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Antioxidant Activity, Total Phenolic, Total Flavonoid and Vitamin C Contents of *Hornstedtia havilandii* (K. Schum.) K. Schum. from Sabah

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ABSTRACT

Hornstedtia is a genus within the Zingiberaceae family, known for its therapeutic properties. However, *Hornstedtia havilandii*, native to Sabah, has received comparatively limited research attention. Therefore, this study aims to conduct phytochemical evaluation and antioxidant activity of *H. havilandii* from Sabah. In this study, water was employed as a solvent, and the maceration method was employed to extract the bioactive compounds from the dried fruits, leaves, and stems of *H. havilandii*. The total flavonoid content (TFC), total phenolic content (TPC), and vitamin C content were quantified using calibration standard curves ranging from 5 to 160 mg/mL and colorimetric titration method respectively. Antioxidant activity expressed as IC_{50}, was determined by conducting diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. The maceration of extract revealed the highest yield in fruits at 10.87%, followed by leaves at 9.03% and stems at 7.98%. Notably, the aqueous fruit extract exhibited significant antioxidant activity with an IC_{50} of 3.31 ± 0.09 µg/mL, surpassing the aqueous leaves extract at IC_{50} of 16.86 ± 0.27 µg/mL and showing lower activity in the aqueous stem extract with IC_{50} of 310.46 ± 0.27 µg/mL. Due to the notable antioxidant activity in the fruit extracts, further quantification of TPC and TFC of the aqueous fruit extract was conducted. The TPC of the fruit extract is 16.17 ± 0.00120 mgGAE/g, and the TFC is 8.54 ± 0.00137 mgRE/g. Given the importance of vitamin C as a nutrient influencing antioxidant activity, quantification revealed that the aqueous fruit extract contained 162.47 ± 0.70 mg/100mL of vitamin C. These findings indicate that *H. havilandii* holds untapped potential as a locally available and cost-effective source of the natural antioxidant agent. Nonetheless, further research is warranted to identify additional natural components and assess their overall efficacy as natural antioxidants.

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INTRODUCTION

In Sabah, approximately 26% of all known Borneon *Zingiberaceae* species serve various purposes, including medicinal, culinary, ornamental, and construction applications [1]. Among the commonly utilized ginger varieties by the locals in Sabah are *Etlingera*, *Alpinia,* and *Zingiber* [2]. The Dusun people in Sabah have a longstanding tradition of consuming various Hornstedtia species, each identified by distinct local names corresponding to different ethnicities, including Telidus, Talirus, Senggang, Taridus, and Tapus. The fruit of *H. havilandii* is a staple in their diet, consumed either raw or cooked. The sweet and sour taste of the fruit is believed to possess fever-curing properties [1].

Studies on various plants, including members from various genera within the Zingiberaceae family, have demonstrated the biological action of their essential oils [3]. However, investigations specifically focused on the genus *Hornstedtia* must be more extensive [4]. Previous research has highlighted phenylpropanoid as the principal chemical compound in the *Hornstedtia* genus, providing insights into its potential antioxidative properties [5]. Furthermore, phytochemical screening of secondary metabolites, such as flavonoids, phenols, and tannins, in *H. havilandii* fruit extract underscores its potential antioxidant properties [6]. Given the growing societal interest in natural and nutraceutical products, exploring the antioxidant potential of *H. havilandii* becomes relevant, as it could offer various health benefits. Additionally, the selection of *H. havilandii* as a study subject aims to raise awareness about its existence, discouraging indiscriminate destruction of the plant.

Therefore, this study aims to conduct a bioprospecting of *Hornstedtia havilandii* from Sabah as a potentially biologically functional food that serves as a natural antioxidant source. This study aims to investigate the antioxidative properties of *H. havilandii* aqueous fruit extract and quantify the key phytochemical constituents, notably phenolic compounds, flavonoids, and vitamin C content, within the extract. To the best of our knowledge, this is the first study to determine the total antioxidant activity and phenolic content of the fruit, leaves, and stems of *H. havilandii* using an aqueous solvent.

EXPERIMENTAL

Materials

The stem leaves and fruit of *Hornstedtia havilandii* were collected from Kampung Pulutan Kiang, Kota Marudu, Sabah, Malaysia, and botanically authenticated by a Sabah Park research officer. Voucher specimens of the plant (SNP 05977) were deposited at the Sabah National Park Herbarium.

Drying and extraction of *Hornstedtia havilandii*

The stems, leaves, and fruits were thoroughly cleaned with running water to eliminate any impurities, followed by sterilization using a 1% sodium hypochlorite solution. Subsequently, the samples were air-dried for three weeks until a constant weight was achieved. The dried stems, leaves, and fruits were then finely ground into a powder, preserving it in air-tight containers until required for subsequent use. The powder was extracted using the maceration method, involving individual soaking of the samples in an aqueous solvent with a ratio of 1:10 for 72 h [7]. The resulting mixture underwent filtration using Whatman No 1 filter paper and was further concentrated. The resulting crude extracts were stored at 4°C until further analysis. The extraction yield for each extract was calculated using Equation 1.

$$\% \text{ Yield of extraction} = \frac{\text{Weight extract (g)}}{\text{Weight dried plant material (g)}} \quad (1)$$

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Antioxidant Activity Testing by Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Assay

The method was carried out as described by [8] with minor modifications. Various extract concentrations were prepared through serial dilution ranging from 0.625 µg/mL to 10 µg/mL. In each test tube, 1.5 mL of the respective extracts were pipetted, followed by adding 1.5 mL of freshly prepared DPPH solution (25 mg/L) in methanol. The mixtures were then thoroughly mixed and incubated for 30 minutes. After the incubation period, the absorbance was measured at 517 nm using a UV-visible spectrophotometer. The capability to scavenge the DPPH radical was calculated using Equation 2. The DPPH solution and ethanol mixture were used as blank, whereas ascorbic acid at the concentration of 0.1 µg/mL was employed as the positive control.

$$\text{DPPH Scavenged (\%)} = \{(Ac - At)/Ac\} \times 100 \quad (2)$$

Where Ac represents the absorbance of the control reaction and at represents the absorbance in the presence of the extracted sample. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} was calculated and determined using the linear equation obtained from the DPPH scavenging assay graph. Values were presented as Mean ± Standard Error Measurement (SEM).

Determination of Total Flavonoids Content

The total flavonoid content of plant extract was determined using the calorimetric assay of [9]. Specifically, 1 mL of the plant extract was added to 300 µL aluminum chloride (10%). The resulting mixture was incubated at room temperature for 5 min before adding 2 mL of 1 mol/L sodium hydroxide. Subsequently, the volume of the reaction mixture was adjusted to 10 mL with distilled water immediately and thoroughly vortexed. The absorbance of the mixture was measured at 510 nm. A standard curve ranging from 5 – 160 mg/mL was prepared using various concentrations of rutin. The total flavonoid content was expressed as milligrams of rutin equivalents per gram dry weight sample (mg RE/g DW) of the extract.

Determination of Total Phenolics Content

Quantifying total phenolic content in the crude extracts followed the Folin-Ciocalteu procedure [4]. In this process, 20 to 100 µL of the tested samples were introduced into test tubes and combined with 1.0 mL of Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate. Following thorough mixing, the tubes were allowed to stand for 30 minutes, and the absorption at 765 nm was measured using a UV-visible spectrophotometer. The total phenolic content was then expressed as Gallic acid equivalents (GAE) in milligrams per gram of the extract's dry weight sample (mg GAE/g DW).

Determination of Vitamin C Content

The determination of Vitamin C employed the redox reaction method [10]. In this methodology, iodine underwent reduction to iodide ions during titration, while ascorbic acid underwent oxidation, forming dehydroascorbic [10]. The iodine solution was prepared by weighing 1.3 g of solid iodine and added with 100 mL of distilled water. The mixture was gently swirled while heating at 20ºC using a hotplate. Once the iodine dissolved, the solution was transferred into a 1 L volumetric flask [11].

A 0.25 g of starch powder was dissolved into 50 mL of distilled water, and the mixture was heated to facilitate dissolution [11]. The titration of the sample solution was carried out against the iodine solution. Before commencing, the titration proceeded until an endpoint was reached, signified by observing a dark blue or grey color in the sample solution [10]. The quantification of vitamin C content followed the method

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outlined by [10], and the amount of vitamin C expressed in mg/100mL was calculated using Equation 3, considering the relative molecular mass (MW) of ascorbic acid, which is 176.12.

$$\text{Mass Vitamin C (g)} = \text{M juice vitamin C} \times \text{V Juice (1)} \times \text{MW vitamin C} \quad (3)$$

Statistical Analysis

The experimental results were expressed as Mean ± Standard Error Measurement (SEM). The determination of IC_{50} was determined using Microsoft Excel.

RESULTS AND DISCUSSION

Yield Of Extraction (%)

The *H. havilandii* samples were extracted using the maceration method, and the resulting extraction yields are presented in Fig. 1. Aqueous extraction was performed on three distinct plant parts, which are the fruits, leaves, and stems. The variation in extract yields among the different plant parts (fruit, leaves, and stems) is attributed to the distinct nature and quantity of the secondary metabolites extracted. Generally, the fruit aqueous extract demonstrated a higher yield, indicative of the solubility of the polar compounds of the secondary metabolites. Additionally, aqueous extraction was employed due to its availability and non-toxic properties, making it suitable for public use. Aqueous extraction is a promising method for extracting bioactive compounds from plants, as water is an economical, secure, and readily available solvent [12]. Also, water-based herbal preparations such as teas, infusions, and decoctions are one of the best ways to extract the health benefits of herbs, as water will extract all the properties of the plant [13]. Therefore, water extraction methods have several advantages, including being cost-effective, ecologically friendly, and safe for public use.

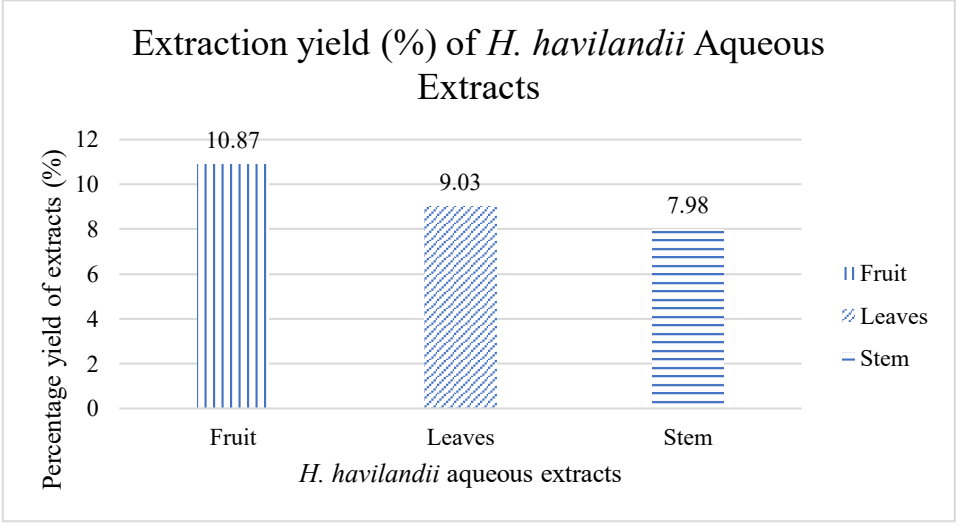

Fig. 1. Yield of Aqueous Extract (%) of *H. havilandii* fruits, leaves, and stems.

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Antioxidant activity

The in vitro antioxidant assay of *H. havilandii* aqueous plant extracts was quantified by the IC_{50} (µg/mL) value. IC_{50} represents the half-maximal inhibitory concentration, measuring the efficacy of a compound or extract in inhibiting a biological or biochemical function. An IC_{50} value below 50 µg/mL indicates high or strong antioxidant activity. In comparison, a value ranging from 70 µg/mL to 100 µg/mL is considered moderate, and a value exceeding 100 µg/mL indicates low or weak antioxidant activity [14]. The study revealed that all extracts exhibited antioxidant activity. As shown in Table 1, it is evident that the aqueous fruit extract exhibited the most potent antioxidant activity with the lowest IC_{50} values of 3.31 ± 0.09 µg/mL, signifying high efficacy in inhibiting oxidative processes. The aqueous leaf extract also demonstrated notable antioxidant activity but to a lesser extent than the fruit extract, as indicated by a slightly higher IC_{50} value of 16.86 ± 0.27 µg/mL. In contrast, the aqueous stem extract displayed the weakest antioxidant activity among the plant parts, with a substantially higher IC_{50} value (310.46 ± 0.45 µg/mL). Ascorbic acid, used as a positive control, exhibited an intermediate level of antioxidant activity with and IC_{50} value of 14.72 ± 0.00 µg/mL, falling between the fruit and leaves extracts.

Table 1. Antioxidant activity of *H. havilandii* fruit, stem, and leaves aqueous extracts.

Solvent extraction	Plant part	% DPPH Radical Scavenging	IC_{50} (µg/mL)[1]	Antioxidant activity
Aqueous	Fruit	65.28 ± 0.00	3.31 ± 0.09	Strong
	Leaves	73.59 ± 0.01	16.86 ± 0.27[a]	Strong
	Stem	82.66 ± 0.00	310.46 ± 0.45	Weak
Ascorbic acid[2]	ASC	90.77 ± 0.00	14.72 ± 0.00[a]	Strong (standard)

Note: [1]Values were IC_{50} (µg/mL) ± SEM; [2]Positive control/Standard; ND (No Data); mean values within a row with no statistically significant difference between extract $P > 0.05$.

The Hornstedtia genus generally exhibits an overall richness in antioxidant activities, as seen in Table 1. Markedly, the stem has lower antioxidant activity compared to other plant parts. The fruits stand out as having the most antioxidant activity, even though the aqueous extract of the leaves and fruits had considerable antioxidant activity. Consequently, the need for further investigation into the antioxidant properties of *H. havilandii* becomes apparent.

Table 2. Comparison of antioxidant activity o*f Hornstedtia* species and other plants

Solvent extraction	Plant / Plant part	IC_{50} (µg/mL)[1]	Antioxidant activity	References
Aqueous	*H. havilandii* / Fruit	3.31 ± 0.09	Strong	(Present study)
Aqueous	*H. havilandii* / Leaves	16.86 ± 0.27[a]	Strong	(Present study)
Aqueous	*H. havilandii* / Stem	310.46 ± 0.45	Weak	(Present study)
n-hexane	*H. leonurus / rhizome*	59.60 ± 0.69	Strong	[15]
methanol	*H. scyphifera / leaves*	35.33 ± 0.210	Strong	[15]
hexane	*H. scyphifera/leaves*	63.4 ± 0.11	Strong	[15]
n-hexane	*H. leonurus/stem*	122.67 ± 0.58	Weak	[15]
methanol	*H. leonurus/stem*	239.63 ± 0.64	Weak	[15]

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Table 2 compares the antioxidant activity of *H. havilandii* with that of other Hornstedtia species, further highlights the robust antioxidant activity *H. havilandii* aqueous fruit extract. The hierarchy of decreasing antioxidant activity is as follows: *H. havilandii* (aqueous fruit extract) > *H. havilandii* (aqueous leaves extract) > *H. scyphifera (methanol leaves extract)* > *H. leonurus (n-hexane rhizome extract)* > *H. scyphifera (hexane leaves* extract) > *H. leonurus (methanol stem extract)* > *H. leonurus (methanol stem* extract) > *H. havilandii* (aqueous stem extract). Importantly, this high antioxidant activity is observed in an aqueous extract, where water is used as the solvent. This is significant as water is a safe and readily available solvent compared to other chemical solvents [16]. Therefore, using water solvent to extract antioxidants from *H. havilandii* makes it a promising and accessible source of natural antioxidants. The high antioxidant activity observed in the aqueous fruit extract of *H. havilandii* is attributed to bioactive compounds recognized for their antioxidant properties. These include flavonoids, phenols, tannins, saponins, and terpenoids [6]. The presence of these bioactive compounds in *H. havilandii* aligns with its potential to serve as a functional food with natural antioxidant properties, as demonstrated by the high antioxidant activity of the aqueous fruit extract.

Total Flavonoid Content (TFC) and Total Phenolic Content (TPC)

Assessing plant extracts' total phenolic and flavonoid content is essential for elucidating their antioxidant potential. Phenolic and flavonoid compounds, classified as plant secondary metabolites, feature an aromatic ring bearing at least one hydroxyl group, rendering them influential electron donors directly contributing to antioxidant action [17]. Numerous studies have consistently reported a positive linear correlation between total phenolic and flavonoid content and antioxidant capacity [17]. Given the robust antioxidant activity exhibited by the aqueous fruit extract of *H. havilandii*, it becomes imperative to analyze total phenolic and flavonoid content to determine the antioxidant potential of the fruit comprehensively.

The total flavonoid content was quantified by conducting the aluminum chloride method. In this method, a stable complex with a carbonyl group at C4, as well as the hydroxyl groups in C3 (flavonols) and C5 flavonols and flavones) will be formed by aluminum chloride. Moreover, aluminum chloride induces the formation of labile acid with hydroxyls in the ortho site in B rings of flavonoids that enable the quantification of flavonoids in extracts [18]. The total flavonoid content of *H. havilandii* fruit extracts was expressed as milligram rutin equivalent per gram dry weight of the sample, and therefore, a standard calibration curve was formed as shown in Fig. 2. The resulting regression equation ($y = 0.0192x + 0.16$; $R^2 = 0.9768$) was utilized for the calculation of total flavonoid content in each extract.

The total phenolic content was quantified by conducting the Folin-Ciocalteu method. In this method, the Folin-Ciocalteu method was an electron transfer-based assay that gave reducing capacity, expressed as phenolic content [19]. The total phenolic content of *H. havilandii* fruit extracts was expressed as milligram gallic acid equivalent per gram dry weight of the sample, and therefore a standard calibration curve was formed as shown in Fig. 3 to obtain the regression equation ($y = 0.0132x + 0.2668$; $R^2 = 0.9609$) required to calculate the total phenolic content of each extract.

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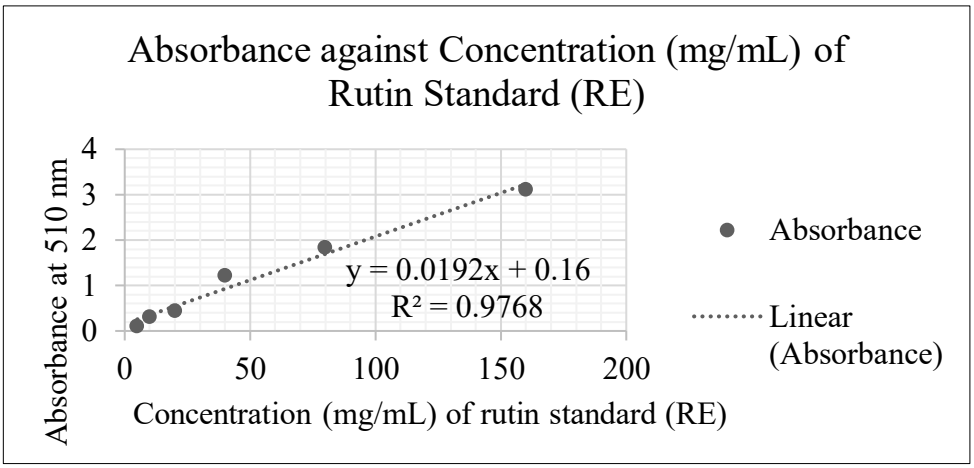

Fig. 2. Absorbance against concentration (mg/mL) of rutin (RE) standard calibration curve for the quantification of TFC.

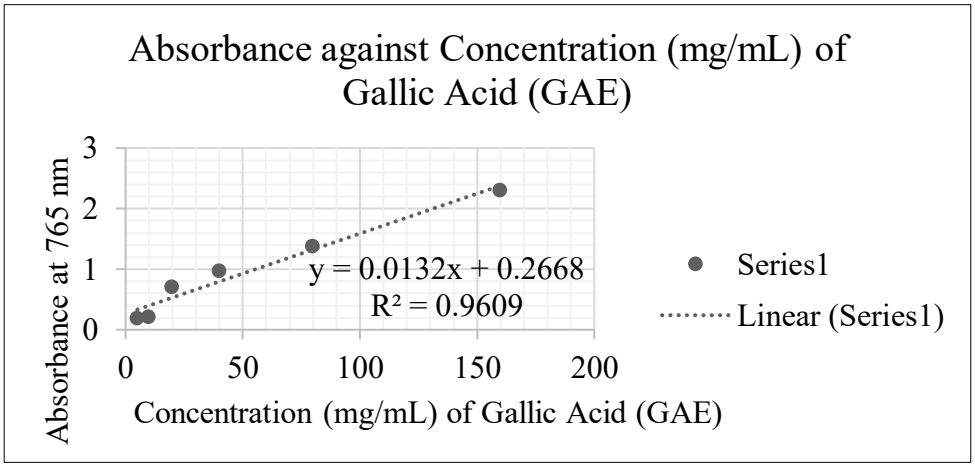

Fig. 3. Absorbance against concentration (mg/mL) of gallic acid (GAE) standard calibration curve for the quantification of TPC.

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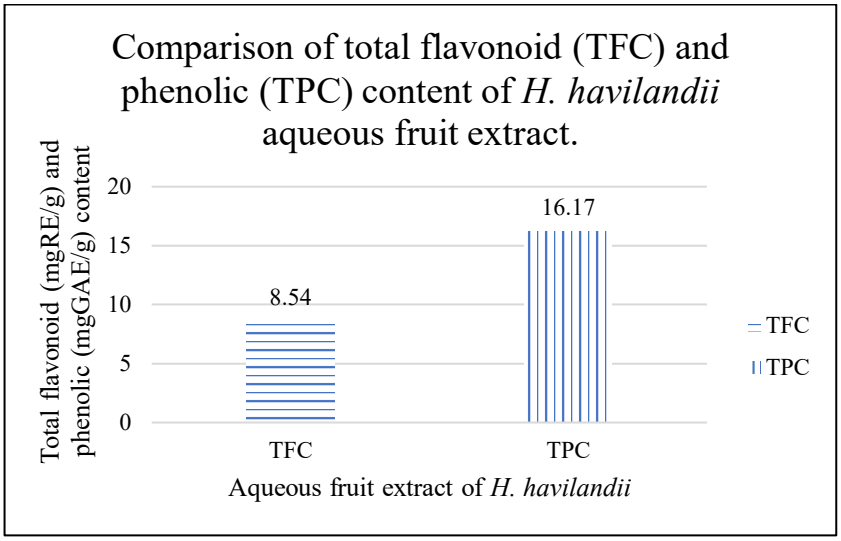

Fig. 4. Absorbance against concentration (mg/mL) of gallic acid (GAE) standard calibration curve for the quantification of TPC.

Referring to Fig. 4, it is evident that the TPC value for the aqueous fruit extract (16.17 ± 0.00025 mgGAE/gDW) surpassed the TFC value (8.54 ± 0.00137 mgRE/gDW). These results suggest that most phenolic compounds in *H. havilandii* fruit extracts are polar. The total flavonoid and phenolic content of *H. havilandii* fruit extracts obtained in this study were then compared with other common plants in Table 3.

Table 3. Total flavonoid content (mg/g) and total phenolic content (mg/g) comparison between *H. havilandii* and other common plants

Extracts	**Total flavonoid content (mg/g)**	**References**
E. elatior aqueous leaves (Daun Kantan)	12.30 ± 0.1000	[20]
H. havilandii aqueous fruit	8.54 ± 0.0014	(present study)
Nephelium lappaceum L. aqueous fruit (Rambutan)	0.015 ± 0.04	[21]
Garcinia mangostana L. aqueous fruit *(Mangosteen)*	0.019 ± 0.03	[21]
Litchi chinensis Sonn. aqueous fruit (Litchi)	0.002 ± 0.01	[21]
	Total phenolic content (mg/g)	
H. havilandii aqueous fruit	16.17 ± 0.0003	(present study)
C. longa aqueous leaves (Daun Kurma)	3.80 ± 0.1000	[22]
Nephelium lappaceum L. aqueous fruit (Rambutan)	0.12 ± 0.0100	[21]
Garcinia mangostana L. aqueous fruit *(Mangosteen)*	0.05 ± 0.1000	[21]
Litchi chinensis Sonn. aqueous fruit (Litchi)	0.19 ± 0.0100	[21]

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The total phenolic content (TPC) and total flavonoid content (TFC) are important parameters used to assess the antioxidant potential and bioactive compound content of plant extracts [12]. The TPC and TFC values can vary widely among different plant species, as demonstrated in studies on medicinal plants, leafy vegetables, and fruit crops [2,21,22]. As shown in Table 3, there is variability in total phenolic and flavonoid content among different plants. Specifically, the TPC and TFC in aqueous extracts of certain fruits are relatively low. However, it is noteworthy that the aqueous fruit extracts of *H. havilandii* exhibited a relatively high level of both TPC and TFC. Therefore, *H. havilandii* aqueous fruit has the highest total phenolic content among the listed samples, followed by *C. longa* aqueous leaves extract, *Litchi chinensis* Sonn. aqueous fruit extract, *Nephelium lappaceum* L. aqueous fruit extract and lastly, *Garcinia mangostana* L. aqueous fruit extract. Regarding the TFC, E. *elatior* aqueous leaf extract has the highest flavonoid content among the listed samples, followed by *H. havilandii* aqueous fruit extract, *Garcinia mangostana* L. aqueous fruit extract, *Nephelium lappaceum* L. aqueous fruit extract and *Litchi chinensis* Sonn. aqueous fruit extract.

Vitamin C content

The antioxidant property of vitamin C is known to aid in preventing the progression of several illnesses, including diabetes, cancer, heart disease, and neurological disorders [22]. The vitamin C content of *H. havilandii* fruit extract was expressed as milligrams per 100 milliliters from 20 milliliters of vitamin C titrated sample. This indicates the potential of *H. havilandii* as a source of vitamin C and its associated antioxidant properties. Studies have shown that bioactive compounds, such as polyphenols and vitamin C, are crucial in reducing oxidative stress and protecting against various diseases. Additionally, the antioxidant effects of vitamin C have been evaluated compared to standard vitamin C concentration, highlighting its significant role in enhancing the antioxidant activity of plant extracts. Therefore, the presence of vitamin C in *H. havilandii* fruit extracts underscores its potential as a natural source of antioxidants, contributing to its beneficial properties.

Table 4. Vitamin C content (mg/100mL) comparison between *H. havilandii* and other underutilized and selected common fruit.

Extracts	Vitamin C content (mg/100mL)	References
P. emblica fruit	529.60 ± 57.5	[23]
A. marmelos fruit	516.60 ± 0.5	[23]
A. occidentale fruit	202.30 ± 2.9	[23]
H. havilandii aqueous fruit	162.47 ± 0.70	(present study)
Orange fruit	107.1 ± 0.00	[24]
Mandarin fruit	63 ± 0.00	[24]
Pomegranate fruit	51.5 ± 0.00	[24]
Lemon	51.33 ± 0.00	[25]
Lime	34.12 ± 0.00	[25]

Table 4 reveals that the vitamin C content in the aqueous fruit extract of *H. havilandii* was measured at 162.47 ± 0.70 mg/100mL. Upon comparison with other commonly known fruits, such as orange, mandarin, pomegranate, lemon, and lime, the vitamin C content of *H. havilandii* aqueous fruit

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extract is found to be higher, followed by orange, mandarin, pomegranate, lemon, and lime fruit. However, when compared with underutilized fruits such as *P. emblica* (529.60 ± 57.5 mg/100 mL), *A. occidentale* (202.30 ± 2.9 mg/100 mL), and *A. marmelos* (516.60 ± 0.5 mg/100 mL), *H. havilandii* fruits had a lower vitamin C content. More specifically, *H. havilandii* fruits contained 162.47 ± 0.70mg/100 mL of vitamin C, significantly less than *P. emblica*, *A. occidentale*, and *A. marmelos*. Despite this, the substantial vitamin C content in *H. havilandii* fruits underscores their potential as a valuable antioxidant agent, offering benefits in combating severe diseases such as cancers and heart disease. Although the observed vitamin C levels in *H. havilandii* fruits, as consumed by the native people of Sabah, were deemed relatively high, these levels were within the tolerable upper intake level for various age groups, as recommended by [14]. The recommended minimum vitamin C intake for children, adolescents, adults, and pregnant and lactating women was 35 mg/day, 65 mg/day, 70 mg/day, 80 mg/day, and 95 mg/day, respectively.

CONCLUSION

The aqueous fruit extracts of *H. havilandii* exhibited a total flavonoid content of 8.54 ± 0.0014 mgRE/g and a total phenolic content of 16.17 ± 0.0003 mgGAE/g. Additionally, the vitamin C content of *H. havilandii* aqueous fruit extract measured 162.47 ± 0.70 mg/100mL. Remarkably, the aqueous fruit extract exhibited the highest antioxidant activity with an IC_{50} value of 3.31 ± 0.09 µg/mL, followed by the aqueous leaves extract with an IC_{50} value of 16.86 ± 0.27 µg/mL) and lastly, the aqueous stem extract with an IC_{50} value of 310.46 ± 0.45 µg/mL. Consequently, the phytochemical analysis and antioxidant assessments of *H. havilandii* aqueous extracts in this study suggest the potential of this underutilized plant, mainly the fruit, as a viable and cost-effective source for developing biological functional foods enriched with natural antioxidants. Nevertheless, further research is imperative to identify additional natural components and evaluate the overall efficacy of *H. havilandii* as a natural antioxidant.

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AUTHOR'S CONTRIBUTION

NM conducted the research, LCW spearheaded the research efforts, FJ guided the phytochemical and antioxidant analysis, and FJ and ES conducted thorough preparation and editing of the manuscript. All authors contributed to the writing and revision of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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