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Research Article

Identification of Flavonoid Compounds in Ethanol Extract of Majapahit plant (*Crescentia cujete*) Leaves and their Potential as Anticancer

Identifikasi Senyawa Flavonoid Ekstrak Etanol Daun Tanaman Majapahit (Crescentia cujete) dan Potensinya sebagai Antikanker

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Abstract

The Majapahit plant is commonly found in Indonesia but is rarely used due to a lack of information about its potential. One of the secondary metabolites commonly found in this plant are flavonoids. Therefore, this study aims to determine the distribution of flavonoid compounds in the Majapahit plant, particularly their potential as anticancer activty. Leaf material from Majapahit plants was extracted using the maceration technique, while the flavonoid compounds in the extract were identified using LCMS (Shimadzu LCMS-8040 LC/MS). The identification results showed that about 97 compounds were detected, including 14 flavonoids. The flavonoid compounds found include Quercetin, Chlorogenic acid, Kaempferol 3-O rhamnoside, Acacetin 7-rutinoside, Fortunellin, Kaempferol 3-[6"-(3-hydroxy-3-methyl glutarl) glucoside], Didymin, Diosmin, Hesperidin, Rutin, Citrusoside C, Citrusoside Narirutin 4'-glucoside, and Kaempferol 3-[6"-(3-hydroxy-3methylglutaryl)glucoside] -7-glucoside. The highest composition of the identified flavonoid compounds was found in Kaempferol 3-O rhamnoside, with a 3.90%

Keywords: ethanol extract; flavonoids; Majapahit plant leaves; LCMS

Abstrak

Tanaman Majapahit banyak ditemukan di Indonesia, tetapi kurang dapat dimanfaatkan oleh masyarakat Indonesia, karena kurangnya informasi mengenai potensi tanaman tersebut. Senyawa metabolit sekunder yang ditemukan salah satunya yaitu flavonoid. Tujuan penelitian ini adalah untuk mengetahui sebaran senyawa flavonoid ekstrak etanol daun tanaman majapahit yang berpotensi sebagai antikanker. Metode yang digunakan untuk ekstraksi daun tanaman majapahit yaitu maserasi, sedangkan identifikasi senyawa flavonoid pada ekstrak daun tanaman majapahit menggunakan LCMS (Shimadzu LCMS-8040 LC/MS). Identifikasi menggunakan LCMS (Shimadzu LCMS-8040 LC/MS) diketahui sekitar 97 senyawa yang terdeteksi, termasuk didalamnya 14 senyawa flavonoid. Senyawa flavonoid yang ditemukan, yaitu; Quercetin, Chlorogenic acid, Kaempferol 3-O rhamnoside, Acacetin 7-rutinoside, Fortunellin, Kaempferol 3-[6"-(3-hydroxy-3-methyl glutaryl) glucoside], Didymin, Diosmin, Hesperidin, Rutin, Citrusoside C, Citrusoside D, Narirutin 4'-glucoside, dan Kaempferol 3-[6"-(3-hydroxy-3-methylglutaryl)glucoside] -7-glucoside. Salah satu senyawa golongan flavonoid yang terdeteksi, senyawa Kaempferol 3-O rhamnoside yang memiliki komposisi tertinggi yaitu 3,90%.

Kata kunci: daun tanaman majapahit; ekstrak etanol; flavonoid; LCMS

1. Introduction

Indonesia is renowned for its abundant natural resources, including a biodiversity of animal and plant species. This biodiversity produces numerous types and quantities of chemical compounds that play a crucial role in supporting life, particularly for humans, such as medicines and antibiotics. One of the chemical compounds that can serve this purpose is a group of secondary metabolites commonly found in plants, including Majapahit.

Majapahit is a native plant to South America [1] but it can also be found in Indonesia. It is a member of the citrus family and is distinguished by enormous, bitter-tasting green fruits. which contributes to its underutilization by Indonesian people. Majapahit receives little attention and is mostly regarded as an ornamental plant. Therefore, it is crucial to conduct a study on the benefits of the plant, with a specific focus on the leaves and constituent compounds, such as flavonoids.

Flavonoids are secondary metabolites abundantly produced in natural subtances. These compounds are concentrated in the leaves, particularly in full exposure sunlight area [2] because they function as UV radiation filters [3]. Flavonoids can be found in the lower and upper epidermis cells as well as the stomata of leaves.

Within plants, flavonoids have various roles, including serving as secondary antioxidants in tissue defense against abiotic and biotic stressors [4], promoting growth, such as auxins [1], and providing color to plants, which can indicate their stress level [5].

The maceration method (soaking) was used to extract Majapahit plant leaves because it requires relatively simple

straightforward equipment and is a process. Compounds in the extract were identified using LCMS (Shimadzu LCMS-8040 LC/MS), which provided information about their structure [6]. Previous studies have been conducted on various parts of the Majapahit plant, including toxicity analysis and anticancer potential of the leaves using maceration with methanol solvent of BSLT [7], identification of potential anticancer compounds and in silico prediction on the stem bark [8], as well as detection and identification of flavonoid compounds in the ethanol extract of the bark using LCMS [9]. The novelty of this study is predicated on identification and classification of flavonoid compounds, which are secondary metabolites in the ethanol extract of Majapahit plant leaves. Furthermore, it aimed to examine the fragmentation patterns of the flavonoid compounds with the highest composition.

2. Research Methods

2.1 Tools and Materials

The main materials used were Majapahit plant leaves, 96% ethanol, methanol, and water, while the materials for the phytochemical test included an alkaline reagent.

The tools used included a maceration tube, a Buchi brand rotary evaporator, and an LCMS (Liquid Chromatography Mass Spectrometry) device, specifically the Shimadzu LCMS-8040 LC/MS model, which was conducted at the Muhammadiyah University of Malang.

2.2 Extraction of Majapahit Plant Leaves

Fresh Majapahit plant leaves were washed thoroughly with running water and then dried. Drying was carried out without direct exposure to sunlight. Subsequently,

The leaves were then sieved through an 80-mesh sieve after being coarsely crushed in a blender. Around 100 grams. sieved material was then macerated with 600 ml of 96% ethanol. This process was carried out in a maceration tube and left for 3 days. The extraction process result was filtered using filter paper and yielded the extract of Majapahit plant leaves. The extract was concentrated using a rotary evaporator.

2.3 Qualitative Test for the Extract of Majapahit Plant Leaves

About 1 mL of the ethanol extract from Majapahit plant leaves was taken and placed into a test tube. Furthermore, 3-5 drops of the alkaline reagent were added and the result was observed. A positive result would produce a yellow color in the sample.

2.4 Identification of Compounds with LCMS

The concentrated extract obtained from the extraction process was dissolved in methanol at a concentration of 20 ppm with a ratio of 2 mg concentrated extract to 10 mL of methanol. Furthermore, 1 µL of the sample was taken and injected into the LCMS-8040 instrument equipped with a binary pump. The eluents used in this process were water and 90% methanol. The LC (Liquid Chromatography) eluent connected to the Quadrupole Time-of-Flight (QTOF) mass spectrometer was set at a total flow rate of 0.5 mL/minute. The mass spectrometry (MS) used was the QTOF system with positive ionization mode.

3. Results and Discussion

3.1 Extraction of Majapahit Plant Leaves

The extraction process of Majapahit plant leaves was conducted using the soaking or maceration method. This extraction employed a semi-polar solvent of 96% ethanol to extract both non-polar and polar compounds. The 96% ethanol was used to inhibit the growth of yeast and mold during the maceration process due to its lower toxicity compared to other organic solvents [10].

In the maceration method, remaceration was carried out to obtain a higher yield of the extract, ensuring that the compounds present in the simplicia were fully extracted. The obtained extract shown in Figure 1 was then concentrated using a rotary evaporator. This process aimed to reduce direct contact between the extract and continuous heat, preventing the degradation or alteration of the constituent compounds.



Figure 1. Concentrated Extract of Majapahit Plant Leaves

3.2 Qualitative Test for the Extract of Majapahit Plant Leaves

The phytochemical analysis was conducted to determine the presence or absence of secondary metabolites in the Majapahit plant, particularly in their leaves. An alkaline reagent was implemented in the procedure to establish the presence of flavonoid compounds.

Samples containing flavonoids were identified by the appearance of a yellow color as shown in Figure 2.



Figure 2. Phytochemical Test of Flavonoid Compounds

3.3 Identification of Compounds with LCMS

The extract of Majapahit plant leaves was discovered to contain 14 chemicals that were thought to be flavonoids based on the identification by LCMS. The included compounds quercetin, chlorogenic acid, kaempferol 3-O rhamnoside, acacetin 7-rutinoside, fortunelin, kaempferol 3-[6"-(3-hydroxy-3methyl glutaryl) glucosidel, didymin, diosmin, hesperidin, rutin, citrusoside C, citrusoside D, narirutin 4'-glucoside, and 3-[6"-(3-hydroxy-3kaempferol methylglutaryl)glucoside]-7-glucoside (Table 1).

As shown in Table 1, the mass spectrum of chlorogenic acid, kaempferol 3-O rhamnoside, kaempferol 3-[6"-(3-hydroxy-3-methyl glutaryl) glucoside], hesperidin, and rutin have the highest content with values of 3.06%, 3.90%, 3.19%, 3.77%, and 3.43% respectively, compared to other flavonoid compounds.

Therefore, the fragmentation patterns of these compounds were determined and the results are presented in Table 2.

The mass spectrum of chlorogenic acid showed peaks at m/z 306, 305, and 304. The molecular ion (M+) at m/z 306 released an H radical, resulting in 2 consecutive fragments at m/z 305 and 304. The molecular ion (M+), as well as the base peak, was observed at m/z 304, corresponding to the molecular weight of the target compound, Chlorogenic acid.

Similarly, the mass spectrum of kaempferol 3-O rhamnoside exhibited peaks at m/z 433, 432, and 431. The molecular ion (M+) at m/z 433 released an H radical, culminating in the generation of 2 consecutive fragments at m/z 432 and 431. The molecular ion (M+), as well as the base peak, was observed at m/z 431, corresponding to the molecular weight of the target compound, kaempferol 3-O rhamnoside.

In the case of kaempferol 3-[6"-(3-hydroxy-3-methyl glutaryl) glucoside], the mass spectrum showed other peaks such as m/z 594, 593, and 592. The molecular ion (M+) at m/z 594 released an H radical, resulting in 2 consecutive fragments at m/z 593 and 592. The molecular ion (M+), as well as the base peak, was observed at m/z 592, representing the molecular weight of the target compound, kaempferol 3-[6"-(3-hydroxy-3-methyl glutaryl) glucoside].

The mass spectrum of hesperidin exhibited peaks at m/z 612, 611, and 610. The molecular ion (M+) at m/z 612 released an H radical, leading to the generation of 2 consecutive fragments at m/z 611 and 610. The molecular ion (M+), as well as the base peak, was observed at m/z 610, corresponding to the molecular weight of the target compound, hesperidin.

For rutin, the mass spectrum showed peaks at m/z 612, 611, and 610. The molecular ion (M+) at m/z 612 released an H radical, resulting in 2 consecutive fragments at m/z 611 and 610. The molecular ion (M+), as well as the base peak, was observed at m/z 610, corresponding to the molecular weight of the target compound, rutin.

The presence of flavonoid compounds in Majapahit plant leaves as shown in Table 1, confirmed its potential as an anti-cancer agent. Kaempferol 3-O rhamnoside was detected at a retention time of 21.429 minutes in the LCMS instrument. A previous study showed that kaempferol 3-O rhamnoside extracted from Schima wallichii Kort leaves can inhibit MCF-7 breast cancer cells [11].

Acacetin 7-rutinoside has been found to induce apoptosis in cancer cells [12], while quercetin has chemopreventive effects on prostate cancer [13]. Quercetin can also act as an anti-metastatic agent in gastrointestinal cancer [14]. In a previous study, hesperidin induced apoptosis in MSTO-211H pleural mesothelioma cells [15]. Another study also showed that hesperidin can induce colon, breast, lung, and liver cancer [16].

In lung cancer cells, rutin has been shown to reduce cell migration and adhesion, leading to inhibited proliferation and decreased ROS production [17].

Acacetin 7-rutinoside, also known as linarin, has the ability to induce apoptosis in human prostate cancer cells, indicating its potential as an anti-cancer agent [12]. Meanwhile, narirutin 4'-glucoside can inhibit the proliferation of HL-60 leukemia cells [1].

Kaempferol 3-[6"-(3-hydroxy-3methylglutaryl) glucoside]-7-glucoside and kaempferol 3-[6'-(3-hydroxy-3methylglutaryl)glucoside] are known to be anti-cancer agents and are derivatives of kaempferol [18]. Kaempferol reportedly inhibited the growth of breast cancer cells [19]. Moreover, diosmin demonstrated great potential against prostate cancer cells and exhibited genotoxic effects. suppressing the proliferation and viability of HepG2 hepatocarcinoma cell cultures [20]. Didymin has potential against neuroblastoma cells as it can kill both p-53 wild-type and drug-resistant p-53 mutant cells [21].

4. Conclusion

In conclusion, LCMS analysis of Majapahit plant leaves revealed the presence of 14 different flavonoid chemicals. 12 of these were discovered to have remarkable anticancer potential. Kaempferol 3-O rhamnoside had the highest concentration compared to other flavonoid compounds.

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Table 1. LCMS Results of Flavonoid Compounds from the Extract of Majapahit Plant Leaves

| No | Compounds | Retention Time (min) | Composition (%) | Analysis | Structure |
|----|---------------------------|----------------------|-----------------|--|--|
| 1 | Quercetin | 11.427 | 1.96925 | Chemical formula: C ₁₅ H ₁₀ O ₇ Molecular weight: 302.0427 m/z: 302.0427 (100%) | HO OH OH |
| 2 | Chlorogenic acid | 12.421 | 3.06210 | Chemical formula: $C_{16}H_{18}O_9$ Molecular weight: 354.0951 m/z: 354.0951 (100%) | HO H |
| 3 | Kaempferol 3-O rhamnoside | 21.429 | 3.90389 | Chemical formula: $C_{21}H_{20}O_{10}$ Molecular weight: 431.0984 m/z : 431.0984 (100%) | HO HIBBOTH OH |
| 4 | Acacetin 7-rutinosid | 33.702 | 2.69590 | Chemical formula: $C_{28}H_{32}O_{14}$ Molecular weight: 592.5500 m/z: 592.1792 (100%) | |

| No | Compounds | Retention Time (min) | Composition (%) | Analysis | Structure |
|----|---|----------------------|-----------------|--|--|
| 5 | Fortunelin | 33.722 | 1.69004 | Chemical formula: $C_{28}H_{32}O_{14}$ Molecular weight: 592.1792 m/z: 592.1792 (100%) | |
| 6 | Kaempferol 3-[6"-(3-hydroxy-3-methyl glutaryl) glucoside] | 33.729 | 3.19042 | Chemical formula: $C_{27}H_{28}O_{15}$ Molecular weight: 592.5060 m/z: $592.1428(100\%)$ | |
| 7 | Didymin | 34.004 | 1.70507 | Chemical formula: $C_{28}H_{34}O_{14}$ Molecular weight: 594.5060 m/z: $594.1428(100\%)$ | |
| 8 | Diosmin | 35.504 | 1.84236 | Chemical formula: $C_{28}H_{32}O_{15}$ Molecular weight: 608.5490 m/z: 608.5490 (100%) | ************************************** |

| No | Compounds | Retention Time (min) | Composition (%) | Analysis | Structure |
|----|---------------|----------------------|-----------------|---|-------------------|
| 9 | Hesperidin | 35.507 | 3.77466 | Chemical formula: $C_{28}H_{34}O_{15}$ Molecular weight: 610.5650 m/z: 610.1741 (100%) | HO THE MENT HOUSE |
| 10 | Rutin | 35.517 | 3.43252 | Chemical formula: $C_{27}H_{30}O_{16}$ Molecular weight: 610.1534 m/z : 610.1534 (100%) | |
| 11 | Citrusoside C | 39.064 | 1.25323 | Chemical formula: $C_{31}H_{40}O_{15}$ Molecular weight: 652.2367 m/z : 654.2367 (100%) | |

| No | Compounds | Retention Time (min) | Composition (%) | Analysis | Structure |
|----|---|----------------------|-----------------|--|--|
| 12 | Citrusosid D | 39.067 | 1.48975 | Chemical formula: $C_{31}H_{40}O_{15}$ Molecular weight: 652.6460 m/z: 654.2434 (100%) | HO H |
| 13 | Narirutin 4'-glucosid | 46.301 | 2.21045 | Chemical formula: $C_{33}H_{42}O_{19}$ Molecular weight: 742.2320 m/z: 742.2320 (100%) | philose p |
| 14 | Kaempferol 3-[6"-(3-hydroxy-3-methylglutaryl)glucosid] -7-glucoside | 46.564 | 2.60072 | Chemical formula: $C_{33}H_{38}O_{20}$ Molecular weight: 754.6470 m/z: 754.1956 (100%) | |

Table 2. Mass Spectrum and Fragmentation of Compounds with the Highest Composition









