Research Article

# Phytochemical screening and antioxidant activity of Tuhau (*Etlingera coccinea*) rhizome and leaf

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**ABSTRACT**

The indigenous people of Borneo have historically utilized the wild ginger *Etlingera coccinea*, locally known as Tuhau, for its medicinal properties. However, most of its medicinal properties are yet to be investigated. Therefore, this study aims to assess the phytochemical profile and antioxidant capacity of Tuhau leaf and rhizome. Phytochemical screening was conducted to assess the presence of flavonoids, glycosides, saponins, proteins and amino acids, tannins, and steroids. Additionally, total phenolic and flavonoid contents were determined. The antioxidant potential of the extracts was evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3 ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays. Results revealed the presence of flavonoids, glycosides, saponins, proteins and amino acids, tannins, and steroids in the leaf extract. Furthermore, the leaf extract exhibited higher total phenolic (61.57 ± 0.29 mg GAE/g dry extract) and flavonoid contents (30.32 ± 0.42 mg CE/g dry extract) compared to the rhizome extract (33.57 ± 0.29 mg GAE/g dry extract and 9.38 ± 0.02 mg CE/g dry extract, respectively). Moreover, the leaf extract demonstrated significantly superior antioxidant activity (71.48 ± 0.53 %, 28.48 ± 0.14 %, and 2.31 ± 0.03 mM $Fe^{2+}$/g dry extract) compared to the rhizome extract in DPPH, ABTS, and FRAP assays, respectively. Consequently, Tuhau leaf exhibited promising potential to be utilized in the food industry.

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## 1. Introduction

Phytochemical screening and evaluation of antioxidant activity are crucial aspects of studying plant materials for potential food applications. The genus *Etlingera* has garnered attention due to its rich

phytochemical composition and potential antioxidant properties (Joseph, 2023). Studies on related Zingiberaceae plants have yielded promising results in phytochemical screenings and antioxidant activities (Ramadanil *et al.,* 2019; Rachkeeree *et al.,* 2018). For instance, the rhizomes of Zingiberaceae plants like *Zingiber officinale var. rubrum* have been explored for essential oils, while *Etlingera elatior* leaves exhibited high phenolic content and radical scavenging activity (Ramadanil *et al*., 2019; Rachkeeree *et al*., 2018). Additionally, research on *Etlingera elatior* grown in different locations has demonstrated variations in secondary metabolite production and antioxidant activities, emphasizing the impact of environmental factors on plant chemistry and medicinal properties (Ghasemzadeh *et al.,* 2015).

Antioxidant activity is one of the primary focus areas in medicinal plant research, as evidenced by studies on ginseng leaves and *Zingiber zerumbet*, where changes in phytochemical levels were found to influence antioxidant properties (Zhang *et al.,* 2014; Ghasemzadeh *et al.,* 2016). Additionally, the correlation between phytochemical content and antioxidant capacity has been emphasized in various studies, highlighting the importance of phytochemical screenings in understanding the antioxidant potential of plant materials (Rachkeeree *et al.,* 2020). The phytochemical diversity of ginger species encompasses compounds such as flavonoids, tannins, and polyphenols, which contribute to their antioxidant activities (Riyanti *et al.,* 2022). *Etlingera coccinea*, wild ginger abundantly found in Borneo including the state of Sabah, is locally known as 'Tuhau'. Tuhau, commonly consumed as salad and pickles is also utilized as a traditional remedy for stomachache, food poisoning and gastric problems (Abang Zamhari and Yong, 2021). Although Tuhau rhizome is a common ingredient in cooking and dishes among Borneo communities, not many studies have been conducted, contributing to the lack of information about this unique traditional food. Therefore, investigating the phytochemical profiles and antioxidant activities of Tuhau, particularly its rhizome and leaf components, holds significant promise for potential applications in the food industry. By building upon existing knowledge from related plant species within the Zingiberaceae family, this research aims to contribute valuable insights into its phytochemical composition and antioxidant potential, aiding in its utilization as a functional food ingredient.

## 2. Materials and Methods

### *2.1 Material and sample extraction*

Fresh Tuhau plants were purchased from a local seller in Kampung Melangkap, Kota Belud. The samples were sent to a botanist for identification. The leaf and rhizome were washed, sliced, and dried using a cabinet dryer until the moisture content of 10%. The samples were ground to obtain uniform particles. Two (2) grams of the powder were weighed and mixed with 80% (v/v) methanol. The mixture was left overnight in a cabinet at room temperature before being centrifuged at 3000 rpm for 15 minutes to obtain a good separation. The supernatant layer was collected by filtering with Whatman filter paper into new centrifuge tubes and subjected to a rotary evaporator to remove the solvent. The yield of the extraction was calculated.

### *2.2 Phytochemical screening*

The rhizome and leaf methanolic extracts were qualitatively tested for flavonoids, glycosides, saponins, proteins and amino acids, tannins and glycosides. Results are expressed as (+) to indicate the presence and (-) for the absence of phytochemicals.

#### *2.2.1 Flavonoid*

The presence of flavonoids was determined following the method by Rajasudha and Mannikandan (2019). One (1) mL of extract was treated with a few drops of sodium hydroxide solution. The resulting yellow color extract was then observed for any color changes upon the addition of diluted hydrochloric acid. A colorless solution indicated the presence of flavonoids in the sample.

### *2.2.2 Glycosides*

The presence of glycosides was assessed according to Rajasudha and Mannikandan (2019). For the presence of cardiac glycosides, the Kellar-Killiani test was conducted. Two milliliters (2 mL) of methanol extracts were mixed with 1 mL of glacial acetic acid, 1 mL of $FeCl_3$, and 1 mL of $H_2SO_4$.

The resulting mixture was observed for formation of bluish-green shade, which confirmed the presence of a glycoside cardiac. To detect the presence of anthranol glycosides, the Borntrager's test was performed. Five milliliters (5 mL) of ammonia solution (10%) were added to 2 mL of methanol extract. The formation of a pink color in the ammonia layer indicated the presence of anthranol glycosides.

### *2.2.3 Saponins*

The test for saponin was carried out according to Sharma *et al.* (2020). To screen for the presence of saponins, the extract was dissolved with 20 mL of distilled water and shaken for 15 minutes. The formation of a layer of foam of 1 cm indicated the presence of saponin.

### *2.2.4 Protein & amino acid*

Xanthoproteic test was carried out to screen for the presence of protein and amino acid (Rajasudha and Mannikandan, 2019). A few drops of concentrated nitric acid were added to the methanol extract. The color change to yellow indicated the presence of protein.

### *2.2.5 Tannin*

Ferric chloride test ($FeCl_3$) was done to screen for the presence of tannin (Sharma *et al.,* 2020). A few drops of 10% ferric chloride were added to 2 mL of methanol extract. The formation of blue color indicated the presence of gallic tannin, while green color indicated the presence of catechic tannin.

### *2.2.6 Steroids*

Salkowski test was carried out to analyze for the presence of plant steroids. The change in color to yellowish on the extract layer following the addition of 0.4 ml of chloroform ($CHCl_3$) followed by sulfuric acid ($H_2SO_4$) concentrate into 0.1 g of extract indicates the presence of steroids.

### *2.3 Total phenolic and flavonoid contents*

For the determination of total phenolic content (TPC), the gallic acid stock solution was prepared following a modified method from Phuyal *et al.* (2020). Specifically, 1 mg of gallic acid was dissolved in 1 mL of methanol. Subsequently, different concentrations of gallic acid solutions were prepared by serial dilution to obtain concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL. To determine the amount of phenolic content in the sample, the method described by Ahmed *et al.* (2019) was employed. First, the gallic acid or sample extract (1 mg/mL) was mixed with 2.25 mL of Folin-Ciocalteu reagent, 2.25 mL of 6% sodium carbonate ($Na_2CO_3$), and 3.16 mL of distilled water. The mixture was left to react for 90 minutes at room temperature in the dark. The absorbance was measured using a UV-Vis Spectrophotometer at 765 nm. The total phenolic content was calculated from the gallic acid standard calibration curve. The results are expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g dry extract).

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method from Ramlan *et al.* (2021). A standard stock solution of catechin was prepared by dissolving 1 mg of catechin in 1 mL of methanol. Different concentrations of catechin solution was prepared to obtain concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL. A total of 1 mL of each concentration of catechin was mixed with 1 mL of 2% aluminum chloride (w/v), and the mixture was incubated for 15

minutes at room temperature. The absorbance was measured using a UV-Vis spectrophotometer at 430 nm. The catechin standard calibration curve was obtained. For Tuhau samples, a total of 1 mL of sample extract (1 mg/mL) was mixed with 1 mL of aluminum chloride (2%) and left for 15 minutes at room temperature. The flavonoid content of samples were determined from the catechin standard curve. The results are expressed in milligrams of catechin equivalent per gram of dry extract (mg CE/g of dry extract).

### *2.4 Antioxidant assays*

#### *2.4.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay*

The free radical scavenging activity was assessed using the DPPH assay, following the method described by Ahmed *et al.* (2019). Firstly, 4 mg of DPPH was dissolved in methanol to a final volume of 100 mL to prepare the solution. Then, 1.5 mL of the sample extract (1 mg/mL) was mixed with 1.5 mL of 0.1 M DPPH solution. The mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was measured using UV-Vis spectrophotometer at 517 nm. In this study, ascorbic acid served as the standard reference. The results are expressed as % scavenging radical activity.

#### *2.4.2 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ) (ABTS)*

ABTS powder (96.02 mg) was dissolved in acetic acid buffer (pH 4.5) to a final volume of 25 mL. Similarly, a 2.45 mmol/L potassium persulfate ($K_2S_2O_8$) stock solution was prepared by dissolving 66.24 mg in acetic acid buffer (pH 4.5) to a final volume of 100 mL. Five (5) mL of the ABTS solution and 5 mL of the potassium persulfate solution were mixed and incubated at room temperature for 12 hours in the dark. Subsequently, 2.80 mL of the resulting solution was diluted with 60 mL of methanol until it reached an absorbance of 0.70 ± 0.01 at 734 nm. Following this, 1 mL of each sample extract was mixed with 2 mL ABTS solution and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 734 nm. Ascorbic acid served as the standard reference and results were expressed as % of ABTS radical inhibition.

#### *2.4.3 Ferric Ion Reducing Antioxidant Power (FRAP) assay*

A total of 80 mg of ferrous sulfate ($FeSO_4$) was dissolved in 100 mL of distilled water to create a stock solution. Solutions of varying concentrations (5, 2.5, 1.25, 0.625, 0.3125, and 0.1563 mM) were then prepared. The FRAP stock solution was prepared according to Ahmed et al. (2019). Firstly, 10 mmol/L TPTZ solution was prepared by dissolving 0.31 g of the powder in 100 mL of HCl. Additionally, 270.03 mg of ferric chloride ($FeCl_3$) was dissolved in 50 mL of water to achieve a concentration of 20 mmol/L. The FRAP stock solution was then prepared by mixing 25 mL of sodium acetate buffer (0.3 mmol/L), 2.5 mL of TPTZ, and 2.5 mL of $FeCl_3$ solution.

Next, 0.1 mL of each $FeSO_4$ solution was mixed with 2 mL of the FRAP solution and incubated in the dark at room temperature for 30 minutes. Absorbance was then measured at 593 nm using a UV-Vis spectrophotometer. For the sample extract, 0.1 mL was mixed with 2 mL of the FRAP solution and processed similarly. Ascorbic acid was used as the standard reference. The ferric ion-reducing antioxidant power was determined from the $FeSO_4$ standard calibration curve, with results expressed in milligrams of ferrous ions per gram of dry extract (mg $Fe^{2+}$/g extract).

### *2.5 Statistical Analysis*

The analyses were performed in triplicate. Statistical analysis utilized IBM Corp.'s SPSS Statistics version 28 software, employing one-way analysis of variance (ANOVA) and independent t-tests to assess mean differences and statistical significance. Pearson correlation analysis was conducted to examine the relationships between TPC, TFC, and the antioxidant activities of samples. A significance threshold of $P < 0.05$ was applied.

# 3. Results and Discussion

## *3.1 Extraction yield*

Tuhau leaves and rhizomes were extracted using methanol as solvent, employing the maceration method to ensure consistent results comparison. The extraction yields of the methanol extract from Tuhau leaves and rhizomes were 8.15 ± 1.32% and 5.85 ± 4.63%, respectively. The differences in extraction yield between the different parts of a plant species can be attributed to various factors. For instance, the bioactive composition of each specific plant part plays a crucial role. The occurrence of different chemical groups, such as aromatic amines, carboxylic acids, ketones, phenols, and alkyl halides, can vary between leaf and rhizome extracts (Konappa et al., 2020). Additionally, studies have demonstrated differences in bioactive compound distribution and antioxidant potential between the rhizome, leaf, and flower parts of the ginger plant (Tanweer *et al.,* 2020).

## *3.2 Phytochemical screening*

Evaluation of the phytochemical profile of the methanol extract of Tuhau leaf and rhizome revealed the presence of several phytochemicals, including flavonoids, glycosides, saponins, proteins and amino acids, tannins, and steroids, as shown in Table 1. Specifically, flavonoids, glycosides, saponins, proteins and amino acids, tannins, and steroids were identified in the leaf extract, whereas only flavonoids, cardiac glycosides, saponins, and steroids were detected in the rhizome extract.

**Table 1.** Phytochemical screening of Tuhau leaf and rhizome

| Phytochemicals | Leaf | Rhizome |
|---|---|---|
| Flavonoid | + | + |
| Cardiac Glycosides | + | + |
| Anthranol Glycosides | + | - |
| Saponin | + | + |
| Protein & amino acid | + | - |
| Tannin | + | - |
| Steroid | + | + |

Results are expressed as (+) to indicate the presence and (-) for the absence of phytochemicals.

Phytochemicals have garnered significant attention due to their molecular structures, which contain hydroxyl groups on aromatic rings. These structures are linked to their antioxidant functions, enabling them to scavenge oxidizing agents (Zengin *et al.*, 2015). Phytochemical screening is valuable not only for identifying the constituents present in plant extracts and determining the dominant ones but also for discovering bioactive agents that can be utilized in medicinal synthesis. A previous study on Tuhau from Brunei Darussalam assessed the presence of alkaloids, cardiac glycosides, steroids, saponins, and anthranol glycosides (Shahid-Ud-Daula *et al.,* 2015). The findings revealed the presence of cardiac glycosides, steroids, and saponins in the leaf extract, whereas only cardiac glycosides and steroids were detected in the rhizome extract. This aligns with the results of the present study, except for the presence of saponins. Interestingly, the current study observed a positive reaction for saponins in the Tuhau rhizome, indicating a deviation from the previous observations.

Flavonoids, which are phenolic compounds commonly found in plants, play pivotal roles as plant pigments. They encompass various subclasses such as flavonones, flavones, isoflavones, flavonols, and anthocyanins, each offering numerous health benefits. Flavonoids found in the leaves and rhizomes of

Tuhau exhibit biological activities including antioxidant, anti-inflammatory, and antimicrobial properties (Rachkeeree *et al.,* 2020). Cardiac glycosides, exclusively present in the leaves, are used in the treatment of cardiac arrhythmias and congestive heart failure. On the other hand, anthranol glycosides, secondary metabolites and glycoside derivatives, exhibit various other beneficial biological activities such as antioxidant, cytotoxic, antimicrobial, antifungal, antitumor, antidiabetic, and antiproliferative effects (Gul *et al.,* 2017).

Both leaves and rhizomes of Tuhau contain saponins, natural detergents known for their ability to bind cholesterol, forming insoluble complexes that prevent cholesterol reabsorption, thereby lowering blood cholesterol levels. Additionally, saponins exhibit anti-inflammatory, immune-stimulating, and antineoplastic properties (Rajasudha and Manikandan, 2019). Furthermore, the presence of steroids in Tuhau leaves and rhizomes provides benefits in treating hypertension due to their steroid cardiotonic properties (Shahid-Ud-Daula *et al.*, 2015). Finally, the presence of tannins in Tuhau leaves holds potential for medicinal applications, particularly in anticancer treatments, potentially owing to their high antioxidant properties (Ahmed *et al.,* 2019).

### *3.3 Total phenolic and flavonoid contents*

TPC and TFC of the Tuhau leaf and rhizome extracts exhibited significant differences ($p < 0.05$), as indicated in Table 2. Notably, the TPC and TFC of the Tuhau leaf extract were higher compared to those of the rhizome extract. Specifically, the TFC value of the Tuhau leaf and rhizome extracts were 30.32 ± 0.42 and 9.38 ± 0.02 mg CE/g dry extract, respectively ($p < 0.05$). Interestingly, the flavonoid content of the leaf extract in our study surpassed that reported in a previous investigation by Shahid-Ud-Daula *et al.* (2015), which recorded a value of only 8.68 ± 0.166 and 1.14 ± 0.104 mg QE/g dry extract for the leaf and rhizome, respectively. This discrepancy highlights significant differences in flavonoid values between the two studies. Differences in extraction techniques, geographical location, sample handling, storage and processing could explain the observed discrepancies in flavonoid content. The variance in flavonoid content between Tuhau leaves and rhizomes can also be elucidated by insights from Alafiatayo *et al.* (2014), who suggested that the production of flavonoid compounds is influenced by light exposure. Consequently, leaves, being exposed to light, tend to accumulate higher flavonoid content compared to rhizomes, which are typically shielded from light as they grow underground. These secondary metabolites accumulate in high constitutive levels within vacuoles of epidermal cells, protecting against UV radiation. Moreover, the positive impact of light on flavonoid biosynthesis has been observed across various plant species (Ni *et al.,* 2020).

**Table 2.** Total phenolic and flavonoid contents of Tuhau leaf and rhizome

| Analysis | Leaf | Rhizome |
|---|---|---|
| TPC (mg GAE/g extract) | 61.57 ± 0.29[a] | 33.57 ± 0.29[b] |
| TFC (mg CE/g extract) | 30.32 ± 0.42[a] | 9.38 ± 0.02[b] |

Values represent mean ± SD (n=3). Different letters (a and b) indicate significant differences at $p<0.05$.
GAE: gallic acid equivalent; CE: catechin equivalent

As secondary metabolites, phenolics and flavonoids are pivotal in plants owing to their antioxidant activity. They function by chelating redox-active metal ions, deactivating lipid chain free radicals, and inhibiting the conversion of hydroperoxides into reactive oxyradicals (Barbosa and Nueva, 2019). Current study highlights the phenolic content of Tuhau compared to other Etlingera species such *as Etlingera araneosa, Etlingera eliator*, and *Etlingera linguiformis* (Rachkeeree *et al.,* 2020). Variations in phenolic compositions may arise from differences in the plant's origin and biological conditions (Nadri *et al.,* 2014). This also highlights the efficacy of methanol as a solvent capable of extracting a greater number of phenolic compounds from plants. Differences in phenolic values among different plant parts of the same plant stem

from the presence of group-dependent phenolic compounds, which elicit varied reactions with the Folin-Ciocalteu reagent. The efficacy of phenolic compound extraction from samples directly correlates with the compatibility of the compound with the solvent, adhering to the principle of 'like-dissolves-like'. The solubility of plant phenolics across solvents of varying polarity contributes to these differences (Othman *et al.,* 2014). It is challenging for a single solvent to extract all types of phenolic compounds from a sample, thereby influencing the presence of phytochemicals, phenolic content, flavonoids, and antioxidant activity in the samples under study. Various factors, including plant age and environmental conditions such as temperature, UV light exposure, and atmospheric $CO_2$ levels, can affect the total phenolic and flavonoid content of plants (Yonekura-Sakakibara *et al.,* 2019). Among the numerous polyphenols found in plants, flavonoids constitute one of the largest groups of compounds present in herbaceous plants. Flavonoids, containing polar hydroxyl groups, demonstrate high extraction yields in methanol, facilitating their extraction (Barbosa and Nueva, 2019). These compounds exhibit a diverse range of biological actions, including antibacterial, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory effects (Ahmed et al., 2019). The antioxidant mechanism of flavonoids is associated with the presence of their hydroxyl groups. Moreover, flavonols and procyanidins interact with membrane phospholipids, preventing the effects of harmful substances and preserving membrane integrity (Nurlaili *et al.,* 2018).

### *3.4 Antioxidant abilities*

The antioxidant activity of Tuhau leaf and rhizome extracts was assessed using DPPH, ABTS, and FRAP assays. Significant differences ($p<0.05$) were observed in the antioxidant activity between Tuhau leaf and rhizome extracts. As shown in Table 3, the antioxidant activity, as measured by the DPPH and ABTS tests, ranged from 28.48 ± 0.14% to 71.48 ± 0.53%, while the FRAP values ranged from 1.05 ± 0.03 to 2.31 ± 0.04 mM Fe2+/g dry extract. Evaluating the antioxidant activity of extracts from various plant parts using different antioxidant tests is essential to capture the diverse endogenous mechanisms involved. Different types of antioxidants, such as polyphenols and reducing agents, vary in solubility, density, redox potential, specificity, and mechanisms (Shahid-Ud-Daula *et al.,* 2015). Understanding the relative contributions of various antioxidant types present in plants, such as hydrophilic components (e.g., ascorbic acid and phenols) and lipophilic compounds (e.g., carotenoids), in both leaves and rhizomes, can provide insights into their overall antioxidant potential (Cano *et al.,* 2002).

**Table 3**. Antioxidant activities of Tuhau leaf and rhizome

| Analysis | Leaf | Rhizome |
|---|---|---|
| DPPH (% scavenging activity) | 71.48 ± 0.53$^a$ | 56.36 ± 0.67$^b$ |
| ABTS (% scavenging activity) | 28.48 ± 0.14$^a$ | 13.06 ± 0.41$^b$ |
| FRAP (% reducing power) | 2.31 ± 0.03$^a$ | 1.05 ± 0.03$^b$ |

Values represent mean ± SD (n=3). Different letters (a and b) indicate significant differences at $p<0.05$.

The variation in the type and amount of antioxidant compounds in the extract is reflected in the results of several antioxidant tests used in this study (Matanjun *et al.,* 2008). Therefore, it is not practical to evaluate the total antioxidant capacity of Tuhau through a single chemical reaction, necessitating the use of multiple antioxidant assays for validation purposes. The DPPH assay utilizes nitrogen free radicals that can be neutralized by antioxidants. It assesses the ability of antioxidants to act as hydrogen donors or proton scavengers (Yasir *et al*., 2013). In this test, the purple color of DPPH changes to yellow after receiving electrons from antioxidants. Antioxidants transfer electrons from hydrogen atoms to DPPH free radicals, thereby neutralizing free radical activity (Senguttuvan *et al.,* 2014). Tuhau leaf extract exhibited strong scavenging activity compared to the rhizome extract, likely due to its high concentration of antioxidant compounds such as phenolic acids and flavonoids. The efficacy of leaf methanol extract in scavenging activity may be attributed to the abundant presence of phenolics and tannins in methanol (Chan

*et al.,* 2014). This assertion finds support in previous research by Shahid-Ud-Daula *et al.* (2015), where the leaf extract exhibited higher antioxidant activity than the rhizome extract, with an IC50 value of 196.72 ± 0.28 µg/mL for the leaf extract and 1380.28 ± 2.37 µg/mL for the rhizome extract. The variance in antioxidant activity was further investigated by comparing the activity of antioxidants from different Zingiberaceae plants. For instance, the DPPH activity of *Zingiber officinale* leaf extract was recorded at 51.12 ± 1.65%, while the rhizome extract exhibited a higher activity of 51.41 ± 0.57% (Ghasemzadeh *et al.,* 2010). Moreover, the methanolic extract of *Alpinia elegans* shoots demonstrated a DPPH antioxidant activity of 14.53% (Nurlaili *et al.,* 2018) while *A. elegans* leaf extract exhibited a significantly higher DPPH radical scavenging activity of up to 95.11%, surpassing that of Tuhau leaf extract. This discrepancy in antioxidant activity could be attributed to the presence of flavonoids in the *A. elegans* leaf extract (Naive *et al.,* 2018).

Compounds with antioxidant properties in Tuhau likely inhibit the activity of potassium persulfate, thereby reducing the production of ABTS (Ling *et al.,* 2013). The results depicted in Table 6 indicate that the leaf extract exhibited a greater ABTS scavenging capacity compared to the rhizome extract, corroborating findings from previous studies (Shahid-Ud-Daula *et al.,* 2015). Specifically, the leaf extract recorded an IC50 value of 106.55 ± 2.59, while the rhizome extract demonstrated a higher IC50 value of 173.14 ± 1.78. Lower IC50 values signify stronger radical scavenging activity, indicating that the leaf extract removes ABTS more effectively than the rhizomes. The ability of the leaf extract to eliminate ABTS radicals can be attributed to its high phenolic content, which is influenced by factors such as molecular weight, the number of aromatic rings, and the properties of hydroxyl group substitution (Sabli *et al.,* 2012). Studies conducted on the rhizomes of *Etlingera araneoso* and *Etlingera eliator* have reported varying values depending on the solvent used. Inhibition was significantly higher with ethyl acetate solvents, yielding values of 159.53 and 127.83 mg TEAC/g dry extract, respectively, while the extracts obtained with n-hexane and ethanol solvents showed lower inhibitory values, around 100 mg TEAC/g extract (Rachkeeree *et al.,* 2020).

In the FRAP test, the presence of antioxidants in leaf and rhizome extracts changes the color of the solution from yellow ($Fe^{3+}$-TPTZ) to blue ($Fe^{2+}$-TPTZ). This indicates that the antioxidant compounds in leaf and rhizome extracts act as electron donors, making them primary and secondary antioxidants (Chan *et al*., 2014). The leaf extract exhibits higher antioxidant activity compared to the rhizome extract. This finding is corroborated by a study conducted by Shahid-Ud-Daula *et al.* (2015), which reported a lower IC50 value for Tuhau leaf extract compared to the rhizome extract, with values of 28.22 ± 0.08 and 64.75 ± 2.65 µg/mL, respectively. A lower IC50 value indicates higher potential antioxidant activity, thus indicating that the leaf extract possesses greater antioxidant activity than the rhizome.

### *3.5 Correlation between total phenolic and flavonoid contents of Tuhau leaf and rhizome with antioxidant activities*

The leaves displayed the highest antioxidant activity across all three antioxidant assays, followed by the rhizomes, likely due to their high polyphenol content. This assertion is supported by the correlation analysis conducted between antioxidant components and antioxidant activity. Our correlation analysis revealed a strong positive correlation between TPC of Tuhau leaves with DPPH and ABTS antioxidant activity, with Pearson's correlation coefficients of 0.946 and 0.990, respectively ($p<0.05$). However, no significant correlation was observed for the leaf extract in terms of TFC and FRAP-reducing activities. For rhizome extract, a strong positive correlation was observed between TPC with DPPH and FRAP antioxidant activity, with correlation coefficients of 0.917 and 0.927, respectively ($p<0.05$). Additionally, a strong positive correlation was found between TFC and ABTS antioxidant activity ($p<0.05$). These findings suggest that the TPC in the Tuhau rhizome extract significantly contributes to the antioxidant activity measured by the DPPH and FRAP assays, while the TFC significantly contributes to the antioxidant activity measured by the ABTS assay.

## Conclusion

The study demonstrates the presence of various phytochemicals in Tuhau extracts, including alkaloids, phenolics, and other bioactive compounds and their potential antioxidant properties, which can capture and neutralize free radicals, potentially offering health benefits. Therefore, Tuhau could serve as a valuable source of antioxidants, potentially contributing to its medicinal properties. In conclusion, this study highlights the potential of Tuhau leaves, which exhibit superior antioxidant activity compared to rhizomes, as functional food ingredients in the food industry. Further research, including chromatographic analyses, is warranted to isolate and characterize the specific compounds responsible for these antioxidant properties. Such investigations will enhance our understanding of Tuhau's mechanisms of action and facilitate the evaluation of its additional medicinal properties.

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