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Antibacterial and phytochemical constituent of *Etlingera rubroloba* A.D. Poulsen extract, an endemic ginger from Wallacea Region, Indonesia

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Abstract. *Andila PS, Nugroho LH. 2022. Antibacterial and phytochemical constituent of* Etlingera rubroloba *A.D. Poulsen extract, an endemic ginger from Wallacea Region, Indonesia. Biodiversitas 23: 3646-3658. Etlingera rubroloba* (Blume) AD Poulsen is one of the wild species of the Zingiberaceae which is a native and endemic species in the Wallacea region and has not been studied well for its biology and phytochemical potential. This study aimed to investigate the potential antibacterial activity of hexane extract from the leaves and rhizome of *E. rubroloba* and study their phytochemical content. The antibacterial activity test was carried out by the agar disc diffusion method against several human pathogenic bacteria and be measured for the Minimum Inhibitory Concentration (MIC) and Zone of inhibition. The phytochemical profile was tested for total phenol content (TPC), total flavonoid content (TFC) and GC-MS analysis. The study results show that TPC of *E. rubroloba* extracts respectively 15.68±0.80 mg *Gallic Acid Equivalent* (GAE)/g sample (leaves) and 11.89±0.76 mg GAE/g sample (rhizomes), while TFC value were 33.99±2.24 mg *Quercetin Equivalent* (QE)/g sample (leaves) and 32.46±0.67 mg QE/g sample (rhizomes). The antibacterial test revealed that *E. rubroloba* extracts were effective against *Bacillus cereus ATCC11778* (with MIC value 0.5 mg/mL using leaves extract and 1 mg/mL in rhizomes), but it was not effective against *Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923* and *Staphylococcus epidermidis ATCC12228.* The hexane extracts of *E. rubroloba* contained a total of 28 and 58 compounds were identified in the leaf extract and rhizome, respectively. The dominant compounds in the leaf extract (Pentadecanoic acid, 14-methyl-, methyl ester; Methyl stearate) and in the rhizome extract (Hexadecanoic acid, ethyl ester; Isopropyl myristate; 9-Octadecenoic acid (Z)-, methyl ester; 9,12- Octadecadienoic acid, methyl ester, (E,E)-) is well known to have antibacterial activity. Based on the results of the antibacterial and phytochemical studies, it can be suggested that *E. rubroloba* has the potential as an antibacterial agent.

Keywords: Antibacterial, *Etlingera rubroloba,* flavonoid, phenolic, phytochemical

Abbreviations: MIC: Minimum Inhibitory Concentration, TPC: Total Phenolic Content, TFC: Total Flavonoid Content

INTRODUCTION

Indonesia has unique geographical and climatic conditions that make Indonesia rich in endemic and endangered species. One of them is the Wallacea Region, which is a geographical line that extends from the Lombok Strait to the Makassar Strait (Van Welzen et al. 2011). The Wallacea region consists of thousands of islands that separate the Sunda Shelf in the west from the Sahul Shelf in the eastern part of the Indonesian archipelago. Based on its geographical history, the Wallacea region is an isolated region for a long time so the speciation process occurs and causes the presence of many native and endemic species in the Wallacea region. Wallacea is also known as Indonesia's biodiversity hotspot, both on land and at sea (Wainwright et al. 2020).

The Wallacea is one of the centers of wild Zingiberaceae diversity in Indonesia and most of them are native and endemic species. Currently, the existence of wild species Zingiberaceae in the Wallacea region is vulnerable due to the conversion of forest land functions and deforestation in the last few decades in the Wallacea region (Supriatna et al. 2020). Wild Zingiberaceae has a potential as a natural resource of the medicinal industry. The previous studies of ethnobotany and pharmacology revealed that most species of the Zingiberaceae family were used as ingredients in traditional medicine and contained various bioactive compounds that have the potential as a source of modern drugs (AlSalhi 2020). Zingiberaceae was reported to have various pharmacology and biology potential, including as an antioxidant (Sompinit et al. 2020; Kawai et al. 2021), antibacterial, anti-inflammatory, anticancer, antitumor, antiarthritis (Zhang et al. 2020), antihelmintic (Lima et al. 2021) antihypertensives (Sompinit et al. 2020) and etch. Several secondary metabolites isolated from Zingiberaceae members have potential as medicinal bioactive compound, for example phenolic compounds and 6-gingerol in *Zingiber officinale* Roscoe. (Mao et al. 2019) and phenolic compounds in *Alpinia zerumbet (*Pers.) B.L.Burtt & R.M.Sm. (da Cruz et al. 2020) was shown to have high antioxidant activity. According to Hartini and Sahromi (2016), *E. elatior* can be used as traditional medicine. The flavonoid and phenolic compounds contained in flower of

Etlingera elatior (Jack) R.M.Sm. showed positive antibacterial activity against *Staphylococcus aureus* (Abdelwahab et al. 2010).

Etlingera rubroloba is one of the wild species of Zingiberaceae that is native and endemic to the Wallacea region, which has not been studied for its biological and pharmacological activities. *Etlingera rubroloba* is known locally as Mandupi (Muna), Kasimpo (Makassar), and Pane (Bugis). *Etlingera rubroloba* is traditionally used by the people of Southeast Sulawesi as a joint pain reliever and to treat gout (Jabbar 2021). Based on scientific data, this species is naturally distributed on Sulawesi Island, Mount Rinjani National Park region, Lombok Island (Ardiyani et al. 2012), and Dompu District, Sumbawa Island (Data Collection from the Bali Botanical Gardens Plant Conservation Center - BRIN). Jabbar et al. (2017) revealed that the stem methanolic extract of *E. rubroloba* contained various types of phytochemical compounds, some of which played a role in the high antioxidant activity. Apart from that, there are no other studies that reveal the phytochemical content or other biological activity studies of *E. rubroloba*. Therefore, based on the background, the current study aimed to investigate the antibacterial activity, and phytochemical profile of the n-hexane extracts from the leaves and rhizomes of *E. rubroloba*.

MATERIALS AND METHODS

Study region and plant material

The plants used in this study were mature leaves and rhizomes of the *E. rubroloba* which had been collected from Pancasila Village, Dompu District, Sumbawa Island, West Nusa Tenggara Province, Indonesia in 1995 (Figure 1). The plant has been planted in the Zingiberaceae plot at the Center for Plant Conservation of the Botanical Gardens "Eka Karya" Bali -BRIN since 1995. The sample materials were collected on 10th September 2021 as a herbarium specimen and for sample material. Validation of the species name has been identified by Zingiberaceae taxonomist Axel D.Poulsen, M.Sc. PhD. from the Royal Botanic Gardens, Edinburgh and a plant taxonomist from the Bali "Eka Karya" Botanical Gardens - BRIN, I Made Sumerta, S.P. and I Nyoman Sudiatna. The herbarium specimens are stored in the Herbarium Tabanan Hortus Botanicus Baliense (THBB) with collector number HBK 718.

Etlingera rubroloba is a wild and endemic species in the Walacceae region and is not naturally distributed on the island of Bali. This is in accordance with the purpose of this paper, which is to explore the potential of wild and endemic plants from Indonesia's biodiversity. Although *E. rubroloba used* in this study has been planted in the Eka Karya Bali Botanical Gardens since 1995, it can be assumed genetically stable and no different with plants from their original habitat because they are planted through vegetative propagation (rhizomes) and isolated in a plot region. Barra (2009) explains that variations in secondary metabolite content in a plant species are influenced by three factors, namely (i) intraspecies genetic variation (ii) differences in plant organs and in the stages of plant organs development, (iii) differences in environmental factor. The effect of genetic variation within a species is in line with research conducted by Asensio et al. (2020) and Wangiyana et al. (2022). Asensio et al. (2020) revealed that genetic and cytogenetic diversity (eg polyploidization) significantly affected the variations of phytochemical content in Bearberry. While Wangiyana et al. (2022) reported that Molecular (genetic) variations in *Gyrinops versteegi*i (Gilg.) Domke (Gyrinops tea) correlated with variations in phytochemical content and morphological variations between accessions.

Figure 1. *Etlingera rubroloba* A.D. Poulsen

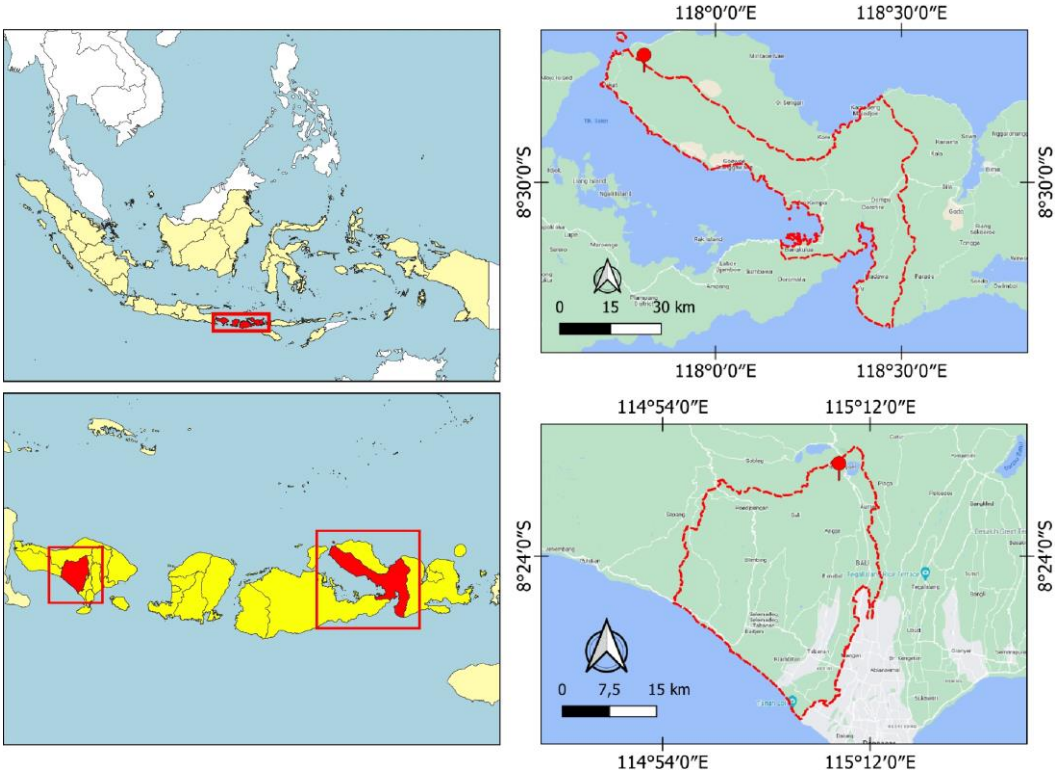

Figure 2. *Etlingera rubroloba* A.D. Poulsen was collected from Pancasila Village, Dompu District, Sumbawa Island, West Nusa Tenggara Province, Indonesia in 1995 (Upper-right picture) and then was planted at "Eka Karya" Bali Botanical Garden on same year (Lower-right picture)

Sample preparation and extraction of *Etlingera rubroloba*

The leaves and rhizomes of *E. rubroloba* were cleaned and dried using the shade-drying method, in which the materials were dried and avoided from direct sunlight (Yuan et al. 2015). completely dry ingredients mashed with a blender until it became a powder. Furthermore, the powdered leaves and rhizomes of *E. rubroloba* were extracted using the maceration extraction method followed by the method of Baehaki et al. (2021) with a slight modification in incubation time and the ratio of the amount of compound and solvent. Fifty grams of powdered plant material of *E. rubroloba* were prepared in 3 replicates each, then each extract was put into an Erlenmeyer flask and soaked in 500 mL n-hexane solvent, then placed at room temperature for 7 days. Hexane is a polar solvent that can bind polar compounds including volatile compounds and terpenes which show strong antibacterial activity (Karadağ et al. 2021). The results of maceration with n-hexane solvent were filtered using Whatman filter paper, then evaporated by a Vacuum rotary evaporator IKA rotary evaporator RV 3 V at 45ºC. The extracts obtained were stored in a refrigerator at 4°C before being used for further analysis. The percentage of extract results is calculated using the following formula:

$$\%\ Yeild = \frac{W1}{W0}\ x\ 100\%$$

W1 is the weight of the extract obtained after evaporation. W2 is the dry weight of the leaf powder (Anokwuru et al. 2011).

Antibacterial n-hexane extract of the *Etlingera rubroloba*

The antibacterial test was conducted using the Agar well diffusion assay based on the Siva et al. (2020) with minor modifications. The pure bacterial cultures used in this study were: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC12228, *Bacillus cereus* ATCC11778 were stored in 30% glycerol at -20°C. A total of 100mL bacterial isolate was cultured in 5 mL of Oxoid Nutrient Broth (NB) media and incubated for 24-48 hours at 37^0 C. Bacterial growth in NB medium was indicated by the presence of turbidity in the test tube. The bacterial cultures contained in the NB media were centrifuged at a rotor rotation speed of 5000 rpm for 5 minutes, then the supernatant (at the top of the test tube) was discarded and the bacterial cell mass was deposited at the bottom. The obtained cell mass was washed 2 times using sterile saline solution (sterile 0.85% NaCl). In the final stage, the bacterial cell mass was dissolved in 3 mL of sterile saline. A total of 100mL of bacterial culture in 5 mL of sterile saline was spread over the surface of the nutrient agar (NA)fμμ media (Oxoid Nutrient Agar Medium) using a glass spreader. In the center of the media, a Whatman paper disc with a diameter of 6 mm is placed. Then, 20mL of extracts with various concentrations (between 0.125 - 5 mg/mL) in dimethyl sulfoxide (DMSO)) were dripped onto the paper disc, then incubated for 24-48 hours at 37°C. After 24 and 48 hours, the diameter of the inhibition zone formed was measured. The measured value of the antibacterial activity is the value of Minimal Inhibitor

Concentration (MIC). MIC shows the lowest concentration value of plant extracts that can still inhibit the growth of bacterial cells (Kowalska-Krochmal and Dudek-Wicher 2021) Each data obtained was repeated 3 times.

Total Phenolic Content (TPC) assay of n-hexane extract of *Etlingera rubroloba*

The total phenolic content (TPC) was tested using the Folin-Ciocalteu Reagent (FCR) method as explained by Singleton et al. (1999) and Ghafoor et al. (2020) with some modifications in determining the concentration of chemical compounds used. For the measurement of total phenol content, four milligram of the extract was dissolved in 4 mL methanol and to obtained a sample concentration of 1000 µg/mL. The test was carried out with the volume samples of 250 µg/mL and 500 µg/mL, with 2 replications each. A total of 500 mL n-hexane extract of the leaves of *E. rubroloba* (1 mg/mL) was mixed with 250 mL FCR and 3.25 mL distilled water. After 8 minutes, 750 µL Na2CO3 20% was added and mixed homogeneously. The mixture was then allowed to stand for 2 hours at room temperature. TPC value of *E. rubroloba* extracts were measured at 765 nm by using a Shimadzu UV-1800 UV-Vis Spectrophotometer. Gallic acid with concentrations of 25-200 mg/mL were used as a standard. Measurements were repeated 2 times so that the phenol content obtained was obtained as *Gallic Acid Equivalent* (mg GAE/g sample).

Total Phenol (GAE mg/g extract) = C x FF x V/m

Where:
C : TFC levels from the curve equation
FF : dilution factor (1 mL/µL volume of measured sample)
V : total volume of stock solution
M : sample weight in stock solution

Total Flavonoid Content assay (TFC) of n-hexane extract of *Etlingera rubroloba*

The Total Flavonoid Content (TFC) in n-hexane extracts of *E. rubroloba* was measured using the colorimetric method of aluminium chloride AlCl3 as reported by Chang et al. (2002) and Baba and Mali (2015) with some modifications to the concentration of the chemical used, such as standard compounds. Four milligrams of Quercetin and samples were dissolved in 4 mL methanol (final concentration 1000 µg/mL). Standard curve measurements: a series of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL standard solutions were prepared by pipetting standard solutions of quercetin into in a test tube. While for measuring the TPC value of the sample, the extract solution was taken as much as 250 mL and 500 mL from the sample stock concentration of 1000 g/mL, then treated the same as the standard. In each of the above test tubes, 2 mL of distilled water and 150 mL of 5% $NaNO_2$ were added, then after 5 minutes, 150mL of 10% $AlCl_3$ was added. After 6 minutes, the solution was added with 2 mL of NaOH (1M), then distilled water was added again until the volume became 5 mL. Finally, the solution was homogenized by vortex and the absorbance of each solution was measured at a wavelength of 510 nm using a UV-Vis spectrophotometer. Based on the absorbance value obtained, a standard solution regression curve was made using Microsoft Excel. The absorption value is determined as the Y coordinate and the concentration of the standard solution is determined as the X coordinate. TFC obtained was defined as a quercetin equivalent (mg QE/g extract and calculated by the following formula:

The flavonoid content (mg QE/g extract) = C x FF x V/m

Where:
C : TFC from the curve equation
FF : dilution factor (1 mL/µL volume measured sample)
V : total volume of stock dilution (1 mL)
M : sample weight in stock dilution (1 mg)

Phytochemical profile analysis of n-hexane extracts of *Etlingera rubroloba* by GC-MS analysis

The phytochemical profile of the n-hexane extract of *E. rubroloba* leaves was carried out by the GC-MS under the conditions described by Andila et al. (2018). The GC-MS was carried out using a GC-MS apparatus model Agilent 7890B (GC) and 5977A (MSD). Column type: Agilent Type 19091S-433:93.92873 DB-5MS UI 5% Phenyl Methyl Silox with a capillary column (30 m x 250µm x 0.25 m). Dimensions 0-325°C. Processing temperature: Initial 40°C, Hold Time 1 min, Post Run 300°C. Injection volume 1mL. Mass spectra fragmentation patterns were used as a reference to identify the chemical contents contained in the extract by comparing the index of retention time, chromatogram peaks and spectral data contained in the WILEY7.LIB computer library. Identified compounds with Similarity Index (SI) -greater than 90 were included as determined compounds from GCMS analysis.

Data analysis

Antibacterial test data were analyzed by One-way Analysis of Variance (ANOVA) with a confidence level of 5%. The significant results were then continued with Duncan's test with a 5% confidence level ($P<0.05$). Meanwhile, TPC and TFC data were analyzed by T Test Independent with a significantly different value at the 5% level ($P<0.05$). Data analyzed using software IBM SPSS Statistics 26.

RESULTS AND DISCUSSION

Yield extract and antibacterial assay of n-hexane extract of the *Etlingera rubroloba*

The hexane extract of *E. rubroloba* showed that the yield of leaf extract was higher than that of rhizome with yield values of 3.04% (leaves) and 2.09% (rhizomes), respectively. The extracts obtained were used for antibacterial test and phytochemical screening. The antibacterial activity assay of *E. rubroloba* leaves and rhizomes extract was carried out using the disc diffusion procedure against four human pathogens bacteria, namely *S. aureus, S. epidermidis, B. cereus*, (Gram-positive

strains) and *E. coli* (Gram-negative strain). The results of the antibacterial test were shown in Table 1.

The test results revealed that the leaves and rhizomes extract of *E. rubroloba* were effective in inhibiting the growth of *B. cereus* (with MIC value 0.5 mg/mL in Leaves and 1 mg/mL in rhizomes) (Figure 3), but were not effective to against *S. aureus, S. epidermidis* and *E. coli*. The MIC results of leaves and rhizomes extracts suggested that *E. rubroloba* extracts could be used as a candidate plant for producing antibacterial agents instead of an antibiotic. Antibacterial activity of the genus *Etlingera* showed varying results and has been reported for several species of *Etlingera*. *Etlingera elatior* (Jack) R.M.Sm. flower extract exhibited a strong antibacterial effect against *Propionibacterium acnes* and *S. epidermidis* (Syafriana et al. 2021), *B. subtilis, S. aureus, E. coli, Listeria monocytogenes, Pseudomonas aeruginosa, and Salmonella typhimurium* (Ghasemzadeh et al. 2015). The rhizomes, stems, and leaves extract of *Etlingera fimbriobracteata* (K.Schum.) R.M.Sm. had positive antibacterial activity against *B. subtilis* and *B. spizini*, but not for *E. coli, P. aeruginosa*, and *S. aureus* (Shahid-Ud-Daula et al. 2019). Meanwhile, leaves extract of several types of Eltingera, including *E. elatior*, *E. fulgens* (Ridl.) C.K.Lim, *E. littoralis* (J.König) Giseke, *E. maingayi* (Baker) R.M.Sm., *Etlingera rubrostriata* (Holttum) C.K.Lim were reported to have an antibacterial effect on the growth of *S. aureus*, *Micrococcus luteus*, and *B. cereus* (Chan et al. 2007).

Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC) of *Etlingera rubroloba* hexane extract

The TPC value in the leaves and rhizomes extracts of *E. rubroloba* were tested by the Folin-Ciocalteu method and using a standard solution of gallic acid. The calibration curve was constructed from the absorbance values of gallic acid with different concentrations. TPC of the *E. rubroloba* extracts was measured by using regression equation of standard solution (namely y = 0.1531x - 0.0536, with the correlation coefficient $R^2 = 0.9862$, shown in Figure 4.) and was defined as mg Gallic Acid Equivalent per gram dry of a sample (mg GAE/g sample).

TPC of the hexane extracts of *E. rubroloba* leaves and rhizomes from higher to lower, respectively 15.68±0.80 mg GAE/g sample (leaves) and 11.89±0.76 (rhizomes) mg GAE/g sample (Table 1). There are not many comparative data that have reported on the total phenolic content in the hexane extract of the *Etlingera* genus. Previous studies have proven that the type of solvent used greatly influences the phenolic and flavonoid content in a plant species. Manurung et al. (2019) reported the TPC content in the hexane extract of the leaves of *Etlingera balikpapanensis* A.D. Poulsen was 29.0 ± 0.009 mg GAE/g sample. This study also reported that the TPC value in the hexane extract of *E. balikpapanensis* leaves was lower than the TPC value in the ethanol and ethyl acetate extracts, namely 57.6 ± 0.004 mg GAE/g sample and 109.8 ± 0.011 mg GAE/g sample.

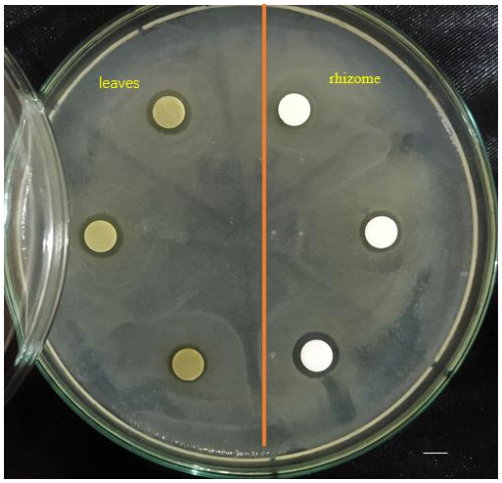

Figure 3. the antibacterial activity test of *Etlingera rubroloba* leaves (left) and rhizome (right) extracts against *Bacillus cereus* ATCC11778 (Bar: 6 mm)

Table 1. The value of the zone of inhibition (cm) at the minimum inhibitory concentration (MIC) in the antibacterial activity test of *Etlingera rubroloba* leaf extract

Organ	Extracts conc. (mg/mL)	Inhibition zone (cm) at the minimum inhibitory concentration (MIC) (mean±SD)			
		Escherichia coli **ATCC 25922**	***Staphylococcus aureus*** **ATCC 25923**	***Staphylococcus epidermidis*** **ATCC12228**	***Bacillus cereus*** **ATCC11778**
Leaves	5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.97±0.12^{c}
	3	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.93±0.06^{c}
	1	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.83±0.06^{bc}
	0.5*	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.87±0.06^{b}*
	0.25	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00^{a}
Rhizomes	5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.97±0.06^{c}
	3	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.93±0.06^{bc}
	1*	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.90±0.10^{bc}*
	0.5	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00^{a}
	0.25	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00^{a}
Control	DMSO	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Note: * MIC value, $p < 0.05$ significant, Alphabet variation means significantly different

While, Chan et al. (2007) and Chan et al. (2013) reported on the content of TPC in leaf methanol extracts of five *Etlingera* genera, namely (from the largest to the smallest) *E. elatior* (35.50 ± 304 mg GAE/g sample), *E. fulgens* (34.80 ± 390 mg GAE/g sample), *E. maingayi* (28.10 ± 242 mg GAE/g sample), *E. littoralis* (25.40 ± 91 mg GAE/g sample) and *E. rubrostriata*. (11.10 ± 93 mg GAE/g sample). Meanwhile, the TPC content in the methanol extract of the leaves of *E. fimbriobracteata* was 7.88 ± 0.25 mg GAE/g sample (Shahid-Ud-Daula et al. 2019) and in *E. coccinea* (Blume) S. Sakai & Nagam 13.49 ± 0.258 mg GAE/g sample (Shahid-Ud-Daula et al. 2015). Sahloul et al. (2014) and Chibane et al. (2019) reported that many phenolic compounds exhibit antibacterial and antioxidant activities significantly.

The results of this study also showed that the TPC content in *E. rubroloba* was higher in the leaf than in the rhizome. These results are in line with the research results of Shahid-Ud-Daula et al. (2019) which reported that the TPC content of the methanolic extract of *E. fimbriobracteata* was higher than that of the rhizome extract (6.27 ± 0.05 mg GAE/g sample). Previously, Shahid-Ud-Daula et al. (2015) also reported that TPC in *E. coccinea* leaf extract was higher than in rhizome methanol extract (2.64 ± 0.242 mg GAE/g sample). TPC in the methanol extract of rhizome *E. vetulina* (Ridl.) R.M.Sm. and *E. belalongensis* also reported by Sabli et al. (2012), namely 25.03 ± 0.46 mg GAE/g sample) and 17.07 ± 0.32 mg GAE/g sample, respectively.

TFC values in the leaves and rhizomes of the hexane extract of *E. rubroloba* were determined by the regression equation of the quercetin (y = 0,0105x + 0,1009, with the correlation coefficient R^2 = 0,9899, shown in Figure 5) and was defined as mg of quercetin equivalent per gram of dry sample (mg QE/g sample).

The results of the TFC (Total Flavonoid Content) hexane extract of *E. rubroloba* extract from different organs respectively, namely: 33.99±2.24 mg GE/g sample (leave) and 32.46±0.67 mg GE/g sample (rhizome), respectively. Observations also proved that the TFC of the *E. rubroloba* extract was lower than that of the hexane extract of the leaves of *E. balikpapanensis* 268.4 ± 0.05 mg QE/g DW (Manurung et al. 2019). Manurung et al. (2019) also reported that the TFC of *E. balikpapanensis* leaves extract with different solvents showed that the TFC of hexane extract was higher than the TFC of ethanol (200.4 ± 0.02 mg QE/g DW) and Ethyl acetate extracts (141.8 ± 0.03 mg) QE/g DW).

There are not many comparative scientific data reported on TFC from hexane extracts of the genus *Etlingera*. However, several previous studies have reported the total phenolic content of *Etlingera* with different solvents and various organs. TFC of methanolic extract of E. coccinea leaves, stems, and rhizomes respectively were 5.33 ± 0.102 mg QE/g DW, 1.87 ± 0.003 mg QE/g DW and 0.84 ± 0.077 mg QE/g DW (Shahid-Ud-Daula et al.2015). Meanwhile TFC of methanolic extracts of *E. fimbriobracteata* leaves, stems and rhizomes were 5.44 ± 0.10 mg QE/g DW, 2.53 ± 0.09 mg QE/g DW, 3.16 ± 0.28 mg QE/g DW (Shahid-Ud-Daula et al. 2019). Sabli et al. (2012) also reported the TFC of methanol extract of *E. belalongensis* with Cathexin standard, namely: 3.77 ± 0.15 mg Cathexin Equivalent (CE)/g (rhizome), 3.57 ± 0.15 mg CE/g (stem), and E. vetulina 7.63 ± 0.06 mg CE/g sample (rhizome), and 2.80 ± 0.2 mg CE/g sample (stem) (Sabli et al. 2012).

The results showed that the TFC in *E. rubroloba* was higher in the leaf extract than in the rhizome. The results of this observation are in line with the report of Shahid-Ud-Daula et al. (2015) and Shahid-Ud-Daula et al. (2019) which revealed that the TPC of *E. coccinea* and *E. fimbriobracteata* was higher in leaf extracts than in rhizome. Flavonoids are well-known potential as an antibacterial agent and have long attracted the interest of scientists as a substitute for antibiotics. The hydroxyl structure of the aromatic ring of flavonoids is thought to play an important role in the effectiveness of the antibacterial activity of flavonoids. The mechanism of action of flavonoids as antibacterial as inhibition of bacterial cellular activity includes inhibition of plasma membrane function, nucleic acid synthesis, metabolism of energy, inhibition of adhesion and formation of biofilm and attenuation processes of pathogen agent (Xie et al. 2015).

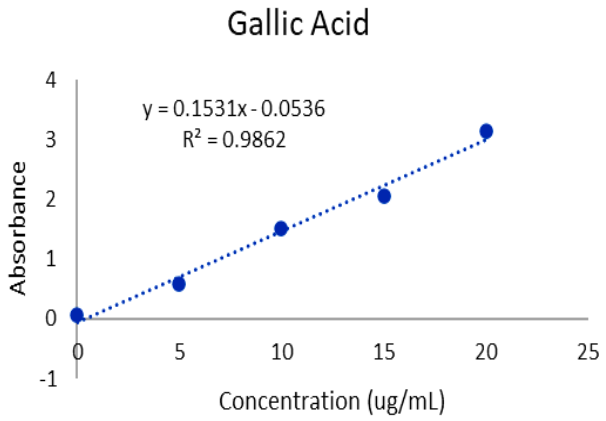

Figure 4. Standard calibration curve of gallic acid

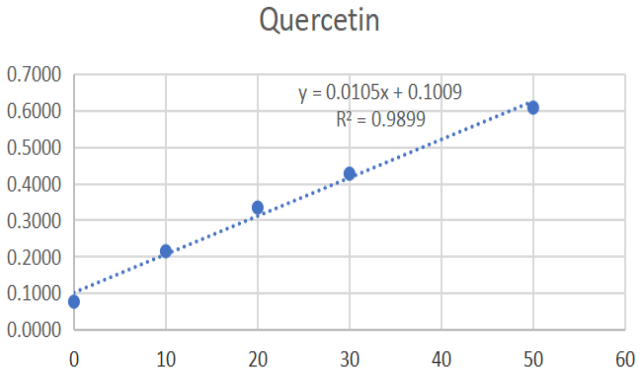

Figure 5. Standard calibration curve of quercetin

Overall, it can be assumed that TPC and TFC of the Zingiberaceae varied greatly in each part of the plant organ and could also be influenced by various factors such as the type of solvent used, species diversity, organ development stage and environmental factors such as altitude affect and microclimate conditions of growth (Ezez and Tefera 2021). In *Zingiber officinale*, the highest TPC content was found in leaves, followed by rhizomes and the lowest in stems (Ghasemzadeh et al. 2010). the same case was reported by Chumroenphat et al. (2021). They reported that TPC values in *Cornukaempferia aurantiflora, C. longipetiolata* and *C. larsenii* (Zingiberaceae) were higher in leaves than in other organs, while higher TFC values were found in rhizomes. The lowest TPC and TFC values are in the stem. Chan et al. (2011) also reported that in *Alpinia galanga, Curcuma longa,* and *E. elatior*, the highest TFC was found in the rhizome and the lowest in the stem. Chan et al. (2007) stated that the TPC content in *Etlingera* is also influenced by altitude. At higher altitudes, the TPC content is also higher than TPC from lower altitudes. It has been reported that the TPC of *E. elatior* at an altitude of 400 m asl was 35.50 ± 304 mg GAE/g sample, while at an altitude of 100 m asl it was lower: 23.90 ± 329 mg GAE/g sample. Likewise for other Elingera species, namely *E. rubrostriata* (TPC value at 300 m asl: 34.80 ± 390 mg GAE/g sample and at 100 m asl: 2430 ± 316 mg GAE/g sample), *E. littoralis* (TPC value in 800 m asl: 28.10 ± 243 mg GAE/g sample and at 100 m asl: 23.40 ± 386 mg GAE/g sample) and *E. fulgens* (TPC values at 400 m asl: 22.70 ± 31 mg GAE/g sample and at 100 m asl: 12.80 ± 143 mg GAE/g sample. While Ghasemzadeh et al. (2015) revelaed that the TPC content of *E. elatior* collected from three different locations in Malaysia differed significantly based on factors of geographical origin and the type of solvent used.

Phytochemical profile analysis of n-hexane extracts of *Etlingera rubroloba* by GC-MS

According to GC-MS results, the hexane extracts isolated from rhizomes and leaves of *E. rubroloba* in this research contained a total of 24 and 65 phytochemical compounds respectively (Tabel 3 and 4). The dominant compounds in leaves and rhizomes were shown in Figure 6 and Figure 7 and the GC-MS chromatograms were shown in Figure 8 and 9. Several compounds contained in the leaves and rhizomes extract of *E. rubroloba* are bioactive compounds that have biological and pharmacological activities. Some of these compounds are known to have potential as antibacterial agents. Antibacterial compounds in leaves extract were γ-Muurolene (Perigo et al. 2016), 1-Tricosene (Susilo et al. 2022), 2,4-Di-tert-butylphenol, 4-Di-tert-butylphenol (Aissaoui et al. 2018) 9-Eicosene, (Lulamba et al. 21021), α-Pinene (Rivas da Silva et al. 2012; Dhar et al. 2014). Cyclohexene, 4-methylene-1-(1 methylethyl)- or β-Terpinene (Guimarães et al. 2019), Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester (Shannon and Abu-Ghannam 2016), D-Limonene (Costa et al. 2019; Han et al. 2020), Ethylbenzene (Bellahcen et al. 2019), Heptadecane, 8-methyl- (Ashraf et al. 2018), Heptadecanoic acid, methyl ester (Mohadjerani et al. 2016), Hexanedioic acid, bis(2-ethylhexyl) ester (alkane); Hexatriacontane (alkane) (Afzal et al. 2014), Isopropyl myristate (Cardoso et al. 2006), Methyl stearate (fatty acid methyl ester) (Agoramoorthy et al. 2007), Nonadecane (Wijayanti and Dewi 2022), Oxalic acid, allyl hexadecyl ester (Dicarboxylic acid)(Zayed and Samling 2016), o-Xylene (Nimbeshaho et al. 2020), Pentadecanoic acid, 14-methyl-, methyl ester (Fatty-acid) (Yoon et al. 2018), p-Xylene (Nimbeshaho et al. 2020), Tetratetracontane (Agarwal et al. 2017, Jamil et al. 2021), and Tritetracontane (Martins et al. 2015). While antibacterial compounds in rhizome were 1HCyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS (3a.alpha., 3b.beta., 4.beta.,7.alpha., 7aS) or β-Cubebene (Constant et al. 2019); 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha., 4a.alpha.,7.beta.,7a.beta.,7b.alpha.) or spatulenol) (Cazella et al. 2019); 1-Hexadecanol (fatty acid) (Togashi et al. 2007), 5-Octadecene, (E)- (Naragani et al. 2016), 9-Hexadecenoic acid, methyl ester, (Z)- (Rahman et al. 2014), α -Pinene (Rivas da Silva et al. 2012; Dhar et al. 2014), Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- or sabibine (Utegenova et al. 2018), Camphene (de Freitas et al. 2020, Copaene (Martins et al. 2015), Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis- or β-Terpinene (Guimarães et al. 2019), Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester (Shannon and Abu-Ghannam 2016), Decane (Kawuri and Darmayasa 2019), D-Limonene (Costa et al. 2019; Han et al. 2020), E-15-Heptadecenal (Supardy et al. 2012), Ethylbenzene (Bellahcen et al. 2019), Eucalyptol (Ghomi and Ahd 2010), γ -Terpinene (Guimarães et al. 2019), Heptadecane, 8-methyl- (Ashraf et al. 2018), Heptadecanoic acid, methyl ester (Köse et al. 2016), Hexadecanoic acid, ethyl ester (Ghareeb et al. 2022), Hexadecanoic acid, methyl ester (Ghareeb et al. 2022), Isopropyl myristate (Cardoso et al. 2006), Nonane (Kawuri and Darmayasa 2019), o-Xylene (Zayed and Samling 2016), Terpinen-4-ol (Cordeiro et al. 2020, Tetradecane (Naragani et al. 2016), Tridecanoic acid, 12-methyl-, methyl ester (Elaiyaraja and Chandramohan 2018), 9,12-Octadecadienoic acid, methyl ester, (E,E)-; 9-Octadecenoic acid (Z)-, methyl ester (Rossellia et al. 2007), Methyl stearate (Fajrih et al. 2022), Hexanedioic acid, bis(2-ethylhexyl) ester; 1-Hexacosene; Tetratetracontane (Kawuri and Darmayasa 2019) and Tritetracontane (Martins et al. 2015).

Table 2. The value of Total Phenol (TFC) (mg /g extract) and Total Flavonoid (mg QE/g extract) of hexane extract of *Etlingera rubroloba* leave

Organ	Total phenol (TFC) (mg GAE/g extract) (mean±SD)	Total flavonoid (mg QE/g extract) (mean±SD)
Leaves	15.68±0.80[a]	33.99±2.24[a]
Rhizomes	11.89±0.76[a]	32.46±0.67[a]

Note: $p > 0.05$ not significant, Alphabet means not significantly different.

Table 3. The result of GCMS analysis of hexane extract of *Etlingera rubroloba* leaves

No.	RT	Chemical compound	% Region	Similarity index
1	5,2089	Ethylbenzene	2.03	95
2	5,3475	o-Xylene	2.22	97
3	5,7256	p-Xylene	0.48	90
4	7,0489	Cyclohexene, 4-methylene-1-(1-methylethyl)-	0.87	94
5	7,1498	α-Pinene	0.93	91
6	7,9816	D-Limonene	0.59	96
7	11,9515	ortho tert-Butyl cyclohexyl acetate	0.44	90
8	13,7916	4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate	0.63	90
9	14,5982	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-methylenetricyclo[4.4.0.02,7]decane-rel-	0.50	99
10	14,7746	2,4-Di-tert-butylphenol	2.41	94
11	15,0141	γ -Muurolene	1.95	94
12	15,8837	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	1.52	87
13	17,8120	9-Eicosene, (E)-	0.50	93
14	18,0640	Heptadecane, 8-methyl-	1.52	91
15	18,1270	Isopropyl myristate	2.28	91
16	18,7068	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	5.84	99
17	19,4251	Pentadecanoic acid, 14-methyl-, methyl ester	47.58	98
18	20,1057	1-Tricosene	1.87	94
19	20,1687	Nonadecane	1.61	91
20	20,4082	Heptadecanoic acid, methyl ester	0.84	97
21	21,0635	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	4.88	99
22	21,1266	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	7.23	99
23	21,3534	Methyl stearate	3.13	97
24	23,6346	Hexanedioic acid, bis(2-ethylhexyl) ester	1.99	91
25	23,7228	Tritetracontane	2.23	90
26	24,5294	Hexatriacontane	1.14	91
27	25,3107	Tetratetracontane	2.20	91
28	26,2812	Oxalic acid, allyl hexadecyl ester	0.58	91
			100.00	

Note: RT: Retention Time, C. Relative: Concentration Relative

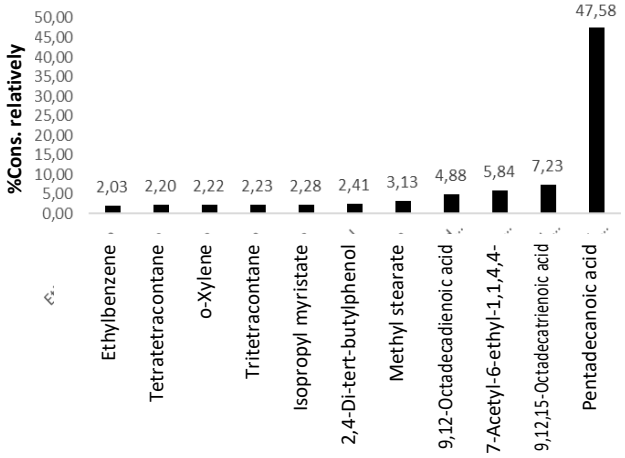

Figure 6. The dominant compounds identified from the hexane extract of *Etlingera rubroloba* leaves

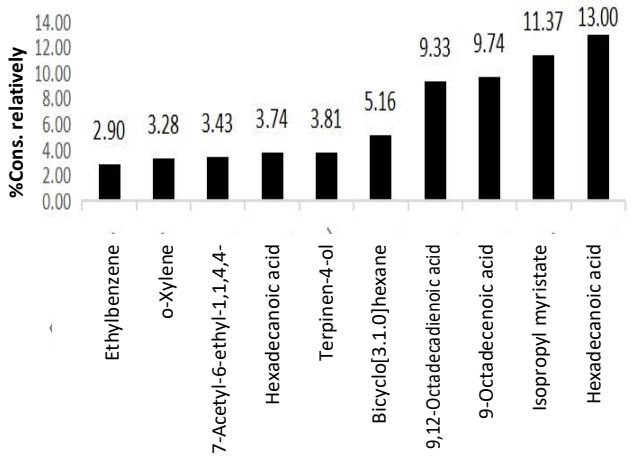

Figure 7. The dominant compounds identified from the hexane extract of *Etlingera rubroloba* rhizomes

Table 4. The result of GCMS analysis of hexane extract of *Etlingera rubroloba* rhizome

No.	RT	Chemical compound	% Region	Similarity index
1	4,2637	Octane	0.56	90
2	5,2089	Ethylbenzene	2.90	95
3	5,3475	o-Xylene	3.28	97
4	5,8012	Nonane	0.66	94
5	6,3936	α -Pinene	1.07	96
6	6,6834	Camphene	0.23	90
7	7,0489	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	5.16	95
8	7,4018	Benzene, 1,2,3-trimethyl-	0.25	95
9	7,4396	Decane	0.91	93
10	7,7295	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	0.32	94
11	7,9816	D-Limonene	2.01	98
12	8,0446	Eucalyptol	0.75	92
13	8,4353	γ -Terpinene	0.60	93
14	8,8764	Benzene, 4-ethyl-1,2-dimethyl-	0.31	93
15	9,0528	Undecane	0.30	90
16	9,1537	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	0.58	96
17	9,3805	Benzene, 1,2,3,5-tetramethyl-	0.19	95
18	9,4309	Benzene, 1,2,3,4-tetramethyl-	0.50	94
19	10,4014	Terpinen-4-ol	3.81	95
20	10,8046	2-Sec-Butylcyclohexanone	0.33	90
21	11,9011	4,7-Methano-1H-indenol, hexahydro-	0.20	95
22	13,1992	Copaene	0.22	97
23	13,3379	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)	0.19	98
24	13,3883	Tetradecane	0.26	95
25	13,7790	4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate	1.28	93
26	14,5856	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	0.46	97
27	15,0015	3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	1.30	99
28	15,7955	E-15-Heptadecenal	0.82	90
29	15,8837	Hexadecane	1.04	96
30	16,4256	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)	0.16	92
31	16,5138	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	1.66	94
32	16,8037	1-Hexadecanol	0.52	91
33	17,2322	E-2-Tetradecen-1-ol	1.14	91
34	17,2952	Tridecanoic acid, 12-methyl-, methyl ester	0.69	95
35	18,0514	5-Octadecene, (E)-	1.29	90
36	18,1270	Heptadecane, 8-methyl-	0.83	93
37	18,3035	8-Ethyl-4,6,6,8-tetramethyl-3,4,6,7-tetrahydro-1H-cyclopenta(G)-2-benzopyran	0.23	95
38	18,3791	Isopropyl myristate	11.37	96
39	18,7068	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	3.43	99
40	19,2235	9-Hexadecenoic acid, methyl ester, (Z)-	1.28	91
41	19,4251	Hexadecanoic acid, methyl ester	13.00	99
42	20,0931	Hexadecanoic acid, ethyl ester	3.74	93
43	20,4081	Heptadecanoic acid, methyl ester	0.51	97
44	21,0635	9,12-Octadecadienoic acid, methyl ester, (E,E)-	9.33	99
45	21,1265	9-Octadecenoic acid (Z)-, methyl ester	9.74	98
46	21,3534	Methyl stearate	1.39	98
47	21,6685	Methyl 9-cis,11-trans-octadecadienoate	1.77	99
48	21,9709	Pentacos-1-ene	1.70	93
49	23,6345	Hexanedioic acid, bis(2-ethylhexyl) ester	2.58	90
50	23,6850	1-Hexacosene	0.84	91
51	24,5294	Tetratetracontane	0.92	91
52	25,3107	Tritetracontane	1.37	91
			100.00	

Note: RT: Retention Time, C. Relative: Concentration Relative

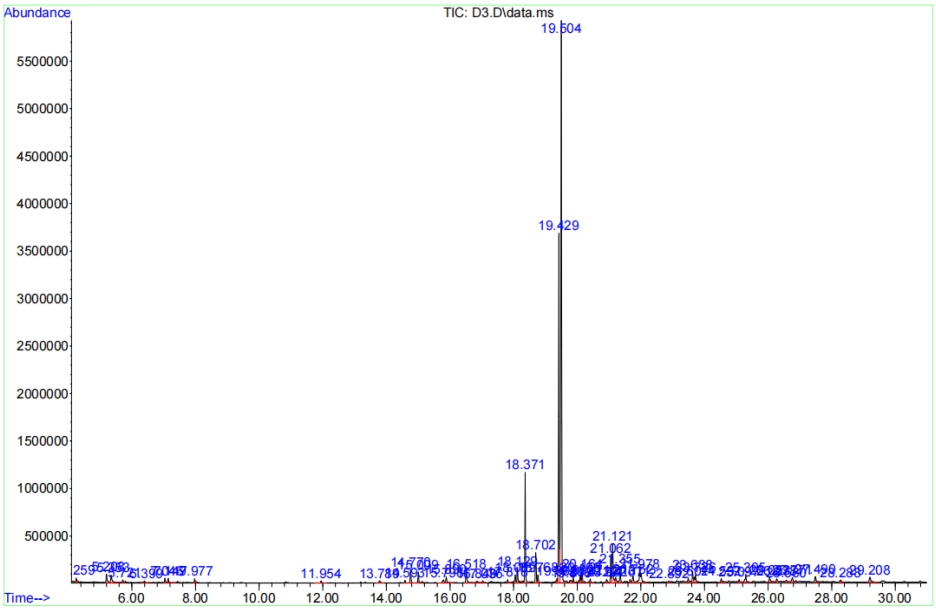

Figure 8. a GC-MS chromatogram of the hexane extract of *Etlingera rubroloba* leaves

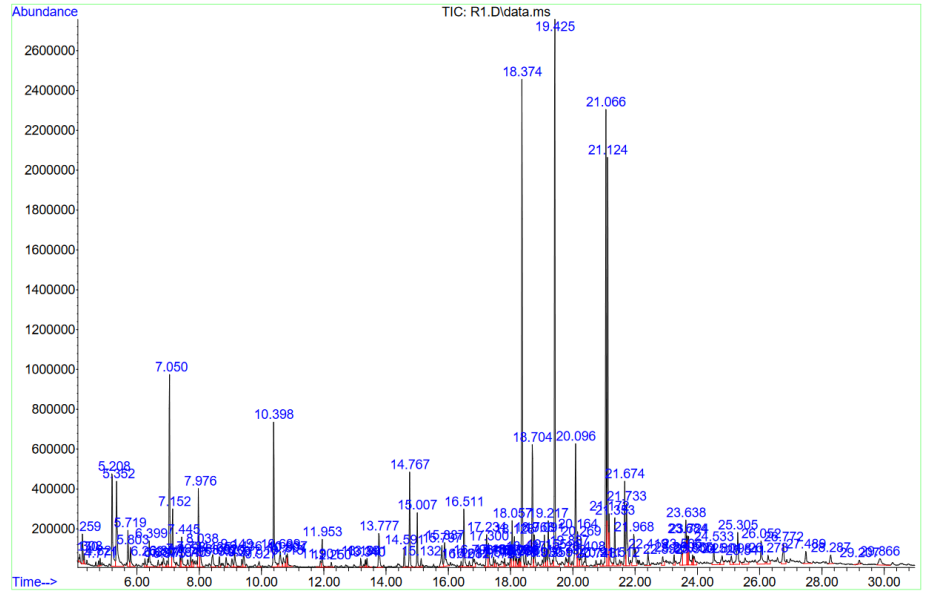

Figure 9. a GC-MS chromatogram of the hexane extract of *Etlingera rubroloba* rhizomes

In conclusion, based on the research, it can be concluded that the hexane extract from the leaves and rhizomes of *E. rubroloba* has a higher TPC and TFC content in the leaf extract than in the rhizome extract. The TPC values in *E. rubroloba* were 15.68 ± 0.80 mg GAE/g sample (leaf) and 11.89 ± 0.76 mg GAE/g sample (Rhizomes) respectively. Meanwhile, the TFC value respectively were 33.99±2.24 mg GE/g sample (leaf) and 32.46±0.67 mg GE/g sample (rhizome. Phenols and flavonoids play a role in antibacterial activity. The antibacterial test showed that hexane extracts had antibacterial activity against B. cereus but not for E. coli .25922, *S. aureus* and *S. epidermidis*. The MIC values in rhizome extract were higher than in leaf extract, namely 1 mg/mL (rhizome) and 0.5 mg /mL (leaves) respectively. The results of the GCMS test showed that the hexane extract of *E. rubroloba* contained a total of 28 and 52 phytochemical compounds identified in the leaves and rhizomes, respectively. Some of these chemical compounds are known to act as antibacterial agents. It can be concluded that the extract of *E. rubroloba* has the potential as a natural antibacterial agent.

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