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*Original Research Article*

# Exploration The Antioxidant and Cytotoxic Activities of Saponins from *Lepisanthes amoena* and *Fordia splendidissima* (Blume ex Miq.) Buijsen

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ABSTRACT

Saponins are secondary metabolites primarily derived from plants, which have gained widespread applications in the pharmaceutical sector due to their medicinal, biological, and pharmacological properties. In the woodlands of East Kalimantan in Melak, the presence of the plants selekop (*Lepisanthes amoena)* and keranyi (*Fordia splendidissima* (Blume ex Miq.) Buijsen) have been extensively reported, with both possessing high saponins content. This study aimed to investigate and compare the bioactivity, saponins content, and molecular characteristics of *L. amoena* and *F. splendidissima* from East Kalimantan's woodlands in Melak. The collected plant samples (stem bark and leaf) were oven-dried, powdered, extracted, and fractionated. The fractions were subjected to antioxidant and cytotoxicity screening using established methods. Results of the screening showed that *L. amoena* and *F. splendidissima* possess significant antioxidant activities against DPPH and are lethal to the brine shrimp nauplii. In addition, the LC-MS findings showed the molecular weight of saponins identified in the active fractions of both plants.

***Keywords***: Saponins, characterization, bioactivity, antioxidant, cytotoxicity

## Introduction

Medicinal plants are known to play an essential role as primary sources in the development of a diverse array of modern drugs and pharmaceuticals, with saponins being among the important bioactive compounds. In addition, the therapeutic and pharmacological significance attributed to medicinal plants arises from the varied bioactive phytochemical constituents, which exert specific physiological effects on the human body.[1] Among these constituents, alkaloids, flavonoids, and saponins have been reported to exhibit various bioactivities.[2] Saponins are glycosides belonging to the family of bioorganic compounds and are abundantly present in plants. These naturally occurring glycosides, when agitated in water-based solutions, exhibit a foaming characteristic similar to soap. Several studies have also shown that saponins comprise a lipophilic triterpene molecule and one or more hydrophilic glycoside sugar moieties.[3,4,5]

In East Kalimantan, one of the indigenous plants is selekop (*Lepisanthes amoena*), which is traditionally used by Dayak and Kutai people for body and facial cleansing.[6] The unique ability of *L. amoena* leaves to produce soap-like foam adds significant value to its use for cleaning purposes. Moreover, empirical evidence supports its effectiveness in addressing various skin issues, including the removal of facial black spots and the healing of acne and smallpox scars.[7,8] The leaves can also be ground into powder for skin protection against sun exposure.[9]

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Therefore, this study aims to determine the saponins content using LC-MS, the cytotoxicity against shrimp nauplii, and the antioxidant activity of *L. amoena* bark and leaves as well as *Fordia splendidissima* (Blume ex Miq.) Buijsen) leaves.

## Materials and Methods

*Plant material and sample preparation*

The leaves and stem bark of *L. amoena* and leaves of *F. splendidissima* were collected in Melak District, West Kutai Regency, East Kalimantan Province, in October 2023. The plant samples were identified by Dr. Ishak Yassir, a botanist at Balai Penelitian Teknologi Konservasi Sumber Daya Alam (Balitek KSDA) Samboja, East Kalimantan, with specimen voucher numbers AA2045 and AA1860, respectively assigned. The stem bark and leaves were cleaned, sliced, and subjected to a 72-hour drying in an oven set at a controlled temperature of 40°C. The plant materials were ground into a powder and kept in air-tight containers until further use.

*Sample extraction and fractionation*

The powdered plant samples were macerated in methanol for 48 hours with intermittent agitation. The mixture was filtered using a Whatman no. 1 filter paper, and the filtrate was concentrated to dryness using a rotary evaporator at 40°C to obtain a viscous crude extract.

The methanol crude extracts were fractionated by vacuum liquid chromatography (VLC) packed with normal silica gel using solvents (n-hexane, ethyl acetate, and butanol) in order of increasing polarity. Each fraction was collected and concentrated to dryness using a rotary evaporator at 40°C

*Qualitative Test for Saponin*

The crude sample extracts (3 mL) were transferred into a test tube and mixed with 10 mL of distilled water, followed by 3 drops of 2 N hydrochloric acid. The test tubes were shaken and allowed to stand for 30 seconds. They were observed for a layer of foam, which was indicative of saponins.[10,11]

*Analysis of the saponins Content by LC-MS*

The saponins content of the plant extracts was determined using UPLC-QToF-MS/MS System (Waters) and MassLynk version 4.1 software for data processing. The profile of UPLC Acquity SDS (Waters) was assessed with column Acquity UPLC BEH C-18 1.7 um, 2.1 x 50 mm; flow rate 0.3 mL/min; injection 5 µL; temperature 40 ºC; eluent water dan 0.1 % formic acid (A); acetonitrile and 0.1 % formic acid (B), eluted by gradient.[12,13]

*Brine Shrimp Lethality Test*

The cytotoxicity potential of the plant extracts was tested against *Artemia salina* nauplii using established protocols. The *A. salina* third instar nauplii was prepared by exposing them to standard *A. salina* hatching medium for 48 h. In the test, each replication contained 10 nauplii that had been selected based on several factors, including how alive or active the samples were. A preliminary test was conducted to determine the test concentrations of the extracts. According to previous studies, it was possible to determine the $LC_{50}$ values for each extract based on concentration.[13]

*Antioxidant activity determination using DPPH assay*

The antioxidant potential of the plant extracts was tested against the DPPH free radical molecule using a previously reported method.[13] The free radical scavenging activity of the extracts of concentrations 1-200 µg/mL was assessed using a UV-VIS spectrophotometer at 517nm. The samples' antioxidant inhibitory concentration ($IC_{50}$) was reported in µg/mL. At $IC_{50}$ value < 50 ppm, the extract is considered to possess very strong antioxidant capacity.[13]

## Results and Discussion

The Saponins content of the samples was examined phytochemically. The presence of saponins in the test samples was established by the formation of a persistent foam that lasted for 30 seconds. There were variations in the thickness of the foam layer formed by the plant extracts. The tests showed several discrepancies between *L. amoena* and *F. splendidissima* extracts. Furthermore, extracts of the stem bark and leaves of *L. amoena* showed a foam layer with a thickness of more than 1 cm. The results showed that the extract of *F. splendidissima* leaves has a foam layer of less than 1 cm. Qualitatively, it could be assumed that the extracts of *L. amoena* had a higher concentration of saponins compared to *F. splendidissima*. The screening results showed that the n-butanol fraction had a similar thickness to saponin foam in the test tube, as indicated in Table 1.

According to the mode of action, antioxidant activity was divided into 3 categories: including hydrogen transfer, electron transfer, and combination mechanism. Depending on the circumstances of the reaction, the combined mechanism comprises varying ratios of hydrogen transfer, electron transfer, and electron-proton transfer. A method that could be used to assess antioxidant activity based on a combination mechanism was the DPPH assay.[8,14]

The DPPH free radical existed as a monomer, soluble in either ethanol or methanol but insoluble in water. The DPPH method uses different concentrations to calculate the percentage of DPPH scavenging activity for each sample. This method relied on antioxidants' ability to transfer electrons to neutralize the unpaired electrons on the DPPH free radicals, accompanied by a decolourization from deep violet to a yellow hue colour with a decrease in absorbance value. The antioxidant activity (DPPH free radical scavenging ability) of the test samples increases with decreasing absorbance.[15-17]

Experimental results from this study as shown in Table 2, revealed that the methanol extract of *L. amoena* stem bark and the methanol fraction of *F. splendidissima* leaves showed better antioxidant activity. This was indicated by the $IC_{50}$ values of 100.25 ppm and 34.83 ppm, respectively. Meanwhile, the lowest activity was shown by the extracts of the leaves of *L. amoena*. This could indicate that the antioxidant phytoconstituents, including saponins in the stem bark of *L. amoena*, exhibit better activity than those in the leaves.

The brine shrimp lethality assay method was used to assess the cytotoxic/safety effects of compounds exposed to shrimp nauplii. $LC_{50,}$ in this method, was defined as the concentration of plant extracts that could kill 50% of nauplii after exposure. According to the results as seen in Table 3, plant extracts having $LC_{50}$ values under 30 µg/mL were highly cytotoxic. In addition, plant extracts with $LC_{50}$ values between 30 and 100 µg/mL were poisonous, but those with $LC_{50}$ beyond 100 µg/mL were considered to have minimal toxicity or being safe.[18,19] In the cytotoxic test for brine shrimp nauplii, the methanol extract of *L. amoena* produced the lowest $LC_{50}$ value with a value of 50.28 µg/mL. The methanol fraction of *F. splendidissima* leaves showed an activity with an $LC_{50}$ value of 193.42 µg/mL. BSLT screening results showed that all samples met the $LC_{50}$ value range of 200-1000 µg/mL. Based on this value, it could be suggested that the sample had pesticidal potential.[20]

The LC-MS chromatogram showed the compound profile of each active fraction (Figure 1-5). N-butanol fraction and methanol fraction of *L. amoena* stem bark; n-butanol fraction of *L. amoena* leaves; n-butanol fraction and methanol fraction of *F. splendidissima* leaves were identified using LC-MS. The chromatogram results showed a molecular weight of > 500 g/mol. The molecular weights of saponins are generally above 500 g/mol (Table 4-8). This further confirmed the results that the active extract in each plant sample contains saponins. Metabolites in plants with a molecular mass above 500 g/mol may include saponins and peptides.

**Table 1:** Phytochemical saponins screening

| Sample | Phytochemical screening | | | | |
|---|---|---|---|---|---|
| | **Methanol extract** | ***n*-hexane fraction** | **Ethyl acetate fraction** | ***n*-Butanol fraction** | **Methanol fraction** |
| *L. amoena* stem bark | + | - | - | + | + |
| *L. amoena* leaves | + | - | - | + | - |
| *F. splendidissima* | + | - | - | + | + |

+ (saponin present); - (no saponin present)

**Table 2:** Antioxidant activity ($IC_{50}$) against DPPH free radical (µg/mL)

| Sample | Antioxidant activity (µg/mL) | | |
|---|---|---|---|
| | **Methanol extract** | ***n*-Butanol fraction** | **Methanol fraction** |
| *L. amoena* stem bark | 100.25 | >200 | >200 |
| *L. amoena* leaves | >200 | >200 | - |
| *F. splendidissima* | 130.21 | 113.38 | 34.83 |

**Table 3:** Toxicity ($LC_{50}$) against brine shrimp nauplii (µg/mL)

| Sample | Toxicity activity (µg/mL) | | |
|---|---|---|---|
| | **Methanol extract** | ***n*-Butanol fraction** | **Methanol fraction** |
| *L. amoena* stem bark | 680.95 | 859.25 | 779.79 |
| *L. amoena* leaves | 50.28 | 672.87 | - |
| *F. splendidissima* | 240.65 | 296.45 | 193.42 |

**Table 4:** LC-MS profile of the n-butanol fraction of *L. amoena* stem bark

| Code | Retention time | Measured (m/z) | Molecule Formula Prediction | Prediction Compounds |
|---|---|---|---|---|
| 01-EFB-KBS | 1.19 | 981.3897 | $C_{47}H_{65}O_{22}^+$ | Unidentified saponins |
| 02-EFB-KBS | 3.38 | 919.5052 | $C_{49}H_{75}O_{16}^+$ | Unidentified saponins |
| 03-EFB-KBS | 5.61 | 961.5592 | $C_{45}H_{85}O_{21}^+$ | Unidentified saponins |
| 04-EFB-KBS | 6.63 | 961.5396 | $C_{48}H_{81}O_{19}^+$ | Unidentified saponins |
| 05-EFB-KBS | 6.99 | 803.5239 | $C_{46}H_{75}O_{11}^+$ | Unidentified saponins |
| 06-EFB-KBS | 8.13 | 859.5804 | $C_{46}H_{83}O_{14}^+$ | Unidentified saponins |
| 07-EFB-KBS | 11.68 | 685.4184 | $C_{36}H_{61}O_{12}^+$ | Unidentified saponins |

**Table 5:** LC-MS profile of methanol fraction of *L. amoena* stem bark

| Code | Retention time | Measured (m/z) | Molecule Formula Prediction | Prediction Compounds |
|---|---|---|---|---|
| 01-EFM-KBS | 1.21 | 979.3526 | $C_{57}H_{55}O_{15}^+$ | Unidentified saponins |
| 02-EFM-KBS | 4.11 | 889.4570 | $C_{47}H_{69}O_{16}^+$ | Unidentified saponins |
| 03-EFM-KBS | 5.64 | 683.5164 | $C_{39}H_{71}O_9^+$ | Unidentified saponins |
| 04-EFM-KBS | 7.12 | 803.4574 | $C_{44}H_{67}O_{13}^+$ | Unidentified saponins |
| 05-EFM-KBS | 7.24 | 803.4753 | $C_{41}H_{71}O_{15}^+$ | Unidentified saponins |
| 06-EFM-KBS | 8.31 | 859.5676 | $C_{42}H_{83}O_{17}^+$ | Unidentified saponins |
| 07-EFM-KBS | 12.05 | 782.5431 | $C_{47}H_{26}O_{12}^+$ | Unidentified saponins |
| 08-EFM-KBS | 13.89 | 758.5499 | $C_{45}H_{25}O_{12}^+$ | Unidentified saponins |

## Conclusion

In conclusion, the activity of saponins observed in samples of *L. amoena* stem bark, leaves, and *F. splendidissima* leaves exhibited varied bioactivity. Phytochemical analysis revealed that both the stem bark and leaves of *L. amoena* demonstrated a moderate content. Meanwhile, antioxidant activity and toxicity test results of *L. amoena* bark extract were not significantly different from those of *F. splendidissima* leaves extract. Methanol extract derived from *L. amoena* leaves exhibited substantial antioxidant activity when compared across all sample groups. LC-MS results revealed the molecular weight of the identified saponins in the active fractions of both *L. amoena* and *F. splendidissima*. Future perspectives could comprise an in-depth exploration of the distinct saponins profiles and antioxidant properties, with potential applications in pharmaceutical and medicinal formulations. Further investigation could also delve into the underlying mechanisms contributing to observed variations in saponins activity between the studied plant parts. Results from this study are expected to address the existing literature void, enhancing the scientific understanding of native plants and creating opportunities for their application in the pharmaceutical and medical fields. This study is also essential for comprehending bioactive compounds present in these Indonesian plants, given the limited existing literature.

## 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.

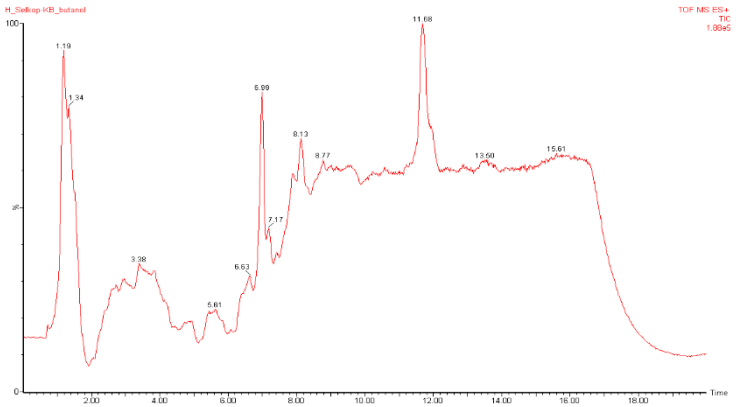


**Figure 1:** *L. amoena* stem bark n-butanol fraction LC-MS chromatogram

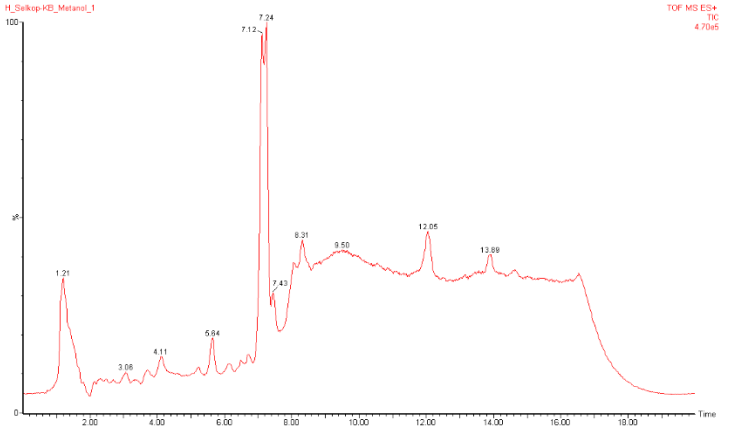


**Figure 2:** *L. amoena* stem bark methanol fraction LC-MS chromatogram

**Table 6:** LC-MS profile of n-butanol fraction of leaf *L. amoena*

| Code | Retention time | Measured (m/z) | Molecule Formula Prediction | Prediction Compounds |
|---|---|---|---|---|
| 01-EFB-DS | 1.21 | 675.4059 | $C_{45}H_{55}O_5^+$ | Unidentified saponins |
| 02-EFB-DS | 1.31 | 965.3620 | $C_{53}H_{57}O_{17}^+$ | Unidentified saponins |
| 03-EFB-DS | 2.47 | 861.4637 | $C_{46}H_{69}O_{15}^+$ | Unidentified saponins |
| 04-EFB-DS | 2.74 | 863.4736 | $C_{46}H_{71}O_{15}^+$ | Unidentified saponins |
| 05-EFB-DS | 3.09 | 949.4988 | $C_{46}H_{77}O_{20}^+$ | Unidentified saponins |
| 06-EFB-DS | 3.69 | 889.4760 | $C_{44}H_{73}O_{18}^+$ | Unidentified saponins |
| 07-EFB-DS | 4.10 | 889.4570 | $C_{47}H_{69}O_{16}^+$ | Unidentified saponins |
| 08-EFB-DS | 5.24 | 961.5460 | $C_{59}H_{77}O_{11}^+$ | Unidentified saponins |
| 09-EFB-DS | 6.15 | 931.5221 | $C_{47}H_{79}O_{18}^+$ | Unidentified saponins |
| 10-EFB-DS | 6.72 | 961.5460 | $C_{59}H_{77}O_{11}^+$ | Unidentified saponins |
| 11-EFB-DS | 7.21 | 803.5113 | $C_{42}H_{75}O_{14}^+$ | Unidentified saponins |
| 12-EFB-DS | 8.08 | 859.5490 | $C_{45}H_{79}0_{15}^+$ | Unidentified saponins |
| 13-EFB-DS | 8.30 | 859.5490 | $C_{45}H_{79}0_{15}^+$ | Unidentified saponins |
| 14-EFB-DS | 9.38 | 619.5460 | $C_{39}H_{71}O_5^+$ | Unidentified saponins |
| 15-EFB-DS | 9.62 | 907.7695 | $C_{57}H_{97}O_{15}^+$ | Unidentified saponins |
| 16-EFB-DS | 10.27 | 699.6144 | $C_{42}H_{83}O_7^+$ | Unidentified saponins |
| 17-EFB-DS | 10.88 | 955.7383 | $C_{55}H_{103}O_{12}^+$ | Unidentified saponins |
| 18-EFB-DS | 12.09 | 685.4064 | $C_{43}H_{57}O_7^+$ | Unidentified saponins |
| 19-EFB-DS | 13.50 | 1208.2412 | $C_{79}H_{163}O_6^+$ | Unidentified saponins |
| 20-EFB-DS | 14.14 | 885.5303 | $C_{48}H_{85}O_{14}^+$ | Unidentified saponins |
| 21-EFB-DS | 16.47 | 1135.9376 | $C_{68}H_{127}O_{12}^+$ | Unidentified saponins |

**Table 7:** LC-MS profile of the n-butanol fraction of *F. splendidissima* leaves

| Code | Retention time | Measured (m/z) | Molecule Formula Prediction | Prediction Compounds |
|---|---|---|---|---|
| 01-EFB-DKr | 1.33 | 439.2077 | $C_{26}H_{31}O_6^+$ | Unidentified saponins |
| 02-EFB-DKr | 1.46 | 857.2282 | $C_{44}H_{41}O_{18}^+$ | Unidentified saponins |
| 03-EFB-DKr | 1.61 | 783.1459 | $C_{47}H_{27}O_{12}^+$ | Unidentified saponins |
| 04-EFB-DKr | 2.52 | 737.2341 | $C_{45}H_{37}O_{10}^+$ | Unidentified saponins |
| 05-EFB-DKr | 3.15 | 595.3539 | $C_{32}H_{51}O_{10}^+$ | Unidentified saponins |
| 06-EFB-DKr | 4.13 | 473.3264 | $C_{29}H_{45}O_5^+$ | Unidentified saponins |
| 07-EFB-DKr | 4.44 | 953.4866 | $C_{52}H_{73}O_{16}^+$ | Unidentified saponins |
| 08-EFB-DKr | 4.69 | 741.4212 | $C_{42}H_{61}O_{11}^+$ | Unidentified saponins |
| 09-EFB-DKr | 5.27 | 1153.6268 | $C_{63}H_{93}O_{19}^+$ | Unidentified saponins |
| 10-EFB-DKr | 6.15 | 721.5573 | $C_{43}H_{77}O_8^+$ | Unidentified saponins |
| 11-EFB-DKr | 6.85 | 885.5622 | $C_{47}H_{81}O_{15}^+$ | Unidentified saponins |
| 12-EFB-DKr | 7.21 | 803.4574 | $C_{44}H_{67}O_{13}^+$ | Unidentified saponins |
| 13-EFB-DKr | 7.84 | 859.5305 | $C_{48}H_{75}O_{13}^+$ | Unidentified saponins |
| 14-EFB-DKr | 8.53 | 873.5585 | $C_{46}H_{81}O_{15}^+$ | Unidentified saponins |
| 15-EFB-DKr | 8.99 | 887.6058 | $C_{48}H_{87}O_{14}^+$ | Unidentified saponins |
| 16-EFB-DKr | 9.37 | 619.5302 | $C_{39}H_{71}O_5^+$ | Unidentified saponins |
| 17-EFB-DKr | 10.29 | 699.5810 | $C_{41}H_{79}O_8^+$ | Unidentified saponins |
| 18-EFB-DKr | 10.95 | 1079.6261 | $C_{61}H_{91}O_{16}^+$ | Unidentified saponins |
| 19-EFB-DKr | 12.09 | 685.4064 | $C_{43}H_{57}O_7^+$ | Unidentified saponins |
| 20-EFB-DKr | 13.06 | 963.7088 | $C_{56}H_{99}O_{12}^+$ | Unidentified saponins |

| | | | | |
|---|---|---|---|---|
| 21-EFB-DKr | 13.48 | 1065.6581 | $C_{54}H_{97}O_{20}^+$ | Unidentified saponins |
| 22-EFB-DKr | 14.67 | 977.6477 | $C_{51}H_{93}O_{17}^+$ | Unidentified saponins |
| 23-EFB-DKr | 16.49 | 1135.9164 | $C_{64}H_{127}O_{15}^+$ | Unidentified saponins |

**Table 8:** LC-MS profile of the methanol fraction of *F. splendidissima* leaves

| Code | Retention time | Measured (m/z) | Molecule Formula Prediction | Prediction Compounds |
|---|---|---|---|---|
| 01-EFM-DKr | 1.00 | 830.2985 | $C_{39}H_{74}O_{18}^+$ | Unidentified saponins |
| 02-EFM-DKr | 1.34 | 1177.6425 | $C_{58}H_{97}O_{24}^+$ | Unidentified saponins |
| 03-EFM-DKr | 1.63 | 783.1459 | $C_{47}H_{27}O_{12}^+$ | Unidentified saponins |
| 04-EFM-DKr | 3.08 | 727.4249 | $C_{38}H_{63}O_{13}^+$ | Unidentified saponins |
| 05-EFM-DKr | 3.60 | 1027.6998 | $C_{56}H_{99}O_{16}^+$ | Unidentified saponins |
| 06-EFM-DKr | 4.15 | 561.3724 | $C_{33}H_{53}O_7^+$ | Unidentified saponins |
| 07-EFM-DKr | 5.22 | 793.5547 | $C_{45}H_{77}O_{11}^+$ | Unidentified saponins |
| 08-EFM-DKr | 6.17 | 953.6627 | $C_{53}H_{93}O_{14}^+$ | Unidentified saponins |
| 09-EFM-DKr | 6.70 | 967.6473 | $C_{57}H_{91}O_{12}^+$ | Unidentified saponins |
| 10-EFM-DKr | 7.19 | 803.4574 | $C_{44}H_{67}O_{13}^+$ | Unidentified saponins |
| 11-EFM-DKr | 8.30 | 859.5305 | $C_{41}H_{79}O_{18}^+$ | Unidentified saponins |
| 12-EFM-DKr | 10.54 | 1185.9646 | $C_{69}H_{133}O_{14}^+$ | Unidentified saponins |
| 13-EFM-DKr | 11.54 | 1211.9889 | $C_{71}H_{135}O_{14}^+$ | Unidentified saponins |
| 14-EFM-DKr | 12.07 | 865.4064 | $C_{40}H_{65}O_{20}^+$ | Unidentified saponins |
| 15-EFM-DKr | 16.56 | 985.6494 | $C_{53}H_{93}O_{16}^+$ | Unidentified saponins |

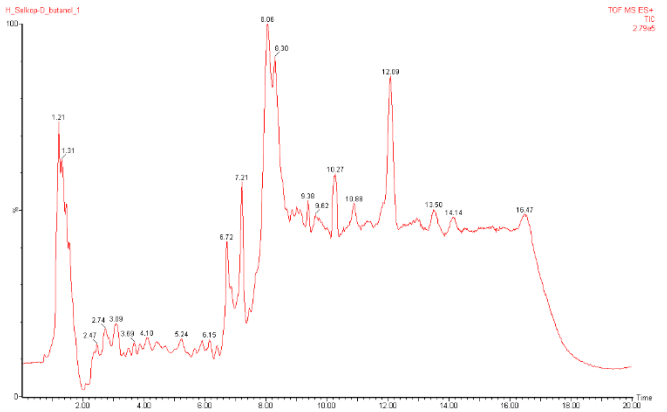

**Figure 3:** *L. amoena* leaf n-butanol fraction LC-MS chromatogram.

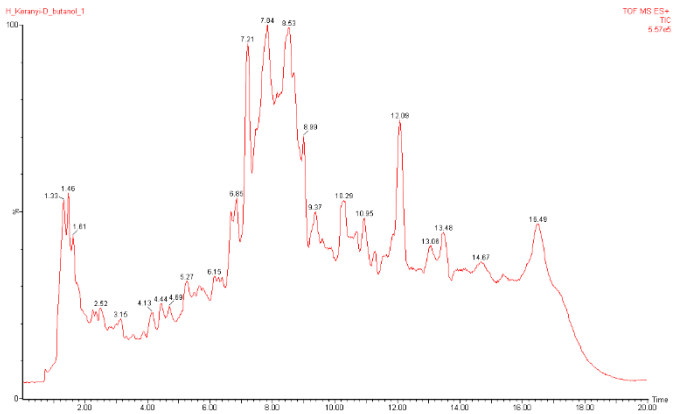

**Figure 4:** *F. splendidissima* leaves n-butanol fraction LC-MS chromatogram.

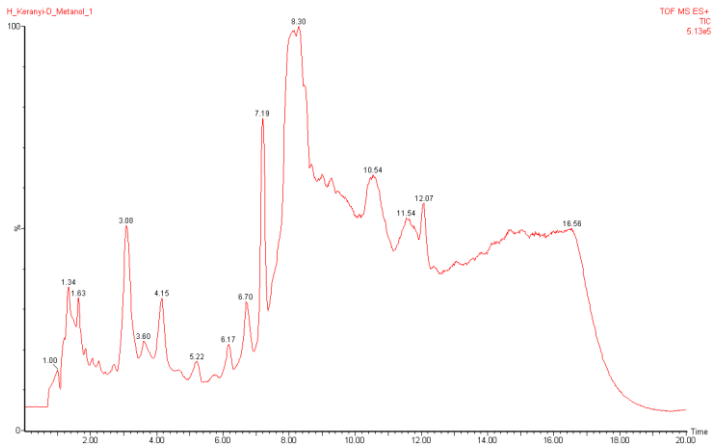

**Figure 5:** *F. splendidissima* leaves methanol fraction LC-MS chromatogram.

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