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LCMS/MS). This study found that ethyl acetate and methanol fractions showed strong antioxidant activity. Separation of the active fractions by gravity column chromatography yielded an active subfraction from the ethyl acetate and the methanol fractions with IC_{50} values of 99.2 µg/mL and 78.8 µg/mL, respectively. A pure white solid compound was obtained from the active ethyl acetate subfraction, which was identified by 1D NMR spectroscopy as betulinic acid. Furthermore, the active subfraction from the methanol fraction was analyzed by LCMS/MS and identified as containing bergenin, quercetin, elemicin, and asarone.

Keywords: *Syzygium myrtifolium*, Antioxidant, Secondary metabolites, Betulinic acid

Introduction

Antioxidants are compounds that prevent and repair cellular damage, especially those caused by exposure to free radicals. Free radicals can damage the DNA structure of cells, induce inflammation, weaken the immune system, and elevate bad cholesterol levels in the body. Excessive and prolonged exposure to free radicals increases the risk of various diseases, including dementia, premature aging, heart disease, high blood pressure, and cancer. Therefore, antioxidants are essential for counteracting the effects of oxidative stress caused by free radicals. Substances with antioxidant properties include polyphenols, flavonoids, lutein. beta-carotene, lycopene, zinc, anthocyanins (pigments found in vegetables and fruits), selenium, and vitamins A, C and E.[1–4]*S. myrtifolium* Walp. or often referred to as red lip is a plant species from the Myrtaceae family. The red lip plant is frequently used by the community to treat digestive ailments.[5]

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In addition to its use for stomach pain, red lip leaves are employed by Balinese Hindus to facilitate childbirth.[6] Furthermore, research indicates that red lip is a promising candidate for hypertension treatment due to the secondary metabolites it contains. The polyphenol compounds found in the *Syzygium* genus are known to exhibit antioxidant activity. [7–10] Based on the results of literature studies, the secondary metabolites in red lip leaves include flavanones, chalcones, triterpenoids, and gallic acid.[11] Moreover, previous research has identified the presence of alkaloids and tannins in red lip plants.[12] Additional studies have reported that red lip leaves contain alkaloids, flavonoids, phenolics, saponins, tannins, and triterpenoid compounds. [13–15] Furthermore, previous studies have also shown that ethyl acetate, ethanol, and methanol extracts from *S. myrtifolium* leaves exhibit significant free radical scavenging activities. At a concentration of 1000 µg/mL, these extracts can scavenge more than 92% of DPPH radicals.[16] Based on these findings, this study aims to examine secondary metabolite compounds isolated from red lip (*S. myrtifolium* Walp.), particularly from its leaves, which can act as antioxidants by isolating antioxidant compounds from red lip (*S. myrtifolium* Walp.).

Materials and Methods

Plant Collection and Identification

The samples used were fresh red lip leaves, collected from the campus of Sriwijaya University, Ogan Ilir, Indralaya, South Sumatra, Indonesia (-3.218525, 104.646329) on May 8, 2023. The plant was authenticated at the Herbarium Andalas University by Dr. Nurainas, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra with no. 536/K-ID/ANDA/VIII/2023 and deposit to Department of Chemistry, Faculty

of Mathematics and Natural Sciences, Sriwijaya University, Palembang, South Sumatera.

Fractionation
Samples of red lip leaves were dried at room temperature (25ºC) and subsequently ground into powder. A 1.4 kg sample of red lip was extracted using a multistage maceration method with n-hexane, ethyl acetate and methanol solvents, up to 5 L for each solvent for 1 × 24 hours. Three repetitions were conducted for each solvent. The filtrate from each repetition was concentrated using a rotary evaporator at 65ºC and 80 rpm to obtain the solid extract.

Antioxidant Test
DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical inhibition was used to assess *in vitro* antioxidant activity using sample concentrations of 500, 250, 125, 62.5, 31.25, 15.625 µg/mL. A volume of 0.2 mL from each concentration was added to 3.8 mL of 0.05 mM DPPH[17], and the solutions were incubated in the dark for 30 min. As a negative control, we used 4 mL of a 0.05 mM DPPH solution in methanol, whereas ascorbic acid was used as a positive control. The absorbance of the solutions was measured using an Orion AquaMate 8000 Ultraviolet-Visible (UV-Vis) spectrophotometer at 517 nm. The percentage inhibition and IC_{50} values were calculated to determine the antioxidant activity.[18]

$$\%Inhibition = \frac{A_k - A_s}{A_s} \times 100\%$$

A_k: control absorbance
A_s: sample absorbance

The samples that showed active antioxidant activity were subjected to LCMS/MS analysis.

Fractionation of Ethyl Acetate and Methanol Extracts
The most promising fractions were separated via gravity column chromatography using a silica gel G60 stationary phase (70-230 mesh). The ethyl acetate and methanol fractions were prepared and pre-absorbed onto silica gel G60 (230-400 mesh) in a mass ratio of 1:1 and then evenly distributed into the chromatography column and eluted with an eluent of increasing polarity, namely n-hexane:EtOAc (10:0 → 0:10) and EtOAc:MeOH (10:0 → 0:10). The eluate was collected at 10-ml intervals. TLC G_{60} F_{254} analysis was then performed, and the spot patterns were viewed under a UV lamp at λ = 254 nm. Eluates showing a similar pattern were combined into a single fraction. This grouping process yielded 9 subfractions (FE1-FE9) from the ethyl acetate fraction and 6 subfractions (FM1-FM6) from the methanol fraction. All subfractions from both ethyl acetate and methanol fraction were subjected to antioxidant assay to determine the active antioxidant subfraction, which was then subjected to further assay.

Isolation and Identification of Compound 1 from Ethyl Acetate Subfraction (FE8)
Subfraction FE8 from the ethyl acetate fraction displayed strong antioxidant activity and was then isolated and purified by column chromatography and eluted using an eluent of increasing polarity (n-hexane: ethyl acetate eluent (8:2 – 1:1)) to obtain pure compounds with a single spot pattern. The structure of the compound was identified using spectroscopic methods, including ^{1}H-NMR (Bruker, 700 MHz, $CDCl_3$) and ^{13}C-NMR (Bruker, 700 MHz, $CDCl_3$), and compared with the NMR data of the same compound from the reference.

*Spectroscopic data of betulinic acid (**1**)*
White solid (31 mg): ^{1}H-NMR (700 MHz, $CDCl_3$) δ 3.00 (1H, m, H-3), 2.26 (1H, m, H-13), 3.19 (1H, m, H-19), 0.93 (3H, s, H-23), 0.75 (3H, s, H-24), 0.82 (3H, s, H-25), 0.97 (3H, s, H-26), 0.96 (3H, s, H-27), 4.74 (1H, s, H-29a), 4.60 (1H, s, H-29b), 1.69 (3H, s, H-30); ^{13}C-NMR (700 MHz, $CDCl_3$) δ 38.9 (C-1), 27.6 (C-2), 79.2 (C-3), 39.0 (C-4), 55.5(C-5), 18.5 (C-6), 34.5 (C-7), 40.9 (C-8), 50.7 (C-9), 37.4 (C-10), 21.0 (C-11), 25.7 (C-12), 38.6 (C-13), 42.6 (C-14), 30.7 (C-15), 32.3 (C-16), 56.4 (C-17), 47.1 (C-18), 49.4 (C-19), 150.6 (C-20), 30.4 (C-21), 37.2 (C-22), 28.2 (C-23), 15.5 (C-24), 16.2 (C-25), 16.3 (C-26), 14.9 (C-27), 179.9 (C-28), 109.9 (C-29), 19.6 (C-30).

LCMS analysis of methanol subfraction (FM6)
Subfraction FM6 from the methanol fraction showed high antioxidant activity. The chemical content was then analyzed by LCMS using the UPLC-MS (Ultra Performance Liquid Chromatography-Mass Spectrophotometer) instrument. Unit (LC: ACQUITY UPLC® H-Class System, Waters, USA) and a mass spectrometer (Xevo G2-S QTof, Waters, USA). This
involved the use of a C18 column (1.8 µm 2.1x100 mm, ACQUITY UPLC®HSS, Waters, USA) at temperatures of 50 °C (column) and 25 °C (room). LC analysis was performed using a mobile phase consisting of water + 5 mM ammonium formate (A) and acetonitrile + 0.05% formic acid (B) at a flow rate of 0.2 ml/min (step gradient) over 23 min (see slide for mobile phase). Injection volume was 5 µl (first filtered through a 0.2 µm syringe filter). Mass spectrometry (MS) was performed using electrospray ionization (ESI) in positive mode with a mass range of 50-1200 m/z and source and desolvation temperatures of 100 °C and 350 °C, respectively. In addition, the flow of cone gas and emissions 0 L / hr and 793 L / hr. As a result, the energy of the collision has changed to 4 to 60 eV. The data was analyzed by the Masslynx 4.1 application, and the predicted molecular formula that had been selected was then searched with the help of the chemspider.com website. [19]

Results and Discussion

Dry powder of *S. myrtifolium* leaves (1.4 kg), extracted through multistage maceration using n-hexane, ethyl acetate, and methanol, yielded extracts of 18.3 g, 43.9 g, and 48.2 g, respectively.

Antioxidant Activity of Fractions from S. myrtifolium leaves
The antioxidant activity of the n-hexane, ethyl acetate, and methanol fractions, along with the antioxidant standard ascorbic acid, was tested using the DPPH assay at concentration of 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL yielding IC_{50} values as shown in Table 1.
Ethyl acetate and methanol fractions exhibited strong antioxidant activity, prompting their separation via column chromatography. The IC_{50} value for ethyl acetate and methanol fractions were 98.2 µg/mL and 93.3 µg/mL, respectively.

Table 1: Antioxidant activity (IC_{50}) of fractions from *S. myrtifolium* leaves and ascorbic acid

No.	Sample	IC_{50} (µg/mL)
1	Fraction n-hexane	482.2 **
2	Fraction ethyl acetate	98.2 ***
3	Fraction methanol	93.3 ***
4	Ascorbic acid	9.5 ****

Note: Antioxidant activity IC_{50} (μg/mL): ****: <20 μg/mL (very strong), ***: 20-100 μg/mL (strong), **: 101-500 μg/mL (moderate); *: >500 μg/mL (weak)[36]

Fractionation of Ethyl Acetate and Methanol Extracts
A total of 5 g of ethyl acetate fraction was grouped into 9 subfractions (FE1-FE9) based on thin-layer chromatography (TLC) spot patterns. Similarly, the 8 g of methanol fraction was grouped into 6 subfractions (FM1-FM6). In total of 15 subfractions from both fractions were tested for antioxidant activity, as summarized in Table 2. Subfractions FE8 and FM6 showed strong antioxidant activity with IC_{50} values of 99.2 µg/mL and 79.8 µg/mL, respectively. Furthermore, FE8 was subjected to 1D NMR assay and FM6 was subjected to LCMS/MS assay. Pure compound **(1)**, which did not show strong antioxidant activity, was isolated from sub-fraction FE8.

Table 2: Antioxidant activity (IC_{50}) of ethyl acetate and methanol subfractions of *S. myrtifolium* leaves

No.	Sample (SubFraction)	IC_{50} (µg/mL)
1	FE1	337.9**
2	FE2	187.9**
3	FE3	209.9**
4	FE4	138.8**
5	FE5	115.0**
6	FE6	253.0**
7	FE7	110.5**
8	FE8	99.2**
9	FE9	148.6**
10	FM1	213.9**
11	FM2	221.7**
12	FM3	173.6**
13	FM4	171.8**
14	FM5	251.1**
15	FM6	79.8***
16	Ascorbic acid	9.5****

Note: FE1-FE9: ethyl acetate subfraction, FM1-FM6: methanol subfraction. Antioxidant activity IC50 (*µ*g/mL): ****: <20 *µ*g/mL (very strong), ***: 20-100 *µ*g/mL (strong), **: 101-500 *µ*g/mL (moderate); *: >500 *µ*g/mL (weak)[36]

This suggests the possibility of synergism between betulinic acid and other components in the sub-fraction that contributed to the observed strong antioxidant activity of this fraction.[39]

Isolation and Identification of Compounds 1

A total of 250 mg of subfraction of FE8, was selected for compound purification because it has strong antioxidant activity. The sample was preabsorbed with silica gel and then subjected to column chromatography using n-hexane: ethyl acetate (8:2 – 1:1) eluent. TLC analysis of each eluent gave three groups of spot patterns that were combined into subfractions FE8.1. Subfraction FE8.1 was rinsed with n-hexane-ethyl acetate solvent mixture (1:1) to obtain a pure compound with a single spot pattern in the form of a white solid (31 mg). Identification of the compound structure using spectroscopic methods including ^{1}H-NMR and ^{13}C-NMR and compared with reference NMR data for the same compound.

The ^{1}H-NMR spectrum of compound **1** (700 MHz, $CDCl_3$) exhibited chemical shifts ranging from δ_H 0.70 to 5.30 ppm, indicating that the isolated compound is a non-phenolate. There are signals that accumulate in the δ_H < 2 ppm region which are typical for triterpenoid or steroid compounds. Furthermore, there are two vinylic proton signals in the δ_H 4 - 5 ppm region and three other methine proton signals in the 2.20 -3.20 ppm region. The 1H-NMR spectrum of compound **1** showed the presence of 6 methyl signals, with strong intensity at δ_H 0.75 (3H, *s*); 0.82 (3H, *s*); 0.93 (3H, *s*); 0.96 (3H, *s*); and 0.97 ppm (3H, *s*). Signals above δ_H 2.00 ppm revealed two typical signals for vinylic protons from the methylene group at δ_H 4.74 (1H, *s*) and 4.60 ppm (1H, *s*), an oxygenated methine proton signal at δ_H 3.00 (1H, *m*), and two additional methine protons at δ_H 2.26 (1H, *m*) and 3.19 ppm (1H, *m*) indicating that compound 1 has an sp^2 methylene group and a hydroxyl group bound to the tertiary carbon which is a typical absorption for a methine proton on a carbon binding a hydroxyl (OH) group commonly found at the C-3 position for triterpenoid class compounds. Comparison of the ^{1}H NMR spectrum of the compound 1 with betulinic acid from the reference shows a high similarity, suggesting that compound 1 is betulinic acid.

The ^{13}C-NMR spectrum of compound 1 shown in Figure 1 shows the presence of 30 carbon signals. There are three *sp*2 carbon signals at δ_C > 100 ppm each indicating a typical carbon signal for carbonyl acids group at δ_C 179.9 ppm and the C=C bond at δ_C 150.6; and 109.9 ppm. Furthermore, there are 27 other carbon signals located in the δ_C < 100 ppm region, correspond to *sp3* carbons, including quaternary carbon, methine (CH), methylene (CH2), and methyl (CH3) groups, typical of the pentacyclic triterpenoid structure. A typical carbon signal at chemical shift δ_C 79.2 ppm indicates the presence of an oxygenated carbon, likely at C-3, the site of the hydroxyl group in the triterpenoid skeleton. Other signals accumulated in the δ_C < 60 ppm region correspond to C-sp3 carbons, characteristic of pentacyclic triterpenoid structures. The ^{13}C-NMR spectrum of the isolated compound is shown in Figure 1.

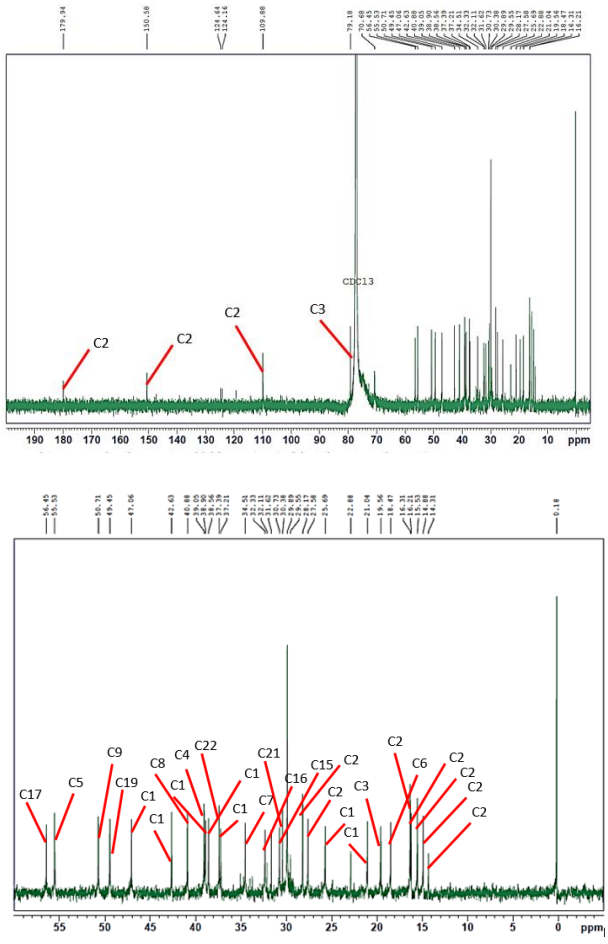

Figure 1: ^{13}C-NMR (700 MHz, $CDCl_3$) spectrum of compound 1

The analysis of ^{1}H-NMR and ^{13}C-NMR data confirms that the isolated compound belongs to the pentacyclic triterpenoid class. There are three sp^2 carbons, namely an sp^2 carbon for an acidic carbonyl group and two other sp^2 carbons for methylene carbon and quaternary carbon. The compound also contains six methyl groups and a hydroxyl group at C3.

Furthermore, the ^{1}H NMR (700 MHz, $CDCl_3$) and ^{13}C NMR (700 MHz, $CDCl_3$) spectral data of compound **1** were compared with betulinic acid reported by Noviany and Osman, H.[37] and Mahato, S.B and Kundu, A.P.[38] The **^{13}**C NMR data of compound 1 show close resemblance to those of betulinic acid. For instance, the C-3 signal, corresponding to a hydroxyl group, appears at δ_C 79.2 ppm for compound 1 and δ_C 78.9 ppm for betulinic acid. Likewise, the carboxylic carbon at C-28 resonates at δ_C 179.9 ppm in compound 1 and δ_C 180.5 ppm in betulinic acid.

The ^{1}H NMR data further support this structural similarity. The proton at C-3 in compound 1 appears as a multiplet at δ_H 3.00 ppm, closely matching the δ_H 2.95–2.98 ppm range reported for betulinic acid. Similarly, the signals at C-13 and C-19 are observed at δ_H 2.26 and 3.19 ppm, respectively, compared to δ_H 2.21–2.24 and 2.91–2.94 ppm in betulinic acid. The vinylic protons at C-29 appear as two singlets at δ_H 4.74 and 4.60 ppm in compound 1, which is consistent with the broad singlets at δ_H 4.56 and 4.69 ppm in betulinic acid. The methyl protons at C-23 to C-27 in compound 1 resonate between δ_H 0.75–0.97 ppm, again very close to the corresponding signals in betulinic acid (δ_H 0.65–0.93 ppm).

Overall, the ^{1}H-NMR and ^{13}C-NMR spectra of compound **1** strongly resemble those of betulinic acid, confirming its identity as betulinic acid. The chemical structure of compound **1** and its carbon atom numbering are shown in Figure 2.

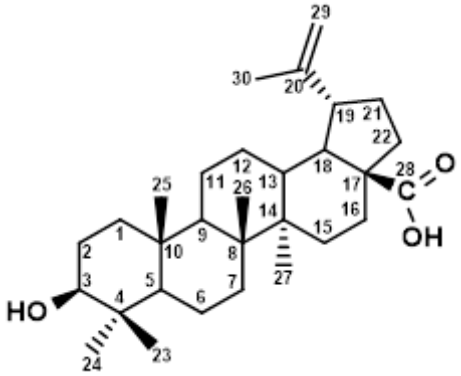

Figure 2: The chemical structure of compound **1** as betulinic acid

LCMS Analysis

Subfraction FM6 from the methanol fraction showed strong antioxidant activity, and its chemical content was analyzed using LCMS with UPLC-MS techniques. The value of measured mass and calculated mass must also be reduced by the mass of 1 H atom, which is 1.0078, due to the addition of H atoms during separation using the column, which comes from the liping of ESI (+) ions. The predicted molecular formula was selected based on a mass difference between the measured and calculated mass of ± 0.0005.[20]

The identification of the chemical structure in the antioxidant active subfraction (FM6) is shown in Figure 3 and Table 3. Figure 3 shows that not all peaks could be assigned tentative chemical structures determined. This is because some peaks had compound structures with % fit conf < 60%, and thus, these compounds were not recommended for selection. In addition, there are also peaks that have not been identified. The compound content selected in Figure 3 are those with a Fit Conf %> 80%, and based on literature studies, the group of compounds has been previously reported.

Syzygium genus plants are one of the abundant native plants from Asia with tropical and subtropical climates. Traditionally, *Syzygium* has health benefits, namely controlling blood sugar, improving skin health because it releases antifungal and antimicrobial substances, maintaining heart health so that it can lower blood pressure, relieving constipation, treating diarrhea, preventing dehydration, selecting bones and teeth because it contains magnesium, phosphorus and potassium.[21–23]

Phytochemicals identified from plants in the *Syzygium* genus have been extensively studied, as shown in Figure 4., Among these compounds, some exhibit biological activity related to antioxidants, namely quercetin (**24**), phloretin (**27**), myrigalon-G (**28**), myrigalon-B (**29**), myricetin-3-O-rhamnoside (**30**), europetin-3-O-rhamnoside (**31**). Phloretin (**27**) has been found in the plants *S. polyanthum* and *S. aqueum*. This compound shows antidiabetic activity with an EC_{50} value of 20 ± 2.2 µM for α-glucosidase enzyme inhibition and 31 ± 5.5 µM for α-amylase enzyme inhibition. Myrigalone G (**28**) and myrigalone B (**29**) were successfully isolated from the leaves of *Syzygium aqueum*. Myrigalone B (**29**) shows stronger inhibitory activity against α-glucosidase with an an EC_{50} value of 7 ± 1.4 µM but weaker activity against α-amylase with an an EC_{50} value of 33 ± 6.6 µM, compared to the same positive control. Meanwhile, myrigalone G (**28**) demonstrates antidiabetic activity with an an EC_{50} value of 19 ± 1.0 µM for α-glucosidase and 8.3 ± 1.3 µM for α-amylase, indicating higher effectiveness against α-amylase compared to acarbose.[24–26]

Table 3: Predicted chemical content of FM6 by LCMS/MS analysis

No.	Retention Time	% Area	Formula	Tentative Identification	% Fit Conf
1	4.33	4.20	$C_{14}H_{16}O_9$	Bergenin	84.14
2	5.80	11.09	$C_{15}H_{10}O_7$	Quercetin	89.52
3	16.62	10.53	$C_{12}H_{16}O_3$	Elemicin	97.80
4	17.16	6.30	$C_{12}H_{16}O_3$	Asarone	94.74

Myricetin-3-*O*-rhamnoside (**30**), isolated from the leaves of *Syzygium aqueum* and *S. guineense*, shows good antioxidant activity, with an EC_{50} value of 3.21 µg/mL in the DPPH assay, which is nearly comparable to ascorbic acid as the positive control, with an an EC_{50} of 2.94 µg/mL. In the FRAP assay, this compound shows an EC_{50} value of 22.9 µg/mL, equivalent to quercetin, which has an an EC_{50} of 23.18 µg/mL. Europetin-3-O-rhamnoside (**31**), isolated from the leaves of *S. aqueum*, also shows significant antidiabetic activity, with an an EC_{50} value of 1.9 ± 0.06 µM for α-glucosidase inhibition and 2.3 ± 0.04 µM for α-amylase inhibition, demonstrating greater effectiveness than the positive control, acarbose.[26,27]

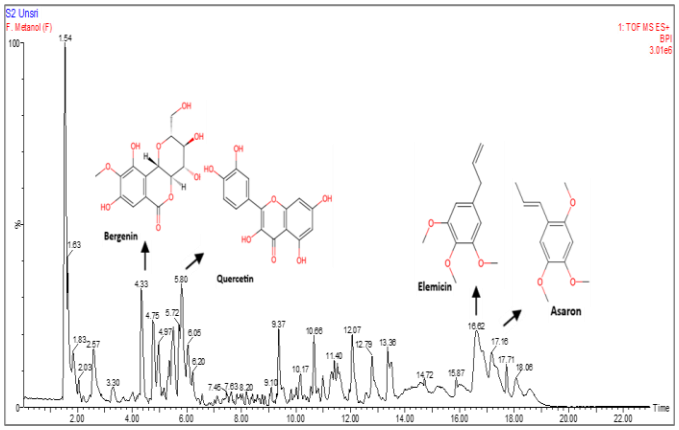

Figure 3: Predicted chemical content of FM6 through LCMS/MS analysis.

Further phytochemicals of *Syzygium* genus plants include steroids, triterpenoids, flavanones, flavanonol glycosides, chalcones, and phenolics, with molecular structures shown in Figure 4. Beyond their antioxidant properties, *Syzygium* plants also exhibit cytotoxic, analgesic, anti-inflammatory, antimicrobial, and anticarcinogenic activities. [16,28–33]

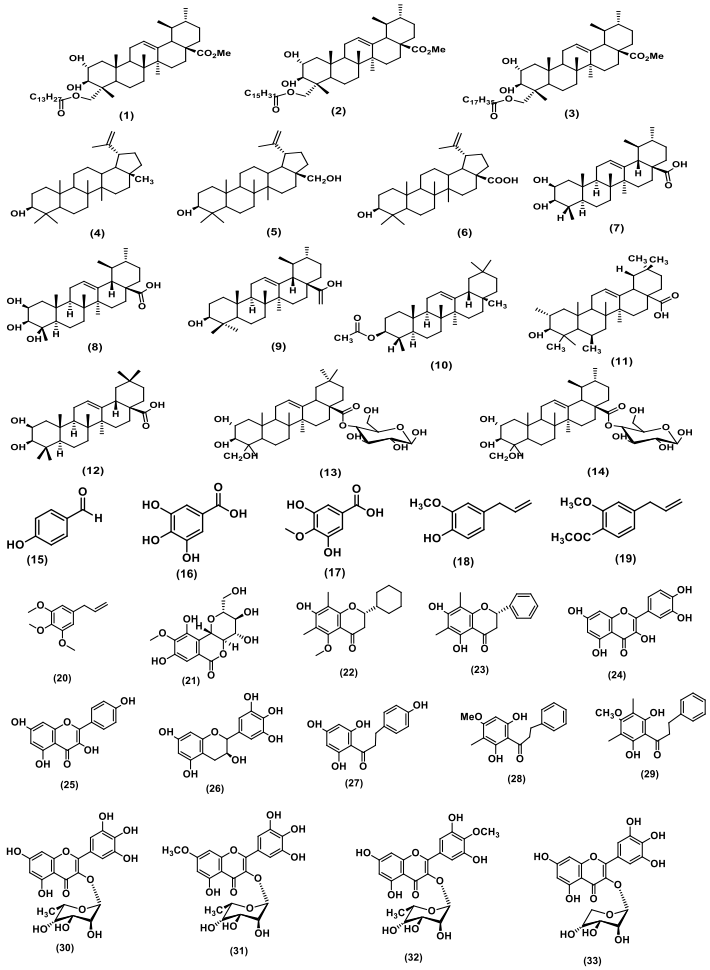

Figure 4: Chemical compounds isolated from some *Syzygium* species

The compounds contained in the antioxidant active subfraction of *S. myrtifolium* leaves in this study are betulinic acid, bergenin, quercetin, elemicin, and asarone. Four of these compounds are common in the *Syzygium* genus, as shown in Table 3. These compounds include betulinic acid (**1**), elemicin (**20**), bergenin (**21**), quercetin (**24**), and asarone, the latter of which is an isomer of elemicin that is rarely found in plants of the genus *Syzygium*. Among the five compounds contained in this antioxidant subfraction, bergenin, quercetin, elemicin, and asarone are known to have antioxidant activity. Especially quercetin, which has very strong antioxidant activity and is often used as an antioxidant standard.[34]

Based on previous research, the isolated flavonoids of *S. aromaticum* exhibited potential antioxidant activity against DPPH radicals at varying concentrations, ranging from 50 to 400 µg/mL. At higher concentrations (400 µg/mL), all extracts of clove buds significantly inhibited 45–93% of DPPH radical scavenging activity compared to 45 mg/mL BHT (95%). Quercetin showed moderate antioxidant activity (46 %) at 400 mg/mL compared to 50 µg/mL BHT (70 %).[35]

Conclusion

The separation of ethyl acetate and methanol fraction using column chromatography revealed strong antioxidant activity in subfractions FE8 and FM6, with IC_{50} values of 99.2 and 79.8 µg/mL, respectively. Betulinic acid **(1)**, which does not have strong antioxidant activity, was isolated from subfraction FE8. LCMS analysis of subfraction FM6 identified chemical compounds such as bergenin, quercetin, elemicin, and asarone. Quercetin has very strong antioxidant activity. Thus, for the utilization of *S. myrtifolium* leaves as a medicinal antioxidant resource, the methanol fraction (FM6) would be the most promising, although stepwise isolation and purification processes will be required to remove inactive components.

Conflicts of interest

The authors declare no conflict of interest.

Authors' Declaration

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

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