ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2021.13.01.179

Research Article

Free radical scavenging activity of methanol extract and compounds isolated from stems of Etlingera rubroloba A.D Poulsen

ASRIULLAH JABBAR[1.3], SUBAGUS WAHYUONO[2*], I SAHIDIN[3], IKA PUSPITASARI[4]
[1]Doctoral Program of the Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281 Indonesia.
[3]Department of Pharmacy, Faculty of Pharmacy, Halu Oleo University, Kendari, 93232, Indonesia
[4]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281 Indonesia
*Corresponding Author
Email: subagusw_fa@ugm.ac.id
Received: 10.08.20, Revised: 13.09.20, Accepted: 22.10.20

ABSTRACT
Etlingera rubroloba (Blume) A.D Poulsen is an endemic plant found in Southeast Sulawesi, Indonesia. So far, there is no report on medicinal usages of this species, therefore expolative study of this species is undertaken. E. rubroloba was subjected to scavenging DPPH free radicals activity method, and then the anti radical active compounds were isolated and identified. Initially this species was extracted, partitioned to give fractions having simple tlc picture, and the compounds were isolated by radial chromatography. The compounds obtained were identified according their spectroscopic (UV, IR, MS, NMR) properties. Two compounds (1 and 2) were isolated and based on spectroscopic data and comparison with reported data, compound 1 was identified as synapyl alcohol diacetate and compound 2 was identified as stigmasterol. The presence of these two compounds were first reported in E. rubroloba. The IC_{50} (μg/mL) values of 1 and 2 were 182.799, 402.397, methanol extract 41.592 and 20.895 μg/mL was for vitamin C as positive control.

Keywords: Etlingera rubroloba, methanol extract, synapyl alcohol diacetate, stigmasterol, DPPH

INTRODUCTION

Etlingera is a plant genus of the Zingiberaceae family that has a large number of species and interesting potential. Etlingera population in the world around 150-200 species, in Java as many as 6 species and in Sulawesi Island grows around 48 species with 7 endemic species in Southeast Sulawesi [1]. The potential of this plant genus is very interesting because it has varied properties as traditional medicine.

Only a few species of Etlingera species are examined from both pharmacological and phytochemical aspects. Of the total Etlingera in Sulawesi, only 2 are reported from 48 species, namely E. elatior [2]–[8] and E. calophrys [9].

From a pharmacological aspect, the researchers reported that E. calophrys stems were antioxidants [9]. The etlingera elatior stem is active as an antioxidant [10]. The leaves, stems and rhizomes of Etlingera coccinea are active as antioxidants [11]. E. elatior active rhizome acts as antibacterial and antioxidant [2], [5]. E. elatior flowers are effective as antibacterial, fungal and antioxidant [4], [6], [7] and the leaves of E. elatior are active as anti-antioxidants, wound medicine. , eliminates body odor, antibacterial and tyrosinase inhibitors [3], [4], [8]. Whereas E. rubrostriata, E.littoralis, E.fulgens are also active as antioxidants and antibacterial [12].

In addition to the pharmacological aspects, the researchers also reported from the phytochemical aspects that E. calophrys stem contains the compound Yakuchinone A, p-Hydroxybenzoic Acid and Stigmasterol [9]. Williams and Harborne, 1977, reported that compounds which were isolated from E. Elatior leaves were quercetin-3-rhamnoside, quercetin-3-glucuronide, quercetin-3-glucoside and kaempferol-3-glucuronide. Whereas E. elatior leaves produce 3-O-caffeoylquinicacid compound, 5-O-caffeoylquinicacid (chlorogenicacid), and 5-O-caffeoylquinic acid methylester [3] . Apart from E.elatior, other researchers have also reported that E.brevilabrum leaves and rhizomes contain β-sitosterol and stigmasterol. The rhizome of E.sphaerochepala

var.grandiflora also produces β-sitosterol, stigmasterol, and paeonol [13]. In addition, it also contains volatile oils from E.brevilabrum, namely o-cymene (7.8%), α-thujene (28.6) and β-pinene (52.6%) [14]. Furthermore, E. elatior contained α-bisabolol (5.9%), L-calamenene (18.0%) and borneol (28.3%) [15].

One of interesting plants to study is E. rubroloba, which is an endemic plant found in Southeast Sulawesi [1]. Efficacy and activity of this plant, has never been reported before. So that this study will provide information about secondary metabolites from the results of isolation and test activity of methanol extracts and isolates of E. rubroloba as antioxidants using the DPPH method.

MATERIALS AND METHODS

Materials

Chromatographic techniques were performed using Kieselgel 60 F254 0.25 mm, silica gel 60 GF254, and silica 60 g (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) plates were derivatized using serium sulfate reagents (Merck, Darmstadt, Germany). DPPH was purchased from Merck (Darmstadt, Germany).

Equipment

The instruments used are UV spectrophotometer, Perkin Elmer Spectrum One fourier transform-IR spectrophotometer, and JEOL ECP 500 nuclear magnetic resonance (NMR) spectrometer (500 MHz for 1H and 125 MHz for ^{13}C).

Sample

E.rubrloba stems used in this study were taken from other sub-districts of South Konawe Regency, Southeast Sulawesi, April 2019. This plant was identified at LIPI Bogor Indonesia.

Extraction and Isolation

E. rubroloba (6 kg) stem powder, macerated with methanol (3 x 15 L) at room temperature, and produced a dark green extract (125 g). Percentage value of extract yield obtained by 3%. This extract was fractionated using liquid vacuum chromatography with silica gel (10 x 5 cm, 125 g), then eluted gradiently with N-Hexan-Ethyl acetate (9: 1 to 0:10), washed with pure methanol, and produced 6 main fractions (F1-F6) weighing each (1 g, 1.4g, 12.4g, 12.6g, 18.4g and 68.4g). The F3 fraction (12.4 g) was fractionated again using liquid vacuum chromatography with silica Gel (5 x 5 cm, 12.4 g), then eluted graded with n-hexane eluent: ethylacetate (5: 5, 8: 2, 9: 1, 9.5: 0.5) then washed with pure methanol and produced 6 sub fractions namely F31 (2.6 g), F32 (3.2 g), F33 (4 g), F34 (2.3 g), F35 (4 g) and F36 (0.9 g). Then the F34 sub-fraction was chromatographed using silica gel Radial Chromatography (RC) with N-hexane-ethyl acetate (7: 3) eluent and washed with pure methanol to produce 1 pure compound (0.058 g). Subsequently the F33 sub-fraction was purified using the recrystallization technique using methanol as a solvent. The results of this recrystallization resulted in pure compound 2 (0.305 g)

Free Radical Scavangging Test.

The antioxidant activity test of E. rubroloba stem isolates was carried out qualitatively and quantitatively using the DPPH method. For qualitative tests based on [16]. using a semi-quantitative approach where the diameter and intensity of yellowish white or pale yellow stains on the purple background plate formed can be known or estimated depending on the amount and concentration of the solution used. The method of doing this is by dipping (dipping technique) the TLC plate that has been dotted with the analyte (known concentration and quantity) into DPPH which has been dissolved in methanol where the stain formed can be determined its activity. The concentration of DPPH solution used was 0.2 mM while the number of analytes used was 25 μg, 12.5 μg, 6.25 μg and 3.125 μg.

Quantitative Test

Meanwhile, the quantitative test can be obtained by observing the color change / decolorization by monitoring the absorbance at the optimum wavelength of DPPH, namely 515 nm in methanol solvent [17] and 540 nm in DMSO solvent [18] . The quantitative test was carried out according to (Clarke et al., 2013). A total of 100 μL of sample solution (concentration of 400 μg / mL in DMSO) was added to the well and then added 100 μL of DPPH solution (concentration 0.2 mM in DMSO) was added. The sample mixture and DPPH were then incubated for 30 minutes at room temperature in the dark and their absorbance was measured at a wavelength of 540 nm. The absorbance obtained is then corrected for the blank absorbance which is the absorbance of 100 μL sample and 100 μL DMSO solvent. The controls in this test were 100 μL DPPH solution and 100 μL DMSO solvent. The test sample that showed% inhibitory activity above 50% was then determined the IC50 value using two series of dilutions at a concentration of 200-1.5625 μg / mL. Ascorbic acid or vitamin C were used as positive controls for this test.

RESULT AND DISCUSSION

Two chemical compounds (1-2) were successfully isolated and identified from the methanol extract of E. rubroloba stem. The structure of this compound is determined based on the physicochemical properties and spectra of IR and NMR spectroscopy. These values are also compared with those reported in previous studies. The spectroscopic data of the compound isolates from the E.rubroloba stem can be seen below.

Sinaphy alchohol diacetate (1);Slightly oily yellow;
^{1}**HNMR** (500MHz, CDCl3)δH(ppm): 6.62 (s, 2H, H-2/H-6), 6.58(d, 1H, 15.87, H-7), 6.22 (dt, 1H,15.87,6.71, H-8), 4.71 (dd, 2H, 6,10, H-9), 2.32 (s, 3H, H-11), 2.10 (s, 3H, H-13), 3.82 (s, 6H, H-14/H-15)
^{13}**CNMR** (125MHz, CDCl3) δC(ppm):
170.98 (C-10), 168.86 (C-12), 152.25 (C-3/C-5), 134,73 (C-1), 134.07 (C-7), 128.64 (C-4), 123.71 (C-8), 103,30 (C-2/C-6), 64,97 (C-9), 56,21 (C-14/C-15), 21.13 (C-11), 20.57 (C-13)
The NMR spectra data are identical to those given in the literature [19].

Stigmasterol (2);white crystals
^{1}**HNMR** (500MHz, CDCl3) δH(ppm): 5.33 (br d, 1H, H-6), 5.15 (dd, J = 15,12 Hz, H-22), 4.98 (dd, J = 15Hz, 1H, H-23), 3.50 (m, 1H, H-3), 2.28 (m, 1H, H-4a), 2.24 (m, 1H, H-4b), 2.03 (m, 1H, H-12), 1.99 (m, 1H, H-20), 1.97 (m, 1H, H-2a),1.92 (m, 2H, H-7, 1.83 (m, 1H, H-2b), 1.81 (m,1H, H-1º), 1.65 (m, 1H, H-25), 1.51 (m, 2H,H-15), 1.48 (m, 1H, H-8), 1.47 (m, 2H, H-11), 1.42 (m, 2H, H-28), 1.22 (m, 1H, H-16), 1.13(m, 1H, H-1b),1.05 (m, 1H, H-17), 1.00 (br s, 3H, H-21), 1.00 (br s, 3H, H-19), 1.00 (br s, 1H, H-26a), 0.98 (m, 1H, H-14), 0.90 (m, 1H, H-24), 0.90 (br d, 1H, H-9), 0.90 (br d, 1H, H-27a), 0.88 (br d, 1H, H-29a), 0.88 (br d, 1H, H-18a), 0.81 (br d, 2H, H-26b), 0.81 (br d, 1H,H-27b), 0.78 (br d, 1H, H-29b), 0.78 (br d, 1H, H-18b),
^{13}**CNMR** (125MHz, CDCl3) δC (ppm): 141.0 (C-5), 138.3 (C-22), 129.7 (C-23), 121.9 (C-6), 71.9 (C-3), 56.9 (C-14), 56.2 (C-17), 51.4 (C-24), 50.2 (C-9), 42.4 (C-4/C-13), 40.7 (C-20), 39.9 (C-12), 37.4 (C-1), 36.6 (C-10), 32.06 (C-7/C-8), 31.8 (C-2), 30.7 (C-25), 28.4 (C-16), 26.1 (C-28), 24.4 (C-15), 21.3 (C-19/C-21), 21.2 (C-11), 19.2 (C-26), 18,8 (C-27), 12.2 (C-29), 12.0 (C-18).The NMR spectra data are identical to those given in the literature [9], [13].

DPPH free radical scavenging activity testing

Testing the activity of the isolates from the etlingera rubroloba stem methanol extract, namely Stigmasterol and Sinafil alcohol diacetate compared to the positive control (ascorbic acid) shows the IC50 can be seen in table 1

DISCUSSION

Isolation and identification and purification of the stem methanol extract of E. rubroloba using silica gel chromatography techniques were carried out to obtain isolates of Sinaphyl alcohol acetate (1) and stigmasterol (2) compounds. To our knowledge, the presence of two compounds in E. rubroloba was reported for the first time in this study in an Etlingera species. For compound 2 is a steroid that is commonly found in plants and has been reported from the leaves of E. brevilabrum and rhizomes [14] and E. sphaerochepala var. Glandiflora rhizome [13]. The structure of this compound is shown in Fig. 1 and 2
The extract of E. rubroloba showed significant free radical scavenging activity compared to ascorbic acid as a positive control. The Sinaphyl alcohol acetate and stigmasterol compounds showed lower free radical scavenging activity than methanol extract. While the antioxidant activity of sinaphyl alcohol acetate is higher than stigmasterol. The activity of free radical scavenging Methanol extract is higher than isolate compounds (1 and 2), this is possible because there are other synergistic compounds that increase free radical scavenging activity. Therefore, both methanol extract and isolate compounds can be further developed as natural antioxidants. IC_{50} values can be seen in Table 1 and Figure 3-6.

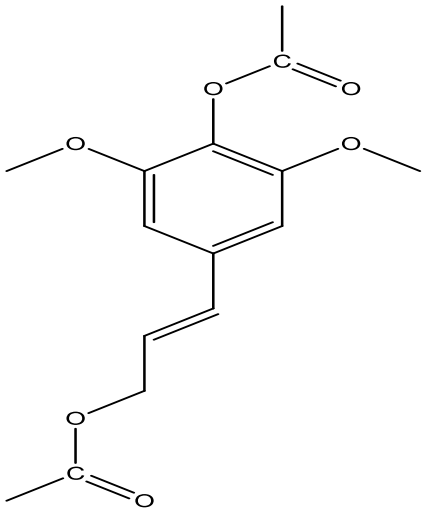

Fig.1: Structural formula for Sinaphyl alcohol diacetate

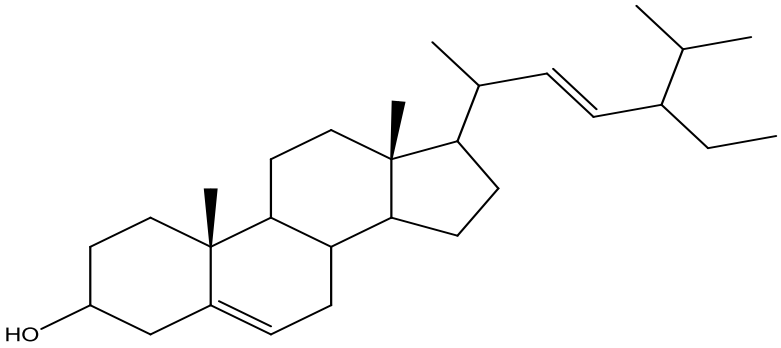

Fig.2: Structural Formula for Stigmasterol

Table 1: IC_{50} values (DPPH) of methanol extract and compounds 1-2

Sample	IC_{50} (µg/mL)
Methanol extract	41,592
Sinaphy alcohol diacetate	182,799
Stigmasterol	402,397
Ascorbic acid (Positive control)	20,895

IC_{50}: Half maximal inhibitory concentration,
DPPH: 2,2-diphenyl-1-picrylhydrazyl

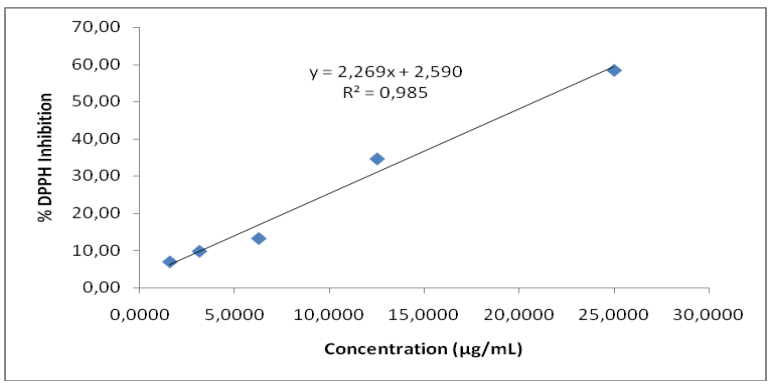

Fig.3: Ascorbic acid (Vitamin C)

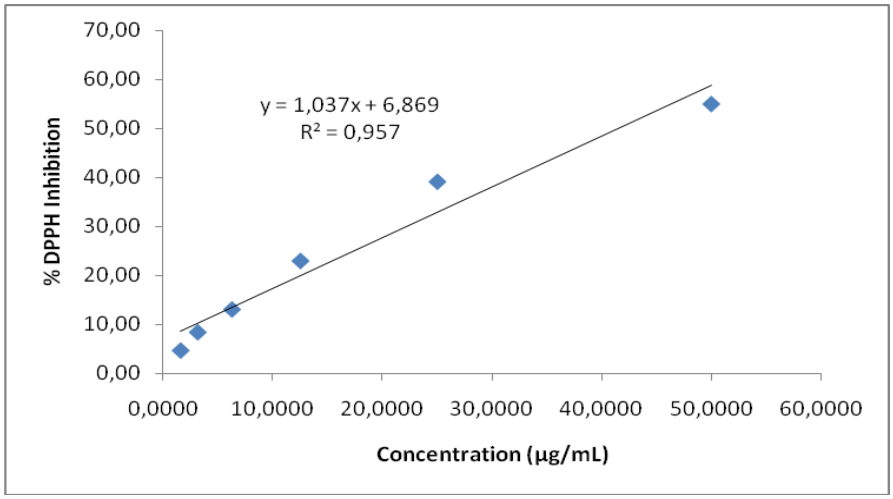

Fig.4: E. Rubroloba extract

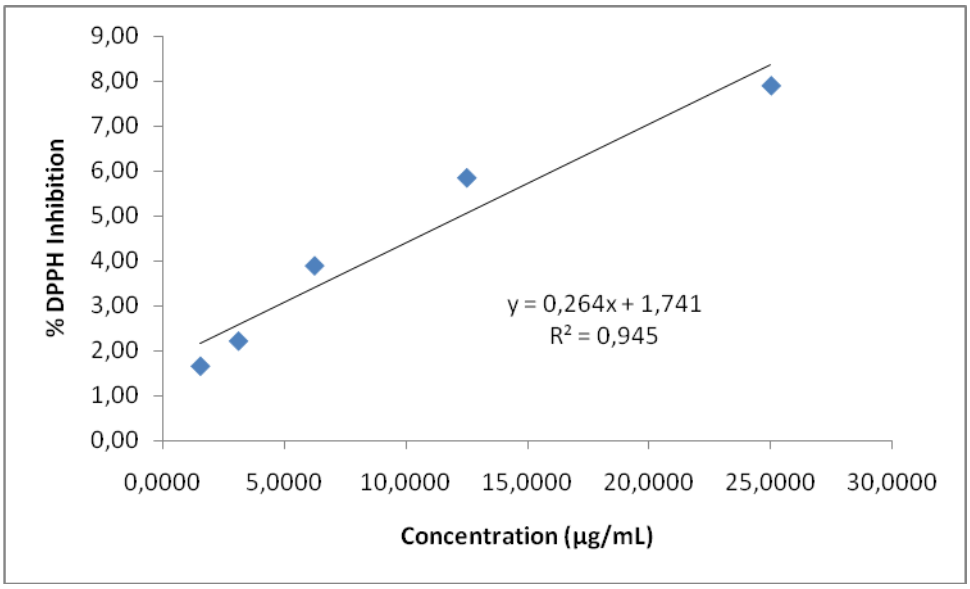

Fig.5. IC_{50} Sinapyl alcohol diacetate

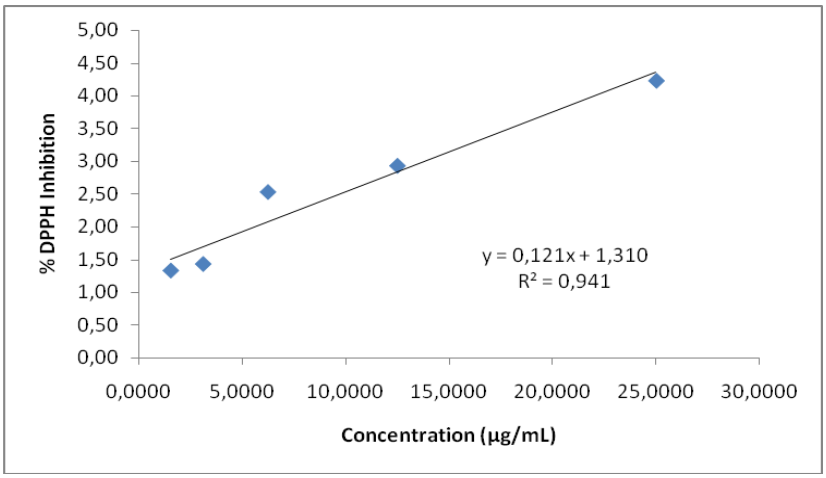

Fig.6: Stigmasterol

CONCLUSION

This study succeeded in isolating two secondary metabolites, namely Sinaphyl alcohol diacetate and Stigmasterol. This study was first reported on the E. rubroloba species. The free radical scavenging activity of the two compounds showed lower activity compared to Methanol Extract and Vitamin C as positive controls. Compounds (1-2) and methanol extract can be developed as natural antioxidants.

ACKNOWLEDGMENT

We would like to thank the Ministry of Education and Culture of the Republic of Indonesia for the grant "Research on Decentralization, National Competitive and Assignment Programs "Penelitian Disertasi Doktor (PDD) Tahun 2020"

AUTHORS CONTRIBUTIONS

AJ collects data, examines samples and prepares manuscripts. SW, IS and IP design research, analyze data, and make critical thinking on research texts.

CONFLICT OF INTERESTS

The author states that there is no conflict of interest, all authors agree that manuscripts are confidential and will not be submitted, or published elsewhere.

REFERENCES

1. A. D. Poulsen, Etlingera Of Sulawesi. England: Natural History Publications (Borneo), 2012.
2. E. Chan et al., Antioxidant and Tyrosinase inhibition properties of leaves and rhizomes of ginger species, vol. 109. 2008.
3. Chan, Y. Y. Lim, S. K. Ling, S. Tan, K. Lim, and M. G. H. Khoo, "Caffeoylquinic acids from leaves of Etlingera species (Zingiberaceae)," LWT - Food Science and Technology, vol. 42, no. 5, pp. 1026–1030, 2009b, doi: 10.1016/j.lwt.2009.01.003.
4. Chan et al., Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species, vol. 113. 2009a.
5. C. E. Ficker, M. L. Smith, S. Susiarti, D. J. Leaman, Ç. Irawati, and J. T. Arnason, "Inhibition of human pathogenic fungi by members of Zingiberaceae used by the Kenyah (Indonesian Borneo)," Journal of Ethnopharmacology, vol. 85, no. 2, pp. 289–293, Apr. 2003, doi: 10.1016/S0378-8741(03)00009-6.
6. S. J. T. Lachumy, S. Sasidharan, V. Sumathy, and Z. Zuraini, "Pharmacological activity, phytochemical analysis and toxicity of methanol extract of Etlingera elatior (torch ginger) flowers," Asian Pacific Journal of Tropical Medicine, vol. 3, no. 10, pp. 769–774, Oct. 2010, doi: 10.1016/S1995-7645(10)60185-X.
7. M. M. J. Wijekoon O., R. Bhat, and A. A. Karim, "Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (Etlingera elatior Jack.) inflorescence," J Food Compos Anal, vol. 24, no. 4–5, pp. 615–619, 2011.
8. C. A. Williams and J. B. Harborne, The leaf flavonoids of the Zingiberales, vol. 5. 1977.
9. I. Sahidin, Wahyuni, M. H. Malaka, A. Jabbar, Imran, and M. A. Manggau, "Evaluation Of Antiradical Scavenger Activity Of Extract And Compounds From Etlingera Calophrys Stems," Asian Journal of Pharmaceutical and Clinical Research, vol. 11, no. 2, p. 238, Feb. 2018, doi: 10.22159/ajpcr.2018.v11i2.22535.
10. I. Sahidin, Wahyuni, M. H. Malaka, A. Fristiohady, A. Saleh, and A. Marianti, "Antibacterial and radical scavenger activities of extract and compounds of Wualae (Etlingera elatior) stems from Southeast Sulawesi," IOP Conf. Ser.: Mater. Sci. Eng., vol. 546, p. 062027, Jun. 2019, doi: 10.1088/1757-899X/546/6/062027.
11. S.-U.-D. Afm, K. As, and Lim Lbl, "Phytochemical Screening, Antioxidant, and Antimicrobial Activities of Leaves, Stems, and Rhizomes of Etlingera coccinea (blume) S. Sakai & Nagam," vol. 7, no. 5, p. 12, 2015.
12. E. Chan, Y. Lim, and M. Omar, Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia, vol. 104. 2007.
13. M. A. A. Yahya, W. A. Yaacob, and I. Nazlina, "Isolation Of Chemical Constituents From Rhizomes Of Etlingera sphaerocephala Var. Grandiflora," vol. 15, no. 1, p. 5, 2011.
14. B. Mahdavi, "Chemical constituents of the aerial parts of Etlingera brevilabrum (Zingiberaceae)," p. 7, 2014.
15. C. S. Vairappan, T. Nagappan, and K. Palaniveloo, "Essential oil composition, cytotoxic and antibacterial activities of five Etlingera species from Borneo.," Nat Prod Commun, vol. 7, no. 2, pp. 239–242, Feb. 2012.
16. A. V. Badarinath, K. M. RAo, C. M. S. Chetty, S. Ramkanth, T. V. S. Rajan, and K. Gnanaprakash, "A Review on In-vitro Antioxidant Methods: Comparisions, Correlations and Considerations," p. 10, 2010.
17. M. S. Blois, "Antioxidant Determinations by the Use of a Stable Free Radical," Nature, vol. 181, no. 4617, pp. 1199–1200, Apr. 1958, doi: 10.1038/1811199a0.
18. G. Clarke, K. N. Ting, C. Wiart, and J. Fry, "High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the Malaysian Rainforest,"

Antioxidants, vol. 2, no. 1, Art. no. 1, Mar. 2013, doi: 10.3390/antiox2010001.

19. Ralph, S.A., Ralph, J., Landucci, L., Landucci, L.L., 2004. NMR database of lignin and cell wall model compounds. US Forest Prod. Lab., Madison, WI (http://ars. usda. gov/Services/docs. htm.