

Phytochemical analysis and antioxidant activity from *Phanera semibifida* stem and leaves extracts using LC-MS/MS

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Abstract. Suhendra M, Juliandi B, Darusman HS, Sadiah S, Fitmawati, Budiarti S, Rianti P. 2025. *Phytochemical analysis and antioxidant activity from Phanera semibifida stem and leaves extracts using LC-MS/MS. Biodiversitas* 26: 500-508. *Phanera semibifida*, a forest plant traditionally used by the Lingga Malay ethnic group for its bioactive metabolites, was the subject of this study. We investigated the phytochemical profile and antioxidant activity of *P. semibifida* stem and leaf extracts. Our process involved extracting samples with 96% ethanol, analyzing them using LC-MS/MS, and assessing their antioxidant potential with the DPPH assay. The stem extract revealed 26 compounds, while the leaf extract contained 24, including carboxylic acids, flavonoids, phenolics, polyphenols, terpenoids, tricarboxylic acids, and humulonoids, with carboxylic acids being the most prevalent. Noteworthy compounds, such as quercetin-3'-glucuronide and glucogallin, were found exclusively in the stem, while salicylic acid was unique to the leaf. This study has identified many secondary metabolites, including supplements, medicines, cosmetics, and fitness products. Other compounds with diverse biological activities, including antiviral, antimicrobial, antitumor, anti-inflammatory, and antidepressant properties, were also found in the *Bauhinia* genus. The stem extract demonstrated the highest antioxidant activity (IC₅₀: 9.09 ppm), while the leaf extract had IC₅₀: 19.38 ppm. That is significant finding that underscores the potential of *P. semibifida* for therapeutic applications due to its flavonoid content and the unique compounds it contains. In conclusion, these findings not only contribute to our understanding of the antioxidant capacity of *P. semibifida* but also pave the way for future research in this area, inspiring further exploration and discovery.

Keywords: Antioxidant, flavonoid, LC-MS/MS analysis, *Phanera semibifida*, phytochemical

INTRODUCTION

Plants are indispensable to human life, serving as essential resources for food, agriculture, cosmetics, and healthcare. In biodiversity-rich regions, traditional societies have extensively utilized plants for their medicinal properties, which are largely attributed to secondary metabolites. In biodiversity-rich regions, the extensive utilization of plants for their medicinal properties by traditional societies is a testament to their profound knowledge and understanding of nature. This knowledge has led to the discovery of secondary metabolites, including polyphenols such as flavonoids, phenolic acids, lignans, and stilbenes, which play a pivotal role in promoting health (Abbas et al. 2017). Polyphenols are particularly valued for their potent antioxidant activity, which helps neutralize free radicals, mitigate oxidative stress, and protect cells from damage (Oroian and Escriche 2015). These functions are crucial in preventing degenerative diseases such as cancer, diabetes, and cardiovascular disorders (Moga et al. 2016). Additionally, flavonoids and phenolic acids exhibit a wide range of pharmacological effects, including antimicrobial, anti-inflammatory, anticancer, and antimutagenic activities,

highlighting their pharmaceutical potential (Ahmed et al. 2016). Globally, there is an increasing demand for plant-derived bioactive compounds to address emerging health challenges, including antimicrobial resistance and the rise of chronic degenerative diseases. Research on phytochemicals offers promising opportunities for discovering novel therapeutic agents that are sustainable and eco-friendly. However, many plant species with significant medicinal potential remain underexplored, especially those used in traditional practices in biodiversity hotspots like Indonesia.

Indonesia, one of the world's most biodiverse countries, is home to approximately 25,000 plant species, many of which are used in traditional medicine (Nursanty et al. 2023). One notable plant is *Phanera semibifida* (Roxb.) Benth., a liana species native to tropical and subtropical forests at altitudes of up to 2,000 meters above sea level. Commonly known for its butterfly-shaped leaves, this plant belongs to the Leguminosae family, which comprises around 300 species distributed across Southeast Asia, including Malaysia, Myanmar, the Philippines, Borneo, Sulawesi, and Sumatra (Fitmawati et al. 2022). Recognized under the Plants of the World Online (POWO) with *Bauhinia semibifida* as its basionym, *P. semibifida* holds

significant therapeutic potential. The Malay Lingga community uses this plant as the main ingredient in *ramuan obat pahit*, a traditional herbal formulation believed to enhance vitality, promote longevity, and improve overall well-being (Hazimi et al. 2018). Despite its widespread traditional use, scientific studies on the phytochemical profile and biological activities of *P. semibifida* remain limited. However, the medicinal application of *P. semibifida* is often combined with other plants, making it difficult to determine the specific effects of this plant when used in single doses. Previous research on methanol extracts of this species identified bioactive compounds using NMR analysis, demonstrating its significant potential for therapeutic applications. Additionally, studies on related species within the *Bauhinia* genus have revealed significant pharmacological properties, such as antioxidant and anti-inflammatory effects, underscoring the need for further investigation into the bioactive compounds of *P. semibifida* (Fitmawati et al. 2017).

Secondary metabolite profiling is a fundamental step in elucidating the pharmacological properties of medicinal plants. Techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) are commonly used for this purpose. While GC-MS is suitable for identifying volatile compounds, LC-MS is particularly effective for analyzing non-volatile and heat-sensitive compounds such as polyphenols (Gao et al. 2016). LC-MS provides detailed molecular data, including structural information, molecular formulas, and weights, which can be matched against chemical databases for accurate compound identification. This method has been successfully applied in many studies to characterize the phytochemical composition of medicinal plants, providing valuable insights into their bioactive

constituents and potential applications (Syarpin et al. 2023; Burhan et al. 2024).

Indonesia's rich biodiversity, which is often referred to as a global hotspot, still needs to be explored for its phytochemical potential. *P. semibifida*, a species with significant traditional medicinal use, represents an example of a plant with untapped pharmaceutical potential. Understanding its phytochemical profile not only sheds light on its biological properties but also highlights the importance of conserving such species for future generations. This study specifically aims to: (i) identify and quantify the secondary metabolites of *P. semibifida* using LC-MS/MS, (ii) evaluate its antioxidant activity using the DPPH assay, and (iii) provide a scientific basis for its traditional use and potential pharmaceutical applications. The findings of this research are expected to provide valuable insights into the potential applications of *P. semibifida* in pharmaceutical and nutraceutical industries, as well as provide information regarding sustainable harvesting and conservation strategies for long-term utilization.

MATERIALS AND METHODS

Plant material

P. semibifida samples were collected in Resun village, Lingga Island, Riau Province, Indonesia, on July 2023 (Figure 1). The plant was identified at botanical characterization laboratories, National Research and Innovation Agency (BRIN), with the voucher number B-3073/II.6.2/IR.01.02/8/2024. The leaf and stem samples were separated, followed by a sorting and drying process.

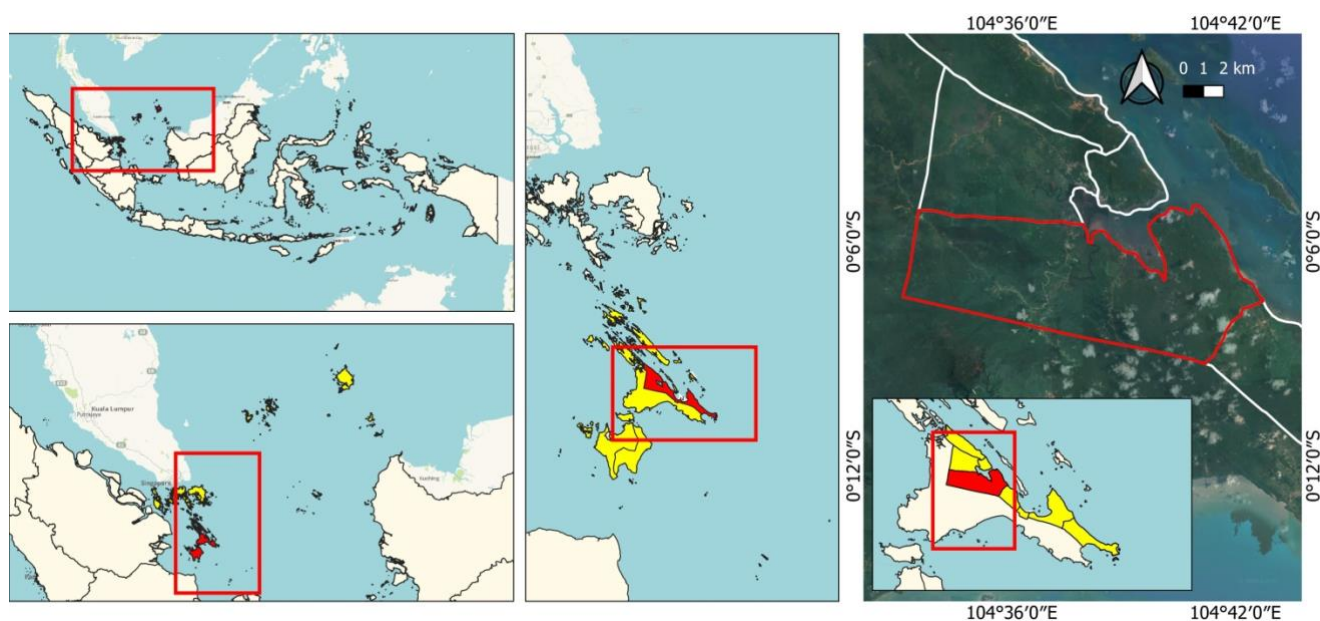


Figure 1. Location of plant collection at Resun Village, North Lingga sub-district, Lingga, Riau Province, Indonesia

Sample preparation

The leaf and stem samples were washed under running water. Wet sorting was carried out to select the best sample. Next, the samples were cut and continued with the drying process. Leaves were dried at room temperature and away from light. Stems were dried under the sunlight. Dried samples were ground into a coarse powder using a dry grinder, then extracted using the maceration method: 96% ethanol (ratio 1:10) for 24 hours at room temperature. Next, the extract was filtered through Whatman filter paper No 2. The samples were then re-macerated in the same solution for 24 hours. After that, they were filtered again using the Whatman No. 2 filter paper. The entire filtrate was subsequently concentrated using a rotary vacuum evaporator at a temperature of 40°C.

Phytochemical analysis

This analysis was conducted in the botany laboratory of the Faculty of Mathematics and Natural Sciences, Riau University. This test was carried out to determine alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids (Sangi et al. 2008).

Identification of secondary metabolites by LC-MS/MS

The identification of secondary metabolites in *kangkang katup* extract was a comprehensive process conducted with thoroughness and attention to detail using LC-MS/MS at the Advanced Laboratory of Bogor Agricultural University, Indonesia. The extract was first filtered using a 0.2 µm PTFE membrane prior to analysis. The LC-MS/MS analysis utilized a UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS from ThermoScientific, and the separation was performed on an Accucore C18 column (100×2.1 mm, 1.5 µm, ThermoScientific). The mobile phase consisted of water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The following gradient elution program was employed: 0-1 min (5% B), 1-25 min (5-95% B), 25-28 min (95% B), and 28-33 min (5% B). The flow rate was maintained at 0.2 mL/min, with the column temperature set to 30°C, and an injection volume of 2.0 µL was carefully controlled. Mass spectra were recorded over a mass-to-charge ratio (m/z)

range of 100-1,500 in negative ionization mode. The Compound Discoverer 3.2 database was used for the qualitative identification of chemical constituents in the extract, and quantitative analysis was based on the peak area of the identified compounds.

Antioxidant activity testing with 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant testing using 2,2-diphenyl-1-picrylhydrazyl (DPPH) was conducted at the Central Laboratory for Biopharmaceutical Studies, Bogor Agricultural University. The assay was performed using the 96-well method on a microplate and measured using a microplate reader. A DPPH stock solution was prepared at a concentration of 10,000 ppm by dissolving 2.50 mg of DPPH in ethanol PA up to a final volume of 50 mL. For the positive control, ascorbic acid was prepared by dissolving 10 mg in 1 mL of DMSO to a concentration of 10,000 ppm. The vitamin C solution was then diluted to concentrations of 100, 20, 10, 5, and 2.5 ppm. Both the DPPH and ascorbic acid solutions were freshly prepared and used on the same day, ensuring the highest level of accuracy.

Next, 10 mg of *P. semibifida* stem and leaf extract were dissolved in 1 mL of DMSO to achieve a concentration of 10,000 ppm. The extract was then meticulously diluted with ethanol PA to final concentrations of 100, 25, 12.5, 6.25, 3.125, and 1.56 ppm. Each sample was analyzed in triplicate. Sample, control, and standard solutions (200 µL) were added, as depicted in Table 1. The solutions were mixed, covered, and allowed to react in the dark for 30 min, after which the absorbance at 517 nm was read.

Calculation of the percentage of inhibition

The DPPH free radical inhibition was calculated using the formula (Zhang et al. 2018):

$$\% \text{ inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100\%$$

Where:

A control: The absorbance of solution without sample

A sample: The absorbance of the sample

Table 1. Template for 96 well assay for DPPH scavenging capacity

	1	2	3	4	5	6	7	8	9	10	11	12
A	DK1.1	DK1.2	DK1.3	DK1K-	K+1.1	K+1.2	K+1.3	K+1.Et	BK1.1	BK1.2	BK1.3	BK1K-
B	DK2.1	DK2.2	DK2.3	DK2K-	K+2.1	K+2.2	K+2.3	K+2.Et	BK2.1	BK2.2	BK2.3	BK2K-
C	DK3.1	DK3.2	DK3.3	DK3K-	K+3.1	K+3.2	K+3.3	K+3.Et	BK3.1	BK3.2	BK3.3	BK3K-
D	DK4.1	DK4.2	DK4.3	DK4K-	K+4.1	K+4.2	K+4.3	K+4.Et	BK4.1	BK4.2	BK4.3	BK4K-
E	DK5.1	DK5.2	DK5.3	DK5K-	K+5.1	K+5.2	K+5.3	K+5.Et	BK5.1	BK5.2	BK5.3	BK5K-
F	DK6.1	DK6.2	DK6.3	DK6K-	K+6.1	K+6.2	K+6.3	K+6.Et	BK6.1	BK6.2	BK6.3	BK6K-
G	DK7.1	DK7.2	DK7.3	DK7K-	K+7.1	K+7.2	K+7.3	K+7.Et	BK7.1	BK7.2	BK7.3	BK7K-
H	BLNK	BLNK	BLNK	BLNK	BLNK	BLNK	BLNK	BLNK	BLNK	BLNK	BLNK	BLNK

Notes: Rows A-G represent the sample concentrations. "D" is the sample code for leaves, and "DK" refers to the leaf sample at concentration 1, with each sample injected three times (DK1.1, DK1.2, DK1.3). "B" is the sample code for stems, and "BK1" refers to stem samples at concentration 1, with replicates 1-3 (BK1.1, BK1.2, BK1.3). DK(1-7)K- represents the negative control for leaf samples (sample + ethanol), while BK(1-7)K- represents the negative control for stem samples (sample + ethanol). "BLNK" is the blank control (DPPH + ethanol). "K+" is a positive control (ascorbic acid), with replicates 1-3 and seven concentration levels

The ability of fraction as DPPH free radical scavenger

This potential was expressed as IC_{50} . The IC_{50} value is the concentration needed to scavenge half of the free radicals. The IC_{50} value was calculated from the linear regression equation $Y = AX + B$ by plotting the concentration of the test solution as the abscissa (X-axis) and the percent inhibition of DPPH as parameters of antioxidant activity as the ordinate (Y-axis). Therefore, in determining the IC_{50} for the sample, the Y value is 50, and the X value obtained is the IC_{50} value. Categories of the antioxidant activity based on IC_{50} value are very strong, strong, moderate, and weak, with values <50, 50-100, 101-150, and 151-200 ppm, respectively (Haerani et al. 2019).

RESULTS AND DISCUSSION

Phytochemical screening of ethanolic stem and leaf extract *P. semibifida*

The stem extract is dark brown, and the leaf extract is green, with a scent similar to herbal medicine. The stem extract is obtained at a concentration of 10.05% on a dry weight basis, with a water correction factor of 5.27%, and the extract from the sample is calculated. Meanwhile, the leaf extract is determined to be 8.89% on a dry weight basis, with a water correction factor of 45%.

The screening of phytochemical content in *P. semibifida* was conducted using the Sangi et al. (2008) method. Overall, the phytochemical composition of the stem and leaves shows no substantial variation (Table 2). However, tannins were more abundant in the stem, as shown by the darker coloration. In contrast, saponin levels were higher in the leaves compared to the stem. This result was thus obtained by Hazimi et al. (2018), who found that the stems of *kangkang katup* in *ramuan obat pahit* detected flavonoids, terpenoids, saponins, and tannins. In similar studies, these compound groups have been utilized for various purposes, including healthcare. Flavonoids can act as antioxidants, antivirals, antimicrobials, and antidiabetics. In a previous study, Flavonoid dan phenolic *Bauhinia strychnifolia* has an antidiabetic effect (Praparatana et al. 2022). Saponin of the aqueous extract *Bauhinia purpurea* leaf has an antiulcer effect (Zakaria et al. 2016), and tannin has an antioxidant effect (Aryantini 2021). Moreover, another compound,

terpenoid, has analgesic, anti-inflammatory, and antitumor effects.

Identification of secondary metabolites stem and leaf ethanolic extract by LC-MS/MS

The identification of secondary metabolite compounds in the ethanol extract was performed using LC-MS/MS analysis. The chromatograms of each sample were analyzed to identify their secondary metabolites (Vinaixa et al. 2016; Yunita et al. 2022). Figure 2 shows the chromatogram of the stem and leaf extract. Differences in the LC/MS chromatograms indicated different chemical compounds. Each peak in the chromatogram has its retention time and molecular weight. In this research, Chromatogram profiles showed a variation in retention time between 0.95 and 32 minutes.

Based on LC-MS/MS analysis (Table 3), 26 compounds were obtained in stems using 96% ethanol solvent. Meanwhile, 24 compounds were obtained in the leaf extract. All compounds were detected using the negative ion. Seven group compounds were discovered: carboxylic acid, flavonoid, phenolics, polyphenol, Tricarboxylic acid, and Humulonoid with 10, 10, 4, 1, 1, and 1 compound, respectively. Previous research shows these group compounds have pharmacological effects like antioxidants, antimicrobials, and antidiabetic. *Bauhinia*'s genera, such as *Bauhinia pulchella*, have antioxidant activities from the carboxylic acid compounds (Monteiro et al. 2022). Furthermore, flavonoid and phenolic compounds from these species also have antioxidants and antidiabetic activity (Farag et al. 2015; Haiyul et al. 2021).

Table 2. Phytochemical screening of *Phanera semibifida* (Sangi et al. 2008)

Group of compounds	Stem	Leaves
Alkaloid	-	-
Flavonoid	+	+
Tanin	++	+
Saponin	+	++
Steroid	-	-
Terpenoid	++	+

Notes: positive symbols (+) are indication of bioactive compounds and the negative (-) are no indication of bioactive compounds

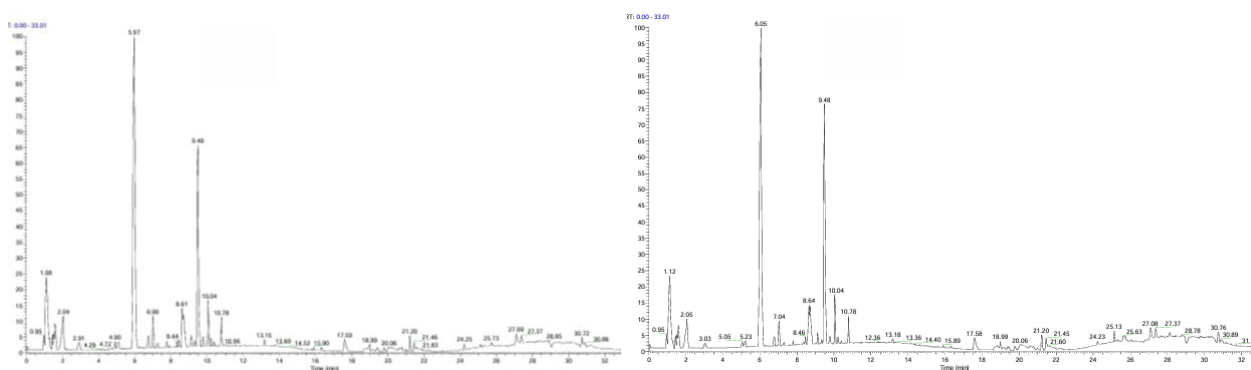


Figure 2. Chromatogram of LCMS/MS. A. Stems, B. Leaves extract ethanol of *Phanera semibifida*

Table 3. Identification of phytochemical compounds in *Phanera semibifida* stems and leaves extract by using LC-MS/MS

Compound name	Molecular formula	Stems		Leaves		Group of compounds	Biological activity
		Retention time (RT) (minutes)	Molecular weight (MW)	Retention time (RT) (minutes)	Molecular weight (MW)		
Quercitrin	C ₂₁ H ₂₀ O ₁₁	9.47	448.11	9.48	448.11	Flavonoid	Antioxidants and antidiabetic (Sharma et al. 2021)
L-(+)-Tartaric acid	C ₄ H ₆ O ₆	1.11	150.02	1.13	150.02	Carboxylic acid	Antimicrobial (Babayan et al. 2020)
Gallic acid	C ₇ H ₆ O ₅	2.01	170.02	2.04	170.	Phenolics acid	Antimicrobial (Buchmann et al. 2022)
DL-Malic acid	C ₄ H ₆ O ₅	1.15	134.02	1.17	134.02	Carboxylic acid	Antimicrobial (Borah et al. 2023)
Ellagic acid	C ₁₄ H ₆ O ₈	8.70	302.01	8.71	302.01	Polyphenol	antioxidant, anti-inflammatory, antimutagenic, and antiproliferative (Sharifi-Rad et al. 2022)
Catalposide	C ₂₂ H ₂₆ O ₁₂	10.04	428.14	10.06	482.14	Flavonoid	Antioxidants and Anticancer (Saracoglu and Harput 2012)
Isoquercetin	C ₂₁ H ₂₀ O ₁₂	8.64	464.09	8.64	464.09	Flavonoid	Anti-inflammatory (Flores-Sánchez et al. 2019), Antidiabetic and Antioxidant (Och et al. 2023)
Phloridzin	C ₂₁ H ₂₄ O ₁₀	10.05	436.14	10.05	436.14	Flavonoid	Antidiabetic (Khanam et al. 2022), Anti-neuro inflammatory (Prabhakar et al. 2018)
1-o-galloylglycerol	C ₁₀ H ₁₂ O ₇	1.95	244.06	1.98	244.06	Phenolics	Antioxidants (Zhang and Akoh 2020)
Citric acid	C ₆ H ₈ O ₇	1.14	192.03	1.16	192.03	Tricarboxylic acid	Antimicrobial (Burel et al. 2021)
Catechin gallate	C ₂₂ H ₁₈ O ₁₀	8.78	442.09	8.79	442.09	Flavonoid	Antiinflammatory, Antimicrobial (Buchmann et al. 2022), Antioxidants (Xu et al. 2021)
Nictoflorin	C ₂₇ H ₃₀ O ₁₅	9.12	594.158	9.13	594.16	Flavonoid	Antioxidants, Neuroprotective, and Hepatoprotective (Zhao et al. 2017)
Quercetin-3'-glucuronide	C ₂₁ H ₁₈ O ₁₃	8.46	478.07	-	-	Flavonoid	Antioxidants and Antitumor (Wu et al. 2018), Antidiabetic (Bule et al. 2019)
Traumatic Acid	C ₁₂ H ₂₀ O ₄	13.17	228.14	13.18	228.14	Carboxylic acid	Antioxidants (Jabłońska-Trypuć et al. 2016), Anticancer (Jabłońska-Trypuć et al. 2019)
Apigetrin	C ₂₁ H ₂₀ O ₁₀	10.22	432.11	-	-	Flavonoid	Anticancer (Guo et al. 2020; Bhosale et al. 2022), Antiinflammatory and antioxidants (Hadrach and Sayadi 2018)
Cianidanol	C ₁₅ H ₁₄ O ₆	6.05	290.08	6.3	290.07	Flavonoid	Anticancer and antiinflammatory (Belmehdi et al. 2023)
Rutin	C ₂₇ H ₃₀ O ₁₆	8.47	610.16	8.48	610.15	Flavonoid	Antioxidants and antidiabetic (Osman et al. 2021)
13S-hydroxyoctadecadienoic acid	C ₁₈ H ₃₂ O ₃	20.05	296.24	20.04	296.23	Carboxylic acid	Antitumor (An et al. 2015)
Juniperic acid	C ₁₆ H ₃₂ O ₃	24.23	272.24	24.23	272.23	Carboxylic acid	Antibacterial (Florenly et al. 2022)
alpha-ketoadipic acid	C ₆ H ₈ O ₅	1.2	160.04	1.54	160.04	Carboxylic acid	Antioxidants (da Silva et al. 2017)
(10E,15Z)-9,12,13-Trihydroxy-10,15 octadecadienoic acid	C ₁₈ H ₃₂ O ₅	12.45	328.23	12.46	328.22	Carboxylic acid	Antioxidants (DellaGreca et al. 2009)
Glucosheptonic Acid	C ₇ H ₁₄ O ₈	1.04	226.07	1.06	226.07	Carboxylic acid	Prebiotics and antimicrobials (Wojciechowska et al. 2020)
Cynarine	C ₂₅ H ₂₄ O ₁₂	10.39	516.13	10.4	516.13	Carboxylic acid	Antioxidants and antibacterial (Thang et al. 2022), Antidepressant (Murlanova et al. 2022), Antiviral (Mamat et al. 2018)
Melilotoside	C ₁₅ H ₁₈ O ₈	6.17	326.11	6.24	362.10	Carboxylic acid	Antiprotzoal (Atay et al. 2016)
Lupulone	C ₂₆ H ₃₈ O ₄	20.77	414.28	20.78	414.28	Humulonoid	Antimicrobial (Cardenas and Çiçek 2023)
Glucogallin	C ₁₃ H ₁₆ O ₁₀	1.63	332.08	-	-	Phenolics	Antidiabetic (Majeed et al. 2022)
Salicylic acid	C ₇ H ₆ O ₃	-	-	10.08	138.04	Phenolics	Antifungal (Neto et al. 2015)

While the stem and leaf extracts share many compounds, the presence of unique compounds in each is particularly intriguing. However, Quercetin-3'-glucuronide, Apigenin, and Glucogallin compounds are only found in stem extract. Salicylic acid is only found in leaf extract. Quercetin-3'-glucuronide and Apigenin were in the flavonoid group compounds, while Glucogallin is exclusive to the stem extract, and Salicylic acid is only found in the phenolic group. This result showed more compounds in stem extract than in leaf extract. Previous research has reported similar compounds in *Bauhinia* genus, such as phlorizin in *Bauhinia semibifida* from Bangkirai Hill, Samarinda, East Kalimantan, Indonesia (Tanjung and Tjahjandarie 2014), quercitrin at *Bauhinia pulla* (Dej-Adisai et al. 2021), catechin and rutin and catechin and rutin in the stem bark of *Bauhinia vahlii* (Narayan et al. 2012).

Plant secondary metabolites, such as those found in *P. semibifida* and *Bauhinia* species, provide a multitude of benefits in human life, including supplements, medicines, cosmetics, and fitness products. *P. semibifida*, for instance, has long-standing tradition of use as an immunomodulatory supplement (Fitmawati et al. 2022) and also exhibits antibacterial properties (Roza et al. 2019). Research by Farag et al. (2015) revealed that five of eight *Bauhinia* species possess antioxidant and antidiabetic activities, largely attributed to flavonoid derivatives such as quercitrin. Gallic acid compounds found in *Bauhinia strychnifolia* have demonstrated antidiabetic effects (Praparatana et al. 2022), while *Bauhinia hookeri* contains ellagic acid, which exhibits hepatoprotective and nephroprotective activities (Al-Sayed et al. 2015). *Bauhinia variegata* also possesses antioxidant and antidiabetic properties attributed to isoquercetin compounds (Abdel-Halim et al. 2020). This study has identified other compounds with diverse biological activities, including antiviral, antimicrobial, antitumor, anti-inflammatory, and antidepressant properties, in addition to the compounds already identified in the *Bauhinia* genus. These findings provide foundational knowledge on *P. semibifida* from the Lingga Islands, further supporting its potential as a traditional medicine. However, further research is essential to overcome the significant challenges associated with its therapeutic applications.

Antioxidant activity of *P. semibifida* ethanolic stem and leaf extract

The DPPH-scavenging capacity is a measure of the sample's antioxidant ability. In this research, DPPH, a stable free radical, is reduced and loses its violet, turning yellow in the presence of oxidizing chemicals. The color loss is proportional to the reducing agent's ability to donate an electron to nitrogen electrons and is determined as absorbance at 517 nm. It is important that the measurement be performed when the reaction between antioxidant and DPPH is finished as shown by no further change in the absorption of the solution (stable).

Phanera semibifida is recognized to have biological activity throughout the plant. In this research, the stem and leaf extracts of *P. semibifida* inhibited free radicals, as evidenced by high percent inhibition values (Figure 3). DPPH radical scavenging activity differed between the

stem and leaf extract at various concentrations. The stem extract showed higher DPPH inhibition capacity than the leaf extract, with inhibition rates of 94.44% and 90.63%, respectively.

The antioxidant activity of *P. semibifida* is measured to assess its potential to block free radicals. The IC₅₀ method for calculating antioxidant activity is simple, sensitive, rapid, and reproducible. This makes it the most convenient and extensively used radical scavenging approach for determining the antioxidant potential of compounds and herbal extracts. Ascorbic acid is utilized as a positive control in this experiment. The metric used is the 50% inhibition concentration (IC₅₀). The IC₅₀ value is calculated using linear regression equation based on the concentrations of *P. semibifida* stem and leaf extracts and their inhibitory activity (%). A lower IC₅₀ value implies stronger antioxidant activity (Table 4).

The IC₅₀ values for ascorbic acid (positive control), ethanol extract of the stem, and ethanol extract of the leaves were 5.86 ppm, 9.09 ppm, and 19.38 ppm. When compared to the antioxidant capacity of other medicinal plants, as reported by (Gulcin and Alwasel 2023), *P. semibifida* has a higher IC₅₀. Based on these findings, both samples' antioxidant capacity falls into the "very strong" category. These findings are also consistent with prior research, in which the IC₅₀ values for methanol extracts of *P. semibifida* roots, stems, and leaves from West Sumatra were categorized as "very strong," with the leaf extract having greater values than the others (Fitmawati et al. 2022).

Table 4. The results of the linear regression equation and IC₅₀ for stems and leaves extract of *Phanera semibifida*

Sample	Regression equation	IC ₅₀ (ppm)	Category
AA	$Y=6.85X+9.82$	5.87	Very strong
Stems	$Y=5.29X+1.87$	9.09	Very strong
Leaves	$Y=2.31X+5.24$	19.38	Very strong

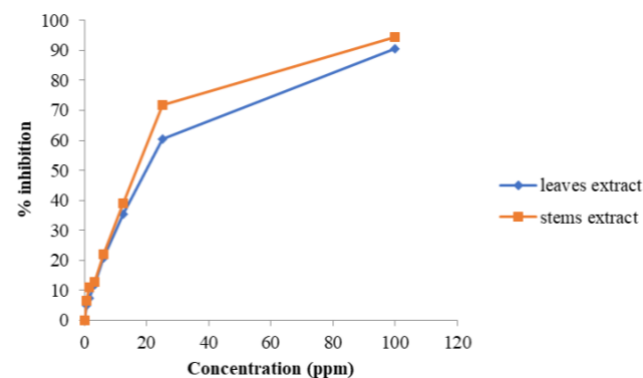


Figure 3. DPPH free radical scavenging activity of *Phanera semibifida* stem and leaf extract

However, in this study, the IC₅₀ value of the stem extract was higher than that of the leaf extract. This difference may be attributed to the variation in sampling locations, as different environmental conditions can influence the secondary metabolite content in plants (Goh et al. 2016). In this study, the variation in antioxidant capacity between the stem and leaf extracts of *P. semibifida* aligns with the phytochemical identification results obtained through LCMS-MS analysis. The presence of polyphenols has a significant impact on antioxidant capacity. Polyphenols are classified into two groups: flavonoids and phenolics (Abbas et al. 2017). In this study, the stem extract contained more flavonoids than the leaf extract, which could partially explain its increased antioxidant potential. These findings are consistent with previous research on *Luvunga sarmentosa*, which found that extracts with higher amounts of bioactive compounds have better antioxidant activity (Syarpin et al. 2023).

In conclusion, our investigation discovered 26 main chemicals in the ethanolic extracts of *P. semibifida* stems and leaves. The in vitro experiment revealed that the extracts had antioxidant activity, as shown by their capacity to block DPPH. According to prior research, the discovered compounds have a number of pharmacological actions, including antioxidant, antibacterial, antiviral, and anticancer/antitumor characteristics. Therefore, *P. semibifida* has significant potential as a medicinal plant and might be used in a variety of sectors, including medicines and healthcare.

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