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Phytochemical composition, antioxidant, and anticancer potential of *Etlingera hemisphaerica* (Zingiberaceae) from the Gayo Highlands, Indonesia

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Abstract. *Ridhwan M, Saudah, Nurman S, Masyudi, Fitriyana L, Yusnaini R. 2025. Phytochemical composition, antioxidant, and anticancer potential of* Etlingera hemisphaerica *(Zingiberaceae) from the Gayo Highlands, Indonesia. Asian J Nat Prod Biochem 23: 101-109.* The increasing global incidence of cancer underscores the urgent need for sefar and more effective alternative therapies. *Etlingera hemisphaerica* (Blume) R.M.Sm. (Zingiberaceae), an andemic medicinal plant from the Gayo Highlands of Indonesia, has traditional uses but remains underexplored for its pharmacological potential. This study investigated the phytochemical composition, antioxidant capacity, and anticancer of *E. hemisphaerica* leaf extracts using methanol, ethyl acetate, and aquadest. Total phenolic and flavonoid contents were determined spectrophotometrically. Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, and anticancer activity was evaluated via the MTT assay against the MCF-7 human breast cancer cell line (ATCC HTB-22), with IC_{50} values calculated through linear regression analysis. Qualitative screening revealed the presence of major phytochemicals including flavonoids, phenolics, tannins, terpenoids, saponins, and steroids. Quantitative analysis showed that the methanol extract contained the highest total phenolic content (506.4 ± 15.41 mg GAE/g), while the ethyl acetate extract had the highest flavonoid content (471.5 ± 20.55 mg QE/g). Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, with the methanol extract exhibiting the strongest radical scavenging capacity IC_{50} (0.40 ppm). Cytotoxic effects were determined by MTT assay against MCF-7 human breast cancer cells (ATCC HTB-22), where the methanol extract showed the most potent activity (IC_{50} = 0.446 ppm; 43.47% inhibition at 200 ppm), outperforming ethyl acetate (IC_{50} (10.45 ppm) and aquadest IC_{50} (4.12 ppm) extracts. These results suggest that *E. hemisphaerica*, particularly in methanol extract, is a promising natural source of antioxidant and anticancer agents. Further bioassay-guided fractionation and in vivo studies are warranted to explore its therapeutic potential.

Keywords: Cancer MCF-7, *Etlingera hemisphaerica*, Gayo Highland, herb medicine, Zingiberaceae

INTRODUCTION

Cancer is one of the leading causes of death globally (Yuan et al. 2022). Despite the availability of anticancer therapies, the incidence of cancer continues to rise steadily. According to the World Health Organization (WHO) and global cancer statistics (GLOBOCAN), the global cancer burden is projected to reach 28.4 million cases by 2040, a 47% increase from 2020 (Sung et al. 2021). Conventional cancer treatments such as surgery, radiotherapy, chemotherapy, and immunotherapy are commonly used but often associated with limitations, including complications, drug resistance, and significant side effects (Mali 2023). Consequently, there is a growing interest in developing alternative or complementary therapies derived from natural sources.

Natural products, especially those derived from plants, have long been used in medical practice. Plants are rich in bioactive compounds and have shown promise as sources of anticancer agents. These include vitamins, minerals, phosphates, and various secondary metabolites such as phenolics, flavonoids, alkaloids, and polyphenols, all of which have demonstrated antioxidant, anti-inflammatory, and chemopreventive properties (Block et al. 2015; Lee et al. 2017; Lichota and Gwozdzinski 2018; Jiang 2019).

Spices are widely used as flavor enhancers and aromatic ingredients in food, as well as traditional remedies for chronic diseases. They contain tannins, alkaloids, flavonoids, and polyphenols with potent biological activities. Clove, cinnamon, and ginger species, in particular, are excellent sources of antioxidants due to their high phenolic content (Jiang et al. 2019). The pharmacological effects of these spices include antitumor, antioxidant, and anti-inflammatory properties (Chin 2016). Moreover, the antioxidant activity of edible and medicinal plant extracts has been proven to counteract reactive oxygen species (ROS)-mediated damage in various human cancers (Sammar et al. 2019).

The Zingiberaceae family, commonly known as the ginger family, is one of the key spice producing families with numerous species used in traditional medicine for cancer prevention. The genus *Etlingera* Giseke, belonging to this family, comprises more than 100 species distributed throughout Southeast Asia, many of which are used in ethnomedicine (Adegoke and Ojo 2017; Saudah et al. 2022). Pharmacologically, studies have demonstrated that *Etlingera* species exhibit antioxidant (Anzian et al. 2017), antibacterial (Shahid-Ud-Daula et al. 2019; Ernilasari et al. 2021), anti-inflammatory (Sahidin et al. 2019), antifungal (Ghasemzadeh et al. 2015), cytotoxic and antiproliferative activities (Mankhong et al. 2019; Shahid Ud-Daula and Mohammad 2019), as well as insect-repellent (particularly against *Aedes aegypti* (Linnaeus, 1762)) (Siregar et al. 2020), anti-aging and antiviral effects (Ruyani et al. 2019).

The Gayo Highlands of Central Aceh, Indonesia, situated at elevations of 1,000-3,000 meters above sea level (masl), form part of the biodiverse Leuser Ecosystem (Cane et al. 2023). Ethnobotanical surveys in this region have documented the traditional use of *Etlingera* species, particularly *Etlingera elatior* (Jack) R.M.Sm. and *Etlingera hemisphaerica* (Blume) R.M.Sm. (Saudah et al. 2021). While *E. elatior* is relatively well studied, *E. hemisphaerica* remains scientifically underexplored. It is a morphologically distinct, highland-adapted species, traditionally used by the Gayo ethnic community for postpartum care, anti-fatigue remedies, and general health support (Saudah et al. 2022; Riyanti et al. 2022). Given that altitude can influence secondary metabolite production and antioxidant activity in plants (Setyawati et al. 2021; Adhikari et al. 2022), the unique ecological niche of *E. hemisphaerica* may contribute to a distinct phytochemical profile.

Although both species share similarities in leaf morphology they differ significantly in flower structure (Lim 2014; Handayani et al. 2020). Taxonomically, *E. hemisphaerica* is a distinct species native to highland environments and traditionally used by the Gayo ethnic community for postpartum care and general health support (Riyanti et al. 2022). Its unique ecological niche in the high-altitude Gayo Highlands is believed to influence its phytochemical composition. Studies have shown that elevation can significantly affect the production of secondary metabolites and antioxidant potential in plants (Setyawati et al. 2021; Adhikari et al. 2022). The high-altitude environment of the Gayo Highlands, characterized by increased UV exposure and temperature variation, may enhance the synthesis of bioactive compounds in *E. hemisphaerica*.

Despite its ethnopharmacological relevance, *E. hemisphaerica* remains scientifically underexplored. No previous studies have reported its phytochemical profile or evaluated its pharmacological activity, particularly with regard to antioxidant and anticancer properties. Given its traditional use, ecological uniqueness, and taxonomic distinction, *E. hemisphaerica* from the Gayo Highlands may serve as a promising candidate for further development in cancer therapeutics. Therefore, this study aims to evaluate the phytochemical composition, antioxidant capacity, and cytotoxic activity of *E. hemisphaerica* leaf extracts collected from the Gayo Highlands, Indonesia. The findings are expected to contribute to the scientific validation of traditional knowledge and support the exploration of novel therapeutic agents from highland tropical biodiversity.

MATERIALS AND METHODS

Study area

Leaves of *E. hemisphaerica* were collected from the Gayo Highlands, Aceh Province, Indonesia, at elevations between 1,000 and 1,500 meters above sea level (Figure 1). The region is characterized by a cool climate and fertile volcanic soil, which are known to support high levels of phytochemical accumulation in local flora (Cane et al. 2023; Lestari et al. 2023). Plant identification was confirmed using the Plants of the World Online database (https://powo.science.kew.org/) (POWO 2023). The collected leaves were washed, air-dried at room temperature for seven days, and then ground into a fine powder using a mechanical grinder (Figure 2).

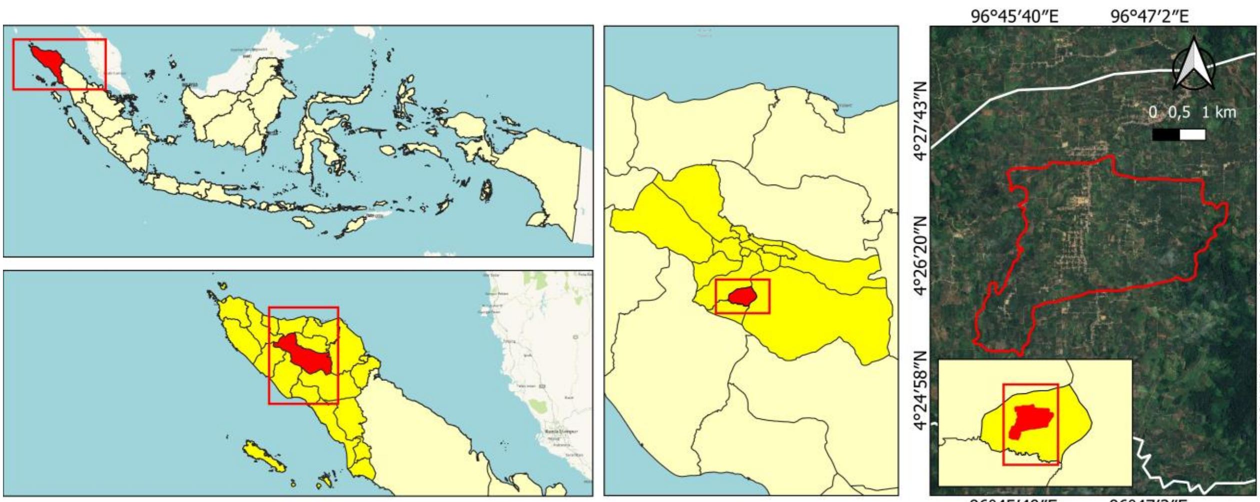

Figure 1. Map of the research location in Gayo Highlands, Central Aceh District, Aceh Province, Indonesia

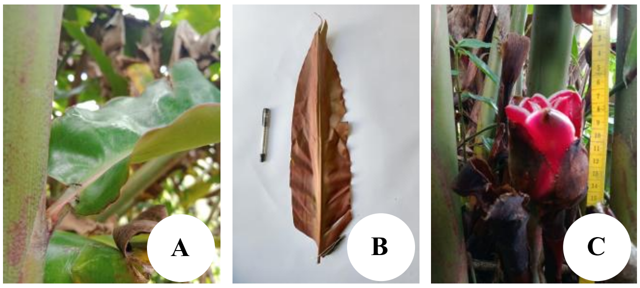

Figure 2. Morphology of *Etlingera hemisphaerica*. A. Stem, B. Leaf, C. Flower

Chemicals and equipments used

The following analytical-grade chemicals and reagents were used in this study: Quercetin, Vitamin C (ascorbic acid), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), methanol, ethyl acetate (Brataco), and distilled water. Other reagents included sodium carbonate, aluminum chloride, sodium nitrite, Folin-Ciocalteu reagent, and sodium carboxymethyl cellulose. For cell culture and cytotoxicity assays, human dermal fibroblast cell line, MCF-7 human breast cancer cell line (ATCC HTB-22), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, penicillin-streptomycin, phosphate-buffered saline (PBS), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Gibco (Thermo Fisher Scientific) or equivalent suppliers.

Equipment

The equipment used in the study included a rotary evaporator (Büchi), water bath (Memmert), UV-Visible spectrophotometer (Shimadzu UV-1800), ELISA reader (Bio-Rad), and standard laboratory glassware such as volumetric flasks, pipettes, and measuring cylinders.

Procedures

Extraction and maceration of Etlingera hemisphaerica *leaf samples*

Fresh leaves *E. hemisphaerica* were collected from the Gayo Highlands, Aceh Province, Indonesia. The leaves were thoroughly washed with distilled water to remove debris and soil, air-dried in a shaded, well ventilated area at ambient temperature (25-30°C) for 7 days, and than ground into a fine powder using an electric grinder. A total of 1000 g of dried leaf powder was used for extraction. The powdered sample was divided equally into three parts (approximately 333 g each) and macerated with three different solvents: methanol, ethyl acetate, and distilled water (aquadest). Each portion was immersed in 1,000 mL of the respective solvent in a clean, dry maceration flask. The maceration was carried out at room temperature (± 27°C) for 72 hours with occasional shaking to maximize extraction efficiency. After 72 hours, the mixtures were filtered using Whatman No. 1 filter paper to separate the extract solution from the plant residues. This process was repeated twice to ensure exhaustive extraction. The filtrates were collected, pooled, and concentrated under reduced pressure using a rotary evaporator at 40°C for methanol and ethyl acetate extracts. The aqueous extract was concentrated using a freeze dryer. The resulting crude extracts of *E. hemisphaerica* from methanol, ethyl acetate, and aqueous were stored in sealed glass containers at 4°C until further analysis. The percentage yield of each extract was calculated based on the weight of the dried extract relative to the original plant material (Fithrotunnisa et al. 2020).

Phytochemical screening procedures

Alkaloid test

A total of 100 mg of plant extract was dissolved in 10 mL of methanol and filtered. Then, 2 mL of the filtrate was acidified with 1 mL of 1% hydrochloric acid (HCl), followed by the addition of 2-3 drops of Dragendorff's reagent. The presence of alkaloids was indicated by the formation of a reddish-brown precipitate (Tiwari et al. 2011). The formation of an orange to reddish-brown precipitate indicated the presence of alkaloids. For the Mayer's test, another 2 mL of the same filtrate was mixed with 1 mL of 1% HCl and treated with 2-3 drops of Mayer's reagent. The presence of a cream or yellowish-white precipitate confirmed the presence of alkaloid compounds (Rahimah et al. 2019).

Triterpenoid and steroid test

Fifty milligrams (50 mg) of the plant extract were dissolved in 5 mL chloroform and filtered or the Liebermann Burchard test, 2 mL of the filtrate was treated with 1-2 mL of acetic anhydride and carefully layered with 2-3 drops of concentrated sulfuric acid (H_2SO_4). The gradual appearance of a red to blue, followed by green color indicated the presence of sterols. In a separate test, 2 mL of chloroform extract was mixed with a few drops of concentrated H_2SO_4 and gently shaken. A reddish to yellow coloration in the acid layer indicated the presence of steroids, while a brownish-red coloration confirmed the presence of triterpenoids (Gadouche et al. 2023).

Saponin test

The extract (approximately 1 g or equivalent extract volume) was diluted with 20 mL of distilled water and heated to boil for 2-3 minutes, then cooled to room temperature. The mixture was then vigorously shaken for 30 seconds. The formation of stable, persistent foam (lasting more than 10 minutes) indicated the presence of saponins (Noviyanty et al. 2020).

Flavonoid test

A measured amount of the extract (approx. 100 mg) was heated with 10 mL of ethyl acetate over a boiling water bath for 3 minutes and filtered. The filtrate was then mixed with 1 mL of 1% ammonia solution and gently shaken. The development of a yellow coloration in the ammonia layer indicated the presence of flavonoids (Kancherla et al. 2019).

Determination of phenolic content (TPC)

The total phenolic content (TPC) of the plant was determined using the Folin-Ciocalteu colorimetric method as described by Ghasemzadeh et al. (2014), with slight modifications. Gallic acid (GA) was used as the reference standard, prepared in distilled water at concentrations ranging from 0.01 to 0.19 mg/mL to construct a calibration curve. A 0.10 mL aliquot of each gallic acid standard solution or plant extract (5.0 mg/mL) was pipetted into a 10.0 mL volumetric flask. Subsequently, 0.5 mL of 10% (w/v) Folin-Ciocalteu reagent was added, followed by a 3-minute incubation at room temperature. Then, 4.0 mL of 7.5% (w/v) sodium carbonate solution was added. The solution was diluted to the mark with distilled water, thoroughly mixed, and incubated at 40°C in a water bath for 30 minutes. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). The TPC was calculated from the standard curve and expressed as milligrams of gallic acid equivalent per gram of dried extract (mg GAE/g), using the formula:

$$\text{TFC (mg GAE/g)} = \frac{\text{C x V}}{\text{M}}$$

Where: C: concentration of gallic acid (mg/mL) obtained from the calibration curve, V: volume of extract used (mL), M: weight of dried extract (g). Results were expressed as milligrams of gallic acid equivalent per gram of dried extract (mg GAE/g).

Determination of flavonoid content

The total flavonoid content (TFC) was measured using a colorimetric method based on the formation of a flavonoid aluminum complex, as described by Ayele et al. (2022). Quercetin was used as the reference standard, prepared in methanol at concentrations ranging from 0.01 to 0.10 mg/mL to construct the calibration curve. For the reaction, 0.5 mL of standard or plant extract (5.0 mg/mL) was transferred into a 10.0 mL volumetric flask. Then, 1.0 mL of 20% aluminum chloride ($AlCl_3$) solution and 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution were added. The volume was adjusted with distilled water to 10.0 mL. After standing for 5 minutes at room temperature, the absorbance was read at 430 nm. The TFC was calculated using the following formula:

$$\text{TPC (mg QE/g)} = \frac{\text{C x V}}{\text{M}}$$

Where: C: concentration of quercetin (mg/mL) from the calibration curve, V: volume of extract used in the assay (mL), M: mass of dried extract used (g). Results were expressed as milligrams of quercetin equivalent per gram of dried extract (mg QE/g), representing the total flavonoid concentration (Ayele et al. 2022).

DPPH antioxidant activity

The antioxidant activity of *E. hemisphaerica* leaf extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, based on the method described by Bello et al. (2024) and Sari et al. (2024), with minor modifications. DPPH is a stable free radical that changes color from violet (DPPH•) to yellow (DPPH-H) upon reduction by an antioxidant. This color change corresponds to a decrease in absorbance at 517 nm and is inversely proportional to the antioxidant capacity of the sample. A 0.004% DPPH solution was freshly prepared in methanol. Extract solutions were prepared in methanol at concentrations of 0, 20, 40, 50, 70, 90, and 100 µg/mL. For each reaction, 200 µL of extract solution was mixed with 800 µL of DPPH solution. The mixture was vortexed and incubated in the dark at 25 ± 2°C for 30 minutes. A negative control was prepared using 200 µL of methanol and 800 µL of DPPH solution. Ascorbic acid, prepared at the same concentration range, was used as the positive control. The absorbance of each sample was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800), and the radical scavenging activity was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} x100$$

Where: A_control: absorbance of the control reaction (DPPH + methanol), A_sample: absorbance of the test sample (DPPH + extract). The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was calculated from the dose-response curve using linear regression analysis.

Anticancer activity using the MTT assay

The cytotoxic potential of *E. hemisphaerica* extract was assessed against MCF-7 human breast cancer cells (ATCC HTB-22) using the MTT assay, following the protocol of Williams et al. (2017) with slight modifications. MCF-7 cells were cultured in RPMI-1640 medium supplemented with 5% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO_2. Cells were seeded in a 96-well plate at a density of 5,000 cells per well in 100 µL of complete medium and incubated for 24 hours to allow attachment. Afterward, cells were treated with various concentrations of the extract (e.g., 10-640 µg/mL) and incubated for 48 hours. On day three, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well, followed by a 4-hour incubation at 37°C. After the formation of formazan crystals, the medium was carefully removed, and 100 µL of ethanol was added to each well to dissolve the crystals. Absorbance was measured at 595 nm using a microplate reader (Bio-Rad) Williams et al. (2017). MTT solution (5 mg/mL) was added to each well at a final concentration of 10 µL and incubated for 4 hours at 37°C. After incubation, formazan crystals formed by viable cells were dissolved using ethanol. The absorbance was measured at 595 nm using a microplate reader. The percentage of inhibition is calculated using the formula:

$$\%Inhibition \frac{abs\ control - abs\ sample}{abs\ control} x\ 100\%$$

Where: abs_control is the absorbance of untreated cells, and abs_sample is the absorbance of treated cells. The IC_{50} value, representing the concentration required to inhibit 50% of cell viability, was determined via linear regression and used to evaluate the antiproliferative potential of the extract.

RESULTS AND DISCUSSION

Phytochemical screening of *Etlingera hemisphearica* leaves

Phytochemical screening of *E. hemisphearica* leaf extract using three different solvents methanol, n-hexane, and ethyl acetate the presence of various secondary metabolites, including flavonoids, phenolics, steroids, tannins, saponins, and alkaloids (Table 1). The methanol extract, representing a polar solvent, was rich in flavonoids, phenolics, saponins, and steroids. The ethyl acetate extract, a semi-polar solvent, contained flavonoids, steroids, saponins, and alkaloids. Meanwhile, the non-polar n-hexane extract primarily exhibited the presence of steroids, with minimal detection of polar compounds. These findings suggest that solvent polarity influences the efficiency of secondary metabolite extraction, with methanol being the most effective in isolating bioactive polar compounds known for their pharmacological potential. The presence of these compounds supports the traditional medicinal use of *E. hemisphaerica* and highlights its potential as a source of antioxidant and anticancer agents.

Total phenolic and flavonoid content (TPC)

The total phenolic content (TPC) and total flavonoid content (TFC) of *E. hemisphaerica* leaf extract were analyzed using a UV-vis spectrophotometer. TPC was determined using the Folin-Ciocalteu reagent, with gallic acid as the standard, and absorbance measured at 745 nm. The results are expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g). TFC was measured using quercetin as the standard and expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g). The type of solvent significantly influenced the amount of bioactive compounds extracted. The methanol extract exhibited the highest TPC value at 506.4 ± 15.41 mg GAE/g, indicating high solubility of phenolic compounds in polar solvents. Conversely, the highest TFC was found in the ethyl acetate extract, with a value of 471.5 ± 20.55 mg QE/g, suggesting better solubility of flavonoids in semi-polar solvents. Aquadest yielded the lowest values for both phenolics and flavonoids, confirming its lower efficiency in extracting these classes of compounds. These findings emphasize the critical role of solvent polarity in the efficiency of phytochemical extraction.

Antioxidant activity using the DPPH method

The antioxidant activity of *E. hemisphaerica* leaf extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The results were expressed as IC_{50} values (mean ± SD, n = 3), representing the concentration of extract required to inhibit 50% of DPPH radical activity. A lower IC_{50} values corresponds to stronger antioxidant potential. As shown in Table 3 and, all extracts exhibited concentration-dependent antioxidant activity. Among the three solvent extracts tested, the methanol extract demonstrated the strongest antioxidant activity, with an IC_{50} value of 0.40 ± 0.03 ppm, significantly ($p < 0.05$) lower than those of the other extracts and the standard. The aqueous extract showed moderate activity with an IC_{50} of 1.07 ± 0.05 ppm, followed by the ethyl acetate extract at 1.97 ± 0.06 ppm. Interestingly, all extracts outperformed ascorbic acid as a positive control, which had an IC_{50} of 6.74 ± 0.12 ppm. These results indicate that solvent polarity significantly affects the extraction of antioxidant compounds. The superior performance of the methanol extract may be attributed to its higher content of phenolic and flavonoid compounds, as previously reported in phytochemical studies. The significant differences among the treatments were confirmed via one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Table 1. Qualitative analysis of secondary metabolites in leaf extracts of *Etlingera hemisphaerica* from the Gayo Highlands using different solvents

Secondary metabolites	Methanol Extract	Ethyl Acetate Extract	Aquadest Extract
Flavonoids	+++	++	+
Terpenoids	+	+	+
Steroids	++	+	+
Tannin	+	-	+
Saponin	++	+	+
Phenolic	+++	+	+
Alkaloids	+	+	+

Note: (-): Not detected, (+): Weakly present, (++): Moderately present, (+++): Strongly present

Table 2. Total phenolic and flavonoid content of *Etingera hemisphaerica* leaf extract

Solvent	Total bioactive content	
	TPC (mg GAE/g)	TFC (mg QE/g)
Methanol	506.4 ± 15.41c	320.4 ± 11.72c
Ethyl acetate	226.1 ± 12.80d	471.5 ± 20.55e
Aquadest	± 3.50d	114.2± 4.68c

Note: Superscript letters (c, d, e) indicate statistically significant differences (p < 0.05) based on post hoc analysis

Table 3. IC_{50} values of antioxidant activity of *Etlingera hemisphaerica* leaf extracts

Solvent	IC_{50} (ppm)
Methanol	0.40 ± 0.03^{a}
Ethyl acetate	1.97 ± 0.06^{b}
Aquadest	1.07 ± 0.05^{c}
Ascorbic acid	6.74 ± 0.12^{d}

Note: Different superscript letters indicate significant differences among groups at p < 0.05 (Tukey HSD)

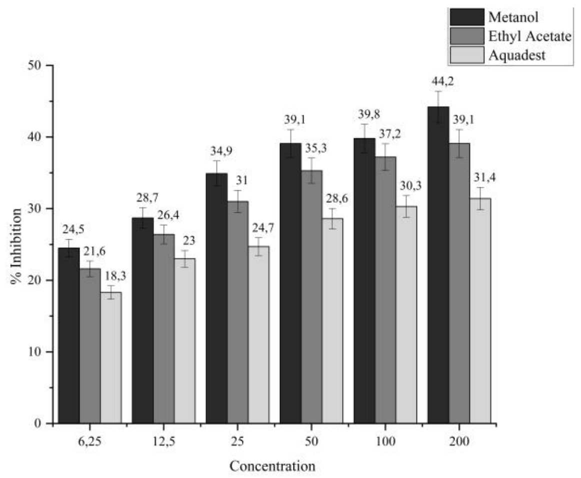

Figure 3. Percentage of MCF-7 breast cancer cell inhibition by *Etlingera hemisphaerica* leaf extracts

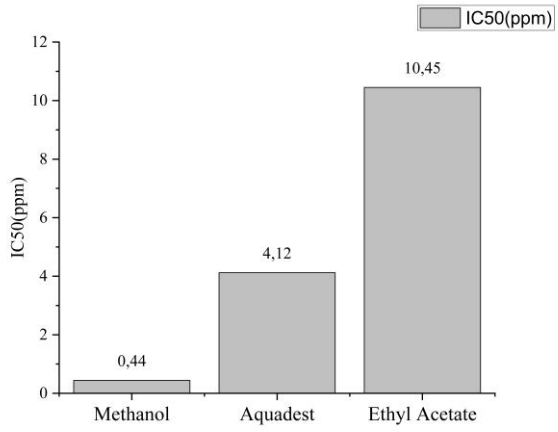

Figure 4. IC_{50} values of *Etlingera hemisphaerica* leaf extracts against MCF-7 breast cancer cells

MTT assay test of *Etlingera hemisphaerica* leaf extract

The cytotoxic activity of *E. hemisphaerica* leaf extracts against MCF-7 breast cancer cells was evaluated using the MTT assay. The percentage of cell inhibition at various extract concentrations (6.25-200 ppm) is shown in Figure 3. The results demonstrate a concentration-dependent inhibitory effect for all extracts tested. At the highest concentration of 200 ppm, the methanol extract exhibited the most potent cytotoxic activity, inhibiting 43.47% of MCF-7 cell viability. As the concentration decreased, the inhibition percentage also declined, reaching 23.21% at 6.25 ppm. This pattern indicates a clear dose-response relationship. The ethyl acetate extract also showed cytotoxic effects, although to a lesser extent than the methanol extract. At 200 ppm, the inhibition was 37.53%, decreasing to 17.59% at the lowest concentration. The aquadest extract displayed the weakest activity, with a maximum inhibition of 30.12% at 200 ppm and 18.21% at 6.25 ppm. These findings suggest that methanol is the most effective solvent for extracting bioactive compounds with anticancer potential from *E. hemisphaerica*. The superior cytotoxicity of the methanol extract correlates with its higher content of flavonoids and phenolics, as previously shown in the phytochemical and antioxidant assays. These compounds are known to exert anticancer activity by inducing apoptosis and inhibiting cancer cell proliferation.

The IC_{50} value of *E. hemisphaerica* leaf extracts against MCF-7 breast cancer cell were determined to assess their cytotoxic potency. The methanol extract exhibited the strongest anticancer activity with an IC_{50} value of 0.446 ppm, indicating that only a very low concentration was required to inhibit 50% of cancer cell viability (Figure 4). This suggests a high cytotoxic potential, likely attributed to the elevated levels of flavonoids and phenolics present in the methanol extract, as previously confirmed in the phytochemical and antioxidant analyses. The ethyl acetate extract showed a markedly weaker effect, with an IC_{50} value of 10.45 ppm, indicating lower anticancer efficacy. In comparison, the aquadest extract displayed moderate cytotoxicity, with an IC_{50} of 4.12 ppm, which was less effective than methanol but more active than ethyl acetate. These findings demonstrate that solvent polarity significantly influences the extraction of cytotoxic compounds, with methanol proving to be the most efficient. The strong inverse correlation between IC_{50} values and phenolic/flavonoid content further supports the role of these compounds in mediating anticancer activity.

Discussion

The phytochemical screening of *E. hemisphaerica* leaf extract from the Gayo Highlands revealed the presence of major classes of bioactive secondary metabolites, including flavonoids, phenolics, terpenoids, steroids, tannins, and saponins (Table 1). These classes of compounds are known to play central roles in mediating various pharmacological effects, particularly antioxidant and anticancer activities. The qualitative phytochemical profile observed aligns with prior reports on other *Etlingera* species such as *E. elatior*, *Etlingera pavieana* (Pierre ex Gagnep.) R.M.Sm., and *Etlingera alba* (Blume) A.D.Poulsen, which exhibit comparable metabolite compositions and traditional medicinal relevance (Iawsipo et al. 2018; Al-Mansoub et al. 2021; Padmanabhan et al. 2024). This also corroborates the findings of Riyanti et al. (2022), confirming the traditional use of the species based on its rich phytochemical content.

Quantitative analyses demonstrated that the methanol extract possessed the highest total phenolic content (506.4 ± 15.41 mg GAE/g), while the ethyl acetate extract had the highest total flavonoid content (471.5 ± 20.55 mg QE/g). These results reflect the solvent-dependent solubility of different phytoconstituents, with methanol being more effective for polar phenolic compounds and ethyl acetate for moderately polar flavonoids. This observation is in agreement with findings from *E. elatior* and *Curcuma longa* L., which highlight solvent polarity as a key factor influencing extraction efficiency (Widyasari et al. 2014; Fithrotunnisa et al. 2020).

Flavonoids and compounds are prominent contributors to antioxidant defense due to their capacity to scavenge free radicals, chelate metal ions, and enhance the activity of endogenous antioxidant enzymes such as catalase and superoxide dismutase. Their electron donating and hydrogen-transfer capabilities make them effective in neutralizing reactive oxygen species (ROS), thereby mitigating oxidative stress a known contributor to cancer, cardiovascular disease, and neurodegenerative disorders (Perez-Vizcaino and Fraga 2018; Kopustinskiene et al. 2020; Rahmiyani et al. 2023). Additionally, flavonoids exhibit anti-inflammatory, antimicrobial, and antiproliferative properties through modulation of NF-κB signaling, inhibition of angiogenesis, and induction of mitochondrial-mediated apoptosis (Sak 2014; Nabavi et al. 2020). Phenolic compounds, synthesized via the shikimate pathway, phenylpropanoid pathways, are similarly associated with cell cycle arrest and tumor-suppressive effects (Maheshwari and Sharma 2023).

The methanol extract displayed the highest antioxidant capacity with an IC_{50} of 0.40 ppm, which was markedly more potent than ethyl acetate (1.97 ppm), aqueous extract (1.07 ppm), and the standard antioxidant ascorbic acid (6.74 ppm). This exceptional activity is likely attributed to the high concentration of phenolics and flavonoids in the methanol extract. Similar solvent-dependent antioxidant trends have been reported in *E. elatior*, *Zingiber officinale* Roscoe, and *C. longa*, further reinforcing the critical role of solvent polarity and phytochemical abundance in antioxidant efficacy (Ghasemzadeh et al. 2015; Chin 2016; Anzian et al. 2017).

In terms of cytotoxic potential, the methanol extract exhibited the most potent inhibition of MCF-7 human breast cancer cells, with an IC_{50} of 0.446 ppm. This strong cytotoxicity at low concentrations suggests a significant presence of antiproliferative constituents. Comparative studies with *E. elatior* and *E. pavieana* have shown similar activity against various cancer cell lines, attributed primarily to flavonoids (quercetin, kaempferol) and diarylheptanoids that induce apoptosis and ROS-mediated DNA damage (Iawsipo et al. 2018; Zhao et al. 2019). Flavonoids are known to activate intrinsic apoptotic pathways via mitochondrial membrane disruption, upregulation of pro-apoptotic proteins, and inhibition of anti-apoptotic proteins (e.g., Bcl-2), culminating in caspase activation and cancer cell death (Nabavi et al. 2020).

The potent cytotoxic effect of the methanol extract may also be attributed to terpenoids and steroids, which have been reported to disrupt cellular homeostasis through membrane destabilization, modulation of cell cycle regulatory proteins, and interference with angiogenesis (Jiang 2019). Such synergistic interactions among phytochemicals likely enhance the therapeutic potential of the extract.

Compared to other Zingiberaceae species, *E. hemisphaerica* appears to demonstrate superior or comparable bioactivity. For example, *C. longa* typically exhibits DPPH IC_{50} values between 2-5 ppm and MCF-7 IC_{50} values ranging from 10-20 ppm (Maheshwari and Sharma 2023), whereas the IC_{50} values of *E. hemisphaerica* are substantially lower, suggesting stronger bioefficacy. The observed activities also support the ethnomedical relevance of *E. hemisphaerica*, indicating that its traditional usage may have a scientific basis grounded in phytochemistry. Moreover, the results provide a comparative framework with related *Etlingera* species, strengthening the claim that this genus represents a rich source of antioxidant and anticancer agents.

In summary, the bioactivities of *E. hemisphaerica* leaf extracts particularly those obtained with methanol are closely linked to their phytochemical composition, especially the high phenolic and flavonoid content. These compounds likely act through multiple cellular mechanisms, including oxidative stress modulation, apoptosis induction, and inhibition of cancer cell proliferation. Given the remarkably low IC_{50} values observed, *E. hemisphaerica* holds strong potential for development as a natural antioxidant and anticancer agent. However, further studies are required to isolate and characterize the active compounds, confirm efficacy in in vivo systems, and assess toxicity and safety for therapeutic use.

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