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Phenolic content, flavonoid, and antioxidant activity of *Etlingera comosa Ardiyani and Ardi* leaf extract endemic to Central Sulawesi

Rasida[1], Annisa Putri Anugraini[2], Satriani[3], Risda Maskur[1], Agustinus Widodo[1], Yonelian Yuyun[1*]

[1]Program Studi S1-Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Tadulako
[2]Program Studi S1-Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Tadulako
[3]Program Studi S1-Kesehatan Masyarakat, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Tadulako
*Corresponding author: yonelian@untad.ac.id

Abstract

Background: The recently identified plant species *Etlingera comosa Ardiyani & Ardi* is endemic to Tentena, Central Sulawesi, where it thrives both terrestrially and as an epiphyte. Nevertheless, the antioxidant activities of *E. comosa* have yet to be investigated, although other Etlingera species are recognized for their antioxidant components.
Objective: This work aims to evaluate the antioxidant capabilities of *E. comosa* leaf extract using the ferric reducing antioxidant power (FRAP) assay, subsequently determining the total phenolic and flavonoid content.
Method: The leaf extract was prepared through maceration with 96% ethanol. The FRAP method was employed to assess antioxidant activity, whereas the Folin-Ciocalteu method was utilized to quantify total phenolic content. The aluminum chloride colorimetric technique was employed to determine the total flavonoid content.
Result: The results indicated that the extract exhibited robust antioxidant activities, with an EC_{50} value of 9.94 µg/mL, whereas vitamin C, serving as the positive control, demonstrated an EC_{50} value of 3.50 µg/mL. The extract comprised 18.86 mg GAE per 100 mg of extract for total phenolics and 3.31 mg QE per 100 mg of extract for total flavonoids.
Conclusion: The antioxidant capacity of *E. comosa* leaf extract is significant, rendering it a promising subject for further pharmacological investigation.
Keywords*:* antioxidant, *Etlingera comosa,* total phenolic, total flavonoid

1. Introduction

The increasing interest in natural antioxidant is driven by the possible side effects of synthetic types which are potentially harmful to health when consumed in high quantities. This has created a greater demand for natural, plant-derived options that are safe and have proven efficacy. Antioxidant activity is found in numerous medicinal and edible plants with a greater abundance of phenolic compounds, which protect cellular constituents from oxidative damage. The genus *Etlingera*, a member of the Zingiberaceae family, provides an abundant but underinvestigated reservoir of natural antioxidant. These plants have been used traditionally within local communities in food and medicine, thereby enhancing the potential as functional ingredients (Elviana *et al.*, 2022; Iglesias & Siregar, 2024).

Free radicals are very reactive molecules that damage the cells by taking electrons from important biomolecules, including proteins, lipids, and DNA. This oxidative damage marks the

initiation of cancer and degenerative diseases (Zulaikhah, 2017). Free radicals can be "silenced" by antioxidant, which help to protect health by keeping the cells in good shape. In general, antioxidant are protective agents that strengthen the body defense system against oxidative stress (Lobo *et al*., 2010).

Phenolic are a class of antioxidant compounds, considered important due to the capability of donating hydrogen atoms or electrons used to counteract free radicals. Flavonoid, phenols, and tannins are the major phenolic compounds, well known for potent antioxidant activity *(Nurcholis et al*., 2012). These substances are found in a variety of plant species and play a role in phytochemistry and pharmacological activity. *Etlingera comosa* (*E. comosa*) is a species that only occurs in Tentena, Central Sulawesi, Indonesia (Ardiyani *et al*., 2021). Although there are few reports for *E comosa*, previous studies on the other species of *Etlingera*, related to antioxidant and anti-inflammatory activities, have shown promising information. For example, the *E. elatior* flowers and leaf ethanol extract showed significant antioxidant activity (Handayani *et al*., 2014). Similarly, extract of *E. sayapensis* (Mahdavi *et al*., 2017) and *E. coccinea* (Jems *et al*., 2021) showed antioxidant and anti-inflammatory activities. These results show that *E. comosa* may have powerful antioxidant activity.

To exploit the therapeutic properties of *E. comosa,* it is important to understand the plant phytochemical profile and quantify antioxidant activity. The estimation of total phenolic content and the active compounds can be useful to provide some evidence of the efficacy. The results of this study will generate scientific data and also help ensure the sustainable use and conservation of Indonesia biodiversity which can be further developed for pharmaceutical, nutraceutical, or cosmetic purposes.

2. Method

2.1. Tools and materials

The tools employed include maceration equipment consisting of a vessel and a beaker glass (Herma), a set of UV-VIS spectrophotometry equipment from Innesa UV-Vis N4S, an analytical balance (Precisa), an oven (Gallenkamp Civilab-Australia), a blender (Miyako), a rotary evaporator (Buchi), and silica GF 254 nm (Merck). Meanwhile, the materials used include leaf of the *E. comosa* plant from the Tentena mountains, Poso Regency, Central Sulawesi. Other ingredients were ethanol 96% (Merck), sodium acetate (Merck), acetic acid glacial (Merck), $FeCl_3$ (Merck), HCl, tripyridyltriazine (Merck), ethanol pro analysis (Merck), acid gallate (Merck), sodium carbonate 7% (Merck), Folin-Ciocalteu reagent (Merck), $AlCl_3$ (Merck), quercetin (Merck), aquadest (Waterone).

2.2. Plant collection and determination

Fresh leaf of *E. comosa* were gathered from Bada Village, Poso District, Central Sulawesi in July 2023. The plant material was selected according to the morphology and local ethnobotanical information. Botanical identification was carried out by a taxonomist at the Celebense Herbarium of Tadulako University. The plant was identified as *E. comosa Ardiyani & Ardi*, and a voucher specimen (No. 28. (UPT-SDHS/LK/2023) was deposited in the herbarium for further reference.

2.3. Liquid-liquid extraction

A total of 498.77 g of *E.comosa* leaf simplicia was macerated with 2.6 L of 96% ethanol as extraction solvent. Extraction was executed for 3 × 24h at room temperature and occasional stirring was performed to provide better contact between the solvent and the plant. After maceration, the solvent was extracted from the plant residue, and the filtrate was concentrated on a rotary evaporator at a lower pressure to create the thick extract (Musnina *et al*., 2019).

2.4. Phytochemical screening

Preliminary phytochemical analysis of extract was completed to detect the major groups of secondary metabolites such as saponins, triterpenoids, steroids, alkaloids, flavonoid, phenolic compounds, and tannins using routine qualitative methods outlined by Harborne (1998), Sofowora (1993), as well as Trease & Evans (2002).

2.4.1 Detection of alkaloids

The crude extract (200-300 mg) was disbanded in 5 mL of ethanol and divided equally into three test tubes. Each tube was exposed to four to five drops of individual alkaloid-identifying reagents namely Dragendorff (tube 1), Mayer (tube 2), and Wagner (tube 3). Alkaloids were thought to be present when orange precipitate appeared with Dragendorff's reagent, white precipitate appeared with Mayer's reagent, and brown precipitate appeared with Wagner's reagent.

2.4.2 Detection of triterpenoids and steroids

Around 20 mg of extract was dissolved in 500 μL ethanol and 5 drops of glacial acetic acid (CH_3COOH) were then included. Eight drops of focused sulfuric acid ($H_2 SO_4$) were included slowly down the wall of the tube to provide a visible interface. Steroids were detected when the solution turned blue or green after mild agitation and standing at room temperature, while triterpenoids presence was detected by the development of a red or purple color.

2.4.3 Detection of saponins

Approximately 20 mg of extract was dissolved in hot purified water in a test tube at room temperature. The solution was shaken violently for about 10 seconds and 1drop of 2 N HCl was

delivered. The development of stable froth 1–10 cm in height which persisted for at least 10 mins denoted the existence of saponins.

2.4.4 Detection of flavonoid

The fraction was re-dissolved in 500 µL ethanol, reacted with a little magnesium powder, and then mixed with 10 drops of conc. HCl. After the material was homogenized, the presence of flavonoid was shown by color (dark red, yellow, orange).

2.4.5 Detection of tannins

About 20 mg of extract was dissolved in 500 µL and was mixture with 1 mL of 10% remedy of ferric chloride ($FeCl_3$). A color of dark blue, blackish blue, or greenish blue showed tannins.

2.4.6 Detection of phenolic compounds

Phenolic substances were found according to the procedure used for tannins. Positive results showed the presence of dark-blue, blackish-blue, or greenish-green color after adding 10% ferric chloride solution.

2.5. Determination of antioxidant activity

Antioxidant activity of extract was measured by the ferric reducing antioxidant power (FRAP) assay, which was performed as characterized by Syarif *et al.* (2015). Extract was prepared in different concentrations of 1, 2, 4, 8, and 16 ppm, with ascorbic acid as a standard reference in the scope of focus 2, 4, 6, 8, and 10 ppm. In the test, 2 mL of each sample (extract or standard solution) was placed in a test tube, and then 6 mL of freshly ready FRAP reagent was added. The solution was vortexed to mix completely and kept to incubate at room temperature for 30 min. At 594.5 nm, the reaction solution's absorbance was measured. The results obtained were disintegrated and analyzed in the form of graphs and tables. Antioxidant activity was expressed as the EC_{50} defined as the sample's concentration that would be required to suppress 50% of the maximum antioxidant activity.

2.6. Determination of total phenolic content

The overall amount of phenolic content of extract was quantified by the visible spectrophotometer based on the experiment by Khadijah *et al.* (2023), using gallic acid as the standard reference. Standard solutions of gallic acid were prepared at concentration levels of 50, 100, 150, 200, and 250 µg/mL. A sample of 10 mg was dissolved in 10 mL of ethanol, and 0.5 mL of the solution was passed into a test tube, then 0.4 mL of Folin-Ciocalteu reagent was added and mixed followed by 5.1 mL of purified water. The mixture was incubated for 5 min and 4 mL of a 7% sodium carbonate (Na_2CO_3) was added. The remedy was subsequently vortexed for 1 min and incubated for an extra 15 min. The absorbance was measured using a UV-visible spectrophotometer at 765.5 nm. Every measurement was made three times. The gallic acid calibration curve was used to convert the

data into gallic acid equivalents. Gallic acid equivalents (μg) per milligram of extract were used to calculate total phenolic. Meanwhile, the concentration of phenolic in the extract was estimated from the equation of the line obtained by graphing the absorbance versus the known concentration of gallic acid.

2.7. Determination of total flavonoid content

Content of total flavonoid was established by visible spectrophotometry according to the method of Haeria *et al.* (2016) using quercetin as a standard. A series of standard quercetin solutions were prepared at the amounts of 20, 40, 60, 80, and 100 μg/mL. In the 0.5 mL of the specimen dissolved in 10 mL of ethanol, 1.5 mL of 96% ethanol, 0.1 mL of 10% $AlCl_3$, and 0.1 mL of sodium acetate 1 M were added, and then 2.8 mL of distilled water. After 1 minute of vortexing, the tubes were left to stand for 15 min. A UV-visible spectrophotometer was used to measure the solution's absorbance at 427.0 nm (λ = 427.0 nm), and all tests were performed in triplicate to ensure accuracy. The outcomes were conveyed in μg/mg of the standard quercetin obtained from the calibration curve. Flavonoid contents in the sample were assessed by absorbance measurement in the spectrophotometer and by the line of regression. Data were averaged from the three repeats to decrease experimental error.

3. Results and discussion

The first phase of the fieldwork was to ensure that the plant species were properly identified, to prevent any mistakes when collecting material. This is important to reinforce the study as strong, trustworthy, and credible. The maceration method was selected for the extraction of bioactive components based on the simplicity and efficiency as extraction method for both thermostable and thermolabile compounds (Tambun *et al*., 2021). The 96% ethanol selected as the solvent was due to the selectivity, non-toxicity, and good absorbability. Ethanol is also well known for high filtration ability, and ability to extract semi-polar, non-polar, and polar compounds. Furthermore, increased ethanol concentration allows for better penetration of the plant cell wall compared to lower concentration (Fitriani *et al*., 2024), resulting in a more concentrated substance. The percentage yield (w/w) of extract by this method was 3.48%. Previous studies have shown significantly varying results of extraction yields among different species of *Etlingera*. For example, an estimated methanol extract yield of about 2.36% was reported for the flowers of *Etlingera elatior* (Maimulyanti & Prihadi, 2015). Low ethanol extraction yield in *Etlingera* leaf might be due to low concentrations of ethanol-soluble compounds in the matrix, mismatch between solvent polarity and target phytochemicals, or geometrical obstacles for the solvent to reach the endogenous compounds of the leaf matrix.

Furthermore, improper circumstances of extraction such as low ethanol concentration, reduced extraction time, or improper sample pre-processing can also lower extracting yield. As a moderately polar solvent, ethanol may not be able to extract highly non-polar and polar compounds which are present in leaf (Azwanida, 2015; Do *et al*., 2014).

The extract's phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, phenols, and tannins, which are known to have a variety of pharmacological properties, such as antibacterial, anti-inflammatory, and antioxidant properties (**Table 1**). The secondary metabolite constituents in *E. comosa* leaf extract comprised alkaloids, flavonoid, steroids, phenolic, and tannins. The alkaloidal nature was ascertained by a positive reaction with Dragendorff's and Wagner's reagent, while the existence of flavonoid was shown by the orange-red precipitates with magnesium and HCl. Sulfuric acid yielded a greenish blue with steroids, and ferric chloride a blackish green with phenolic compounds and tannins but saponins did not, as showed by the lack of form production. These results show that the extract has an excellent potential for use in the field of medicine, specifically for antioxidant and anti-inflammatory purposes, but specific bioactive compounds should be isolated and evaluated. Similarly, extract of *E. elatior* usually contain flavonoid, phenolic, steroids and saponins but are commonly deficient in alkaloids (Jackie *et al*., 2011; Maimulyanti & Prihadi, 2015). *E. brevilabrum* contains abundant flavonoid, phenolic acids, and sterols, while special assays for alkaloids or saponins are limited. Flavonoid, phenolic, and steroid are generally found in all the *Etlingera* species, while alkaloids and saponins vary (Novitasari, 2023). Specific secondary metabolites vary in Etlingera taxa primarily due to genetic diversity as well as plant part used and environmental factors. Extraction methods and solvents used are also important factors for the compounds measured.

Table 1. Phytochemical screening results

Test	Reagent	Information	Result
Alkaloid	Dragendroff	Orange red sediment	+
	Wagner	Orange red sediment	+
Flavonoid	Mg + HCl	Orange red sediment	+
Saponin	H_2O + HCl	Not in the form of foam	-
Steroid	H_2So_4 + $C_4H_6O_3$	Greenish blue color	+
Phenolic	$FeCl_3$	Blackish green color	+
Tanin	$FeCl_3$	Blackish green color	+

Description :
+ = positive contains the tested compound
- = negative, no compound found

Antioxidant activity of *E. comosa* leaf ethanol extract was assessed by FRAP method which determines the compound's capacity to decrease Fe^{3+} to Fe^{2+}. This method is well known for simplicity and utility in evaluating the total assay of a given sample. The half maximal effective

concentration (EC_{50}: 9.94 ppm) showed that the ethanol extract possesses very strong antioxidant activity. Compared to other studies of *Etlingera* plant species, the EC_{50} of the *E. comosa* exract was less than *E. elatior*. In a study by Solihah *et al*. (2024), the ethanol leaf extract of *E. elatior* showed EC_{50} value of 58.82 ppm, which is is charge of the increased antioxidant activity (**Table 2**). However, antioxidant capacity of *E. comosa* extract is a little stronger than that of *E. rubroloba*. In a study by Jabbar *et al*. (2022), the ethanol extract of *E. rubroloba* stem had an IC_{50} value of 12.720 ± 0.12 µg/mL (about 12.72 ppm) which confirmed a high antioxidant activity. Antioxidant potential of *E. comosa* leaf extract is slightly lower than vitamin C (EC_{50} approximately 2.80ppm). This implies that although the *E. comosa* extract possessed very high antioxidant activity, it is lower than that of vitamin C. However, being naturally derived from *E. comosa* leaf, extract can be a good source of antioxidant in health or industrial products.

Table 2. EC_{50} values of *E. comosa* extract and vitamin C

Sample	EC_{50} value (ppm)
E. comosa	9.94
Vitamin C	3.50

As a major class of secondary plant metabolites, phenolic compounds have got a lot of scientific interest due to the strong antioxidant activities and potential drug development. These molecules have several bioactivities including anti-inflammatory, antimicrobial, anticancer, and anti-viral, showing the potential as a good candidate for drug (Supritha & Radha, 2018). The objective of this report was to evaluate obtained data on the total phenolic value of *E. comosa* leaf extract measured by the Folin-Ciocalteu method and to discuss the result in the context of available data on plant phenolic compounds, particularly *Etlingera* species. The colorimetric technique known as the Folin-C is used to ascertain the total phenolic content of plant extract. In this assay, phenolic hydroxyl compounds present in extract, undergo a redox reaction with Folin-Ciocalteu reagent, a mixture of phosphomolybdic and phosphotungstic acids. With an alkaline medium created by sodium carbonate addition, heteropolyacids are reduced by phenolic compounds in the reagent and a blue complex is formed. The intensity of the blue color, quantified spectrophotometrically at a wavelength namely c.a 760 nm, is directly proportional to the quantity of phenolic compounds present in the sample (Lawag *et al*., 2023). The total of phenolic content in extract amounted to 18.31 mg of gallic acid equivalents per 100 mg extract (**Table 3**). Compared to a more common unit of measurement, the total phenolic content was 183.1 mgGAE/g extract. For comparison of this value, it can be contrasted with the total phenolic content range from other medicinal plants that had been previously published. Studies of different *Etlingera* species have shown significant variations in phenolic content among plant parts. For instance, *E. coccinea* leaf extract had significantly higher total

phenolic (11.69 mg GAE/g sample) than the rhizomes (0.58 mg GAE/g sample) (Mendez *et al.*, 2022). Similarly, *E. pubimarginata* presented a higher content of phenolic in leaf (27.25 mg GAE/g dried sample) compared to rhizome (0.76 mg GAE/g dried sample) (Mendez *et al*., 2022). This observation is in agreement with the higher phenolic content in *E. comosa* leaf compared to *E. elatior* rhizomes. The profile of phenolic compounds throughout the plant is determined by several factors such as the unique metabolic pathways in each tissue and responses to environmental signals. The selection of extraction solvent is also very important. In this study, ethanol extract from *E. comosa* leaf was used, but the solvent applied was different from the one used by Chan *et al*. (2011b) for *E. elatior* rhizomes. Ethanol is a common and efficient solvent for the extraction of various phenols. Differences in geographic location can also be a factor in variations in phenolic concentration and the environment, as well as the stage of plant development and time of harvest.

Table 3. Total phenolic content in ethanol extract of *E. comosa* leaf

Replication	Phenolic content (GAE mg/ 100 mg extract)	A verage of phenolic content (GAE mg/ 100 mg extract)
R1	18.55	18.31
R2	18.60	
R3	17.93	

Flavonoid are the largest group of bioactive mixtures distributed ubiquitously in the plant world. These molecules, with well-documented pharmacological activities, are significantly endowed with strong antioxidant activity. Flavonoid are also known as subclasses of phenol which have a specific amount of other supplementary aiming compounds (Safrina *et al.*, 2022). The flavonoid content of 3.28 mg quercetin equivalent per 100mg of *E.comosa* leaf extract (3.28% w/w QE) (**Table 4**) is within the range reported for other *Etlingera*, although there is marked interspecific variation in the genus. For example, *E. elatior* leaf flavonoid contents ranged from 0.46% w/w QE to 4.86% w/w CE and even higher in some fractions (Utami *et al*., 2024). In contrast, *E. coccinea* leaf has been reported with values ranging between 0.15% w/w QE to 5.33% w/w QE (Safrina *et al*., 2022). Leaf of *E. rubroloba* had a higher content of flavonoid of 3.399 % w/w QE (Andila & Nugroho, 2022). This shows that the amount of flavonoid differs significantly among the *Etlingera* species or even within the same part of the plant that is to be analyzed.

Table 4. Total flavonoid content in ethanol extract of *E. comosa* leaf

Replication	Flavonoid content (QE mg/ 100 mg extract)	Average of flavonoid content (QE mg/ 100 mg extract)
R1	3.62	3.28
R2	3.10	
R3	3.11	

4. Conclusion

In conclusion, leaf extract of *Etlingera comosa Ardiyani & Ardi* showed potent antioxidant activity (EC_{50} of 9.94 ± 1.03 ppm) based on the FRAP method. Moreover, the extract was found to have a substantial amount of bioactive constituents as reflected by the total amount of phenolic content of 18.31 mg GAE/100 mg extract and total flavonoid content of 3.28 mg QE/100 mg extract. These results suggest that leaf of *E. comosa* has strong antioxidant and potential utility in pharmaceutical or nutraceutical fields. Further studies are needed to identify active compounds and the potential biological activities *in vivo*.

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