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Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity of Tigarun (*Crateva magna* DC.) Leaf Extract

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ABSTRACT

Introduction*: Tigarun (Crateva magna DC.) is a medicinal plant traditionally used in South Kalimantan to treat various ailments. Its leaves contain bioactive compounds such as phenolics and flavonoids with known antioxidant properties. However, scientific data on the antioxidant capacity and quantitative composition of these compounds, particularly from local populations, remain limited. This study therefore evaluates the antioxidant activity and quantifies the phenolic and flavonoid contents of Tigarun leaf extracts to support its traditional use scientifically.* ***Aims****: This study aimed to determine the total phenolic and flavonoid content and evaluate the antioxidant activity of tigarun leaf extract.* ***Methods****: The dried tigarun leaves were extracted by maceration using 70% ethanol. Total phenolic content was analyzed using the Folin–Ciocalteu method with gallic acid as the standard, while total flavonoid content was determined by the aluminum chloride method using quercetin. Antioxidant activity was evaluated using the DPPH method and expressed as Ascorbic Acid Equivalent Antioxidant Capacity (AEAC).* ***Results****: The total phenolic content of the extract was 5.741 g GAE/100 g extract, and the total flavonoid content was 2.685 g QE/100 g extract. The antioxidant activity was 209.772 ± 2.432 mg AEAC/g extract, indicating strong free radical scavenging ability.* ***Conclusion****: The ethanolic extract of Tigarun (Crateva magna DC.) leaves showed notable phenolic and flavonoid contents with strong antioxidant activity. Compared with related species such as Crateva nurvala, these levels are within a comparable range, supporting the potential of Tigarun as a natural antioxidant source for pharmaceutical or nutraceutical applications.*

KEYWORDS*: Antioxidant, Crateva magna, flavonoid, phenolic, tigarun*

INTRODUCTION

Oxidative stress is a key factor in the development of chronic diseases such as cardiovascular disorders, diabetes mellitus, neurodegenerative diseases, and cancer (Sharifi-Rad et al., 2020). The modern lifestyle (characterized by processed food consumption, chemical exposure, and limited physical activity) further increases oxidative stress, leading to cellular damage

Figure 1. Tigarun flower (left) and fermented Tigarun (Jaruk) as traditional food (right) (Ellya et al., 2022).

through free radical reactions. To counter this, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are widely used; however, their excessive or improper use may cause cytotoxicity, endocrine disruption, and carcinogenic effects (Xu et al., 2021). Therefore, the search for safe, natural antioxidant sources is essential.

Indonesia's vast biodiversity provides great potential for developing natural antioxidants, yet the country still depends heavily on imported pharmaceutical raw materials (Widia & Wathoni, 2017). One promising native plant is Tigarun (*Crateva magna* DC.), a member of the Capparidaceae family traditionally used in South Kalimantan for treating bloating, fever, menstrual disorders, and as an antipyretic and tonic (Bhattacharjee et al., 2012; Bhattacharjee et al., 2016). The plant also serves culinary and medicinal purposes, its flowers are fermented into a local food product called jaruk (Figure 1), while decoctions of its leaves are used to relieve flatulence and menstrual discomfort.

Previous studies reported that Tigarun leaf extract exhibits sedative, anxiolytic, anti-inflammatory, and antinociceptive activities, associated with approximately 48 phytochemical constituents that interact with the GABAergic, opioid, and glutamatergic systems (Moniruzzaman & Imam, 2014). Despite these findings, scientific data on the antioxidant capacity and quantitative composition of Tigarun's bioactive compounds remain limited, particularly for populations in South Kalimantan. Despite the extensive traditional use and preliminary scientific evidence of its pharmacological potential, comprehensive phytochemical and functional evaluations of Tigarun, particularly its antioxidant capacity, remain scarce. Given these considerations, further

scientific exploration of Tigarun leaves is essential to validate their antioxidant potential.

To date, most studies on Tigarun (*Crateva magna* DC.) have primarily focused on its antibacterial and other pharmacological properties, while investigations on its antioxidant potential remain limited, this study aims to determine the total phenolic and flavonoid contents and evaluate the antioxidant activity of Tigarun leaf extract using the DPPH method. The findings are expected to strengthen the scientific basis for utilizing Tigarun as a natural antioxidant source in the development of herbal-based pharmaceutical products.

METHODS

Chemicals and Reagents

The materials used in this study included *Crateva magna* DC. (Tigarun) leaves, collected from Kabupaten Banjar, South Kalimantan, Indonesia. Ethanol 70% (technical grade; Medika, Indonesia), gallic acid (≥98% purity; Sigma-Aldrich, Merck, Germany), quercetin (≥95% purity; Sigma-Aldrich, Merck, Germany), Folin–Ciocalteu reagent (Sigma-Aldrich, Merck, Germany), sodium carbonate (Na_2CO_3; Merck, Germany), aluminum chloride ($AlCl_3$; Merck, Germany), sodium acetate (Merck, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich, Merck, Germany), and Ascorbic acid (Sigma-Aldrich, Merck, Germany) were used. All solvents employed were of analytical grade unless otherwise specified.

Preparation of Simplicia

Fresh *Tigarun* (*Crateva magna* DC.) leaves (5 kg) were collected, cleaned, and air-dried at room temperature (±25°C) until constant weight. Dried leaves were ground using a blender to obtain simplicia powder. The yield of simplicia was calculated based on the dry weight obtained (Sidabutar et al., 2023).

Extraction

The simplicia powder was macerated with 70% ethanol at a ratio of 1:10 (w/v) in a closed container. The mixture was kept at room temperature (±25°C) for 3 × 24 hours with occasional stirring. The filtrate was collected, and the residue was re-macerated twice under the same conditions until the filtrate became nearly colorless. All filtrates were combined and evaporated at 40°C using a rotary evaporator to obtain a thick extract. The extract yield was calculated relative to the initial simplicia weight. (Padmasari et al., 2013).

Determination of Total Phenolic Content

Gallic acid (60–130 µg/mL). Mix 50 µL standard/sample with 500 µL of 10% Folin–Ciocalteu reagent. Add 400 µL of 1 M sodium carbonate. Incubate for 15 minutes at room temperature. Measure absorbance

at 765 nm using a UV–Vis spectrophotometer. Methanol with reagents served as blank. Analyses were performed in six replicates. Results were expressed as grams of gallic acid equivalents per 100 grams of extract (g GAE/100 g) (Pourmorad et al., 2006).

Determination of Total Flavonoid Content

Quercetin (40–110 µg/mL). Mix 100 µL standard/sample with 300 µL methanol, 560 µL distilled water, 20 µL of 10% $AlCl_3$, and 20 µL of 1 M sodium acetate. Incubate for 30 minutes at room temperature. Measure absorbance at 415 nm using a UV–Vis spectrophotometer. Analyses were performed in six replicates. Results were expressed as grams of quercetin equivalents per 100 grams of extract (g QE/100 g) (Chang et al., 2002).

Antioxidant Activity by DPPH Method

Ascorbic acid (20 mg/100 mL methanol). Prepare serial dilutions (5–22.5 µL stock solution) and adjust volume to 125 µL with methanol. Add 750 µL of 0.1 mM DPPH solution and incubate in the dark for 30 minutes. Measure absorbance at 517 nm using a UV–Vis spectrophotometer. Extract samples were prepared and analyzed under the same conditions. All measurements were performed in six replicates. Antioxidant activity was expressed as milligrams of Ascorbic Acid Equivalent Antioxidant Capacity (mg AEAC/g extract) (Celep et al., 2015).

RESULTS AND DISCUSSION

Total Phenolic Content

The absorbance was measured at a wavelength of 765 nm using gallic acid as a standard. Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic compound known for its strong antioxidant activity. The total phenolic content (TPC) in the ethanol extract consists of phenolic compounds with the ability to donate hydrogen atoms, making the antioxidant activity of phenolics related to their ability to neutralize free radicals (Tosun et al., 2009).

TPC in plants is expressed in GAE, which is the milligram equivalent of gallic acid per gram of sample. The calibration result of gallic acid against the Folin–Ciocalteu reagent. TPC was calculated based on the GAE value (g GAE/100 g extract) using the regression equation $y = 0.004x + 0.0903$ with an R^2 value of 0.9989, indicating that the absorbance value was highly influenced by concentration, while the remainder was affected by other factors such as temperature, light, storage, and chemical substances.

The results showed in Table 1 that Tigarun leaf extract exhibited a high total phenolic content (TPC) of 5.741 g GAE/100 g extract, calculated using a gallic acid

Table 1. Total phenolic content (TPC) of *Crateva magna* DC. leaf extract

Concentration (ppm)	Absorbance	Calculated concentration (ppm)	TPC (g GAE/100g extract)	Average	SD	%RSD
1000	0.231	58.78	5.878	5.741	0.196	0.034
1000	0.233	59.18	5.918			
1000	0.225	57.58	5.758			
1000	0.230	58.58	5.858			
1000	0.219	56.38	5.638			
1000	0.207	53.98	5.398			

standard calibration curve with an R^2 value of 0.9989. This indicates that polar compounds contained in Tigarun leaves were well dissolved in 70% ethanol. The total phenolic content in the sample was determined using the Folin–Ciocalteu method, based on the ability of phenolic compounds in the extract to react with phosphomolybdic–phosphotungstic acid complexes present in the Folin–Ciocalteu reagent. This reaction results in the formation of a blue-colored molybdenum–tungstate complex. The higher the intensity of the blue color produced, the greater the total phenolic content in the sample (Marjoni et al, 2018).

The reaction between phenolic compounds and the Folin–Ciocalteu reagent occurs only under alkaline conditions. To achieve this, 10% sodium carbonate (Na_2CO_3) was used to allow the dissociation of protons from the phenolic compounds, resulting in phenolate ions (Rizvi et al., 2023). Under alkaline conditions, the hydroxyl groups in the phenolic compounds react with the Folin reagent to form a blue complex with an unknown structure, which can be detected using a spectrophotometer (Singleton et al., 1999). The intensity of the blue color formed is directly proportional to the concentration of phenolate ions present in the solution (Ainsworth, E. A., & Gillespie, K. M., 2007).

Total Flavonoid Content

From the calibration curve, the regression equation obtained was y = 0.0042x + 0.2262. Based on this regression equation, the total flavonoid content of Tigarun leaf extract was calculated as 2.685 g QE/100 g extract, expressed as quercetin equivalent (Table 2).

The results presented in the Table 2 indicate that tigarun leaf extract contains a high level of phenolic and flavonoid compounds, which correspond to strong antioxidant potential. These findings support the traditional use of this plant and suggest that Tigarun has promising value as a source of natural antioxidants. Further studies are recommended to explore its potential in pharmaceutical formulations.

Table 2. Result of total flavonoid content of tigarun leaf extract

Concentration (ppm)	Absorbance	Calculated concentration (ppm)	TFC (gQE/100g extract)	Average	SD	%RSD
1000	0.238	28.521	2.852	2.685	0.364	0.136
1000	0.210	22.688	2.269			
1000	0.240	28.938	2.894			
1000	0.256	32.271	3.227			
1000	0.217	24.146	2.415			
1000	0.219	24.563	2.456			

The determination of total flavonoid content was carried out using the colorimetric method based on complex formation with $AlCl_3$. In this analysis, quercetin (QE) was used as a reference standard. Quercetin is a strong flavonol-type flavonoid containing a keto group at carbon-4 and adjacent hydroxyl groups at carbon-3 or carbon-5. Flavonols are widely used as indicators of flavonoid presence due to their abundance in plant species (Erwiyani et al., 2021).

Sodium acetate was added to function as a buffer to maintain the optimal pH for the Al^{3+}–flavonoid complex formation. In the aluminum chloride method, an inappropriate pH, either too low or too high, may alter the ionization state of flavonoids, affecting complex formation and the resulting absorbance value. Sodium acetate ($NaCH_3COO$) also assists in identifying hydroxyl (–OH) groups at position C-7 of the flavonoid structure, which plays an important role in the biological activity of the compound (Panche et al., 2016). It facilitates detection of these groups through color changes or shifts in wavelength. Additionally, sodium acetate helps stabilize the measurement within the visible light range, ensuring consistent and accurate absorbance readings when measured by spectrophotometry (Aryal et al., 2019).

Flavonoids are phenolic components known for their antioxidant activity. This activity is related to the number and position of hydroxyl groups on the phenol ring, which are responsible for hydrogen donation and free radical scavenging (Rahmi et al., 2016).

Antioxidant Activity

The antioxidant capacity was expressed in terms of ascorbic acid equivalence (AAE), which quantifies the antioxidant potential of a sample by comparing it to the activity of ascorbic acid (vitamin C). In the DPPH method, the percentage of DPPH reduction at a given sample concentration is used to determine antioxidant activity. A lower absorbance reading indicates a higher radical scavenging percentage. Antioxidant activity is calculated by substituting the reduction percentage into the regression

Table 3. Result of antioxidant activity of tigarun leaf extract

Concentration (ppm)	Absorbance	% Inhibition	Antioxidant Activity (mg AEAC/g extract)	Average	SD
200	0.398	70.970	208.808	209.772	2.432
200	0.393	71.335	210.415		
200	0.397	71.043	209.129		
200	0.383	72.064	213.631		
200	0.406	70.387	206.236		
200	0.393	71.335	210.415		

equation derived from the ascorbic acid standard.

The result is expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg AEAC/g sample. The higher the AEAC value, the greater the antioxidant activity of the sample. The antioxidant activity of the 70% ethanol extract of Tigarun leaves using the DPPH method is shown in Table 3.

The antioxidant activity of the 70% ethanol extract of Tigarun leaves, as evaluated by the DPPH method, was 209.772 ± 2.432 mg AEAC/g extract. In this study, antioxidant activity was expressed as milligrams of ascorbic acid equivalent per gram of extract (mg AEAC/g extract). A higher AEAC value indicates a greater antioxidant capacity. This result suggests that 1 gram of Tigarun leaf extract possesses an antioxidant capacity equivalent to 209.772 mg of ascorbic acid.

The strong antioxidant activity observed is in line with the extract's total phenolic content. It is well established that the higher the phenolic content, the stronger the antioxidant activity of a plant extract (Tungmunnithum et al., 2018). Phenolic compounds serve as essential plant constituents, largely due to their robust capacity to scavenge free radicals—a function inherently linked to the presence and positioning of hydroxyl (–OH) groups within their molecular structure. These phenolic groups act as potent antioxidants by donating electrons or hydrogen atoms to neutralize reactive oxygen species (ROS), thereby mitigating oxidative damage and effectively reducing their toxicity in biological systems (Gülçin, 2025). In line with this, Gülçin (2025) emphasizes that the thorough screening of these antioxidant properties necessitates the use of appropriate, standardized methods that specifically address the underlying chemical mechanisms and reaction kinetics. Because different plant-derived compounds interact with radicals in unique ways, a single test is often insufficient. This systematic approach is vital for the development of stable food products and effective pharmaceutical formulations, ensuring they are better equipped to counteract oxidative stress-mediated deterioration and prevent various patholo-

gical processes (Gülçin, 2025).

This finding reinforces the potential use of Tigarun leaf extract as a natural antioxidant. The relatively high phenolic and flavonoid contents may contribute to its strong DPPH radical scavenging activity, which is consistent with the antioxidant mechanisms reported in other *Crataeva* species. However, variations in extraction yield and antioxidant capacity could be influenced by environmental factors, leaf maturity, or solvent polarity, which were not extensively evaluated in this study. Future research should therefore include compound isolation and in-depth antioxidant mechanism studies using multiple assay systems (e.g., ABTS, FRAP), as well as cytotoxicity evaluation to ensure its safety for potential development into health supplements or functional food products.

CONCLUSION

This study confirms that the ethanolic extract of *Crateva magna* DC. (tigarun) leaves possesses notable antioxidant activity, supported by substantial phenolic and flavonoid contents. These results strengthen the scientific basis for developing Tigarun as a local natural antioxidant source for pharmaceutical or nutraceutical applications. Future research should include metabolite profiling and in vivo evaluations to better understand its bioactive constituents and therapeutic potential.

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