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# Characterization of essential oils and biological activities of *Etlingera* spp. from different agroecology in Southeast Sulawesi, Indonesia

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**Abstract.** *Tee SA, Alam S, Sastyarina Y, Yodha AWM, Reymon, Setiawan MA, Musdalipah. 2025. Characterization of essential oils and biological activities of* Etlingera *spp. from different agroecology in Southeast Sulawesi, Indonesia. Biodiversitas 26: 1653-1665.* Genus *Etlingera*, a part of Zingiberaceae, shows potential due to secondary metabolites influenced by agroecological factors, enhancing its therapeutic uses. The study aimed to characterize essential oils and biological activities of *Etlingera elatior*, *Etlingera rubroloba*, and *Etlingera calophrys* rhizomes collected from South Konawe, Wakatobi, and Muna District. Gas Chromatography-Mass Spectrometry (GC-MS) profiled the essential oils, while phenolic (TPC) and flavonoid (TFC) concentrations were determined using Folin-Ciocalteu and aluminum chloride methods. Antioxidant activity was assessed using DPPH and ABTS assays, and toxicity testing was performed on shrimp larvae. The research found that multiple species grow in areas with annual rainfall ranging from 1,800-2,100 mm/year, temperatures of 23-30°C, humidity levels of 78-84%, topography of 9-72 masl, and pH of 4.9-6.5. The essential oil content includes fatty acids (20.1-42.48%), phenolic (6.28-29.52%), and terpenoid (1.24-2.39). The $IC_{50}$ of antioxidant activity (ABTS) for *E. elatior*, *E. rubroloba*, and *E. calophrys* were 1.3 mg/mL, 1.5 mg/mL, and 1.1 mg/mL, compared to vitamin C at 2.23 mg/mL. The DPPH assay IC50 values were 2.09 mg/mL, 2.17 mg/mL, and 2.42 mg/mL, compared to vitamin C at 2.78 mg/mL, respectively. TPC values were 113.63 mgGAE/g, 26.56 mgGAE/g and 143.00 mgGAE/g, respectively. TFC values were 62.03 mgQE/g, 21.07 mgQE/g, and 34.51 mgQE/g, respectively. Toxic $LC_{50}$ values for extracts were 6.98 mg/L, 18.72 mg/L, and 120.86 mg/L compared to the positive control at 3.24 mg/L. The Structural Equation Models (SEM) approach demonstrates the direct and indirect influence of climatic and soil factors on secondary metabolites and biological activity of the genus *Etlingera*. In conclusion, the genus *Etlingera* is a promising source of antioxidants and anticancer agents. It is recommended for cultivation in suitable locations in Southeast Sulawesi, instilling hope and optimism about the future of this research.

**Keywords:** Agroecology, essential oils, *Etlingera calophrys*, *Etlingera elatior*, *Etlingera rubroloba*

## INTRODUCTION

Zingiberaceae is one of the important plant families thathas high potential biological activity for various diseases (Chan et al. 2011; Sharifi-Rad et al. 2017; Effendi et al. 2019; Dash et al. 2020). Globally, species of Zingiberaceae have been widely studied worldwide, including in Indonesia. *Etlingera* is a genus within the Zingiberaceae family, known for its many species with medicinal potential (Mahdavi et al. 2017; Imran et al. 2022; Ilyas et al. 2023). There are about 150-200 species of *Etlingera* globally, and 48 of them are native to Sulawesi (Wahyuni et al. 2021a). Previous studies have explored and shown the potential of *Etlingera* as a nutraceutical and medicinal ingredient and proven in various countries globally, including Malaysia, Thailand, Singapore, and Indonesia (Juwita et al. 2018). Zingiberaceae plants have been well-known since ancient times for their economic benefits and diverse uses, including medicinal purposes (Lianah et al. 2021; Nugroho et al. 2022). Newly discovered generations of Zingiberaceae have continued to be located, including *Alpinia*, *Cinnamomum*, *Meistera*, and *Wurfbainia* (de Boer et al. 2018). *Alpinia monopleura* K.Schum., an endemic medicinal plant in Southeast Sulawesi, continues to be studied (Yodha et al. 2023), as well as *Meistera chinensis* (Chun ex T.L.Wu) Škorničk. & M.F.Newman (Musdalipah et al. 2021a, 2021b, 2021c, 2022; Tee et al. 2021), Polygonum (Ahmad et al. 2018), and *Etlingera* (Sahidin et al. 2018, 2019a, 2019b; Aswan et al. 2020; Fristiohady et al. 2020). For its medicinal potential, the *Etlingera* that is endemic to Southeast Sulawesi has proven to show pharmacological activity, such as an extract from *E. elatior* fruit, which has shown functions as hepatoprotector, antibacterial, antioxidant, and antidiabetic (Sahidin et al. 2019a; Fristiohady et al. 2020), stem of *E. calophrys* as an antioxidant (Sahidin et al. 2018; Megawati et al. 2021), rhizome of *E. alba* as an antioxidant, anticancer (Wahyuni et al. 2021b, 2022), fruit and stem of *E. rubroloba* as antioxidant, anticancer, antidiabetic, anti-inflammatory (Ilyas et al. 2023; Jabbar et al. 2021, 2024).

Antioxidants play an important role in scavenging free radicals in biological systems by eliminating reactive

oxygen and nitrogen. Nitrogen is essential for the synthesis of amino acids, which serve as the fundamental units of proteins and are vital for numerous metabolic processes. Plant secondary metabolites such as phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids, and alkaloids are known to neutralize oxidative stress due to their antioxidant activity (Barbosa and Nueva 2019). Genetic factors and environmental conditions, such as soil characteristics and climate, influence the synthesis of antioxidant compounds in plants. Environmental factors regulating the production of bioactive compounds are crucial for enhancing plant-derived antioxidant sources and maximizing their pharmacological benefits (Zargoosh et al. 2019; Pant et al. 2021).

Environmental conditions, including soil properties and climate, are detrimental species growth and distribution and exert various influences on the physical, chemical, and biological characteristics of plants (Cornara et al. 2023). Variations in soil conditions influence crop production (Alam et al. 2020) due to differences in nutrient availability, soil texture, pH, moisture levels, and organic matter content. All of these factors are essential in determining plant growth, including medicinal compounds in the form of secondary metabolites as treatment therapies (David et al. 2015; Radha et al. 2021). Soil nutrient cycling, a key factor in agroecosystem sustainability, has been reported as a major determinant of secondary metabolite production in plants (Jan et al. 2021; Mosa et al. 2022; Cornara et al. 2024). Soil properties, including nutrient availability, pH, texture, and organic matter, play a crucial role in determining both the chemical profile and the yield of essential oils (Karimi et al. 2020; Laftouhi et al. 2023).

Plants of the *Etlingera* genus have great potential to be developed as medicinal raw materials (Sahidin et al. 2018). Therefore, a more comprehensive understanding of their interaction with the environment is needed to achieve optimal productivity (Wirabuana et al. 2021). Selecting new planting sites for medicinal plants must consider regional variations in agroecological conditions to ensure optimal yield and quality (Subaryanti et al. 2021). We hypothesize that *E. elatior*, *E. rubroloba*, and *E. calophrys* growing in different locations can estimate the presence of specific compounds, such as essential oils, phenolics, and flavonoids, and affect their antioxidant activity. Until present, no studies have reported on the specialized metabolites and antioxidant activity of these *Etlingera* species in different locations. Therefore, this study aimed to characterize the essential oils and biological activity of rhizome extracts from *E. elatior*, *E. rubroloba*, and *E. calophrys* based on varying agroecological conditions.

## MATERIALS AND METHODS

### Study area

The experiment survey was conducted at three different locations in Southeast Sulawesi Province, Indonesia (Figure 1), i.e., (i) South Konawe District at 30 m asl., an average rainfall of 2,148.68 mm/year, the average daily temperature of 23°C with a minimum of and a maximum of 30°C, relative humidity of 84.19%, soil characters like texture i.e., sand (9%), silt (55%) and clay (36%), pH 4.9, organic-C content of 1.06%, and $CaCO_3$ of 0%. (ii) Wakatobi District at 72 m asl., the average rainfall of 1,625.51 mm/year, the average daily temperature of 25°C with a minimum of and a maximum of 30°C, relative humidity of 78.01%, so characters, like texture, i.e., sand (1%), silt (46%) and clay (53%), pH 6.5, organic-C content of 3.73%, and $CaCO_3$ of 2.66%. (iii) Muna District at 9 masl, the average rainfall of 1,805.20 mm/year, an average daily temperature of 24°C with a minimum of and a maximum of 30°C, relative humidity of 82.41%, soil characters like texture, i.e., sand (43%), silt (32%) and clay (25%), pH 5.6, organic-C content of 2.25%, and $CaCO_3$ of 0.45%.

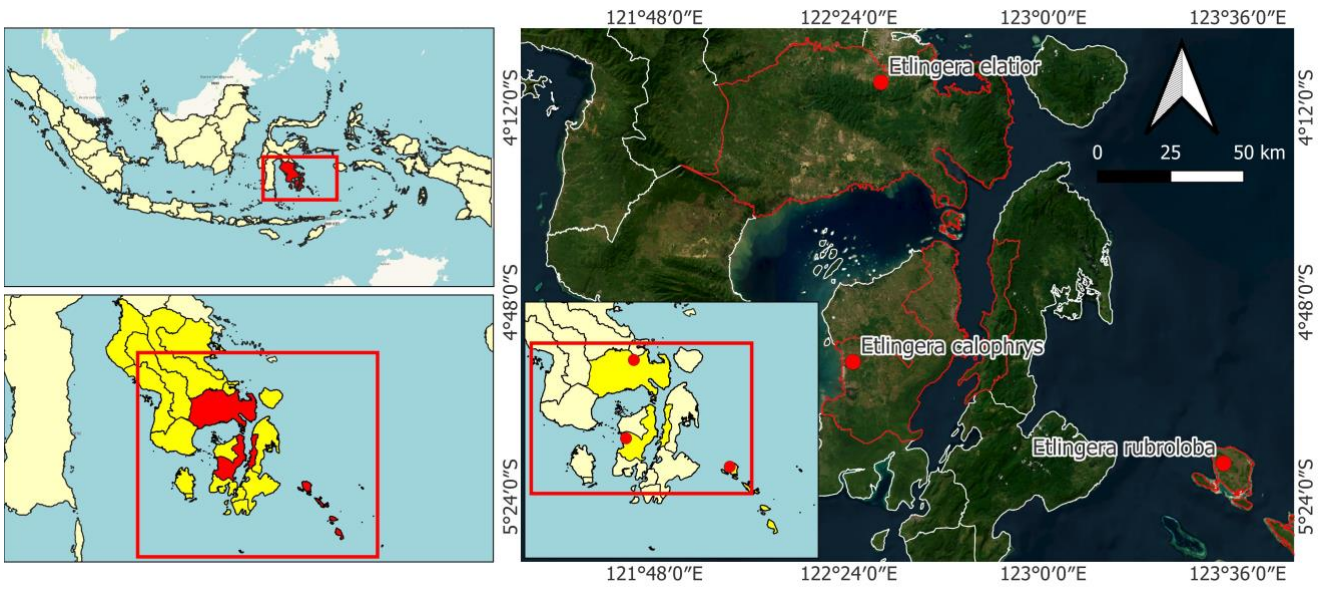


**Figure 1.** Ecology *Etlingera* in Southeast Sulawesi, Indonesia

**Plant materials**

Plants were identified at the Research Center for Biosystematics and Evolution, National Research and Innovation Agency/BRIN (Formerly Research Center for Biology, Indonesian Institute of Sciences/LIPI), Cibinong, Bogor, Indonesia, under a certificate of 957/IPH.1.01/If.07/V/2019. The rhizome of *E. elatior* was obtained from Konda Sub-district, South Konawe District, Southeast Sulawesi Province (4.121281° S, 122.499933° E). The rhizome of *E. rubroloba* was obtained from Wangi-Wangi Sub-district, Wakatobi District, Southeast Sulawesi Province (5.296182° S, 123.557015° E). The rhizome of *E. calophrys* was obtained from Kabangka Sub-district, Muna District, Southeast Sulawesi Province (4.983394° S, 122.413467° E) (Figure 1). The rhizomes were collected, washed, sorted, and dried at 45°C for three days. The dried simplicia was ground, and stored in a clean and dry container that was protected from the light.

**Procedures**

*Extraction*

A total of 500 g of dried simplicia was macerated in methanol for three consecutive 24-hour periods (3 × 24 hours). The filtrate was separated and evaporated under vacuum at 45°C using a Stuart RE300 rotary evaporator (USA) with a rotor speed of 80 rpm, yielding a thick extract. The condensed extracts of *E. elatior*, *E. calophrys*, and *E. rubroloba* rhizomes were analyzed for their chemical compound content and evaluated for antioxidant activity, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and toxicity (Hamsidi et al. 2024; Karmilah et al. 2024).

*Extraction of essential oil*

Essential oil extraction and chemical compound analysis were conducted using Gas Chromatography-Mass Spectrometry (GC-MS). Fresh rhizomes of *E. elatior*, *E. calophrys*, and *E. rubroloba* (500 g each) were subjected to hydrodistillation using a Clevenger apparatus. The rhizomes were placed in a 3-L round-bottom flask containing distilled water, and the distillation process was carried out for 6 hours. The condensed essential oils were collected and dried with anhydrous sodium sulfate to remove excess water, and then stored at 4°C for further analysis (Loying et al. 2021).

*GC-MS analysis*

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze and identify the volatile compounds present in essential oil. The analysis was conducted using a Shimadzu QP-2010 Ultra GC-MS system equipped with an AOC-20i autosampler and an SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The injection temperature was set to 250°C in non-split mode. The column oven temperature program began at 70°C and was maintained for 2 minutes, followed by a temperature increase to 200°C at a rate of 10°C/min, and then to 280°C at 5°C/min, where it was held for 9 minutes. The ion source and interface temperatures were set at 200°C and 280°C, respectively. Chromatograms were identified by comparison with the NIST and Wiley databases (Elgamal et al. 2021; Zubair et al. 2021).

*Total Phenolic Content (TPC)*

The Total Phenolic Content (TPC) of the selected Zingiberaceae species extracts was analyzed using a modified spectrophotometric method. Gallic acid standard solutions were prepared at gradient concentrations (10, 20, 30, 40, and 50 mg/L). A mixture of 3,160 µL ultrapure water and 40 µL of the extract or gallic acid standard solution was combined with 200 µL of 10% Folin-Ciocalteu (FC) reagent. After 6 minutes of incubation, 600 µL of 20% (w/v) sodium carbonate ($Na_2CO_3$) solution was added. The mixture was incubated in the dark for 2 hours, and the absorbance was measured at a wavelength of 765 nm using ultrapure water as the blank. Results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE $g^{-1}$ DW) (Ivanovic et al. 2021).

*Total Flavonoid Content (TFC)*

In brief, a 5 mL sample extract was mixed with 0.3 mL of a 5% sodium nitrite solution in a labeled test tube and stirred thoroughly for 5 minutes. Following this, 0.3 mL of a 10% aluminum chloride solution was added, and after 6 minutes, 2 mL of NaOH solution was added to stop the reaction. The absorbance of the solution was measured immediately at a wavelength of 510 nm using a UV spectrophotometer, with ultrapure water as the blank. The standard solution used for calibration was quercetin. The average TFC concentration, obtained from triplicate analyses, was expressed as milligrams of quercetin equivalent per 100 g of dry sample (mg QE/100 g) and presented as the mean±Standard Deviation (SD) (Musdalipah et al. 2024).

**Antioxidant activity**

*ABTS radical scavenging*

The radical scavenging activity for ABTS was assessed according to the method of Whangsomnuek et al. (2019) with some modifications. Next, the ABTS+ solution was prepared with the utmost care, mixing 7 mM ABTS and 2.45 mM potassium persulfate in a 1:1 (v/v) ratio, and the mixture was kept in the dark at room temperature for 12 hours. Before testing, the ABTS+ solution was diluted to achieve a final absorbance of 0.7±0.5 at 734 nm. About 200 µL of the extract (at various concentrations) was mixed with 1,800 µL of the ABTS+ solution and incubated at room temperature for 6 minutes, with distilled water as the control. The absorbance was measured at 734 nm, and Trolox was used as the reference substance. Results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in mg per gram of dry extract (mg TEAC/g extract), calculated using the Trolox calibration curve (Daula et al. 2019).

*DPPH radical scavenging*

The free radical scavenging activity of the extract was evaluated by its ability to stabilize 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, following the method described by Al Bashera et al. (2021). Plant extracts or standards (catechins) at concentrations of 200 mg/L, 400

mg/L, and 800 mg/L (0.5 mL) were mixed with 3.5 mL of a 0.002% DPPH solution in methanol. After 30 minutes of incubation, the absorbance was measured at 517 nm.

The percentage of inhibition was calculated using the formula:

$$\frac{A_0 - A_1}{A_0} \times 100\%$$

Where:
$A_0$ : Absorbance of the control
$A_1$ : Absorbance of the sample (extract or standard)

The $IC_{50}$ value was determined from the equation of the regression line plotted with concentration (mg/L) against the percentage of inhibition (Yodha et al. 2023).

**Toxicity test**

The toxicity of the plant extracts was evaluated by using the Brine Shrimp Lethality Test (BSLT). *Artemia salina* (Linnaeus, 1758) larvae (10 larvae in each well), 48 hours post-hatching, were mixed with the extracted sample in 5 mL of seawater. The concentrations tested were 10, 100, 250, 500, and 750 µg/mL, with one negative control (DMSO) included. Larval mortality was observed 24 hours later, and the percentage of mortality was calculated. This procedure was repeated three times for each concentration. The level of toxicity was determined based on the $LC_{50}$ value, representing the concentration required to cause 50% mortality in the larvae (Erwin et al. 2020).

**Data analysis**

The $IC_{50}$ values for DPPH and ABTS antioxidant activity, as well as the $LC_{50}$ values for toxicity, were calculated through the following steps: (a) The percentage of inhibition (y-axis) was plotted against the concentration (x-axis) (100, 50, 25, 15.5, 6.3, and 3.3 mg/L), (b) the regression y = ax + b, and (c) the concentration of 50% inhibition or toxicity was determined by substituting y = 50 into the regression equation. SEM was used to analyze the hypothesized pathways and structural relationships between latent variables representing agroecological factors and essential oil. The SEM approach allows for the evaluation of complex relationships, including direct and indirect effects. The assessment of SEM involves steps: i) model specification (constructing a path diagram), ii) measurement model assessment by reliability analysis (Cronbach's alpha ≥0.70) and validity analysis (assessed by Average Variance Extracted (AVE)≥0.50), iii) structural model assessment (testing hypothesized relationships latent variables), iv) model fit evaluation (P values (<0.05)). We set up SEMs for each region using Partial Least Squares (PLS) regression with Warp PLS 6.0. In the SEM analysis, we established paths from climatic factors (rainfall, temperature, humidity), topography, soil analysis (soil texture: clay, silt, and sand, pH, $CaCO_3$, and organic carbon) directly and indirect effects to secondary metabolites, essential oils, and pharmacological activity.

## RESULTS AND DISCUSSION

**Agroecological description of the study area**

The agroecological conditions in the different study areas include climate data (rainfall, temperature, humidity), soil characteristics (soil texture, pH, organic carbon, and $CaCO_3$ content), and topography. Tables 1 and 2 present the agroecology conditions and soil character at three locations.

The climatic conditions in South Konawe District range from 23°C to 30°C, with a relative humidity of 84.19%, a total annual rainfall of 2,148.68 mm, and a topography of 9 masl. In Wakatobi District, temperatures range from 25-30°C, with a relative humidity of 78.01%, total rainfall of 1,625.51 mm/year, and a topography of 72 masl. Meanwhile, in Muna District, temperatures range from 24-30°C, with a relative humidity of 82.41%, total rainfall of 1,805.20 mm, and a topography of 9 masl. Wakatobi District has lower total rainfall and humidity compared to the other regions.

The soil texture in South Konawe District is classified as silty clay loam, Wakatobi District as silty clay, and Muna District as loam. The soil pH in South Konawe is acidic, while the other two locations are slightly acidic. The organic carbon (C-organic) content in South Konawe is low (1.06%), in Wakatobi, it is high (3.73%), and in Muna, it is moderate (2.25%). The highest $CaCO_3$ content is found in Wakatobi District, followed by Muna District and South Konawe District.

**Chemical composition of essential oil of *E. elatior*, *E. calophrys*, and *E. rubroloba* identified by GCMS**

The data of the essential oils extracted from *E. elatior*, *E. calophrys*, and *E. rubroloba*, obtained from GC-MS, including retention time, relative area (%), relative height (%), chemical compound, and classification, are presented in Table 3 and Figure 2. The chromatogram of each compound is further shown in Figure 3. The main component of *E. elatior* essential oil, with the highest percentage area, is cis-vaccenic acid (25.13%), while the component with the lowest percentage area is 3,11-tetradecadien-1-ol (0.12%). Other major components of *E. elatior* are hexanedioic acid, bis(2-ethylhexyl) ester (14.37%), phthalic acid, di(2-propylpentyl) ester (9.84%); 1-hexadecanol (8.05%); 1-dodecanol (7.21%); 2-(2,5-dimethoxy-4-ethylphenyl) ethanol acetate (6.51%); and 2-dodecen-1-yl(-) succinic anhydride (5.1%). The major compounds of *E. rubroloba* rhizome essential oil including cis-vaccenic acid (25.78%), hexanedioic acid, bis(2-ethylhexyl) ester (19.84%), 2-(2,5-dimethoxy-4-ethylphenyl) ethanol acetate (19.6%), phthalic acid, di(2-propylpentyl) ester (14.09%), and trans-13-octadecenoic acid methyl ester (7.41%). The major compounds of *E. calophrys* rhizome essential oil including 1-heptatriacotanol (48.01%), cis-vaccenic acid (16.78%), gingerol (10.50%), trans-13-octadecenoic acid methyl ester (5.7%), and hexanedioic acid, bis(2-ethylhexyl) ester (4.26%).

The chromatogram from the GC-MS analysis demonstrates the separation of chemical compound peaks in each extract (Figure 3), observed within the Retention Time (RT) range of 0-30 minutes. This separation occurs due to the differing migration rates of each compound in specific phases of the mobile and stationary phases (Fonmboh et al. 2020).

**Table 1**. Agroecology description in the study area

| Location | Altitude (m asl.) | Rainfall (mm/year) | Temp. (°C) | Relative humidity (%) |
|---|---|---|---|---|
| South Konawe District | 30 | 2,148. 68 | 23-30 | 84.19 |
| Wakatobi District | 72 | 1,625.51 | 25-30 | 78.01 |
| Muna District | 9 | 1,805.20 | 24-30 | 82.41 |

**Table 2**. Soil character in the study area

| Location | Soil texture (%) | | | pH | Organic-C (%) | $CaCO_3$ (%) |
|---|---|---|---|---|---|---|
| | Sand | Silt | Clay | | | |
| South Konawe District | 9 | 55 | 36 | 4.9 | 1.06 | 0.00 |
| Wakatobi District | 1 | 46 | 53 | 6.5 | 3.73 | 2.66 |
| Muna District | 43 | 32 | 25 | 5.6 | 2.25 | 0.45 |

**Table 3.** Chemical composition of essential oil of *Etlingera* species by GCMS

| Retention time (min) | *E. elatior* | | *E. rubroloba* | | *E. calophrys* | | Chemical compound | Classification |
|---|---|---|---|---|---|---|---|---|
| | Relative area (%) | Relative height (%) | Relative area (%) | Relative height (%) | Relative area (%) | Relative height (%) | | |
| 11.693 | 2.37 | 3.59 | - | - | - | - | Dodecanal | Other |
| 12.771 | 7.21 | 10.78 | - | - | - | - | 1-Dodecanol | Other |
| 12.819 | 1.35 | 0.72 | - | - | - | - | 1-Undecanol | Other |
| 14.781 | 0.12 | 0.13 | - | - | - | - | 3,11-Tetradecadien-1-ol | Other |
| 14.89 | 1.58 | 2.46 | 0.55 | 0.80 | - | - | Lauryl acetate | Other |
| 14.961 | 1.47 | 2.20 | 0.22 | 0.34 | 0.62 | 1.04 | Tetradecana | Other |
| 15.92 | 8.05 | 9.78 | - | - | - | - | 1-Hexadecanol | Other |
| 16.522 | 0.31 | 0.42 | 1.60 | 2.46 | - | - | Phenol 2,6-dimethoxy-4-(2-propenyl)- | Phenolic |
| 17.029 | 0.32 | 0.31 | 0.73 | 0.73 | - | - | Octanal, 2-(phenylmethylene)- | Other |
| 17,672 | - | - | 0,42 | 0,69 | - | - | 4-(1-Acetoxyallyl)-2-methoxyphenyl isobutyrate | Phenylpropanoids |
| 17.791 | 1.10 | 1.65 | 0.08 | 0.11 | - | - | 1-Tetradecyl acetate | Fatty acid |
| 17.913 | 0.31 | 0.42 | 0.34 | 0.50 | 2.43 | 3.56 | E-2-Tetradecen-1-ol | Other |
| 19.369 | 1.76 | 1.95 | 3.87 | 4.71 | 3.02 | 3.12 | Hexadecanoic acid methyl ester | Fatty acid |
| 19.832 | 6.51 | 8.89 | 19.06 | 27.06 | 1.35 | 1.70 | 2-(2,5-Dimethoxy-4-ethylphenyl) ethanol acetate | Phenolic |
| 19.886 | 0.29 | 0.35 | 0.78 | 1.00 | - | - | 1-Diphenylsilyloxypentadecane | Other |
| 21.512 | 1.42 | 1.04 | 0.73 | 1.24 | 1.85 | 2.39 | E,E,Z-1,3,12-Nonadecatriene-5,14-diol | Terpenoid |
| 21.583 | 3.20 | 2.71 | 7.41 | 4.54 | 5.70 | 4.85 | trans-13-Octadecenoic acid methyl ester | Fatty acid |
| 22.097 | 25.13 | 12.07 | 25.78 | 9.97 | 16.78 | 8.51 | cis-Vaccenic acid | Fatty acid |
| 23.362 | 0.76 | 0.46 | 2.32 | 0.68 | 0.70 | 1.03 | trans-13-Octadecenoic acid | Fatty acid |
| 23.855 | 1.60 | 1.16 | 0.77 | 0.77 | 0.05 | 0.05 | Glycidyl palmitate | Other |
| 24.77 | - | - | 0.65 | 0.73 | 48.01 | 56.93 | 1-Heptatriacotanol | Other |
| 24.981 | 14.37 | 19.01 | 19.84 | 28.14 | 4.26 | 4.88 | Hexanedioic acid bis(2-ethylhexyl) ester | Other |
| 25.1 | 0.74 | 0.40 | - | - | - | - | Stigmasterol | Sterol |
| 25.148 | 0.35 | 0.29 | - | - | - | - | Cholesta-22,24-dien-5-ol, 4,4-dimethyl | Steroid |
| 25.807 | 0.69 | 0.84 | - | - | - | - | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- | Fatty acid |
| 25.858 | 1.01 | 0.88 | 0.76 | 0.71 | 1.05 | 1.09 | Glycidyl palmitoleate | Other |
| 26.831 | 9.84 | 9.81 | 14.09 | 14.81 | 2.60 | 2.59 | Phthalic acid di(2-propylpentyl) ester | Fatty acid |
| 26.977 | 5.10 | 4.44 | - | - | - | - | 2-Dodecen-1-yl(-) succinic anhydride | Other |
| 27.151 | - | - | - | - | 1.09 | 0.74 | Ethyl iso-allocholate | Other |
| 28.821 | - | - | - | - | 10.50 | 7.51 | Gingerol | Phenolic |
| 29.358 | 0.61 | 1.29 | - | - | - | - | ß-Sitosterol | Sterol |
| 29.385 | 1.87 | 1.48 | - | - | - | - | Stigmastan-3-en-6-ol | Sterol |
| 29.964 | 0.59 | 0.47 | - | - | - | - | 2,5-Furandione 3-dodecyl | Other |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | | |

The appearance of compound peaks on the chromatogram evidences the separation. The peaks formed at each retention time are further analyzed using MS/MS to determine their molecular weights and fragmentation patterns, facilitating the identification of the detected molecules by their names and structures (Hamsidi et al. 2024). The classification of secondary metabolite compounds is grouped into fatty acids, phenolics, phenylpropanoids, sterols, steroids, and terpenoids (Table 2).

Based on Table 4 shows that *E. rubroloba* has a higher relative percentage of fatty acid metabolites (53.55%), compared to *E. elatior* and *E. calophrys*. *E. elatior* has a relative percentage of sterol (3.22%) that is higher than the others. The highest relative percentage of phenolic compounds is found in *E. rubroloba* (24.64%), while terpenoid compounds are most abundant in *E. calophrys* (1.85%).

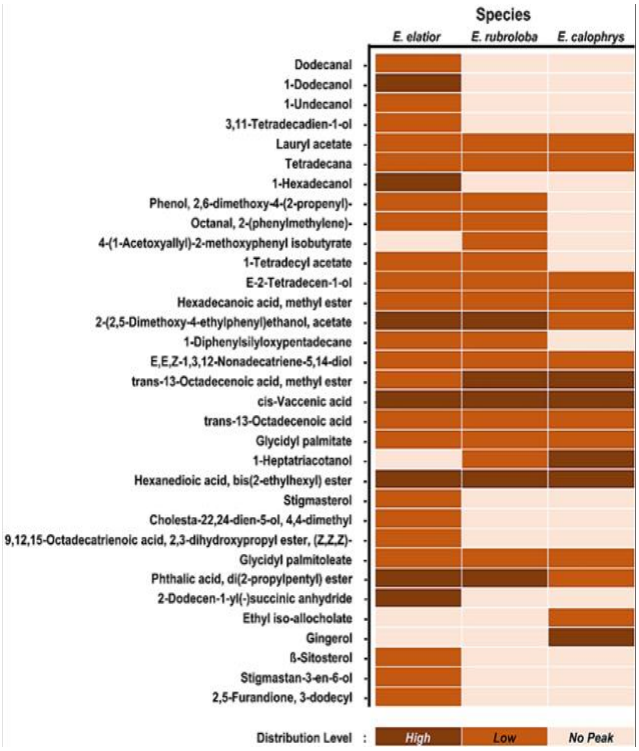


**Figure 2**. Chemical compound distribution of *Etlingera* species

**Table 4.** Group of secondary metabolites of *Etlingera* species in Southeast Sulawesi

| Secondary metabolites | *E. elatior* | *E. rubroloba* | *E. calophrys* |
|---|---|---|---|
| | % Relative | % Relative | % Relative |
| Fatty acid | 42.48 | 53.55 | 28.79 |
| Phenolic | 6.82 | 24.64 | 11.85 |
| Phenylpropanoids | 0 | 0.69 | 0 |
| Sterol | 3.22 | 0 | 0 |
| Steroid | 0.35 | 0 | 0 |
| Terpenoid | 1.42 | 1.24 | 1.85 |
| Other | 45.74 | 24.64 | 57.51 |

**Total Phenolic Content (TPC)**

Gallic acid equivalents (GAE) as an expression of the Total Phenolic Content (TPC) of the extracts ranged from 32.90 mgGAE/g to 232.71 mgGAE/g. The total phenolic content of the rhizome extracts of *E. elatior*, *E. rubroloba*, and *E. calophrys* were 119.93 mgGAE/g, 232.71 mgGAE/g, and 32.90 mgGAE/g, respectively. The highest TPC was observed in *E. rubroloba*, sourced from Wakatobi District (Table 5). The high total phenolic content in *E. rubroloba* aligns with the secondary metabolite content of the essential oils obtained (24.64%).

**Total Flavonoid Content (TFC)**

Flavonoid content is measured in milligrams of rutin equivalents per gram. The total flavonoid content of the rhizome extracts of *E. elatior*, *E. rubroloba*, and *E. calophrys* were 90.5 mgQE/g, 60.64 mgQE/g, and 43.48 mgQE/g, respectively. The highest TFC was observed in *E. elatior* sourced from South Konawe (Table 6).

**Antioxidant activity: ABTS and DPPH radical scavenging**

An antioxidant activity test was conducted quantitatively on the rhizome extracts of *E. elatior*, *E. calophrys*, and *E. rubroloba*. The percentage inhibition of ABTS and DPPH radical scavenging expressed the antioxidant activity, calculated by the difference in absorbance between the ABTS and DPPH solutions in ethanol and the absorbance produced by the sample (Figure 4). The differences in absorbance reflect the free radical neutralizing effectiveness of the extract present in the assay, which is then calculated into the $IC_{50}$ value that represents the sample concentration required to inhibit 50% of ABTS and DPPH free radicals. A small $IC_{50}$ value of a compound indicates better neutralization of free radicals (Imran et al. 2022; Jabbar et al. 2024), compared to vitamin C as a positive control due to its strong antioxidant properties. The rigorous methodology employed in this research instills confidence in the validity of the results.

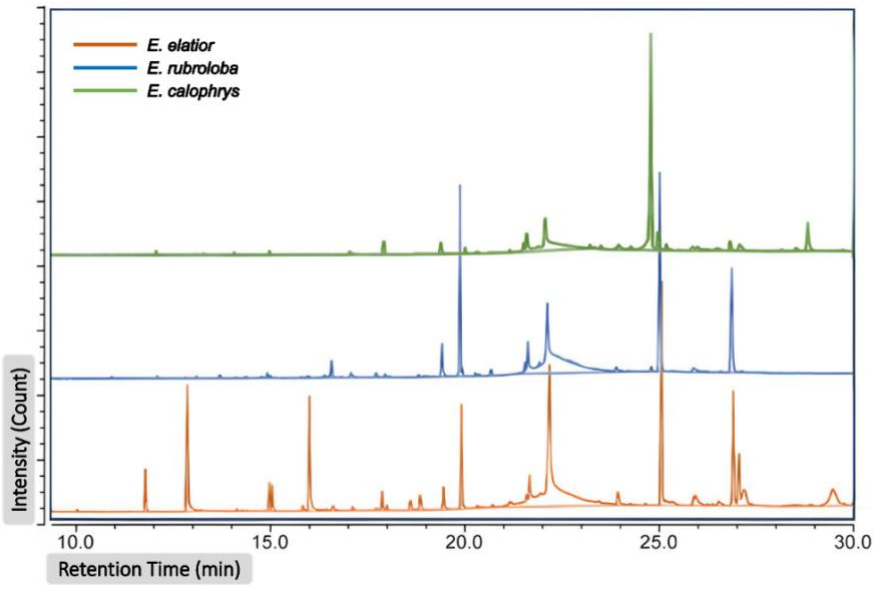


**Figure 3**. GC-MS chromatogram of the chemical compound in *Etlingera* species

**Table 5**. Total phenolic content of *Etlingera* species in Southeast Sulawesi

| Species | Sample location | Replicate of total phenolic content | | | Average | SD | Average total phenolic content (mgGAE/g) |
|---|---|---|---|---|---|---|---|
| | | I | II | III | | | |
| *E. elatior* | South Konawe District | 120.65 | 121.92 | 117.22 | 119.93 | 2.431 | 119.93±2.431 |
| *E. rubroloba* | Wakatobi District | 233.25 | 234.55 | 230.33 | 232.71 | 2.161 | 232.71±2.161 |
| *E. calophrys* | Muna District | 33.26 | 34.07 | 31.36 | 32.90 | 1.391 | 32.9±1.391 |

**Table 6**. Total flavonoid content of *Etlingera* species in Southeast Sulawesi

| Species | Sample location | Replicate of total phenolic content | | | Average | SD | Average total phenolic content (mgGAE/g) |
|---|---|---|---|---|---|---|---|
| | | I | II | III | | | |
| *E. elatior* | South Konawe District | 89.23 | 89.59 | 91.92 | 90.5 | 1.460 | 90.253±1.460 |
| *E. rubroloba* | Wakatobi District | 59.87 | 60.74 | 61.32 | 60.64 | 0.730 | 60.64±0.73 |
| *E. calophrys* | Muna District | 42.56 | 44.65 | 43.23 | 43.48 | 1.067 | 43.48±1.067 |

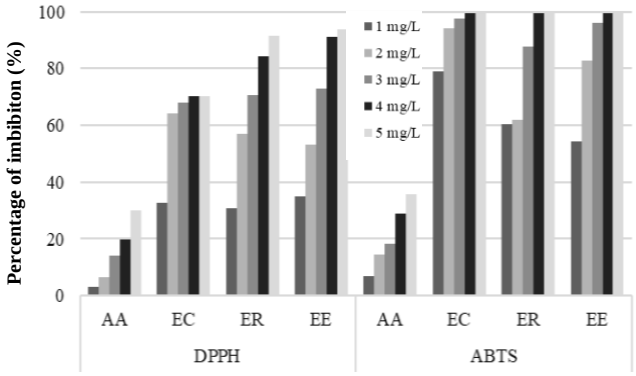


**Figure 4.** Inhibition activity of *Etlingera* species extracts in differing concentrations. AA: Ascorbate Acid; EC: *E. calophrys*; ER: *E. rubroloba*; EE: *E. elatior*

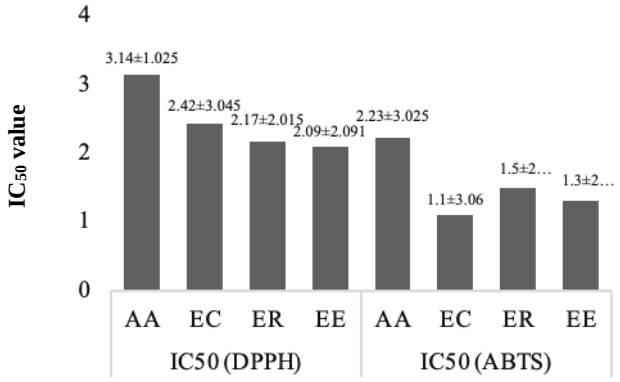


**Figure 5.** The $IC_{50}$ values of DPPH and ABTS radical scavenging activity by *Etlingera* species extract. AA: Ascorbate Acid; EC: *E. calophrys*; ER: *E. rubroloba*; EE: *E. elatior*

The $IC_{50}$ results (ABTS) for *E. elatior*, *E. rubroloba*, and *E. calophrys* were 1.3 mg/L, 1.5 mg/L, and 1.1 mg/L, respectively, compared to vitamin C at 2.23 mg/L. The $IC_{50}$ results (DPPH) were 2.09 mg/L, 2.17 mg/L, and 2.42 mg/L for *E. elatior*, *E. rubroloba*, and *E. calophrys*, respectively, compared to vitamin C at 2.78 mg/L. *E. calophrys* exhibited the highest antioxidant activity in the ABTS method, while *E. elatior* showed the highest activity for DPPH (Figure 5). The $IC_{50}$ values indicate that the *Etlingera* species possess very strong antioxidant activity (ABTS and DPPH).

**Toxicity assay: Brine shrimp lethality assay**

The toxicity of *Etlingera* species rhizome extracts was evaluated using the BSLT method (Fauziah et al. 2022). The mortality percentage is shown in Figure 6. The test results showed that the highest larval mortality rate was achieved at concentrations of 1,000 mg/L, 500 mg/L, 250 mg/L, 125 mg/L, followed by 62.5 mg/L, 31.25 mg/L, 15.625 mg/L, and 7.8125 mg/L. Various concentration levels of the extract were used to examine the relationship between the test solution and the larval mortality rate of shrimp.

$LC_{50}$ reflects the concentration of a compound that is toxic enough to cause 50% mortality of the organism. The toxicity test on larval mortality showed the $LC_{50}$ values of *E. elatior*, *E. calophrys*, *E. rubroloba* rhizomes, and the positive control were 6.98 mg/L, 120.86 mg/L, 18.72 mg/L, and 3.24 mg/L, respectively. These values belong to the categories of highly toxic (≤30 ppm) and toxic ($LC_{50}$≤1,000 mg/L).

**Structural Equation Models (SEM)**

Structural Equation Modeling (SEM) is a statistical approach that explains the relationship between environmental factors (climate, soil, and topography) to the secondary metabolites, essential oil, and pharmacological activities of *Etlingera* species. The relationship is presented in Figure 7 and Table 5.

Direct effect refers to the immediate influence of one latent variable on another latent variable without involving a mediator variable. The relationship is represented by a direct path in the structural model (Figure 7). Table 7 shows that climate has a significant effect on the essential oil content of three *Etlingera* species, while soil significantly affects essential oil and secondary metabolites. Essential oils influence pharmacological activity, and secondary metabolites, in turn, affect the content and pharmacological activity of essential oils.

The indirect effects of agroecology on essential oils, secondary metabolites, and pharmacological activities are intricate and diverse. Agroecological factors, including soil properties, climatic conditions, altitude, and geographical location, substantially influence plant growth and metabolic processes. Table 8 shows climate, and soil had a significant effect on the content of secondary metabolite, essential oils and pharmacology activity (P value<0.05). Although these factors do not directly generate bioactive compounds, they affect the plant's physiological and biochemical mechanisms responsible for the biosynthesis of essential oils and secondary metabolites.

## Discussion

Agroecology, the study of ecological dynamics within agricultural systems, reveals that the quality and growth of medicinal plants are profoundly influenced by abiotic factors such as rainfall, temperature, soil, and topography. The significance of a plant's growth location cannot be overstated, as it establishes a unique combination of environmental, soil, and climatic conditions that directly impact its metabolism and the production of secondary metabolites, such as essential oils. Climate conditions play a pivotal role in shaping the physical, chemical, and biological properties of plants. Factors like sunlight duration, rainfall, temperature, and humidity variations have a direct bearing on the compound content in medicinal plants. Soil, the provider of essential nutrients for plant growth, also influences the composition of medicinal compounds. It's fascinating to note that plants growing under different soil conditions have the potential to produce medicinal compounds with varying therapeutic values, which can be utilized in treating various diseases. Soil nutrients are known to affect the production of secondary metabolites in plants, which, in turn, influence nutrient cycling and enhance the sustainability of agroecosystems (David et al. 2015; Li et al. 2021; Radha et al. 2021). The environment highly influences the accumulation of secondary metabolites. External environmental factors, such as light, temperature, groundwater, soil fertility, and salinity, significantly impact most plants' growth and development, which relates to the synthesis of secondary metabolites. These factors lead to overall changes in the phytochemical profile and impact the production of bioactive substances (Chaouqi et al. 2023; Qaderi et al. 2023).

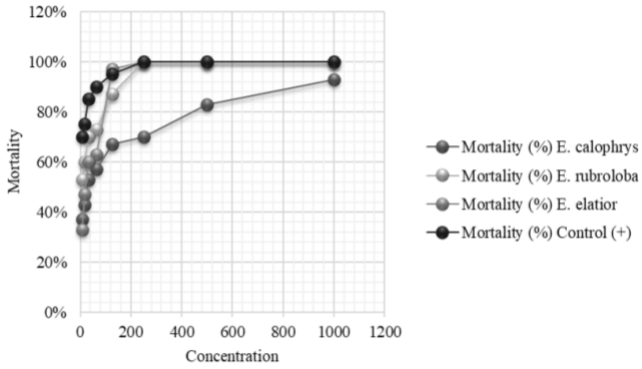


**Figure 6**. Toxicity of *Etlingera* species and positive control on shrimp larval mortality

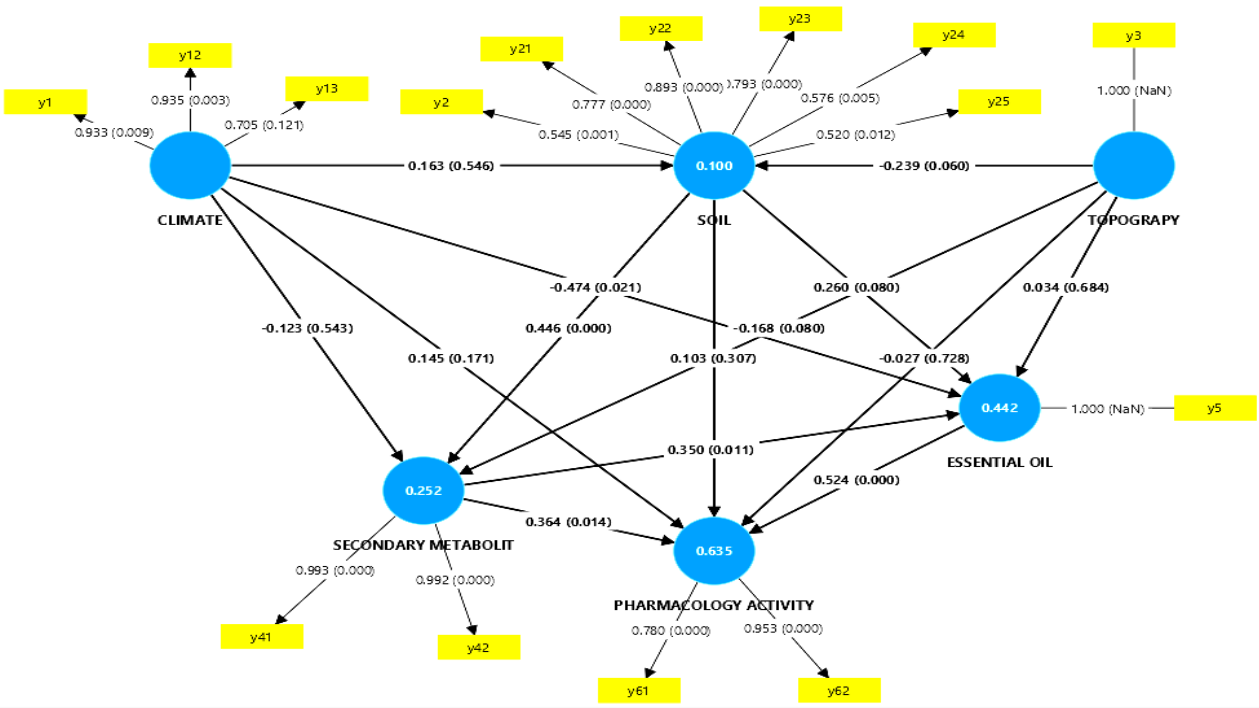


**Figure 7**. The corrected model of the relationship between agroecological conditions and essential oils, secondary metabolites, and pharmacological activity of *Etlingera* from Southeast Sulawesi (PLS output with factor loading values). Note: Y11: Rainfall; Y12: Temperature; Y13: Relative humidity; Y2: Sand; Y21: Silt; Y22: Clay; Y23: pH; Y24: Organic C; Y25: $CaCO_3$; Y3: Topography: Y41: Flavonoid; Y42: Phenolic; Y5: Essential oil; Y61: Antioxidant (DPPH and ABTS); Y62: Toxicity

**Table 7**. The direct effects of agroecology on essential oils, secondary metabolites, and pharmacological activities

| Direct effect | Sample (O) | Mean (M) | Standard deviation (STDEV) | T statistics (O/STDEV) | P values (<0.05) |
|---|---|---|---|---|---|
| Climate → essential oil | -0.474 | -0,414 | 0.205 | 2.312 | **0.021** |
| Climate → pharmacology activity | 0.145 | 0.119 | 0.106 | 1.370 | 0.171 |
| Climate → secondary metabolite | -0.123 | -0.096 | 0.201 | 0.609 | 0.543 |
| Climate → soil | 0.163 | 0.122 | 0.271 | 0.604 | 0.546 |
| Soil → essential oil | 0.260 | 0.269 | 0.148 | 1.754 | 0.080 |
| Soil → pharmacology activity | 0.103 | 0.112 | 0.100 | 1.022 | 0.307 |
| Soil → secondary metabolite | 0.446 | 0.400 | 0.092 | 4.843 | **0.000** |
| Topography → essential oil | 0.034 | 0.050 | 0.084 | 0.407 | 0.684 |
| Topography → pharmacology activity | -0.027 | -0.010 | 0.077 | 0.348 | 0.728 |
| Topography → secondary metabolite | -0.168 | -0.182 | 0.096 | 1.755 | 0.080 |
| Topography → soil | -0.239 | -0.226 | 0.127 | 1.888 | 0.060 |
| Essential oil → pharmacology activity | 0.524 | 0.481 | 0.124 | 4.223 | **0.000** |
| Secondary metabolite → essential oil | 0.350 | 0.372 | 0.137 | 2.550 | **0.011** |
| Secondary metabolite → pharmacology activity | 0.364 | 0.397 | 0.148 | 2.467 | **0.014** |

**Table 8**. The indirect effects of agroecology on essential oils, secondary metabolites, and pharmacological activities

| Indirect effect | Sample (O) | Mean (M) | Standard deviation (STDEV) | T statistics (O/STDEV) | P values (<0.05) |
|---|---|---|---|---|---|
| Climate → essential oil → pharmacology activity | -0.249 | -0.203 | 0.122 | 2.041 | **0.042** |
| Climate → soil → pharmacology activity | 0.017 | 0.008 | 0.043 | 0.388 | 0.698 |
| Climate → soil → secondary metabolite → essential oil | 0.026 | 0.019 | 0.043 | 0.597 | 0.551 |
| Climate → soil → secondary metabolite → pharmacology activity | 0.027 | 0.020 | 0.043 | 0.611 | 0.541 |
| Climate → soil → essential oil → pharmacology activity | 0.022 | 0.024 | 0.050 | 0.441 | 0.659 |
| Climate → soil → essential oil | 0.042 | 0.048 | 0.101 | 0.422 | 0.674 |
| Climate → soil → secondary metabolite | 0.073 | 0.049 | 0.095 | 0.770 | 0.442 |
| Climate → secondary metabolite → essential oil | -0.043 | -0.034 | 0.087 | 0.491 | 0.624 |
| Climate → secondary metabolite → pharmacology activity | -0.045 | -0.041 | 0.089 | 0.500 | 0.618 |
| Climate → secondary metabolite → essential oil → pharmacology activity | -0.022 | -0.016 | 0.038 | 0.596 | 0.551 |
| Climate → soil → secondary metabolite → essential oil →pharmacology activity | 0.013 | 0.008 | 0.018 | 0.729 | 0.466 |
| Soil →secondary metabolite → essential oil → pharmacology activity | 0.082 | 0.69 | 0.030 | 2.729 | **0.007** |
| Soil → essential oil → pharmacology activity | 0.136 | 0.138 | 0.092 | 1.486 | 0.138 |
| Soil → secondary metabolite → essential oil | 0.156 | 0.151 | 0.069 | 2.270 | **0.024** |
| Soil → secondary metabolite → pharmacology activity | 0.163 | 0.163 | 0.079 | 2.048 | **0.041** |
| Topography → soil → secondary metabolite | -0.107 | -0.093 | 0.057 | 1.882 | 0.060 |
| Topography → secondary metabolite → pharmacology activity | -0.061 | -0.076 | 0.056 | 1.091 | 0.276 |
| Topography → secondary metabolite → essential oil | -0.059 | -0.071 | 0.050 | 1.181 | 0.238 |
| Topography → soil → essential oil | -0.062 | -0.057 | 0.047 | 1.309 | 0.191 |
| Topography → soil → secondary metabolite → pharmacology activity | -0.039 | -0.038 | 0.030 | 1.294 | 0.196 |
| Topography → soil → secondary metabolite → essential oil | -0.037 | -0.035 | 0.026 | 1.447 | 0.149 |
| Topography →secondary metabolite → essential oil → pharmacology activity | -0.031 | -0.032 | 0.021 | 1.474 | 0.141 |
| Topography → soil → essential oil → pharmacology activity | -0.032 | -0.030 | 0.029 | 1.132 | 0.258 |
| Topography → soil → pharmacology activity | -0.024 | -0.024 | 0.028 | 0.862 | 0.389 |
| Topography → soil → secondary → metabolite essential oil → pharmacology activity | -0.020 | -0.016 | 0.012 | 1.588 | 0.113 |
| Topography →essential oil → pharmacology activity | 0.018 | 0.021 | 0.039 | 0.460 | 0.646 |
| Secondary metabolite → essential oil → pharmacology activity | 0.183 | 0.170 | 0.059 | 3.118 | **0.002** |

The agroecological conditions across the various study areas, including climate data, soil properties, and topographical features, are crucial for plant growth. In terms of climate, the total rainfall in South Konawe is classified as high. High rainfall can cause the leaching of nutrients from the soil, particularly basic ions such as $Ca^{2+}$, $Mg^{2+}$, $K^{+}$, and $Na^{+}$. When these ions are washed away from the topsoil to deeper layers, the soil loses its capacity to neutralize hydrogen ions ($H^{+}$), leading to increased soil acidity. This factor is one of the indicators that the soil pH in these areas is acidic (4.9). The optimal soil pH, which is crucial for the growth and development of plants in the Zingiberaceae family, falls within an ideal range of 5.5 to 6.5 to achieve maximum growth potential. This underlines the significance of soil pH in plant growth. Nevertheless, these plants also exhibit high tolerance to lower or higher pH levels under various soil conditions (Fisher et al. 2023).

Soil characteristics play a crucial role in the growth, development, and metabolite content of *Etlingera* species. Soil organic carbon content serves as an energy source for soil microorganisms, influencing soil structure and its Cation Exchange Capacity (CEC). The soil in this study varies in organic carbon content for planting *Etlingera* species, ranging from low to high categories. Locations with high organic carbon, such as *E. rubroloba* in Wakatobi District, can support growth by increasing nutrient availability. Vegetation type, soil management practices, and climate can all influence differences in soil organic carbon content. Soil texture significantly affects the growth and yield of the Zingiberaceae family, particularly in relation to rhizome development. Soil texture, determined by the proportion of sand, silt, and clay, influences various soil properties such as water retention, aeration, and nutrient availability, all crucial for optimal plant growth (Supriya et al. 2020). Lime in the soil supports the biosynthesis of secondary metabolites, such as flavonoids and alkaloids, by providing calcium as a cofactor for metabolism and stress signaling. Calcareous soil influences metabolite production by altering nutrient availability and creating environmental stress, resulting in higher levels of compounds like flavonoids, phenolics, terpenoids, alkaloids, and saponins as adaptive responses. These results align with the phenolic metabolite content in *E. rubroloba* (29.52%), which has a higher lime content compared to the other two species.

The chemical composition of essential oils is highly diverse, encompassing fatty acids, phenolics, phenylpropanoids, sterols, steroids, and terpenoid structures. The volatile components typically found in essential oils include aromatic terpenes, aldehydes, ketones, phenols, volatile acids, and esters (Subaryanti et al. 2021). Previous research identified essential compounds in the Zingiberaceae family, such as those found in *Zingiber officinale* Roscoe (ginger), which consist of sesquiterpenes, monoterpenes, esters, and aldehydes (Oforma et al. 2020). Table 1 presents several compounds that share structural similarities among *Etlingera* species, such as cis-vaccenic acid, phthalic acid di(2-propylpentyl) ester, and 2-(2,5-dimethoxy-4-ethylphenyl) ethanol acetate (Table 1). These compounds can be used as markers for identifying plants within the *Etlingera* genus. Previous research revealed that the chemical compounds in the essential oil of *E. elatior* rhizomes include dodecanal (36%), n-hexane (16.90%), dodecanoic acid (15.54%), and cyclododecane (5.07%). The chemical composition of *E. calophrys* rhizome essential oil includes n-decanal (34.09%), dodecanoic acid (14.5%), n-hexane (13.79%), acetone (5.49%), and dodecyl acetate (4.62%). Volatile compounds in the rhizomes of *Etlingera elatior* and *Etlingera calophrys* primarily include dodecanal, α-pinene, and caryophyllene (Aswan et al. 2020). The difference in essential oil composition from the *Etlingera* species in Southeast Sulawesi, as observed in the study by Aswan et al. (2020), can be attributed to several factors such as climate, soil composition and altitude.

Table 2 presents the distribution of secondary metabolite groups in *Etlingera* species from different locations. Closely related plants typically exhibit similarities in the types and compositions of chemical compounds, particularly secondary metabolites, which act as marker compounds. The difference in the composition of compounds in *Etilingera* species can be attributed to differences in agroecological factors such as soil and climate conditions (Cornara et al. 2023; Qaderi et al. 2023). Previous investigations have revealed marker compounds, such as yakuchinone A and B, found in all samples of *E. calophrys*, *E. canarina*, and *E. echinulata* (Hamsidi et al. 2024). Zingiberaceae species from Southeast Sulawesi, such as the leaves and fruits of *Alpinia monopluera* (locally known as *wundu watu*), contain major compounds such as α-caryophyllene, β-pinene, limonene, α-pinene, β-caryophyllene, and caryophyllene oxide (Yodha et al. 2023; Karmilah et al. 2024). Walay (*Meistera chinensis*) rhizome identified compounds, including caryophyllene, hydroquinone, z-(13,14- epoxy) tetradec-11-en-1-ol acetate, cis-vaccenic acid, and copaene (Musdalipah et al. 2024).

Figure 2 highlights the major compounds in *Etlingera* species, which are often the primary focus due to their significant contribution to biological activities. According to literature reviews, some major essential compounds, such as cis-vaccenic acid, gingerol, and phthalic acid di(2-propylpentyl) ester, possess anticancer, anti-inflammatory, and antioxidant properties (Huang et al. 2021; Yücel et al. 2022; Scott et al. 2024; Yazıcı 2024). Phenolic and flavonoid compounds, which are abundantly found in *Etlingera* species (Table 5 and Table 6), significantly contribute to their pharmacological activity, such as antioxidant and anticancer (Imran et al. 2022; Jabbar et al. 2024). These bioactive compounds are closely linked to the plant's therapeutic potential by efficiently scavenging free radicals, minimizing oxidative stress, and regulating inflammatory processes. Therefore, analyzing the phenolic and flavonoid content in *Etlingera* species is crucial for assessing their pharmacological effectiveness. Figure 3 provides detailed views of the peaks listed in Table 1, illustrating the spectrogram of the detected compounds. A total of thirty-four compounds were identified in the *Etlingera* species. In the chromatogram shows that Stigmastan-3-en-6-ol and 2,5-Furandione 3-dodecyl have the highest retention values in *E. elatior*. These compound is a sterol derivative that has a structure similar to stigmasterol. Biosynthesis sterols on the plant is crucial for responses to abiotic stress. *E.elatior* grows in areas with high rainfall (Table 1). The predominant presence of Stigmastan-3-en-6-ol compounds is an adaptive mechanism to maintain water balance and protect cells from oxidative stress (Du et al. 2022). The results of antioxidant tests (DPPH and ABTS) (Figure 4 and Figure 5) and toxicity (Figure 6) on the rhizomes of *Etlingera* species from three different locations show that all three species possess strong antioxidant activity and are toxic. A compound is considered to have very strong antioxidant activity if its $IC_{50}$ value is <50 mg/L, strong if the $IC_{50}$ value is between 50-100 mg/L, moderate if the $IC_{50}$ value is between 100-150 mg/L, and weak if it is between 151-200 mg/L (Molyneux 2004). Musdalipah et al. (2024) categorized extract toxicity levels based on $LC_{50}$ values as follows: extracts with $LC_{50}$ values≤30 mg/L are considered highly

toxic, $LC_{50}$ values≤1,000 mg/L are classified as toxic, and $LC_{50}$ values>1,000 mg/L are regarded as non-toxic.

The metabolites identified in this study are believed to be strongly influenced by the environmental conditions of each location both directly and indirectly ($P<0.05$). These relationships illustrate how agroecological factors, particularly climate and soil, impact the synthesis of secondary metabolites and essential oils, which in turn determines their pharmacological properties. SEM analysis allows for the quantification of these direct and indirect effects, providing valuable insights into the complex interactions between environmental conditions and plant bioactivity (Table 7). Climate plays a critical role in influencing various aspects of plant growth, metabolism, and medicinal value. It directly affects soil quality, secondary metabolite production, essential oil composition, and pharmacological activities. Temperature, rainfall, and humidity determine the quantity and quality of essential oils and secondary metabolites like terpenoids, flavonoids, phenolics, sterols and fatty acid. Variations in climate conditions, such as drought or high temperatures, can enhance the synthesis of bioactive compounds as adaptive responses, thereby influencing their pharmacological properties. Additionally, climate impacts soil properties, including moisture content, nutrient availability, and pH, which indirectly affect metabolite production. In indirect effect (Table 8) show that the composition of essential oils is influenced by the presence and concentration of secondary metabolites. These essential oils, which are abundant in bioactive compounds, demonstrate distinct pharmacological activities that can be harnessed for medicinal purposes. Investigating essential oils offers valuable insights into the therapeutic potential of plants and their potential applications in various medical treatments.

Variations in climate and environmental growing conditions significantly influence the phytochemical diversity of *Etlingera* species. Consequently, the variability in the chemical profiles of the studied populations presents an opportunity to select optimal locations for plant breeding. Other factors, such as harvest time greatly affect the yield and chemical profile of essential oils. The biosynthesis and accumulation of essential oil compounds are closely linked to the developmental stage of the plant. Therefore, identifying the optimal harvesting time is essential to achieve the desired chemical composition and maximize essential oil yield. Our approach to identifying environmental predictors for essential oil content with high levels of specific compounds can aid in determining appropriate regions for sampling plant materials with the desired chemical profiles. The chemical interactions between plants and their surroundings are largely governed by the biosynthesis of secondary metabolites, which serve as adaptive responses to environmental challenges. Such interactions frequently lead to variations in the production of plant metabolites.

In conclusion, these insights can be applied to identify ideal locations and ecosystems for cultivating medicinal plants. This approach aims to produce high-value products with superior quality and enhanced bioactive properties. To produce abundant secondary metabolites such as fatty acids, phenolics, and terpenoids, we can develop the cultivation of *E. rubroloba.* Flavonoid and sterol compounds can be obtained through the cultivation of *E. elatior*. These findings can serve as a basis for developing efficient cultivation strategies by modifying agroecological factors, such as environmental and soil conditions, to optimize the productivity of secondary metabolites.

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