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Phytochemical diversity and anticancer potential of *Etlingera elatior* fruits across ecological regions in Aceh, Indonesia

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Abstract. *Asmilia N, Sari W, Nursanty R, Saudah, Zumaidar. 2025. Phytochemical diversity and anticancer potential of* Etlingera elatior *fruits across ecological regions in Aceh, Indonesia. Biodiversitas 26: 5423-5429. Etlingera elatior* (Zingiberaceae) fruit is a traditional Acehnese medicinal plant known for its diverse bioactive compounds. This study investigated the phytochemical diversity, antioxidant properties, cytotoxicity, and anticancer potential of *E. elatior* fruits collected from three regions of Aceh: Pidie, the Gayo Highlands, and Simeulue Island. Qualitative and quantitative screenings were performed to assess secondary metabolites, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC). Antioxidant capacity was performed by the DPPH assay, cytotoxicity by the Brine Shrimp Lethality Test (BSLT), and anticancer activity by the MTT assay against MCF-7 breast cancer cells. The results revealed clear regional differences in phytochemical profiles and bioactivities. Simeulue fruits exhibited the highest TPC and TFC, while extracts from the Gayo Highlands showed the strongest antioxidant and cytotoxic activities. Notably, the Gayo extracts also demonstrated the most potent anticancer activity, significantly inhibiting MCF-7 cell proliferation. These findings highlight the ecological influence on phytochemical diversity and biological activity of *E. elatior*, with altitude and geographic conditions shaping metabolite accumulation. Overall, the study demonstrates that *E. elatior* fruits, particularly those from the Gayo Highlands, possess considerable anticancer potential. This underscores the importance of conserving local biodiversity and supports the development of *E. elatior* as a natural source for novel anticancer agents. Further research should prioritize isolation of active constituents, molecular pathway analysis, and in vivo validation to advance its development for nutraceutical and phytopharmaceutical applications.

Keywords: Antioxidant, cytotoxicity, MCF-7, traditional spice, Zingiberaceae

INTRODUCTION

Plant-derived products are valuable for human health due to their rich bioactive metabolites such as phenolic, flavonoids, alkaloids, terpenoids, and essential minerals. These phytochemicals help prevent or delay chronic diseases like cancer through antioxidant, anti-inflammatory, and antiproliferative effects (Lee et al. 2017). Among plant-based resources, spices occupy a central role, functioning not only as culinary ingredients but also as traditional remedies across different cultures. Well-documented examples include turmeric (*Curcuma longa*), cloves (*Syzygium aromaticum*, and cinnamon (*Cinnamomum* spp.), all of which exhibit high phenolic content and remarkable antioxidant capacity (Zahra et al. 2016; Jiang 2019; Sammar et al. 2019). The Zingiberaceae family comprises important medical and aromatic plants, notably *Zingiber officinale* (ginger), which exhibits antimicrobial, antioxidant, and anticancer properties through apoptosis inducing metabolites (Aryal et al. 2019; Alfuraydi et al. 2024). While ginger is well studied, many other ethnomedicinal species in this family remain underexplored, offering potential sources of novel anticancer compounds.

One underexplored member is *Etlingera elatior*, known as torch ginger or *kecombrang*. Native to Southeast Asia and widespread in Indonesia, it holds notable importance in traditional medicine and food culture. In Aceh Province, for example, its fruits are commonly used as both a spice and an herbal remedy. Ethnobotanical records indicate that different parts of *E. elatior* are used in the treatment of wounds, diarrhea, cough, abdominal pain, ear infections, postpartum disorders, and general health maintenance (Fristiohady et al. 2019; Gulati et al. 2021). Modern pharmacological studies have corroborated many of these uses, reporting antioxidant (Adegoke and Ojo 2017; Tee et al. 2025), antibacterial (Shahid-Ud-Daula et al. 2019; Ernilasari et al. 2021), antifungal, immunomodulatory (Safriani et al. 2021), antidiabetic (Zumaidar et al. 2024), and cytotoxic (Mankhong et al. 2019) activities. These diverse bioactivities are strongly associated with its rich phytochemical profile, which includes flavonoids, terpenoids, saponins, tannins, carbohydrates, and anthocyanins (Lestari et al. 2018). Reviewing the traditional uses of *E. elatior* is vital to recognize the balance between traditional knowledge and scientific validation. Traditional practices offer valuable ethnopharmacological insights, however, they should not overshadow the need for evidence-based approaches, which are key aspect of this study. Emphasizing scientifically validated findings is essential to ensure efficacy and safety, thereby supporting the responsible integration of traditional remedies into modern medical applications. A comprehensive approach that values both traditional and scientific perspectives can ultimately lead to more robust and effective therapeutic practices. Collectively, these findings highlight *E. elatior* as a promising species for further investigation in the context of drug discovery and functional food development.

Despite the growing number of pharmacological studies, research on *E. elatior* has mostly focused on materials collected from single or restricted geographic regions. This limited approach overlooks the potential influence of ecological variation on metabolite composition and biological activity. In Aceh Province, which is home to 12 indigenous ethnic groups, ethnobotanical surveys have revealed the widespread use of *E. elatior* as well as noticeable morphological differences among populations from distinct regions (Saudah et al. 2022). Such variation suggests that ecological parameters—including altitude, soil type, and microclimatic conditions—may significantly affect the biosynthesis of secondary metabolites. This hypothesis is consistent with findings in other medicinal plants, where altitude and geography were shown to influence phytochemical accumulation and related bioactivities (Pant et al. 2021; Rayhannisa et al. 2024). A comprehensive understanding of these ecological determinants is therefore essential for both conservation strategies and the development of high-quality herbal products.

Recently, no systematic study has directly compared the phytochemical composition and anticancer potential of *E. elatior* fruits collected from ecologically diverse regions in Aceh. Addressing this gap is vital for elucidating the ecological-phytochemical linkages that underlie the therapeutic potential of this culturally significant species. It also provides a scientific basis for the conservation and sustainable utilization of regional plant resources. Therefore, the present study aimed to evaluate the phytochemical composition, antioxidant properties, cytotoxicity, and anticancer activities of *E. elatior* fruits obtained from three contrasting regions of Aceh—Pidie (500-700 masl, East Coast), the Gayo Highlands (1,000-1,500 masl), and Simeulue Island (0-400 masl, Southwest Coast). We hypothesized that altitude and geographic origin strongly influence the secondary metabolite profiles and biological functions of *E. elatior* fruits, thereby identifying promising ecological sources of bioactive compounds with anticancer potential.

MATERIALS AND METHODS

Study area and sample collection

Fresh fruits of *Etlingera elatior* were collected from three regions of Aceh, Indonesia: Pidie (East Coast, 500-700 masl), the Gayo Highlands (1,000-1,500 masl), and Simeulue Island (Southwest Coast, 0-400 masl) (Figure 1). Voucher specimens were identified by a taxonomist at the Department of Biology, Universitas Syiah Kuala, and deposited in the Herbarium Acehense (ID no. 1283/UN11.F8.4/TA.00.03/2024). Mature fruits (7-12 cm long, yellow to reddish) were harvested in the morning and used for extraction. This was done to ensure that samples collected from different locations were standardized based on their morphological characteristics and collected at the same time of day.

Sample extraction

Fresh fruit *E. elatior* (2 kg) was washed with destilled water, air-dried at room temperature for seven days, and ground into a coarse powder. Extraction was performed by maceration using 96% ethanol, following Sruthi and Indira (2016), with slight modifications. Briefly, 100 g powdered sample was soaked in 500 mL of ethanol for 24 hours at room temperature and repeatedly extracted until the solvent became colorless. The resulting filtrates were concentrated through reduced pressure using a vacuum rotary evaporator (Büchi Rotavapor R-300, R-220 Pro, Switzerland) at 40-50°C, and the crude ethanolic extract was stored at 20°C in an amber bottle for further analyses. This extract is hereafter referred to as EEFEE (Ethanolic Extract of *E. elatior* Fruits).

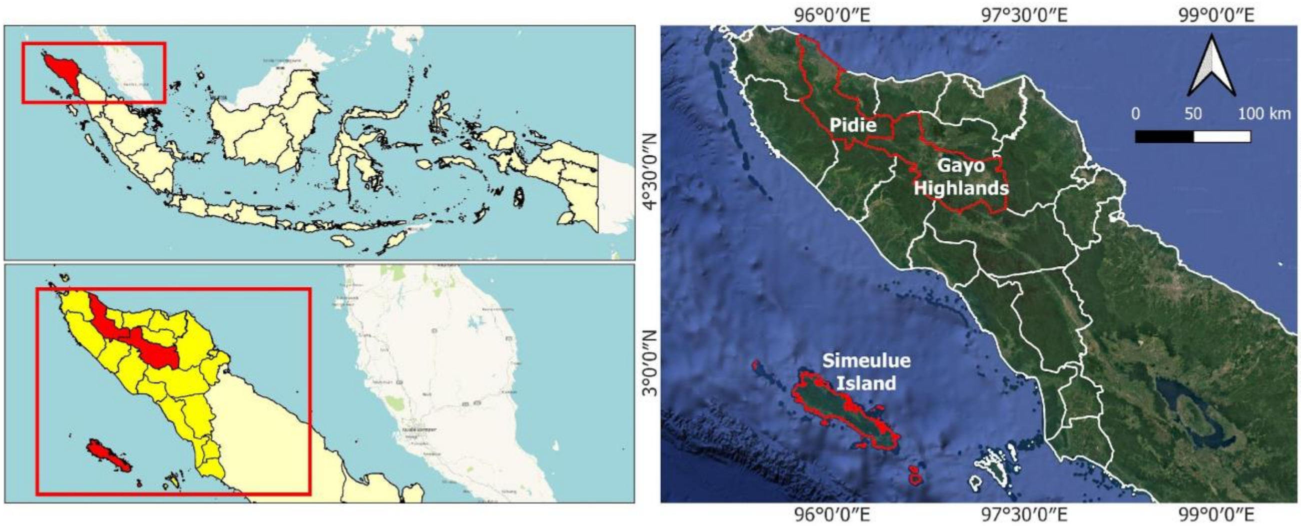

Figure 1. Sampling location of *Etlingera elatior* fruit in Aceh Region, Sumatra-Indonesia

Phytochemical screening

Qualitative phytochemical screening of *E. elatior* Ethanolic Fruit Extracts (EEFEE) from Simuelue, Pidie, and Gayo Higlands was conducted to detect major classes of secondary metabolites, including flavonoids, phenolics, tannins, terpenoids, saponins, and alkaloids. Standard colorimetric and precipitation assays were performed according to Nuraskin et al. (2020) with slight modifications. Flavonoids were identified using the Shinoda test (red-pink coloration after reaction with magnesium and concentrated HCl), phenolics by ferric chloride test (blue-black or green coloration), and tannins by gelatin and ferric chloride tests (precipitation or blue-black coloration). Terpenoids were detected using the Salkowski method (reddish-brown interface after treatment with chloroform and concentrated H_2SO_4), saponins by the froth test (persistent foam formation), and alkaloids by Dragendorff's and Mayer's reagents (orange-brown or cream precipitate). The presence (+) or absence (-) of each metabolite class was recorded for all regional extracts, providing preliminary insights into phytochemical diversity.

Total Phenolic Content (TPC)

Total phenolic content of EEFEE was determined using the Folin-Ciocalteu method (Jayaprakasha and Patil 2007). Briefly, 200 µL of extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent (Merck, Germany) and allowed to stand for 10 min. Subsequently, 4.0 mL of sodium carbonate (Na_2CO_3, Merck, Germany) was added, and the mixture was incubated at room temperature for 30 min. Absorbance was performed at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-2550, Japan). Results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DW), based on calibration curves prepared with gallic acid standards.

Total Flavonoid Content (TFC)

Total flavonoid content of EEFEE was determined following Ghasemzadeh et al. (2015) with slight modifications. Briefly, 500 µL of extract was mixed with sodium nitrite ($NaNO_2$, Merck, Germany) in 4 mL of methanol and incubated for 6 min. Then, 0.3 mL of 10% aluminum chloride ($AlCl_3$, Merck, Germany) was added and left to react for another 6 min, and then another 2 mL of 1 M NaOH (sodium hydroxide) (Merck, Germany) was added. After 10 min, absorbance was performed at 510 nm using a UV-Vis Spectrophotometer (Shimadzu UV-2550, Japan). Results were described as milligrams of quercetin equivalents per gram of dry extract (mg QE/g DW), based on calibration curves prepared with quercetin standards.

Antioxidant activity 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) assay

The antioxidant activity of EEFEE was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay following Molyneux (2004) and Sulaiman and Ooi (2012) with slight modifications. A 0.4 mM DPPH solution was prepared in methanol, and extract solutions were tested at concentrations of 3.125-100 µg/mL. Ascorbic acid (Merck, Germany) served as the positive control. After mixing 1 mL of DPPH solution with the extracts and adjusting the volume mL with methanol to 5 mL, samples were incubated for 30 min at room temperature. Absorbance was measured at 515 nm using a UV-Vis spectrophotometer (Shimadzu UV-2550, Japan). Antioxidant capacity was expressed as IC_{50}, defined as the concentration required to scavenge 50% of DPPH radicals, calculated from triplicate measurements.

Cytotoxicity (Brine Shrimp Lethality Test)

Cytotoxicity of EEFEE was evaluated against *Artemia salina* larvae using the Brine Shrimp Lethality Test (BSLT) following Yahya et al. (2022) with slight modifications. Extracts were dissolved in 5% Dimethyl Sulfoxide (DMSO, Merck, Germany) and diluted with seawater to prepare concentrations of 20, 100, 250, 500, and 1000 µg/mL. 10 nauplii were transferred into each test solution, while seawater with 5% DMSO served as a negative control. After 24 h incubation, larval mortality (%) was calculated, and LC_{50} values were determined by probit analysis using linear regression of mortality versus log concentration. The mortality rate of larvae by percentage was calculated according to the following equation:

$$\%\ \text{Mortality} = \frac{\text{The number of dead larvae}}{\text{The number of larvae test}} \times 100\%$$

Anticancer activity (MTT assay) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

The anticancer activity of EEFEE was evaluated against MCF-7 human breast cancer cells (ATCC HTB-22) using the MTT assay, conducted at the Primate Research Center, Institut Pertanian Bogor, Bogor, Indonesia. Cells were cultured in RPMI-1640 medium supplemented with 5% Fetal Bovine Serum (FBS) and 100 µg/mL streptomycin at 37°C in a humidified 5% CO_2 atmosphere. Cells were seeded into 96-well plates at a density of 1×10^4 cells/well and allowed to adhere for 24 h before treatment. Extracts were applied at concentrations of 40, 80, 160, 320, and 640 µg/mL and incubated for 48 h. Subsequently, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The resulting formazan crystals were dissolved in 100 µL of 96% ethanol, and absorbance was measured at 596 nm using a microplate ELISA reader. Doxorubicin (1 µg/mL) served as the positive control, while untreated cells were used as the negative control. All treatments were performed in triplicate (n = 3). Cell viability (%) was calculated relative to the control, and IC_{50} values were obtained by nonlinear regression analysis.

$$y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (x / IC_{50})^\wedge\text{HillSlope})$$

Where:

Bottom : 0 (or the minimum observed *y* value)

Top : 100 (or the maximum observed *y* value)

HillSlope : 1 (if the curve is very steep, try values between 1-3; if it is shallow, try around 0.5)

LogIC_{50} : LOG10 of the midpoint concentration range

IC_{50} (non-log) : The median concentration value

Data analysis

The data were analyzed using a one-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). For significance, a p-value≤0.05 was used to determine statistical significance. All data examinations were performed utilizing SPSS version 25 software.

RESULTS AND DISCUSSION

Phytochemical properties

Qualitative screening revealed consistent classes of secondary metabolites across regions, including flavonoids, phenolics, terpenoids, saponins, and alkaloids, though with varying intensities (Table 1). Extracts from the Gayo Highlands showed the strongest presence of flavonoids, phenolics, and tannins, indicating greater phytochemical richness compared with Pidie and Simeulue. Saponins were abundant in both Pidie and Gayo, whereas steroids were uniquely detected in Pidie. These findings suggest that ecological conditions, particularly altitude, influence metabolite profiles.

Quantitative phytochemical content

Quantitative assays revealed substantial variation in Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) (Table 2). Simeulue fruits recorded the highest TPC and TFC, while Gayo Highlands and Pidie exhibited lower values. Interestingly, higher TPC and TFC in Simeulue did not correspond to stronger antioxidant or cytotoxic activity, suggesting that qualitative composition and metabolite interactions play a more critical role than absolute quantities.

Antioxidant activity

The DPPH assay showed that extracts from Gayo Highland exhibited the strongest antioxidant activity, with an IC_{50} of 23.8 µg/mL, surpassing the positive control ascorbic acid (16.43 µg/mL). Simeulue extracts also displayed strong activity (47.89 µg/mL), while Pidie extracts were the weakest (72.33 µg/mL). These results highlight a clear ecological influence, where high-altitude Gayo samples were the most potent radical scavengers (Table 3).

Cytotoxicity

Cytotoxic screening using BSLT confirmed that Gayo Highland extracts were the most toxic, with an LC_{50} of 13.28 µg/mL, classifying them as highly toxic (≤30 µg/mL). Simeulue (76.39 µg/mL) and Pidie (62.72 µg/mL) extracts were moderately toxic (Table 4). This pattern is consistent with their qualitative phytochemical richness, particularly in flavonoids and tannins.

Anticancer activity (MTT assay)

All extracts inhibited MCF-7 breast cancer cell proliferation in a dose-dependent manner (Figure 2). However, regional variation was pronounced. The Gayo Highlands extract was the most effective, with an IC_{50} of 7.35 µg/mL, approaching the potency of doxorubicin (2.08 µg/mL). In contrast, Pidie (365.39 µg/mL) and Simeulue (452.18 µg/mL) extracts exhibited weaker activity, requiring higher concentrations for significant inhibition. These findings indicate that altitude-driven phytochemical diversity strongly influences anticancer potential.

Table 1. Qualitative phytochemical screening of ethanolic fruit extracts of *Etlingera elatior* from three regions of Aceh, Indonesia

Secondary metabolites	Simeulue Island	Pidie	Gayo Highland
Flavonoids	+	++	+++
Terpenoids	+	+	++
Steroids	-	+	-
Tannin	-	-	++
Saponin	+	++	++
Phenolic	+	++	+++
Alkaloids	-	+	+

Note: +: Low presence, ++: Moderate presence, +++: Strong presence, -: Not detected

Table 2. Total Phenolic Content (TPC) and Flavonoid Content (TFC) of *Etlingera elatior*

Testing	Ethanol extract of *Etlingera elatior* fruit (mg/100 g DM)		
	Pidie	Gayo Highland	Simeulue Island
Total Phenolic (TPC)	73.5±3.20	126.4±10.44	250.0±10.52
Total Flavonoid (TFC)	35.0±2.68	22.4±15.80	118.0±5.72

Table 3. DPPH Antioxidant activity of EEFEE

Samples	Concentration (µg/mL)	Average	Abs sample	Inhibition (%)	IC_{50} (µg/mL)
Pidie	100	0.11	0.05	91.09	72.33±0.14**
	50	0.11	0.05	92.18	
	25	0.14	0.08	86.32	
	12.5	0.25	0.19	68.04	
	6.25	0.39	0.33	42.88	
	3.125	0.48	0.41	28.76	
Gayo Highland	100	0.08	0.03	92.58	23.8±0.04***
	50	0.09	0.04	89.52	
	25	0.11	0.06	82.10	
	12.5	0.14	0.09	73.29	
	6.25	0.19	0.13	60.93	
	3.125	0.22	0.16	51.93	
Simeulu Island	100	0.12	0.06	88.48	47.89±0.11***
	50	0.11	0.06	89.93	
	25	0.11	0.05	90.17	
	12.5	0.17	0.12	79.04	
	6.25	0.30	0.25	55.55	
	3.125	0.41	0.36	34.54	
Ascorbic acid	100	0.06	0.03	95.60	16.43±0.20***
	50	0.17	0.13	79.43	
	25	0.26	0.23	64.46	
	12.5	0.37	0.33	47.77	
	6.25	0.55	0.52	18.73	
	3.125	0.635	0.60	5.76	

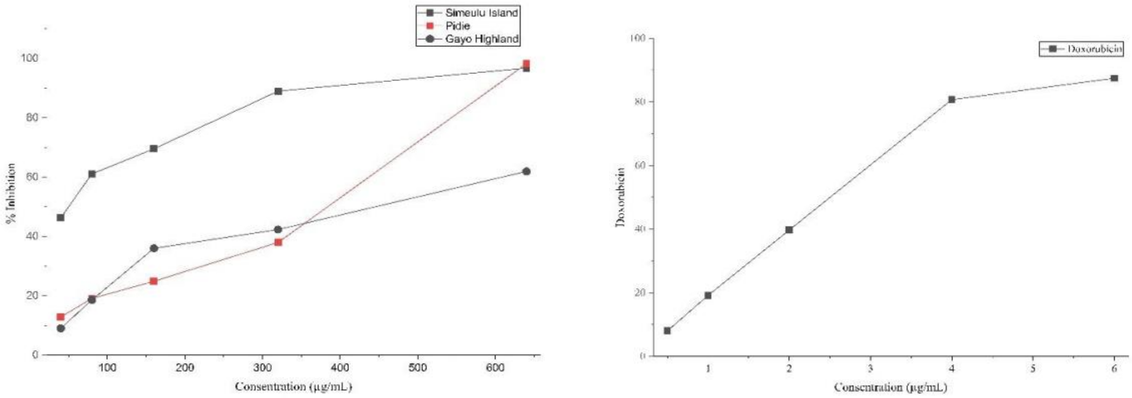

Figure 2. Dose response curves of *Etlingera elatior* fruit extracts from Pidie, Gayo Highlands, and Simeulue compared with doxorubicin against MCF-7 cells. The Gayo Highlands extract demonstrated the strongest activity

Table 4. Cytotoxicity of EEFEE determined by the BSLT method

Region	Concentration (µg/mL)	Death (%)	LC_{50} (µg/mL)
Pidie	1000	90.00	62.72
	500	83.32	
	250	73.33	
	100	46.71	
	20	36.74	
Gayo Highland	1000	83.30	13.28
	500	80.00	
	250	76.72	
	100	53.30	
	20	60.03	
Simeulue Island	1000	93.30	76.39
	500	76.71	
	250	70.04	
	100	40.02	
	20	36.70	

Table 5. Anticancer activity (MTT assay) of ethanolic fruit extracts of *Etlingera elatior* against MCF-7 breast cancer cells

Location	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Simeulu Island	40	46.26±3.11^{b}	452.18
	80	61.09±2.02^{a}	
	160	69.54±3.47^{a}	
	320	88.99±1.10^{c}	
	640	96.75±0.42^{c}	
Pidie	40	19.32±2.15^{a}	365.39
	80	23.89±4.40^{a}	
	160	28.11±3.14^{a}	
	320	37.64±3.99^{b}	
	640	81.46±3.17^{c}	
Gayo Highland	40	9.01±1.84^{a}	73.51
	80	18.56±2.01^{a}	
	160	35.99±0.81^{c}	
	320	42.32±2.15^{c}	
	640	61.94±1.21^{c}	
Doxorubicin	0.5	8.01±6.6^{a}	2.08
	1	19.16±3.7^{a}	
	2	39.72±2.5^{a}	
	4	80.72±3.3^{a}	
	6	87.48±3.4^{a}	

Discussion

The phytochemical screening of *Etlingera elatior* Ethanolic Fruit Extracts (EEFEE) revealed clear regional variation, demonstrating the significant role of ecological factors in shaping metabolite diversity. Although fruits from Simeulue contained the highest levels of total phenolics and flavonoids, extracts from the Gayo Highlands consistently exhibited stronger antioxidant, cytotoxic, and anticancer activities. This contrast indicates that pharmacological potency is not determined solely by the abundance of metabolites but is also influenced by their qualitative diversity and the presence of highly bioactive subclasses. Comparable findings in other Zingiberaceae species have shown that compounds such as quercetin, kaempferol, and curcumin derivatives may exert strong biological activities even at relatively low concentrations (Ghasemzadeh et al. 2015; Aryal et al. 2019; Ahmad et al. 2024).

Ecological conditions provide a plausible explanation for these differences. Plants growing in high-altitude environments such as the Gayo Highlands are exposed to greater ultraviolet radiation, lower temperatures, and hypoxic stress, which are known to stimulate secondary metabolite biosynthesis (Pandey et al. 2018; Pant et al. 2021). These stress factors can upregulate pathways associated with flavonoid and phenolic production, thereby enhancing the accumulation of compounds with strong antioxidant capacity (Cai et al. 2012; Rondevaldova et al. 2024). However, variations in secondary metabolite profiles are not solely determined by ecological conditions. Genetic diversity among plant populations can also play a significant role in shaping metabolic outcomes, as genotypic differences influence gene expression related to biosynthetic pathways (Rai et al. 2022; Yan et al. 2024; Chen et al. 2025). Thus, both environmental and genetic factors interactively contribute to the observed chemical diversity. By contrast, sea-level conditions in Simeulue may favor the bulk accumulation of phenolics and flavonoids, yet these compounds are likely of less potent subclasses, explaining the mismatch between their high quantitative levels and relatively weaker biological activity.

The antioxidant data strongly support this ecological-phytochemical link. Extracts from the Gayo Highlands demonstrated exceptional radical scavenging activity (IC_{50} = 23.8 µg/mL), while Simeulue and Pidie extracts were markedly weaker. These results align with earlier observations that altitude enhances the synthesis of redox-active flavonoids with superior electron-donating capacity (Leorita et al. 2019; Indiarto et al. 2024).

Cytotoxicity assays provide further evidence of this pattern. The Gayo extract showed an LC_{50} of 13.28 µg/mL, categorizing it as highly toxic, whereas Simeulue and Pidie extracts fell within the moderately toxic range. Comparable toxicity profiles have been reported in other Zingiberaceae species such as *Etlingera hemisphaerica* and *Alpinia galanga*, where high levels of flavonoids and terpenoids are associated with membrane disruption and apoptosis induction (Musdalipah et al. 2021; Utami et al. 2023).

Most importantly, the anticancer activity of the Gayo extract against MCF-7 breast cancer cells was remarkable, with an IC_{50} of 73.51 µg/mL, approaching the potency of doxorubicin (IC_{50} = 2.08 µg/mL). In contrast, Simeulue and Pidie extracts required much higher concentrations to achieve significant inhibition. The superior efficacy of the Gayo extract suggests that synergistic interactions among multiple phenolic and flavonoid compounds produce a broad spectrum of anticancer effects, including apoptosis induction, modulation of oxidative stress, and MAPK signaling pathways and inhibition of PI3K/Akt (Mankhong et al. 2019; Situmorang et al. 2024). Such multi-target mechanisms are advantageous compared to single-compound chemotherapeutics, as they may reduce both toxicity and the risk of resistance (Widowati et al. 2018; Sammar et al. 2019).

Overall, these results establish a consistent relationship between altitude-driven phytochemical enrichment and enhanced biological activity. The findings validate ethnopharmacological knowledge in Aceh, where *E. elatior* has long been valued as a medicinal spice (Saudah et al. 2022; Rayhannisa et al. 2024), while also providing new scientific evidence for its potential as a natural anticancer agent. The novelty of this study lies in demonstrating how ecological variation, particularly altitude, can directly shape metabolite profiles and pharmacological outcomes in *E. elatior* fruits.

Future studies should focus on isolating and structurally characterizing the most active compounds, while also conducting mechanistic research to elucidate molecular pathways underlying antioxidant and anticancer activities. In vivo validation and safety assessments are crucial steps toward establishing therapeutic viability. Moreover, genetic variation among populations should also be considered, as it may influence the metabolite profile and consequently the biological activity of *E. elatior*. .From an applied perspective, these findings highlight the importance of developing geographically informed cultivation and standardization strategies, where ecological origin is treated as a critical determinant of product quality. In response to potential skepticism that the antioxidant and anticancer activities observed in *E. elatior* fruits may not directly translate to human applications, it is important to highlight the need for further research, including clinical studies, to validate these findings. Laboratory-based assays provide a fundamental understanding of biological effects, but human clinical trials are indispensable for confirming efficacy and safety. Advocating for such rigorous testing ensures that any potential health benefits can be accurately verified and effectively utilized in practical or therapeutic contexts.

In conclusion, ecological variation strongly influences the phytochemical composition and pharmacological activity of *Etlingera elatior* fruits across Aceh, Indonesia. The Gayo Highland population exhibited superior antioxidant, anticancer, and cytotoxic properties despite lower total phenolic and flavonoid content compared with Simeulue fruits. These findings suggest that environmental stress at higher altitudes promotes the accumulation of bioactive compounds with greater therapeutic potency. The pronounced anticancer activity of Gayo extracts underscores the potential of *Etlingera elatior* as a promising source of novel anticancer agents and emphasizes the significance of biodiversity conservation in supporting drug discovery and nutraceutical development. However, further in vivo investigations are required to validate these findings and to elucidate the underlying mechanisms of action in a biological system.

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