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Phytochemical Profiling of *Hippobroma longiflora* Leaf Extract Using LC-MS/MS Analysis and Pharmacological Potential

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Abstract

Hippobroma longiflora is a weed plant with diverse metabolites, including alkaloids, phenolics, tannins, and saponins. The community in several tropical regions uses this plant as medicine, but information about the metabolite profile contained in its leaves is still quite limited. Therefore, this research was conducted to identify the metabolite content in *Hippobroma longiflora* leaf extract and investigate its potential pharmacological effects to ensure its use in healthcare. The method used was LC-MS/MS, followed by inputting the results of LC-MS/MS into MS Dial and MS Finder applications to identify the essential compounds. The analysis resulted in 29 compounds identified: phenolic, terpenoid, and alkaloid compound groups. *Hippobroma longiflora* contains several potent antioxidants, antibacterial or antiviral compounds, and anticancers. In conclusion, *Hippobroma longiflora* leaf extract is rich in compounds that can be utilized by the community in medicine and healthcare, in particular for certain infectious diseases and anticancer.

Keywords: Secondary metabolites, traditional medicine, weed plant

1. Introduction

Over the past few decades, the significant role of herbs has increasingly manifested itself in the healthcare sector. It is believed that a large majority of the world's population, with a percentage of around 70-90%, relies on herbs to address various health issues [1]. Hence, herbal medicine has become an essential part of the healthcare industry [2]. Plants are one of the sources of metabolites that can be utilized as functional food and medicine. Conventionally, secondary metabolites are obtained by extracting directly from plant organs. One of the plants that is used as a medicine is *Hippobroma longiflora.*

Hippobroma longiflora (L.) G. Don is a wild plant originating from Jamaica, found growing in South America (Brazil, Bolivia, and Ecuador), the Pacific Ocean islands (Fiji, Carolina Islands, and Cuba), as well as in the Asian continent i.e., India, Sri Lanka, and Taiwan, including Indonesia [3], especially in habitats that have sufficient moisture, such as streams and bushes [4]. It belongs to the family Campanulaceae. It has several popular names, such as Star of Bethlehem or Madam Fate; in Indonesia, it is called Kitolod or Kembang Bintang. Although currently considered a weed, empirically, Hippobroma longiflora has been used as an antibacterial [5] and anticancer [6],eye medicine to reduce cataracts, and medicine to cure toothache, asthma, bronchitis, wound, and laryngitis [7]. Various studies on *Hippobroma longiflora* have found that it contains phytochemical compounds, but the reported

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results varied. The presence or absence of a particular phytochemical compound depends on the type of solvent used, the nature of the extraction process, or the environmental conditions in the region where the sample was taken [8]. *Hippobroma longiflora* extracted in chloroform and methanol solvents (1:1) possessed secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids,and steroids [9,10]. Some compound activities, such as anticancerand antioxidant [8,11],antibacterial [12], anti-inflammatory, and antifungal, have been scientifically proven through experimental testing and can help treat glaucoma and hyperlipidemia [10]. Although *Hippobroma longiflora* has long been utilized as a medicinal plant in Indonesia and several other countries, information on the composition of secondary metabolites in the leaf extracts still needs to be available. Therefore, this study aims to reveal any potential active compounds in the leaves through the LC-MS/MS method. Findings regarding active compounds that have potential are also further explained.

Material and Methods

Plant Material

Hippobroma longiflora samples were collected in Bogor, West Java (6033'48.8"S106042'57.3"E) on June 20, 2023. The plant was identified at the Biopharmaca Cultivation Conservation Unit (UKBB) of the Tropical Biopharmaca Study Center of IPB University, with the voucher number BMK 0282102016. The leaves and stems were separated and washed using running water to remove dirt from the plant material. Next, the samples were cut into small pieces.

Preparation of Extracts

The leaf samples were dried in an oven at 40 °C. After the sample was dehydrated, 0.2 kg was extracted using the maceration method: 96% ethanol was used as the distilling liquid for five days. The dregs (solid material) were separated through a Whatman No.1 filter paper. The filtrate was then concentrated using a rotary vacuum evaporator at a temperature below 45 °C. Furthermore, the extract was dried using a pressurized dryer until a solid form was obtained [13].

Measurement by LC-MS/MS

The dried extract (10 mg) was mixed with 10 mL methanol, then the extract in methanol was taken with a microsyringe as much as 5 µl for further injection. qTOF LC-MS/MS analysis was performed by using ultra-performance liquid chromatography (UPLC) (LC: Acquaity UPLC H-class system, Waters, USA) and mass spectrometer (Xevo G2-S QTof, waters, USA). The separation applied an Acquaity UPLC® HSS, Waters, C18 column (2.1 x 100 mm 1.8 µm) at 50°C (column) and at 25 °C room temperature. LC analysis used a mobile phase consisting of water + 5 mM ammonium formate (A) and acetonitrile + 0.05% formic acid (B), with a flow rate of 0.2 mL min-1, for 23 min and an injection volume of 5 µL. The delivery system was at a constant rate of 200 µL min-1, and the mobile phase used 70% acetonitrile and 1 mmol of 30% HCOOH. The sample injection volume was 10 µL, with 3 replicates (n=3). The MS/MS operation used electrospray ionization (ESI) in positive and negative modes with a mass range of 50-1200 m/z, and the source and desolvation temperatures were 100 and 350 °C. In addition, the cone and desolvatization gas rates were 0 L/h and 793 L/h, respectively.

Data Analysis

The qualitative analysis of the compounds was conducted using MS-DIAL version 3.82, which included peak discrimination, filtering, and alignment processes. MS raw data in *.raw format were imported into Abf Converter 4.0.0 to convert them into *.abf format and MS FileReader 2.2.62 were also utilized [14]. The parameters for peak detection included a minimum peak height of 10,000 (Orbitrap), a smoothing method using a linear weighted moving average, a smoothing level of, and a minimum peak width. The identification used an in-house database (MSP File) in MS/MS positive and negative modes (http://prime.psc.riken.jp/compms/msdial/main.html#MSP)(accessed on August 16, 2023), with a 70% score cut off, adduct types $[M + H]^+$ and $[M-H]^-$, and the alignment was performed using reference quality control. We then calculated the mass error value (10 ppm) as the filtering criterion for the compounds identified in the scanning experiments.

Results and Discussion

Profile of Active Compounds of Hippobroma longiflora leaf

Based on the interpretation of metabolites profile data (Table 1), 29 compounds were obtained in leaves using 96% ethanol solvent. This chromatogram profile shows apparent variations in retention time between 1.1 and 15.6 minutes in the analyzed samples. The variation of sample ion chromatogram profiles at this retention time can be distinguished based on the number of peaks detected, peak height, and area. In Table 2, several groups of compounds, such as phenolics, flavonoids, terpenes, and alkaloids, were obtained. It is worth mentioning the presence of fisetin, hypericin, orcinol, and vindoline, which have been reported for their pharmacological effects. These results follow those obtained by Savira [15] and Hastuti [12] in Hippobroma longiflora leaf extracts, in which secondary metabolites of flavonoids, alkaloids, and steroids were detected. The compound groups mentioned above have been carried to further research for their utilization as active ingredients, such as food supplements, nutraceuticals, and medicines. The benefits of natural material as biochemicals producers have been widely reported, i.e., several phenolic compounds have antioxidant and antimicrobial activities, and alkaloids are anticancer [16]. Apart from that, alkaloids also have several functions, such as tachycardia, anti-malaria, anti-diabetes, and anti-hypertension. Another biochemical group, terpenes, has analgesic, anti-inflammatory, and antitumor effects. In addition to being utilized in the healthcare industry, several groups of compounds are also beneficial in the cosmetic industry; some examples are phenolics, alkaloids and terpenes, methylxanthines, coumarins, carotenoids [16].

Table 1. Active compound profile in *Hippobroma longiflora* leaves

No	Metabolite Name	Molecular Weight (G/Mol)	Retention Time	Type
1	(-)-Gallocatechin-3-O-gallate	459.001	1.344	$[M+H]^+$
2	1.2-Didehydromiltirone	280.992	1.148	$[M+H]^+$
3	2'.4'.6'.3.4-Pentahydroxychalcone	287.002	1.190	$[M-H]^-$
4	2-Aminophenol	110.008	1.169	$[M+H]^+$
5	3-Amino-1.2.4-triazole	85.0096	15.519	$[M+H]^+$
6	3-Aminopropionitrile	71.006	15.540	$[M+H]^+$
7	4-Methylpyrazole	83.0058	15.540	$[M+H]^+$
8	Acrylate	71.006	15.540	$[M-H]^-$
9	Aminophylline	178.995	1.365	$[M-H]^-$
10	Apiopaeonoside	459.001	1.344	$[M-H]^-$
11	Chloratranol	185.008	1.633	$[M-H]^-$
12	Cimetidine	253.005	1.303	$[M+H]^+$
13	Creatinine	112.004	15.469	$[M-H]^-$
14	Cytosine arabinoside	241.998	1.148	$[M-H]^-$
15	Dihydrostreptomycin	541.995	1.344	$[M+H]^+$
16	Fisetin	287.002	1.190	$[M+H]^+$
17	Glabrol	391.001	7.961	$[M-H]^-$
18	Glucuronate	192.998	1.211	$[M-H]^-$
19	Hispaglabridin B	391.001	7.961	$[M+H]^+$
20	Homoeriodictyol	192.998	1.211	$[M+H]^+$
21	Hypericin	503.006	6.266	$[M-H]^-$
22	Isonicotinamide	123.007	4.332	$[M+H]^+$

23	Lucidenic acid A	457.009	1.303	$[M-H]^-$
24	Man2XylManGlcNAc2	1192.01	17.803	$[M+H]^+$
25	Mefenamate	241.998	1.148	$[M+H]^+$
26	Orcinol	123.007	4.332	$[M-H]^-$
27	Peonidin 3-(6''-acetyl)glucoside	505.01	5.801	$[M]^+$
28	Vindoline	457.009	1.303	$[M+H]^+$
29	Lobeline A	338.210	5.760	$[M+H]^+$

Table 2. Molecule formula and ontology of *Hippobroma longiflora* leaves

No	Metabolite Name	Molecule Formula	Ontology	Ref.
1	(-)-Gallocatechin-3-O-gallate	$C_{22}H_{18}O_{11}$	Phenolics	NCBI
2	Cyclofenil diphenol	$C_{19}H_{20}O_2$	Diterpenes	NCBI
3	2'.4'.6'.3.4-Pentahydroxychalcone	$C_{15}H_{12}O_6$	Phenol	NCBI
4	2-Aminophenol	C_6H_7NO	Anilines	NCBI
5	3-Amino-1.2.4-triazole	$C_2H_4N_4$	Aromatic Amin	NCBI
6	3-Aminopropionitrile	$C_3H_6N_2$	Aliphatic nitrile	NCBI
7	4-Methylpyrazole	$C_4H_6N_2$	Diazole	NCBI
8	Acrylate	$C_3H_3O_2^-$	Carboxylic acids	NCBI
9	Aminophylline	$C_{16}H_{24}N_{10}O_4$	Xanthine	NCBI
10	Apiopaeonoside	$C_{20}H_{28}O_{12}$	Glycoside	NCBI
11	Chloratranol	$C_8H_7C_lO_3$	Phenol	NCBI
12	Cimetidine	$C_{10}H_{16}N_6S$	Diazole	NCBI
13	Creatinine	$C_4H_7N_3O$	Imidazolidines	NCBI
14	Cytosine arabinoside	$C_9H_{13}N_3O_5$	Pyrimidines	NCBI
15	Dihydrostreptomycin	$C_{21}H_{41}N_7O_{12}$	Amino cyclitol glycoside	NCBI
16	Fisetin	$C_{15}H_{10}O_6$	Hydroxyflavone	NCBI
17	Glabrol	$C_{25}H_{28}O_4$	Flavans	NCBI
18	Glucuronate	$C_6H_{10}O_7$	Glucurinic acid	NCBI
19	Hispaglabridin B	$C_{25}H_{26}O_4$	Benzopyrans	NCBI
20	Homoeriodictyol	$C_{16}H_{14}O_6$	Hydroxyflavanone	NCBI
21	Hypericin	$C_{30}H_{16}O_8$	Carbocyclic	NCBI
22	Isonicotinamide	$C_6H_6N_2O$	Pyridines	NCBI
23	Lucidenic acid A	$C_{27}H_{38}O_6$	Terpenoid	NCBI
24	Man2XylManGlcNAc2	$C_{39}H_{66}N_2O_{30}$	Amino hexasaccharide	NCBI
25	Mefenamate	$C_{15}H_{15}NO_2$	Aromatic amino acid	NCBI
26	Orcinol	$C_7H_8O_2$	Phenol (Benzenediols)	NCBI
27	Peonidin 3-(6''-acetyl)glucoside	$C_{24}H_{25}O_{12}$	Flavonoid glycosides	NCBI
28	Vindoline	$C_{25}H_{32}N_2O_6$	Vinca alkaloids	NCBI
29	Lobeline A	$C_{22}H_{27}NO_2$	Alkyl-phenyl ketones	NCBI

Biological Activities of Hippobroba longiflora Leaf Compounds

Plant metabolites have biological activities that can benefit or harm human beings. Severalcompounds in a single plant exert diverse physical exercises. However, medicinal plants often display various metabolites and their content in every organ, giving different effects in the therapeutic uses. It has been reported that Hippobroma longiflora has effects as an antioxidant [17], antibacterial [18], anticancer [11], and antiglaucoma [19]. The pharmacological activities of each compound found in the leaf extract of this species are presented in Table 3.

In a study conducted by Martiningsihet al.[20], it was shown that Hippobroma longiflora leaf extract in ethanol demonstrated a high antioxidant activity attributed to 2-aminophenol. This metabolite is a base compound for forming antioxidant molecules [21]. This study revealed the same compound and some other compounds were (-)-gallocatechin-3-O-gallate and fisetin, which are classified as phenolics functioning well as antioxidants [22]. Also, hispglabridin B and homoeriodictyol were known for their antioxidant activity. In addition, Also, hispglabridin B and homoeriodictyol were known for their antioxidant activity. In addition, several compounds were found to have antibacterial activity, including acrylate, glabrol, and hispaglabridin B. Hippobroma longiflora is also reported to have substances with anticancer/antitumor activity. They are represented by compounds 2',4',6',3,4- entahydroxychalcone, fisetin, hypericin, homoeriodictyol, and vindoline. Many compounds having potential as anticancer contained in this plant render developing this species as an alternative medicine for anticancer.Further research is going to be a great challenge to do. This study discovered other compounds with different medical activities, such as anti-Alzheimer, anti-diabetic, and anti-inflammatory. It proves that Hippobroma longiflora has excellent potential as a traditional medicine.

Table 3. Biological activities of the active compounds in*Hippobroma longiflora* leaves

No	Metabolite Name	Biology Activity
1	(-)-Gallocatechin-3-O-gallate	Antioxidants [22]. Antiviral [23]. proteosome inhibition [24]. Immunebooster [25]
2	Cyclofenil diphenol	Alzheimer disease. Cholinesterase Inhibition [26].
3	2'.4'.6'.3.4-Pentahydroxychalcone	Antitumor activity [27]
4	2-Aminophenol	Antioxidants [28]
5	3-Amino-1.2.4-triazole	Carcinogenic activity [29]
6	3-Aminopropionitrile	Thoracic aortic dissection [30]. Antiviral [31].Peyronie's disease [32]
7	4-Methylpyrazole	Acute kidney injury [33]. Gastroprotection [34]. Antidotum methanol/ethylene [35]
8	Acrylate	Hemolytic Activity [36]. Antibacterial [37]
9	Aminophylline	Analogs for ischemic [38] . Acute asthma [39]
10	Apiopaeonoside	No activity
11	Chloratranol	No activity
12	Cimetidine	Anticolinergic [40]
13	Creatinine	No activity
14	Cytosine arabinoside	Acute myeloid leukemia [41]
15	Dihydrostreptomycin	Antibiotic [42]
16	Fisetin	Anticarcinogenic. Antioxidant [43]
17	Glabrol	Antibacterial [44]
18	Glucuronate	No activity

19	Hispaglabridin B	Antimicrobial [45]. Antioxidant [46]
20	Homoeriodictyol	Antioxidant. Antitumor. Antidiabetic [27]
21	Hypericin	Anticancer [47]
22	Isonicotinamide	No activity
23	Lucidenic acid A	Cytotoxic activity [48]
24	Man2XylManGlcNAc2	No activity
25	Mefenamate	Anti-inflamatory [49]
26	Orcinol	Anxiolityc [50]
27	Peonidin 3-(6"-acetyl)glucoside	Parkinsons deaseas [51]
28	Vindoline	Antitumor [52]
29	Lobeline A	Drug addiction [53]

CONCLUSION

There were 29 main compounds identified in Hippobroma longiflora leaves, extracted in 96% ethanol. The leaves phytochemical profile demonstrated several compounds, namely phenolics, terpenoids, and alkaloids. Most identified compounds have pharmacological activities such as antioxidants, antibacterial, antiviral, and anticancer/antitumor. Therefore. Hippobroma longiflora leaves have great potential as medicinal plants.

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