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Exploring The Bioactive Potential of Cultivated Ashitaba (*Angelica keiskei*) in Indonesia: A Chemical Profiling Study

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Abstract

The scientific research related to identifying active compounds in medicinal plants is an integral part of developing new drugs and understanding the healing properties of plants. The Ashitaba plant (*Angelica keiskei*) originates from Japan. The ashitaba is known as a long-lived plant in Japan because it has been used for generations as a therapeutic herb that helps improve health issues (Ohkura et al., 2018). This plant has also been cultivated in Indonesia since 2002 in the Mojokerto region. The potential of the ashitaba plant as a medicinal herb is not widely known due to the limited research regarding its compound identification and applications. This research aimed to identify bioactive compounds in Ashitaba leaf specifically cultivated in Trawas, Mojokerto, Indonesia. The identification process involved creating extracts from Ashitaba leaves as samples. The identification method used was LCMS/MS-Q-TOF. The results indicated 20 flavonoid compounds and ten phenolic compounds successfully identified from the Ashitaba leaf. Some of the flavonoid compounds found in the highest fragment numbers include Kaempferol-3-glucuronide, Quercetin-3-O-α-L-arabinofuranoside, (-)-Epiafzelechin-3-O-(6"-O-acetyl)-β-D-allosepyranoside, Bavachinin, Quercetin-3-O-α-L-arabinofuranoside dan Quercetin-7-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranoside]. As for the phenolic compounds, they are Methyl-5-O-caffeoylquinate, 2-Hydroxybenzyl-3-hydroxybenzoate, and dan Mulberrofuran D.

Keywords: Ashitaba Leaf, Flavonoids, Phenolics, Bioactive Compounds

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1. Introduction

Scientific research related to identifying active compounds in medicinal plants is an integral part of developing new drugs and understanding plants' healing properties. As a country rich in biodiversity, Indonesia possesses various traditional medicinal plants that local communities have used for healthcare and treatment. One medicinal plant that has captured the attention of researchers is Ashitaba (*Angelica keiskei*), known for its potential in the health field.

The Ashitaba plant (*Angelica keiskei*) originates from Japan. In Japan, the Ashitaba plant is a long-lived plant because it has been traditionally used for generations as a medicinal herb that can improve health conditions (Ohkura et al., 2018). This plant has also been cultivated in Indonesia since 2002 in the Mojokerto region. Ashitaba leaves possess antioxidant, anticancer, and anti-inflammatory properties, making them attractive as a potential ingredient for developing new medications (Kil et al., 2017). However, despite its significant potential, the active compounds in Ashitaba leaves cultivated in Indonesia have not been fully identified and understood. Recent studies have highlighted the importance of identifying active compounds in medicinal plants, including Ashitaba, to understand their mechanisms of action and potential in treatment. Therefore, this research aims to identify active compounds in Ashitaba leaves sourced from Indonesia. The information obtained is crucial for exploring the natural healing potential of this plant. It will provide a profound knowledge base for further research in the development of plant-based medications. This research used the LCMS/MS Q-TOF detection method to determine the presence of flavonoid and phenol compounds in Ashitaba leaves from Indonesia.

2. Materials and methods

2.1 Plant Materials

The plant used in this research is Ashitaba (*Angelica keiskei*), cultivated in Trawas Mountain, Mojokerto, Indonesia. The part of the plant used in the study was the leaf. The harvested plants were sorted, and only high-quality plants that were free from plant diseases and parasites were selected. The chosen plants also had healthy leaves with a fresh green color.

2.2 Extracts Preparation

The Ashitaba was washed and air-dried to remove dust and other organic compounds. The leaf part was separated from the whole plant. The leaves were then subjected to natural drying for 5 hours. After drying, the leaves were finely ground using a blender, and an extraction was carried out using 70% ethanol through the maceration method for 3x24 hours. It was followed by evaporation to remove the solvent until Ashitaba leaf extract was obtained.

2.3 LCMS/MS-Q-TOF Detection

The identification test of these bioactive compounds was conducted at PT. Saraswanti Indo Genetech laboratory in Bogor, Indonesia, with contract number SIG.CI.L2023.18132921. The method used involved LCMS/MS-Quadrupole Time-of-Flight (QTOF). The test began with the preparation of biotin and chloramphenicol standards. A 1 mg/L biotin standard was prepared by dissolving 25 µL of the 1000 mg/L biotin standard in a 25 mL volumetric flask. The mixture was homogenized and calibrated to a volume of 25 mL. A 1 ppm chloramphenicol standard was prepared by dissolving 25 µL of the 1000 mg/L chloramphenicol standard in a 25 mL volumetric flask. The mixture was homogenized, and the volume was adjusted to 25 mL. A 0.1 g sample was weighed and dissolved in ethanol to a volume of 10 mL. The mixture was homogenized, filtered with a 0.22 µm GHP/PTFE membrane filter, and injected into the LC-QTOF-MS/MS system. Each sample was tested in duplicate. The LC settings included a C18 column, column temperature of 40°C, autosampler temperature of 15°C, and injection volume of 10 µL. The mobile phase consisted of 0.1% formic acid in acetonitrile distilled water. The flow rate was 0.6 mL/min with a gradient. The MS settings involved TofMSE operation mode, ESI (-) /ESI (+) ionization, and an acquisition range of 50-1200 Da.

2.4 Data Analysis

The active substance screening process using LCMS/MS-QTOF was carried out using UNIFI software, which includes a mass spectrum library of active natural substances from the Waters database. UNIFI software can identify the mass spectrum of compounds in the sample and match them with the mass range available in the library. Bioactive compounds were identified if they met the following criteria: i) Mass reading error of the analyte < 5 ppm; ii) Isotope conformity MZ RMS < 6 ppm and isotope conformity MZ RMS % < 10%; iii) Analyte intensity ≥ 300; and iv) There is one fraction with a break value < 4 in the fragment matching elution system.

3. Results and Discussions

The analysis results conducted using LC-MS/MS equipped with a Q-TOF high-resolution analyzer showed the identification of 20 flavonoid compounds and ten phenolic compounds. Different bioactive compounds were observed based on the positive and negative ESI chromatograms (Figure 1).

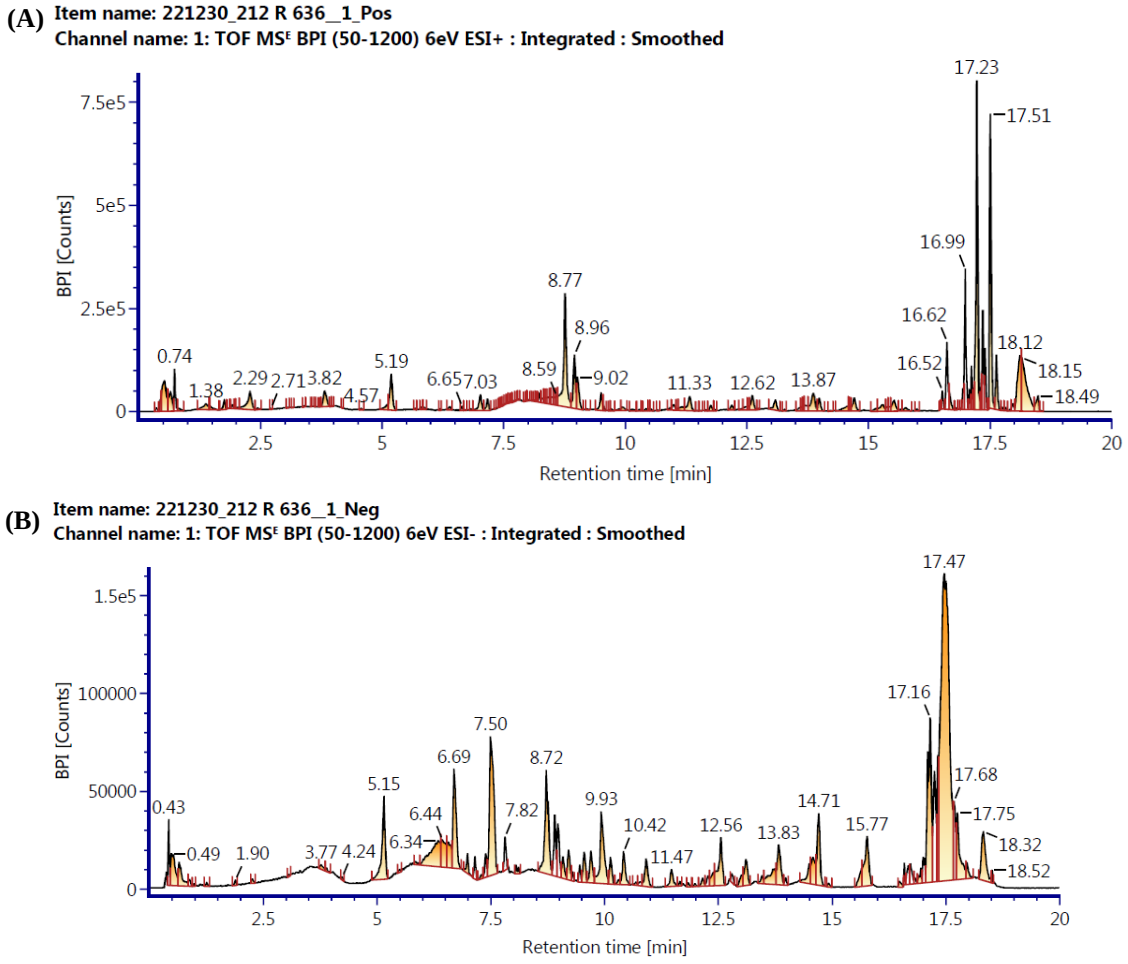

Figure 1: LCMS-MS chromatograms from Ashitaba leaf extracts: (A) Chromatogram Positif ESI; (B) Chromatogram Negative ESI

Eight flavonoid compounds and two phenolic compounds were identified in the positive ESI chromatogram, whereas in the negative ESI chromatogram, 12 flavonoid compounds and seven phenolic compounds were identified. Flavonoids have complex chemical structures and often contain hydroxyl groups, which can provide proton charges in positive ESI chromatogram observations. On the other hand, phenols are aromatic compounds with hydroxyl groups (-OH) attached to the aromatic ring. These hydroxyl groups allow phenols to acquire proton charges in positive ESI mode. Phenols can also exist as phenolates with proton loss, which can be detected in negative ESI mode.

Flavonoid and phenolic compounds are essential constituents that play a role in antioxidant activity. The antioxidant potential of these compounds depended on the number and position of free hydroxyl groups, facilitating free radical trapping activities (Shahid et al., 2023). Based on the research findings, flavonoids are bioactive compounds with many benefits, such as antioxidant activity, heart protection, and anti-inflammatory and antibacterial properties. Flavonoids also show significant differences in inhibiting various types of enzymes, including α-amylase and α-glucosidase enzymes (Zhu et al., 2020). The identification of compounds in Ashitaba leaves revealed the presence of 20 flavonoid compounds (Table 1). The identified flavonoid compounds outnumbered the phenolic compounds. Among the flavonoid compounds captured, those with the highest number of detected fragments included Kaempferol-3-glucuronide, Quercetin-3-O-α-L-arabinofuranoside, (-)-Epiafzelechin-3-O-(6”-O-acetyl)-β-D-allosepyranoside, Bavachinin, Quercetin-3-O-α-L-arabinofuranoside, and Quercetin-7-O- [β-D- glucopyranosyl (1→6)-β-D-glucopyranoside] Additionally, other compounds identified were Luteolin-7-O-glucoside, Glabrol, Kaempferol 3-O-β-D-glucuronopyranosyl methyl ester, Quercetin-3-O-α-L-arabinofuranoside dan Trifolin.

The identified flavonoid compounds in Ashitaba leaves are known to have several benefits. Kaempferol-3-glucuronide is a glucuronidated form of kaempferol. Kaempferol is a flavonoid compound known for its antioxidant and anti-inflammatory properties. Glucuronidation, on the other hand, enhances the solubility and excretion of compounds in the body, aiding in the detoxification of harmful compounds within the body (Chen et al., 2012). Quercetin-3-O-α-L-arabinofuranoside is a flavonoid compound classified as a glycoside. Quercetin is a crucial flavonoid known for its antioxidant, anti-inflammatory, and anticancer properties. In its glycoside form, quercetin is bound to arabinose sugar, which enhances its solubility and stability in the body (Suzuki et al., 2023).

Table 1. Flavonoid compound identified of ethanol eluant from ethanol leaf extracts of *Angelica keiskei* by LCMS/MS-Q-TOF

No	*Flavonoid*	Compound Formula	Total Fragments Found	Observed Rate Time (min)	Error (ppm)
1.	(-)-Epiafzelechin-3-O-(6”-O-acetyl)-β-D-allosepyranoside	C23H26O11	25	14.00	-0.3
2.	Bavachinin	C21H22O4	24	16.80	-2.1
3.	Galangin (Norizalpinin)	C15H10O5	3	14.28	-3.2
4.	Kaempferol	C15H10O6	5	8.23	-1.5
5.	Kaempferol-3-glucuronide	C21H20O12	48	8.77	-0.2
6.	Luteolin-7-O-glucoside	C21H20O11	16	9.03	0.5
7.	Maltol	C6H6O3	1	2.71	-4.2
8.	Quercetin-3-O-α-L-arabinofuranoside	C20H18O11	31	9.51	0.6
9.	Quercetin-7-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranoside]	C27H30O17	20	5.90	0.7
10.	Undulatoside A	C16H18O9	8	5.19	-1.8
11.	6-Hydroxykaempferol-3-O-glucoside	C21H20O12	23	8.72	1.3
12.	Glabrol	C25H28O4	15	16.80	-2.0
13.	Kaempferol 3-O-β-D-glucuronopyranosyl methyl ester	C22H20O12	15	6.34	-1.9
14.	Kushenol A	C25H28O5	8	16.73	-1.8
15.	Naringenin-4’,7-dimethyl ether	C17H16O5	1	15.94	-3.1
16.	Patuletin	C16H1208	4	10.34	-4.7
17.	Quercetin-3-O-α-L-arabinofuranoside	C20H18O11	12	9.46	-0.9
18.	Sec-O-glucosylhamaudol	C21H26O10	6	12.51	-1.5
19.	Smiglanin	C15H16O9	1	4.42	-3.9
20.	Trifolin	C21H20O11	12	8.98	-0.5

According to a study by Dong et al., 2020, Bavachinin can protect liver cells (HepaRG cells) from palmitic acid-induced death by inhibiting fat accumulation and cholesterol synthesis. It is achieved by inhibiting the activity of the enzyme squalene synthase (SQS), which is regulated by the FDT1 gene.

A total of 10 phenolic compounds were identified in the Ashitaba leaf extract in this study (Table 2). Among the phenolic compounds identified, those with the highest number of fragments include Methyl-5-O-caffeoylquinate, 2-Hydroxybenzyl-3-hydroxybenzoate, and Mulberrofuran D. Methyl-5-O-caffeoylquinate is a phenolic compound classified as a chlorogenic acid derivative. This compound exhibits antioxidant activity, which is beneficial in combating oxidative stress and protecting body cells from damage caused by free radicals (Li et al., 2012). The compound 2-Hydroxybenzyl-3-hydroxybenzoate is a compound that has two hydroxyl (OH) groups on the benzene ring.

This compound is a derivative of benzoate and hydroxybenzyl. Compounds with such structures tend to have antioxidant activity because the hydroxyl groups enable the combination to interact with free radicals and prevent cell damage (Wu et al., 2013). On the other hand, Mulberrofuran D is a stilbenoid compound, a type of phenolic compound. Stilbenoids are found in various plants and possess strong antioxidant properties. Mulberrofuran D has antioxidant potential due to its structure, which allows it to capture free radicals and prevent cell damage (Batiha et al., 2023).

Table 2. Phenol compound identified of ethanol eluant from ethanol leaf extracts of *Angelica keiskei* by LCMS/MS-Q-TOF

No	*Phenol*	Compound Formula	Total Fragments Found	Observed Rate Time (min)	Error (ppm)
1.	2,4,7-Trihydroxy-9,10-dihydrophenanthrene	C14H12O3	3	11.00	-3.1
2.	2-Hydroxybenzyl-3-hydroxybenzoate	C14H12O4	12	16.61	-1.3
3.	Cassialactone	C16H16O6		12.22	
4.	Dihydrooxyresveratrol	C14H14O4	3	11.33	-3.3
5.	Dihydroresveratrol	C14H14O3	2	12.53	0.0
6.	Meliadanoside A	C16H24O10	6	6.62	0.6
7.	Meliadanoside B	C15H20O8	4	5.68	-2.9
8.	Methyl caffeate	C10H10O4	3	9.91	-3.1
9.	Methyl-5-O-caffeoylquinate	C17H20O9	19	6.99	-2.0
10.	Mulberrofuran D	C29H34O4	8	17.97	-4.1

Recent studies have revealed that various biological activities, such as antioxidant, antidiabetic, and anti-inflammatory, are associated with phenolic compounds in fruits and vegetables. In recent times, the food industry and consumers have shown increased interest in plant-based materials rich in phenolic compounds due to their ability to slow down oxidative lipid degradation, enhance both the quality and nutritional value of food and promote human well-being and health (Peixoto Araujo et al., 2020).

4. Conclusions

Twenty flavonoid compounds and ten phenolic compounds were successfully recognized in the Ashitaba leaf extract. Among the flavonoid compounds with the highest fragment numbers were Kaempferol-3-glucuronide, Quercetin-3-O-α-L-arabinofuranoside, (-)-Epiafzelechin-3-O-(6”-O-acetyl)-β-D-allosepyranoside, Bavachinin, Quercetin-3-O-α-L-arabinofuranoside, and Quercetin-7-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranoside]. As for the phenolic compounds, they included methyl-5-O-caffeoylquinate, 2-Hydroxybenzyl-3-hydroxybenzoate, and Mulberrofuran D.

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