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Chemotaxonomy in the *Etlingera* Genus from Sulawesi, Indonesia: Design and molecular docking of antioxidant marker

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Abstract. *Hamsidi R, Karmilah, Daud NS, Malaka MH, Yodha AWM, Musdalipah, Arfan, Sahidin. 2024. Chemotaxonomy in the* Etlingera *Genus from Sulawesi, Indonesia: Design and molecular docking of antioxidant marker. Biodiversitas 25: 449-457. Etlingera* is one of the important genera in the Zingiberaceae family because of its potential uses. Several studies have been conducted on the Genus *Etlingera* as nutraceuticals and drugs. Recently new species have been found in the Sulawesi Region, Indonesia, namely *E. canarina* A.D.Poulsen and *E. echinulata* A.D.Poulsen. Based on literature review through sciencedirect and springer link, both species unknown for their chemical and pharmacological content, so it is necessary to continue to reveal the deeper potential. The fruits of *E. calophrys* (K.Schum.) A.D.Poulsen, *E. canarina* and *E. echinulata* were extracted with methanol by maceration method. The chemical content of each extract was analyzed using a Liquid Chromatography Mass Spectrometer (LCMS). Antioxidant activity was tested based on the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) method. The ability as an antioxidant is proven by the molecular interactions of marker compounds in inhibiting xanthine oxidase. The secondary metabolites contained in the fruit extracts of *E. calophrys*, *E. canarina* and *E. echinulata* are E-p-Coumatic acid, 1,5-Dimethyl citrate, Trimethyl citrate, Crocetin, Tanshinone IIA, Ethinyl Estradiol, Yakuchinone B, Anisalacetone, Yakuchinone A, Nobilin, α-Estradiol, Momor-cerebroside I, Spinasterone, 12-Hydroxy-methyl abietate, Spinasterol, 2,3-Dihydroxypropyl oleate, 11-Octadecenoate acid methyl ester, Stigmastan-3,6-dione, β-Sitosterol- 3-O-β-D-glucopyranoside, Trilaurin, 28-O-Hexahydrophthalate and Fasciculol C. Yakuchinone A and Yakuchinone B are chemotaxonomic marker compounds identified in all species. The antioxidant properties of each extract of 42.27±0.53, 46.59±0.81 and 35.66±0.73 mg/L have been proven by the molecular interactions of the marker compounds in their ability to act as xanthine oxidase inhibitors. The compounds Yakuchinone A and Yakuchinone B are thought to be responsible for the pharmacological activity of the species *E. calophrys*, *E. canarina* and *E. echinulata*. Yakuchinone A and Yakuchinone B are marker compounds because they are distributed in all samples.

Keywords: Chemotaxonomy, *Etlingera calophrys*, *Etlingera canarina*, *Etlingera echinulata*, Yakuchinone A and Yakuchinone B

INTRODUCTION

Several scientific studies have reported the bioactivity of medicinal plant (Awang-Jamil et al. 2021). Nevertheless, scientific studies on reviewing the chemical and pharmaceutical aspects of Sulawesi culture's traditional medicinal plants are still very limited. Several endemic medicinal plants of Sulawesi that have been studied include *Alpinia monopleura* K.Schum. (Yodha et al. 2023), *Meistera* (Musdalipah et al. 2021a,b), *Polygonum* (Sahidin et al. 2019) and *Etlingera* (Sahidin et al. 2018; Hamsidi et al. 2021; Wahyuni et al. 2021a,b; Sahidin et al. 2022; Wahyuni et al. 2022). *Etlingera* is one of the important genera in the Zingiberaceae family because of its potential uses. Several studies have been conducted on the Genus *Etlingera* to explore the potential of this genus as nutraceuticals and pharmaceuticals. Globally, research on *Etlingera* has been conducted in various countries successively such as Malaysia, Thailand, Singapore and Indonesia (Juwita et al. 2018). Plants belonging to the Zingiberaceae family are well known for their versatility in supporting and improving people's welfare. This plant has been used for medicinal purposes since ancient times, especially in traditional health systems in Indonesia (Elfahmi et al. 2014; Fathir et al. 2021).

Medicinal plants are an important resource because they have the property of curing disease. This plant has fewer side effects than chemical drugs, so many people use it (Nugroho et al. 2022; Nayaka et al. 2023). Recently, chemical and pharmaceutical studies of the Genus *Etlingera* of the species *E. alba* have been found to contain chemical compounds such as 1,7-diphenyl-6-heptene-3-one, sitostenone, sinapyl alcohol diacetate, and sinapyl alcohol acetate. These four compounds were able to provide anticancer activity against MDA-MB 231 cells. Meanwhile, nine chemical compounds were found from other species, namely *Etlingera calophrys* (K.Schum.) A.D. Poulsen such as 1,7-diphenylhept-6-en-3-one, 1,7-diphenylheptan-3-one, stigmast-4-en-3-one, β-sitosterol, stigmasterol, Yakuchinone A, 7-(4-hydroxy-3-methoxyphenyl)-1-phenylhept-4-en-3-one, 1-(4-hydroxyphenyl)-7-phenylheptan-3-one and Yakuchinone B

as well able to provide good activity as an antimicrobial in inhibiting the growth of pathogenic bacteria and as an antioxidant in inhibiting free radicals. Most of the antioxidants found in fruits. Some of them are derivatives of polyphenolic compounds. Single antioxidants like ascorbic vita have also been shown to reduce the risk of coronary heart disease and cancer (Breaud et al. 2022).

This description shows that the *Etlingera* plant has many interesting and important chemical variations and properties. However, little has been explored for these plants chemical content and efficacy compared to their populations in Sulawesi. The quantity of chemical markers can be used as a determinant of quality factors of medicinal plants. The composition and bioactive content of medicinal plants are influenced by various factors: internal, external, and genotype (Batubara et al. 2020; Asyhar et al. 2023). In Sulawesi, Indonesia, the *Etlingera* population grows to around 48 species, such as Central Sulawesi, Southeast Sulawesi, South Sulawesi and North Sulawesi, from 150-200 species worldwide. Therefore, to reveal the potential of the *Etlingera* plant in more depth, it is important to carry out chemical and pharmacological studies of other species. Species *Etlingera canarina* A.D.Poulsen found in East Luwu District, South Sulawesi and *Etlingera echinulata* A.D.Poulsen found in Buol District, Central Sulawesi. They are endemic plants of Sulawesi whose chemical and pharmacological properties have not discovered until now. Based on a review of plant morphological characters, it was explained that *E. calophrys* has close taxonomic kinship with *E. canarina* and *E. echinulata* (Droop 2012).

Currently, the development of plant classification can be carried out through the science of chemotaxonomy which is based on the use of phytochemical (Bamigboye et al. 2020). Chemotaxonomy is a branch of plant taxonomy based on chemical information to find plant classifications. To complete the morphological characters of plants, phytochemical elements and molecular markers are needed with analytical techniques to identify, differentiate and compare plant species (Thirumurugan et al. 2018). Plants with a kinship relationship will have similar types and homologies of chemical compound content, especially their secondary metabolites as marker compounds (Sahidin et al. 2006). This chemical content similarity will also be related to similar pharmacological activities so that all of the three species can be utilized together. In this study, marker compounds have been found in three different *Etlingera* species. These compounds are secondary metabolites that are important in providing antioxidant activity.

MATERIALS AND METHODS

Plant material

The *E. calophrys* fruit obtained from Southeast Sulawesi Province, Indonesia 4°2'55.2"S 122°23'23.9"E, 180 m, *E. canarina* fruit obtained from South Sulawesi Province 2°16'0"S 120°47'17"E, 1300 m and *E. echinulata* fruit obtained from Central Sulawesi Province, 0°59'30.5"N 121°36'11.1"E, 40 m (Figure 1). The plant sample was identified by The BRIN Research Center for Biology, Indonesia determined the sample and registered it with Number 1535/IPH.1.01/If.07/VIII/2019. The collected fruits were cleaned of soil using water and cut into small pieces. The fruits were dried in a shady place and pulverized using a grinder. The dried samples were then extracted.

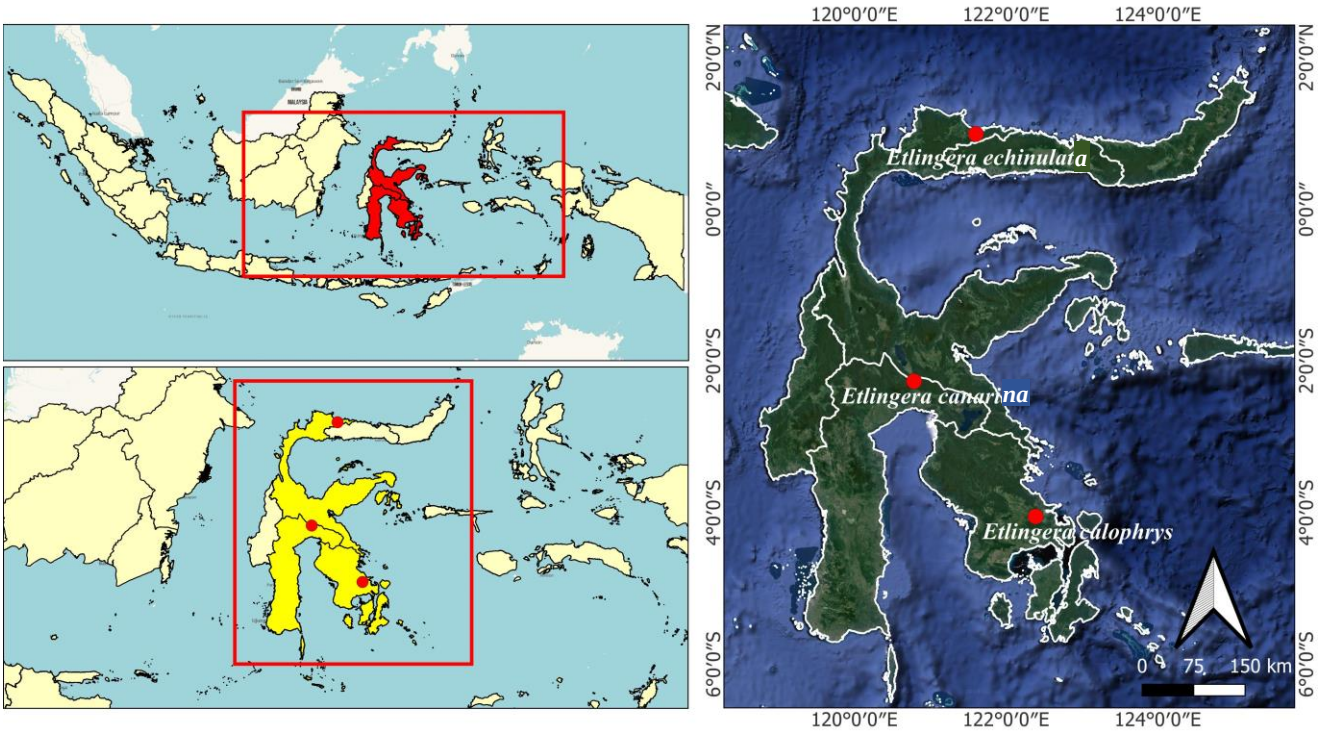

Figure 1. Ecological distribution of *Etlingera* plants in Sulawesi, Indonesia

Extraction method

About 500 g of each fruit powder was dissolved with methanol in a sealed glass container. The maceration process was carried out for three days and filtered and replaced the solvent. The filtrate was collected and concentrated using a vacuum rotary evaporator (Stuart RE300, USA) at 45°C at 80 rpm until a thick extract was obtained. Each condensed extract of *E. calophrys* fruit, *E. canarina* fruit and *E. echinulata* fruit was analyzed for its chemical compounds and antioxidant activity (Yodha et al. 2023).

Chemical compound analysis

The secondary metabolites of each extract were analyzed using Liquid Chromatography Quadrupole Time of Flight Mass Spectrometer (LCMS) (Waters, USA). The stationary phase used a reversed phase column (HSS T3 C18) at 40°C. The mobile phase consisted of A (0.1% Formic Acid in Water (v/v), Pierce) and B (0.1% Formic Acid in Acetonitrile (v/v), Pierce). Gradient elution was carried out at a flow rate of 0.300 mL/min with an injection volume of 1 µL. The gradients are as follows: 5% B (0-1 min), 40% B (8-13 min) and 100% B (13-16 min). The data range is between 50-1200 m/z. The separated peaks were detected with a mass detector. The mass experiments were carried out in positive mode Electro-Spray Ionization (ESI), according to the acquisition parameters: capillary voltage, 1.5 kV; cone voltage, 30 V; source temperature, 120°C; desolvation temperature, 500°C; cone gas flow, 50 L/h; desolvation gas flow, 1000 L/h. The mass range for the MSE function was m/z 100-1200. MSE (function 1) was run at a cone voltage of 30 V and collision energy of 6 eV. Meanwhile, MSE (function 2) was performed at a cone voltage of 30 V and impact energy from 10 eV to 40 eV. The mass-to-charge ratio (m/z) values of all peaks obtained by LC-MS/MS analysis were identified by UNIFI software by applying MSE identification. The values were analyzed using databases in Metlin, Metfrag, Massbank North America, Massbank Japan, mzCloud, ChemSpider, and PubChem (Sahidin et al. 2020; Wahyuni et al. 2021a,b).

Determination of antioxidant activity

Antioxidant properties of fruit extracts of *E. calophrys*, *E. canarina* and *E. echinulata* were determined based on their ability to scavenge free radicals using the DPPH (1,1-diphenyl-2-picrylhydrazyl). A total of 1 mL of 0.1 mM DPPH solution (HIMEDIA) in methanol was mixed with 2 mL of extract solutions in methanol at different concentrations (10-50 µM). The mixture was then incubated at room temperature for 30 minutes in the dark. The absorbance was measured against methanol as a blank at 517 nm using UV-Vis spectrophotometer (Jenway, 6800 Double Beam Spectrophotometer, UK). The use of methanol as a blank because it is the solvent used to dissolve DPPH and pure compounds. Higher DPPH free radical inhibitory activity is indicated by decreasing absorbance value of the reaction mixture. The positive control used was ascorbic acid. Samples were prepared and measured in 3 repetitions. The percentage of DPPH radical inhibitory activity of each compound was calculated as % DPPH inhibition (I%) using the following equation (Sadarun et al. 2022; Asyhar et al. 2023):

$$I\% = \left|\frac{(A^{o} - A^{s})}{A^{o}}\right| \times 100\%$$

Where:

A^{o} : Absorption of the control

A^{s} : Absorption of the sample solution

The IC_{50} of each *Etlingera* Genus was determined by: (a) the inhibitory activity (y) is plotted against the concentration (x) at six points (60, 50, 40, 30, 20, and 10 g/mL), (b) the regression line equation (y = ax + b) is determined, and (c) the sample concentration (x) is calculated by replacing y = 50 in the regression equation (b) (Wahyuni et al. 2021a,b).

Molecular docking

The target protein employed in the research is Xanthine Oxidase (XO) (PDB ID 3NRZ) (Chekol 2019; Dong et al. 2020; Hille 2023) obtained from the RCSB website (https://www.rcsb.org/). The downloaded target protein was subsequently prepared using AutoDock Tools v1.5.6, removing chains A and B, bound ligands, and water molecules from the protein structure. Finally, hydrogen atoms were added to the polar groups of the target protein, along with Kollman charges (Morris et al. 2008). The ligands used in this study are Yakuchinone A, Yakuchinone B, and Trolox (as comparative compound), with their three-dimensional structures sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). These ligands were then prepared by setting to their maximum torsional conformation, adding hydrogen atoms, and applying Gasteiger charges using AutoDock Tools v1.5.6.

The molecular docking process was conducted using AutoDock 4.2, employing a cubic grid with dimensions of 40 x 40 x 40 Å and a center coordinate aligned with the position of hypoxanthine in XO. The molecular docking parameters utilized the Genetic Algorithm (GA) with 100 conformational searches and a maximum of 2,500,000 evaluations[18]. The molecular docking method was validated by redocking the natural ligand Hypoxanthine (HPA) onto XO. The docking method is considered reliable if the re-docked conformation of the natural ligand exhibits a Root Mean Square Deviation (RMSD) value of ≤2 Å when compared to its crystallographic conformation (Arfan et al. 2022). The molecular docking evaluation was based on choosing the ligand configurations with the most favorable binding free energy (ΔG). The resulting ligand-receptor complexes were visualized using the Discovery Studio Visualizer software v17.2.0.16349.

RESULTS AND DISCUSSION

The Genus *Etlingera* has been shown to produce a variety of structurally unique chemical constituents. several compounds have been isolated from the Genus *Etlingera* such *E. calophrys* and *E. elatior* including, Yakuchinone A, p-hydroxybenzoic acid and stigmasterol (Chan et al. 2011; Sahidin et al. 2019). *Etlingera* species have been used traditionally and commercially as food, spices, medicines, and ornamental plants; and also play an important role in the

understory layer as a source of animal food. With promising medicinal properties and activities, these species have great potential to be developed into natural preservatives and herbal products, which can be applied to the food, cosmetic, and nutraceutical industries (Trimanto and Hapsari 2018).

Chemotaxonomic strategies have successfully identified markers that play a role in secondary plant metabolism. A number of morphological and chemical studies over the past decades have established the taxonomic position of the genus Zingiberaceae. Antioxidants protect cells by converting ROS into non-radicals. Plants are natural sources of exogenous antioxidants such as phenolics and vitamins. The Genus *Etlingera* is one of the herbal plants which belonging to the Zingiberaceae family with interesting potential.

The results showed that the fruit extracts of *E. calophrys*, *E. canarina*, and *E. echinulata* were obtained with yield values of 3.4%, 3.7%, and 4.1%, respectively. The yield value is influenced by the extraction method and the solvent used. The maceration method allows contact between the sample and the solvent for a long time to maximize the transfer of compounds from the sample into the extraction solvent. In addition, the methanol solvent used is capable of extracting many classes of secondary metabolites such as anthocyanins, tannins, totarols, terpenoids, saponins, xanthoxyllines, quassinoids, phenones, polyphenols, lactones and flavones (Fonmboh et al. 2020; Meng et al. 2020). Mostly, methanol is used to extract various polar compounds, although there are some nonpolar groups that are quite soluble in this solvent. In addition, methanol has the lowest boiling point among all types of alcohols. Therefore, extraction and concentration of bioactive compounds can be more efficiently carried out using this solvent (Panphut et al. 2020).

The chromatogram of the results of the LCMS analysis showed the peak separation of the chemical compound content of each extract (Figure 2), which was observed from the Retention Time (RT) 0-16 minutes. The separation occurs because each chemical compound contained will have a different migration rate in certain mobile and stationary phases (Fonmboh et al. 2020). Chromatogram of Liquid Chromatography (LC) separation results, compounds contained in each sample (*E. calophrys*, *E. canasia*, *E. echinulata*) are separated based on different retention times (0-16 minutes). The separation results are characterized by the formation of compound peaks. The peaks that appear on each Rt, translated by MS / MS through molecular weight and fragmentation so that molecules can be known (Name and Structure). The chromatogram also explains the similarity of chemical content, based on the similarity of retention time. Based on the chromatogram, Base Peak Intensity (BPI) and compound (high or low content) can also be determined.

The data in Table 1 presents the diversity of compounds in *E. calophrys*, *E. canarina* and *E. echinulata* extracts. As many as 22 types of compounds were distributed in each extract with different concentrations (Figure 3). Differences in compound content between the spesies of etlingera depends in climatic conditions, soil types, and fertility levels of the plant's growth site (Mahdavi et al. 2017; Zandalinas et al. 2022). The number of concentrations identified provides an indication and confirmation that the compound is a major component of the *Etlingera.* The Yakuchinone A compound that appeared at a retention time of 9.51 minutes with m/z 313.1802 was a compound with high levels in each of the extracts analyzed. In addition, Yakuchinone A and Yakuchinone B (Figure 4) were identified in all samples, making them marker compounds in the studied *Etlingera* species. A significant compound present in the *Etlingera* Genus is yakuchinon A (Figure 3).

Table 1. Chemical composition based on LC-MS/MS data analysis

RT (min)	Observed $[M+H]^+$ (m/z)	Experimental neutral mass (Da)	Compounds
5.36	165.0546	164.0473	E-p-Coumatic acid
5.65	220.0472	219.0505	1,5-Dimethyl citrate
7.31	235.0627	234.0740	Trimethyl citrate
7.75	329.1751	328.1675	Crocetin
8.72	295.1340	294.1556	Tanshinone IIA
9.35	297.1854	296.1776	Ethinyl Estradiol
9.41	311.1644	310.1569	Yakuchinone B
9.46	177.0903	176.0837	Anisalacetone
9.51	313.1802	312.1725	Yakuchinone A
9.62	347.1446	346.1780	Nobilin
9.62	273.1682	272.1776	α-Estradiol
9.65	844.6837	843.6799	Momor-cerebroside I
9.78	411.3604	410.3549	Spinasterone
10.14	333.2235	332.2302	12-Hydroxy-methyl abietate
10.16	413.3753	412.3705	Spinasterol
10.19	357.2802	356.2927	2,3-Dihydroxypropyl oleate
11.03	297.2779	296.2715	11-Octadecenoate acid methyl ester
11.18	429.324	428.3654	Stigmastan-3,6-dione
11.39	577.4274	576.4389	β-Sitosterol-3-O-β-D-glucopyranoside
11.54	639.6370	638.5485	Trilaurin
12.47	597.4498	596.4441	28-O-Hexahydrophthalate
13.80	573.4494	572.4441	Fasciculol C

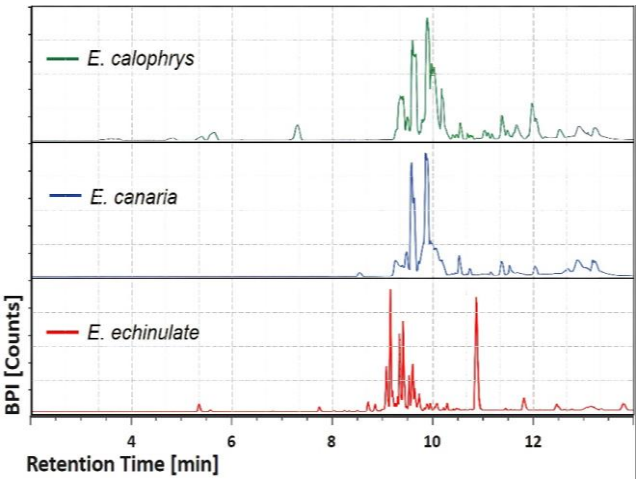

Figure 2. LCMS-MS chromatogram of the chemical constituents

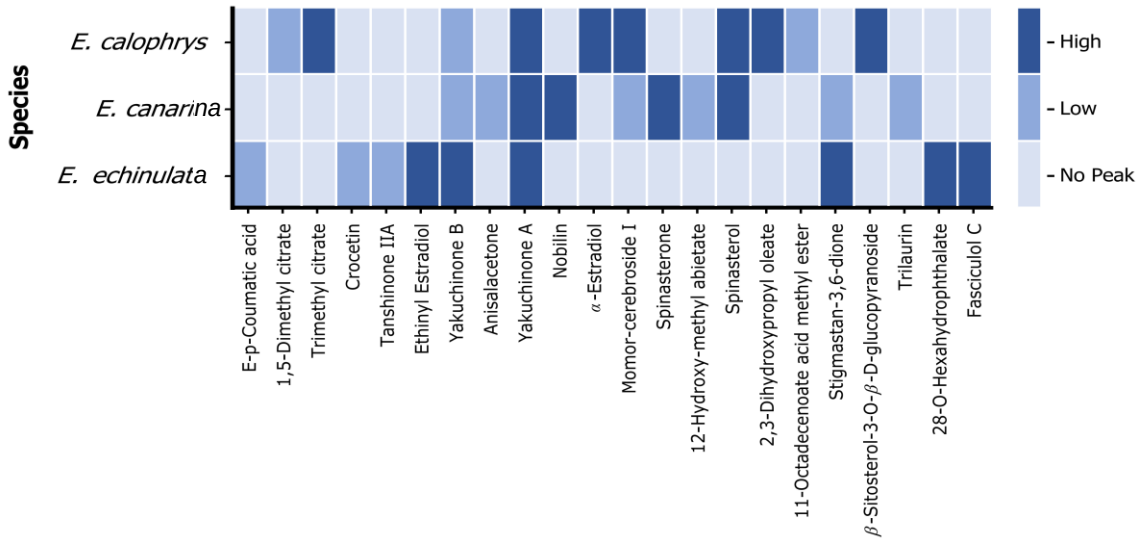

Figure 3. Distribution of compounds in the species studied

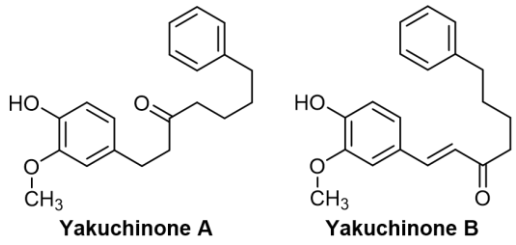

Figure 4. Structure of chemical compounds distributed in *E. calophrys*, *E. canarina* and *E. echinulata*

The data in Figure 4 presents the results of the antioxidant test using the DPPH method. The radical inhibition values of the fruit extracts of *E. calophrys*, *E. canarina*, *E. echinulata*, and Trolox were 42.27±0.53, 46.59±0.81, 35.66±0.73, and 16.23±0.64mg/L, respectively. The ability to reduce free radicals is expressed as IC_{50}. IC_{50} values of *Etlinegera* species showed IC_{50} value <50 µg/mL. IC_{50} values of >200 µg/mL have no activity, >150-200 µg/mL are weak, >100-150 µg/mL are quite strong, >50-100 µg/mL are strong and <50 µg/mL are very strong (Yodha et al. 2023). It is known that *Etlingera* species have antioxidant with very strong ability. The antioxidant activity of the tested extracts is thought to be influenced by the content of Yakuchinone A and yakuchinone B as marker compounds, which are phenolic group compounds. Phenol (Ar-OH) is known to reduce the rate of oxidation of organic compounds by transferring the H atom (from its OH group) to the DPPH radical to become DPPH-H (Kamal et al. 2022; Warsito et al. 2022). Phenol also suppresses the formation of ROS so that damage caused by free radicals can be avoided (Wahyuni et al. 2021a).

The assessment of antioxidant activity involves measuring the capacity of compounds to neutralize free radicals or reduce oxidative stress, as demonstrated through in vitro testing using the DPPH radical and computational methods employing molecular docking with the enzyme xanthine oxidase. Correlation of antioxidant activity in vitro with the DPPH method showed linear results against in silico simulations through molecular docking. A simulation was conducted to assess the antioxidant activity of the *Etlingera* extract against xanthine oxidase. Xanthine oxidase yields Reactive Oxygen Species (ROS) as byproducts during its normal catalytic cycle. These ROS, including superoxide radicals and hydrogen peroxide, can contribute to oxidative stress and damage cellular components like lipids, proteins, and DNA (Altemimi et al. 2017; Schmidt et al. 2019). Identifying compounds capable of inhibiting the excessive activity of XO, which generates radicals, serves as a foundation for developing compounds with antioxidant properties. Molecular docking is a computational approach at the molecular level to evaluate interactions of compounds from natural products to macromolecular targets such as enzymes. In this study, molecular docking was conducted on two compounds, namely yakuchinone A and yakuchinone B, identified from fruit extracts of *E. calophrys*, *E. canarina*, and *E. echinulata*, showcasing their potential as antioxidants. The docking process used in this study was meticulously executed, yielding a Root Mean Square Deviation (RMSD) value of 0.514 Å (as depicted in Figure 5). This precision in docking ensured a reliable representation of the interactions between the extract's components and xanthine oxidase.

A comprehensive analysis of the docking results showed a notable correlation between the compounds' binding energies and antioxidant activity. The analysis of molecular docking results is based on the binding free energy value (ΔG). A lower value indicates a stronger bond between the compound and the macromolecule target, reflecting increased stability and strength of non-covalent interactions. A low energy value promotes spontaneous bonding between the ligand and the macromolecule, as the ligand requires minimal energy for binding or affinity to the target (Motiejunas and Wade 2007). Specifically, both Yakuchinone A and Yakuchinone B exhibited significantly

lower binding energies in comparison to HPA (with a binding energy of -5.73 kcal/mol) and Trolox (with a binding energy of -3.0 kcal/mol). The binding energies for Yakuchinone A and Yakuchinone B were calculated to be -7.54 kcal/mol and -8.17 kcal/mol, respectively. This number indicates a higher affinity of Yakuchinone A and Yakuchinone B towards xanthine oxidase, signifying their potential effectiveness in counteracting radicals. The correspondence between the favorable binding energies and the antioxidant activity highlights the likelihood of these compounds to be effectively neutralizing radicals produced by xanthine oxidase, thus contributing to their potential therapeutic application as natural antioxidants.

HPA generally forms hydrogen bond interactions with the Glu802, Arg880, and Thr1010 residues on the xanthine oxidase active site (Figure 6). Meanwhile, the carbonyl group of Yakuchinone A only forms a hydrogen bond with Ser876. Remarkably, this compound shows hydrogen bonds not observed in other compounds, specifically Ala1079 and Glu1261, forming from the methoxy and hydroxy groups of the compound, respectively (Figure 7.A). Yakuchinone B exhibits similar interactions to HPA, where its carbonyl group forms hydrogen bonds with the Arg880 and Thr1010 residues (Figure 7.B). The differences in hydrogen bond interactions between Yakuchinone A and Yakuchinone B are influenced by the presence of double bonds in Yakuchinone B, causing the phenyl ring substituted by methoxy and hydroxy groups to be less flexible in rotating at the XO's active site.

Meanwhile, Trolox, a reference compound in this study, only exhibits a single hydrogen bond interaction with the amino acid Ser876, formed by the oxygen atom in its chromane ring (Figure 8).

Furthermore, all compounds form hydrophobic interactions with the Phe914, Ala1078, and Val1011 residues. In the case of Yakuchinone B, hydrophobic interactions are observed with Leu873 and Ala910 involving its benzene ring, an interaction absent in the other compounds. This benzene ring also contributes to forming pi-anion interactions with the Glu802 and Glu1261 residues (Figure 7.B). Meanwhile, the benzene ring in Yakuchinone A establishes four distinct hydrophobic interactions with the Leu648, Phe1013, and Leu1014 residues (Figure 7.A). On the other hand, the chromane ring of the reference compound (Trolox) demonstrates hydrophobic interactions with the Leu648, Phe649, Leu873, Phe1009, and Pro1076 residues (Figure 8). Both compounds (Yakuchinone A and Yakuchinone B) exhibited tight binding to critical amino acids, particularly those involved in substrate binding (Arg880, Val1011) and catalysis (Glu802, Glu1261), potentially inhibiting xanthine oxidase enzymatic activity. If the compounds compete with substrates for binding or disrupt the catalytic mechanism, they could effectively reduce the conversion of xanthine to uric acid and Reactive Oxygen Species (ROS) (Xue et al. 2023). Furthermore, Yakuchinone A and Yakuchinone B each possess a hydroxyl group on their benzene ring. The antioxidant mechanism involves a hydrogen atom transfer, donating H atoms to free radical substrates, generating non-radical substrate species (RH, ROH, or ROOH), and free radical antioxidants (Gutiérrez-Del-Río et al. 2021). The summarized interactions can be observed in Table 2.

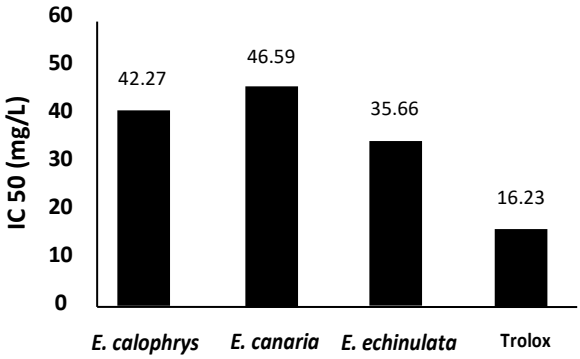

Figure 5. Determination of antioxidant capacity in *E. calophrys*, *E. canarina* and *E. echinulata*

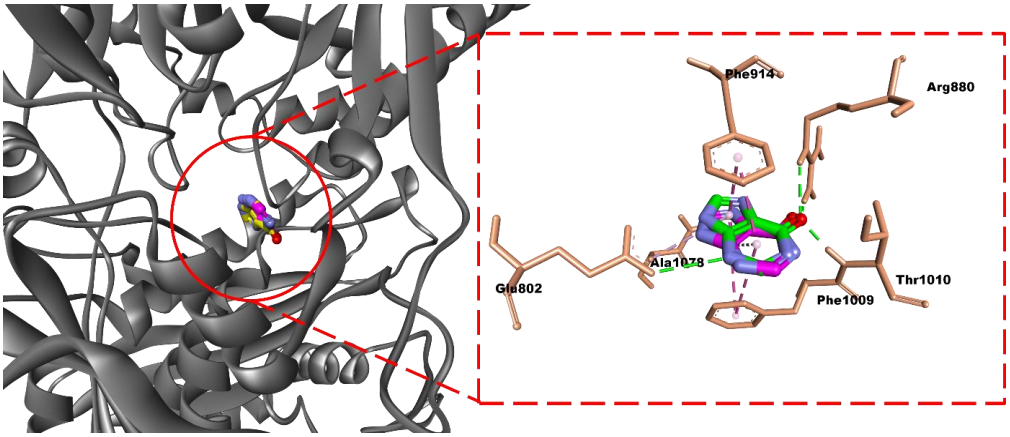

Figure 6. The superimposition of the HPA crystallographic conformation (green) and the re-docking conformation (pink) on the xanthine oxidase active site

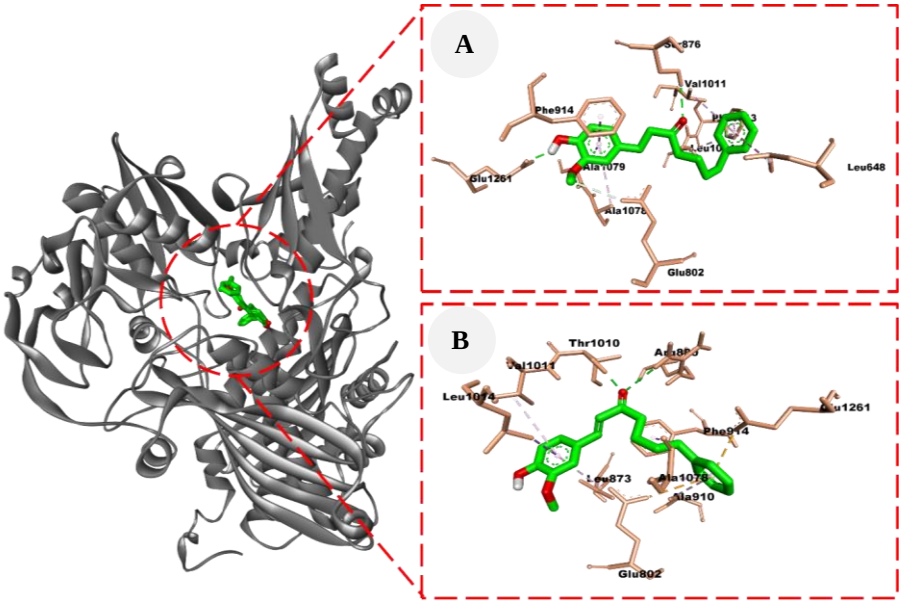

Figure 7. Molecular interactions of: A. Yakuchinone A and B. Yakuchinone B on the xanthine oxidase active site

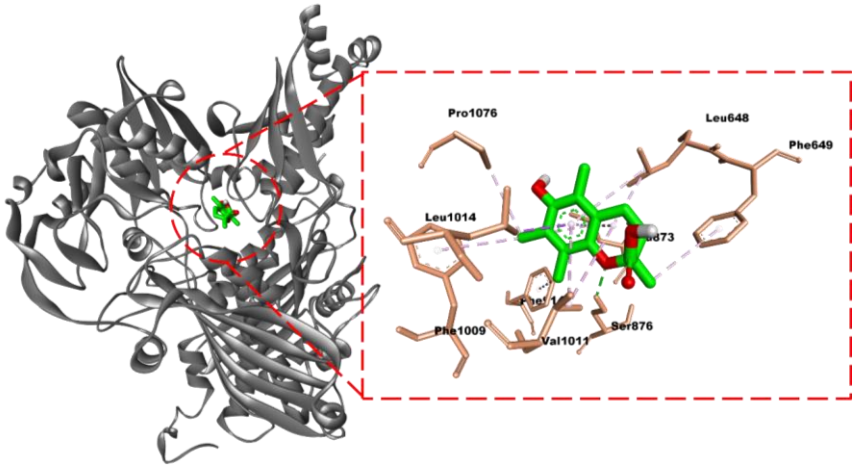

Figure 8. Molecular interactions of Trolox on the xanthine oxidase active site

Table 2. Summary of molecular interactions of the identified compounds in *Etlingera* extract against xanthine oxidase

Compunds	Binding energies (kcal/mol)	Hydrogen bonds	Hydrophobic interactions
Hipoxanthine	-5.73	Glu802, Arg880, Thr1010	Phe914, Phe1009, Ala1078, Ala1079
Trolox	-3.0	Ser876	Leu648, Phe649, Leu873, Phe914, Phe1009, Val1011, Leu1014, Pro1076
Yakuchinone A	-7.54	Glu802, Ser876, Ala1079, Glu1261	Leu648, Phe914, Val1011, Phe1013, Leu1014, Ala1078
Yakuchinone B	-8.17	Arg880, Thr1010	Leu873, Ala910, Phe914, Val1011, Ala1078, Glu802, Glu1261

The binding energy analysis and examination of molecular interactions provide valuable insights into the antiradical potential of Yakuchinone A and Yakuchinone B as xanthine oxidase inhibitors. Both compounds exhibited notably lower binding energies than reference compounds HPA and Trolox. Moreover, the intricate network of hydrogen bonds and hydrophobic interactions formed by Yakuchinone A and Yakuchinone B with key residues highlights their promising affinity and potential as antiradicals through xanthine oxidase inhibition. The inhibition of xanthine oxidase is linked to its antioxidant effects, as the enzyme generates Reactive Oxygen Species (ROS) in the process of converting xanthine to uric acid. This inhibitory action has the capability to reduce the production of ROS, thereby presenting itself as a possible antioxidant (Rullo et al. 2023). This study underscores the significance of these compounds in combatting radicals, offering a promising avenue for further exploration in developing novel antioxidants.

In conclusion, a total of 22 types of chemical compounds were identified from the fruit extracts of E. *calophrys*, *E. canarina*, and *E. echinulata*. Two of them, Yakuchinone A and Yakuchinone B, were marker compounds distributed in all the analyzed species. The extract has the ability as an antioxidant with IC_{50} values of 42.27±0.53, 46.59±0.81, and 35.66±0.73 mg/L, respectively. Yakuchinone A and Yakuchinone B, as chemotaxonomic marker compounds, have a significant role as xanthine oxidase inhibitors through evidence of molecular interactions.

This study found antioxidant compounds as chemotaxonomic markers in *E. calophrys*, *E. canarina*, and *E. echinulata* species. These antioxidant compounds will play a role in utilizing traditional medicines from *Etlingera* species to be used together and is the basis for further analysis in finding new, more potential drugs.

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