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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.807.814

Research Article
Phytochemical Screening, Toxicity Activity and Antioxidant Capacity of Ethanolic Extract of *Etlingera alba* Rhizome

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Abstract

Background and Objective: *Etlingera alba* (Blume) A.D. Poulsen is one of the plants of the genus *Etlingera* which is commonly found in Southeast Sulawesi. The research is still lacking, thus, we assumed other species related to *E. alba,* specifically from the genus *Etlingera* that provides antioxidant and radical scavenging activity, namely *Etlingera elatior* (Jack) R.M. Smith. Thus, this study aimed to assess the antioxidant and toxicity activity as well as its secondary metabolites. **Materials and Methods:** *Etlingera alba* rhizome was extracted with 96% ethanol. The radical scavenging activity was assayed with 1,1-diphenyl-2-picrylhydrazyl (DPPH) and antioxidant activity was assayed with 2,2′-azino-bis-[3-ethylbenzothiazoline sulphonate (ABTS) assay for radical cation decolourization *in vitro*. Both Ascorbic Acid (AA) and Trolox were used as positive control. The secondary metabolites were identified by Thin Layer Chromatography (TLC) and LSMS/MS analyzed the difference between compounds. According to results performed with TLC and LCMS/MS. **Results:** The extract exhibited antioxidant properties using both DPPH and ABTS method. The LC_{50} of the extract was 608.42±18.31 mg L^{-1}. *Etlingera alba* rhizome extract contains alkaloids, flavonoids, terpenoids and steroids. The compounds detected in the extract were E-p-Coumaric acid aschantin, 2-Methoxyanofinic acid, Chavicol-β-D-glucoside, Myristicanol B, ent-16α,17-Hydroxy-19-kaurenoic acid, 5-Hydroxy-7,8,2'-trimethoxyflavone, Methyl ursolate and Spinasterol. **Conclusion:** *Etlingera alba* rhizome contains several compounds that might be responsible for antioxidant activity and the extract itself classified as medium toxic.

Key words: Etlingera alba, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, 2,2′-azino-bis-[3-ethylbenzothiazoline sulphonate (ABTS) assay, antioxidant activity, flavonoids, thin layer chromatography, decolourization

Citation: Wahyuni, A. Diantini, M. Ghozali, A. Subarnas, E. Julaeha, R. Amalia and I. Sahidin , 2021. Phytochemical screening, toxicity activity and antioxidant capacity of ethanolic extract of *Etlingera alba* rhizome. Pak. J. Biol. Sci., 24: 807-814.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oxidative stress is a vital risk factor in numerous chronic diseases due to cell, protein and DNA damage, including inflammation, diabetes, cancer, atherosclerosis, ageing and neurodegenerative diseases such as Parkinson's and Alzheimer's disease[1]. Reactive Oxygen Species (ROS) are responsible for the production of oxidative stress. ROS is a waste of metabolism in the human body and produced by cells as free radicals. The human body has endogenous antioxidants as compensation for these free radicals, such as Superoxide Dismutase (SOD), catalase and glutathione peroxidase. However, the human body is not always compensating for free radicals. Thus, an exogenous antioxidant is required[2].

Antioxidant protects cells by conversing the ROS into non-radical species. Plants are natural sources of exogenous antioxidants such as phenolic compounds, vitamins and carotenoids. Indonesia is abundant in terrestrial natural products and Indonesian people are using many plants as traditional medicines empirically. Zingiberaceae is a herb that is mainly utilized by people for traditional medicine. *Etlingera* is one of the genera that belongs to the Zingiberaceae family, with many species and interesting potencies[3,4].

Many pharmacological activities of *Etlingera* have been reported, namely *Etlingera elatior*. The leaves part is used deodorant, wound healing agent, antioxidant, antibacterial, antifungal and tyrosinase inhibitor, the flower part is used as an antimicrobial against bacterial and fungal as well as antioxidant and the rhizome part are showing antioxidant capacity and cytotoxic capacity[3,5]. Other species reported are leaves and stem part of *E. brevilabrum* in decreasing cholesterol levels, the leaves part of *E. fulgens* as antibacterial, methanol extract of *E. calophrys* stem has the antioxidant capacity as well as *E. paviena* and *E. punicea* for anticancer[3,6-11]. The Zingiberaceae family's abundant potencies, specifically from the *Etlingera* genus, interest authors to investigate the potency of the *Etlingera* genus. *Etlingera alba* (Blume) A.D. Poulsen is one of the *Etlingera* genus commonly found in Southeast Sulawesi. The research itself is still limited but locally it is used as spices as well as traditional medicine.

Thus, we investigate the antioxidant activity and toxicity of *E. alba* and the chemical constituents in it.

MATERIALS AND METHODS

Study area: This study was conducted at Laboratorium Farmasi, Fakultas Farmasi UHO, Indonesia from June, 2020-February, 2021.

Plant material: *Etlingera alba* rhizome was obtained in Punggaluku Village, Laeya District, South Konawe of Southeast Sulawesi (4019'26"S 122028'58"E, 325 m). The sample was dried at a temperature below 40°C and avoided from sunlight. The dried sample was then ground into the ideal size and put in a sealed container for further analysis.

Materials: Materials used were ethanol 95%, $FeCl_3$, NaOH, H_2SO_4, HCl, 2'-Azino bis-3-ethylbenzothiazoline-6-Sulphonic acid ascorbic acid, Trolox, potassium persulfate, potassium dichromate and dimethyl sulfoxide was obtained from Merck®, Darmstadt, Germany. Mayer's reagents were obtained from Mercuric-Iodide TS, LabChem Inc, USA. Chloroform was obtained from full-time, PT MDK, East Jakarta, Indonesia. 0.1% formic acid in water and acetonitrile in 0.1% formic acid were obtained from Thermo Fisher Scientific, Rockford, US. 1,1-Diphenyl-2-Picrylhydrazyl was from HIMEDIA, Mumbai, India). Brine Shrimp Eggs *Artemia salina* was obtained from (Brine Shrimp Direct, USA).

Extraction method: Dried *E. alba* rhizome was macerated with 95% ethanol (500 mL×3). The filtrate obtained was concentrated using a rotary evaporator (Rotavapor (R) RII, Buchi, Switzerland) at 40°C to obtain the concentrated extract. The concentrated extract was used for further phytochemical identification and biological activity assay.

Qualitative phytochemicals screening: Qualitative phytochemical screening was carried by colourimetric methods to detect the presence of secondary metabolites in the extract using standard methods[12]:

- **Test for phenols and tannins:** The 2% solution of $FeCl_3$ (2 mL) was mixed with the crude extract. A blue-green or black colouration indicates the presence of phenols and tannins
- **Test for flavonoids:** The 2% solution of NaOH (2 mL) was mixed with the crude extract. The presence of flavonoids was indicated by an intense yellow colour formed, which turned colour less by adding a few drops of diluted acid
- **Test for steroid:** Chloroform (2 mL) was mixed with the crude extract and concentrated H_2SO_4 was added side wise. The presence of steroids was indicated by a red colour produced in the lower chloroform layer. Another test was performed by mixing the crude extract with 2 mL of chloroform. Then 2 mL of each of concentrated H_2SO_4 and acetic acid was poured into the mixture. The development of a greenish colouration indicates the presence of steroids

- **Test for terpenoids:** Chloroform (2 mL) was used to dissolved the crude extract and evaporated it into dryness. To this, 2 mL of concentrated H_2SO_4 was added and heated for 2 min. A greyish colour indicated the presence of terpenoids
- **Test for alkaloids:** The 1% HCl (2 mL) was mixed with the crude extract and heated gently. Mayer's reagents were added to the mixture. The turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids

Liquid chromatography-mass spectrometry mass spectrometry (LCMS-MS): Secondary metabolites from ethanol extracts were determined using an LC-MS-MS analysis, Xevo G2-XS QTOF (Waters Corporation, Milford, USA) equipped with an Electrospray Ionization (ESI) source was coupled to a UPLC analysis was performed using a Waters Acquity Ultra Performance LC system. A reverse-phase HSS T3 C18 column (2.1×100 mm, 1.8 µm particle size) was used and maintained at 40°C. The mobile phases consisted of A (0.1% formic acid in water) and B (acetonitrile in 0.1% formic acid). Gradient elution was conducted at a flow rate of 0.3 mL min^{-1} with an injection volume of 1 µL. The gradient was as follows: 5% B (0-8 min), 40% B (8-11 min) and 100% B (11-16 min). The data range was from 50-1200 m/z. The source temperature applied was 120°C and the desolvation gas flow was at 1000 L h^{-1} with a desolvation temperature of 500°C. The capillary voltage was at 2.0 kV and the cone voltage was 30 V. Alternative low and high collision energy scans were used to obtain data-independent acquisition (known as MSE mode). The low collision energy scan was set at 6.00 eV and the high collision energy scan was set at a ramp energy scan from 10-40 eV. All LC-MS data was processed, peak picked and analyzed using the UNIFI informatics platform. A 3-Dimensional (3D) peak detection algorithm was used to detect the peak apexes of all the ion responses based on their 3D shapes to obtain cleaner spectra and more accurate peak volumes than 2D extracted ion chromatograms. The total intensity of each ion was normalized to the total ion count to generate a matrix consisting of the m/z value, Retention Time (RT) and normalized peak area. The variables of interest were then identified using the discovery tools within UNIFI software[13].

Determination of antioxidant capacity

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay: The assay was conducted according to the modified by Shahidi and Zhong[14]. About 1 mL of 0.1 mM solution of DPPH (HIMEDIA) in ethanol was mixed with 2 mL of the aqueous extracts at different concentrations (10-50 mg mL^{-1}). The mixture was then incubated at room temperature for 30 min in the dark. Mix 1 mL of DPPH solution with double distilled water to prepared control. The absorbance was measured against a blank at 517 nm using a spectrophotometer (Jenway, 6800 Double-Beam Spectrophotometer, UK). Higher DPPH free radical scavenging activity is indicated by the lower absorbance of the reaction mixture. The standard used was Ascorbic acid and Trolox (Sigma-Aldrich). Samples were prepared and measured in 3 replications (triplicates). The percentage of scavenging activity of each extract on DPPH radical was calculated as % inhibition of DPPH (I%) using the following Eq.[14]:

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

A^o is the control's absorption and A^s is the tested extract solution's absorption.

ABTS [2,2'-azino bis (3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity assay: This assay was conducted using the modified method of Moniruzzaman *et al.*[15]. The ABTS (Sigma Aldrich) aqueous solution (7 mM) reacted to 2.45 mM aqueous solution of potassium persulfate (Merck) in equal quantities is for preparing the $ABTS^{\bullet+}$ stock solution, the mixture was allowed to stand in the dark at room temperature for 12-16 hrs prior use. The working solution of $ABTS^{\bullet+}$ was obtained by diluting the methanol's stock solution to provide an absorbance of 0.70±0.02 at 734 nm. Continued by, 2 mL of $ABTS^{\bullet+}$ solution was mixed with 1 mL of the aqueous extracts at different concentrations (10-50 mg mL^{-1}). Continued by the mixture was incubated at room temperature for exactly 10 min in the dark. The $ABTS^{\bullet+}$ solution (2.0 mL) was mixed with 1 mL of double distilled water to prepare the control. A spectrophotometer (Jenway) was used to measure the absorbance of a blank (734 nm). Ascorbic acid and Trolox (Sigma-Aldrich) was used as the standard. The samples were prepared and then measured in triplicates. The percentage of scavenging activity of each extract on ABTS[15]:

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

where, A^o is the absorption of control and A^s is the absorption of the tested extract solution.

Calculation of IC_{50}: The IC_{50} of each analytical sample was calculated according to the following procedure: (a) Inhibition ratios (y) were plotted against the sample concentrations (x) at all six points and the separate regression line (y = ax+b) was drawn. The regression line was not required to pass through the origin. In this step, we verified that all of the measurement points were basically on the regression line. It was also verified that two points around the 50% inhibition did not have a deviation from the regression line. Because the inhibition curve is not entirely straight yet slightly curved, thereby we decided to calculate the IC_{50} value by using the interpolation method by joining the two points around the 50% inhibition with a straight line as follows, (b) Two points enclosing a 50% inhibition ratio were selected and a regression line (Y = AX+B) was drawn. The regression line was not required to pass through the origin and (c) X (sample concentration) was calculated when Y in the regression equation of (b) was substituted with 50[16].

Toxicological evaluation brine shrimp lethality assay: The method described by Waghulde *et al.*[17] was adopted to study the general toxicity of the ethanol extract. The eggs of *Artemia salina* were hatched in a transparent container containing seawater. A light bulb with a power supply of 40-60 watt was provided in the hatching process to prevent the temperature within 25-30°C. Oxygen was supplied by using a blower. The larvae with a 48 hrs life span were used in the toxicity test. The concentrations used in BSLT were 10, 100, 250, 500, 750 µg mL^{-1} and negative control. A stock solution of dense extract was prepared by balancing 5 mg of the extract, which was then dissolved in 5 mL ethanol to produce 1.000 ppm. The stock solution was then pipetted and put in vials. Each concentration has three replications (triplicates). The vials were aerated to dry the samples put in them. The vials were added DMSO 1% for water-undissolved fractions. Five millilitres of seawater was added to the vials. The process was followed by adding samples to the vials to make concentrations of 10, 100, 250, 500 and 750 µg mL^{-1}. The control had no sample in it. Ten larvae of *Artemia salina* were added to each vial. The observation was conducted for 24 hrs. The toxicity test was determined according to the number of death larvae[17].

The toxicity test was assessed by determining the LC_{50} score. For obtaining the LC_{50} score, the mortality rate of larvae after 24 hrs of exposure was assessed first:

$$\text{Mortality (\%)} = \frac{\text{Total larvae mortality}}{\text{Total larvae}} \times 100\%$$

By having that mortality rate, probit analysis was carried out to find the LC_{50} score. LC_{50} score is defined as the concentration where the compound produced 50% death. After doing probit analysis, LC_{50} was counted by using the linear regression equation y = a+bx. The toxicity level of a compound is classified according to the LC_{50} score[18]. LC_{50} score in the range of $\leq$30 µg mL^{-1} is defined as highly toxic. LC_{50} in range of 31-1000 µg mL^{-1} is classified as medium toxic, while >1,000 µg mL^{-1} as low toxic.

RESULTS

According to the results, the yield ethanol extract of *Etlingera alba* rhizome is 2.9% and revealed the contents of phenolic compounds, flavonoids, steroids, terpenoids and alkaloids, qualitatively. The data in Table 1 is showing the phytochemical screening of extract, qualitatively.

The data in Table 2 presents the result of the DPPH and ABTS assay. The DPPH value of extract ascorbic acid and Trolox were 43.61±0.69, 13.59±0.79 and 17.30±0.69 mg L^{-1}, respectively. Similar results to ABTS value, which extract ascorbic acid and Trolox were 50.57±0.83, 15.43±0.43 and 20.30±1.13 mg L^{-1}, respectively.

According to toxicological evaluation (Table 3), the *E. alba* rhizome extract with LC_{50} 608.42±18.31 mg L^{-1} is classified as medium toxic (LC_{50} between 31-1000 mg L^{-1}). Compared to the control used, potassium dichromate with LC_{50} 2.24±0.23 and is classified as highly toxic. It is potentially a source of cytotoxic agents, although it is medium toxic.

LCMS-MS profiling exhibited the peak of chemical constituents of *Etlingera alba* rhizome extract (Fig. 1) observed from the Retention Time (RT) of 5.37-13.66 min. Compound 1 in positive mode ions appeared at a time of 5.37 min with

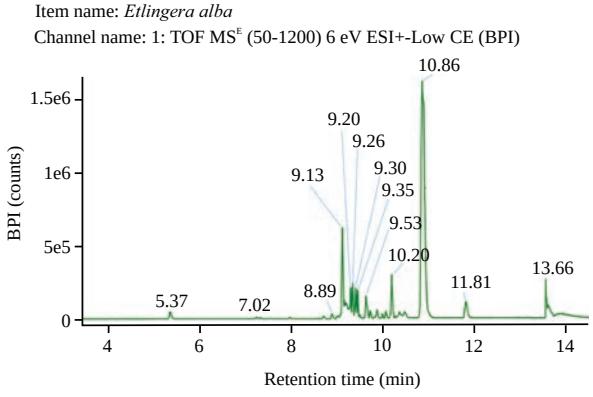

Fig. 1: LCMS-MS chromatogram of the chemical constituents of *E. alba* extract

peak $[M+H]^+$ at m/z 165.0548, confirmed with 164 as its molecular weight, concluding with a mass spectrophotometer that its molecular structure is $C_9H_8O_3$. Fragments that appeared at m/z 147.0441 $[M]^+$ dan m/z 119.0493 $[M]^+$, respectively, indicate the release of hydrogen dioxide and carbon monoxide units (Fig. 2). Compound 1 exhibited ion fragments at m/z 165.0548, thus concluded that compound 1 is E-p-Coumaric acid. The LCMS-MS exhibited varied types of compounds contained in *E. alba* rhizome ethanol extract. The data of Table 4 present the molecular structure of identified compounds from *E. alba* rhizome extract which are these compounds are phenylpropanoid (such as E-p-coumaric acid and chavicol-β-D-glucoside that appeared at m/z 165.0548 at 5.37 and 319.1153 at 9.20 min), terpenoids (ent-16α,17-hydroxy-19-kauranoic acid, 2-methoxyanofinic acid and methyl ursolate that appeared at m/z 343.2221 (9.42 min), m/z 235.0967 (9.13 min) and m/z 477.3662 (10.20 min), respectively), steroid (spinasterol, appeared at m/z 413.3784 at 13.66 min), flavonoids (5-hydroxy-7,8,2'-trimethoxyflavone, appeared at m/z 329.1022 at 9.53 min) and lignans (myristicanol B and aschantin, that appeared at m/z 427.1704 (9.26 min) and m/z 401.1588 (7.02 min), respectively) are present in Fig. 3. Spinasterol and Methyl Ursolate are the highest retention time and neutral mass observed, respectively.

Table 1: Yield and qualitative phytochemical screening of extracts ethanol

Metabolites	Results	Yield (%)
Phenolic	+	2.9
Flavonoid	+	
Steroid	+	
Terpenoid	+	
Alkaloid	+	

Table 2: Determination of antioxidant capacity

	IC_{50} (mg L^{-1})	
Sample	DPPH method	ABTS method
Extract ethanol	43.61±0.69	50.57±0.83
Ascorbic acid	13.59±0.79	15.43±0.43
Trolox	17.30±0.69	20.30±1.13

Table 3: Toxicological evaluation

Sample	LC_{50} (mg L^{-1})
Extract ethanol	608.42±18.31
Potassium dichromate	2.24±0.23

Table 4: Chemical composition of extract ethanol

No. structure	Observed RT (min)	Observed (m/z) [(+)-ESI]	Neutral mass (Da)	Formula	Identified compounds
1	5.37	165.0548 $[M+H]^+$	164.0474	$C_9H_8O_3$	E-p-coumaric acid
2	7.02	401.1588 $[M+H]^+$	400.1522	$C_{22}H_{24}O_7$	Aschantin
3	9.13	235.0967 $[M+H]^+$	234.0892	$C_{13}H_{14}O_4$	2-Methoxyanofinic acid
4	9.20	319.1153 $[M+Na]^+$	296.1260	$C_{15}H_{20}O_6$	Chavicol-β-D-glucoside
5	9.26	427.1704 $[M+Na]^+$	404.1835	$C_{22}H_{28}O_7$	Myristicanol B
6	9.42	343.2221 $[M+Na]^+$	320.2351	$C_{20}H_{32}O_3$	ent-16α,17-Hydroxy-19-kaurenoic acid
7	9.53	329.1022 $[M+H]^+$	328.0947	$C_{18}H_{16}O_6$	5-Hydro-7,8,2'-trimethoxyflavone
8	10.20	477.3662 $[M+Na]^+$	454.3811	$C_{31}H_{50}O_2$	Methyl ursolate
9	13.66	413.3784 $[M+H]^+$	412.3705	$C_{29}H_{48}O$	Spinasterol

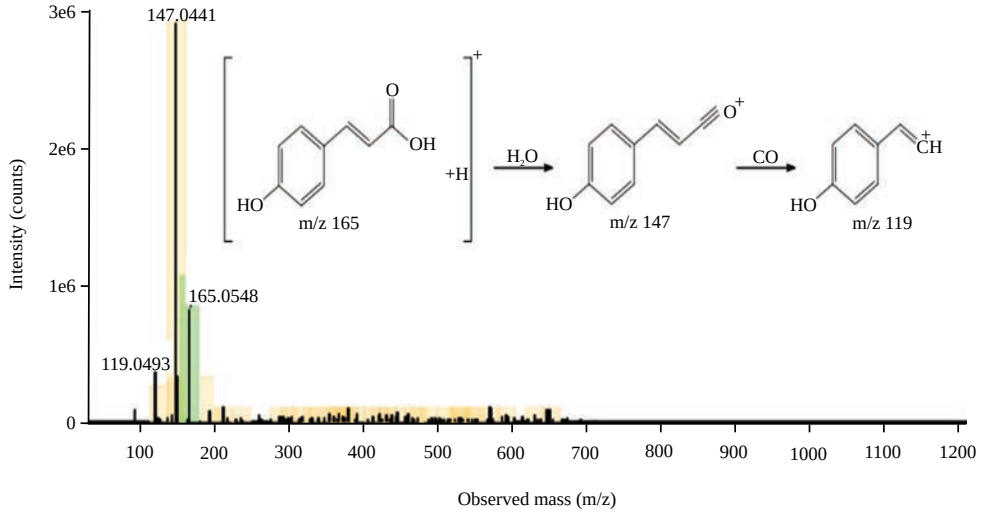

Fig. 2: ESI-MS spectra of E-p-coumaric acid (1)

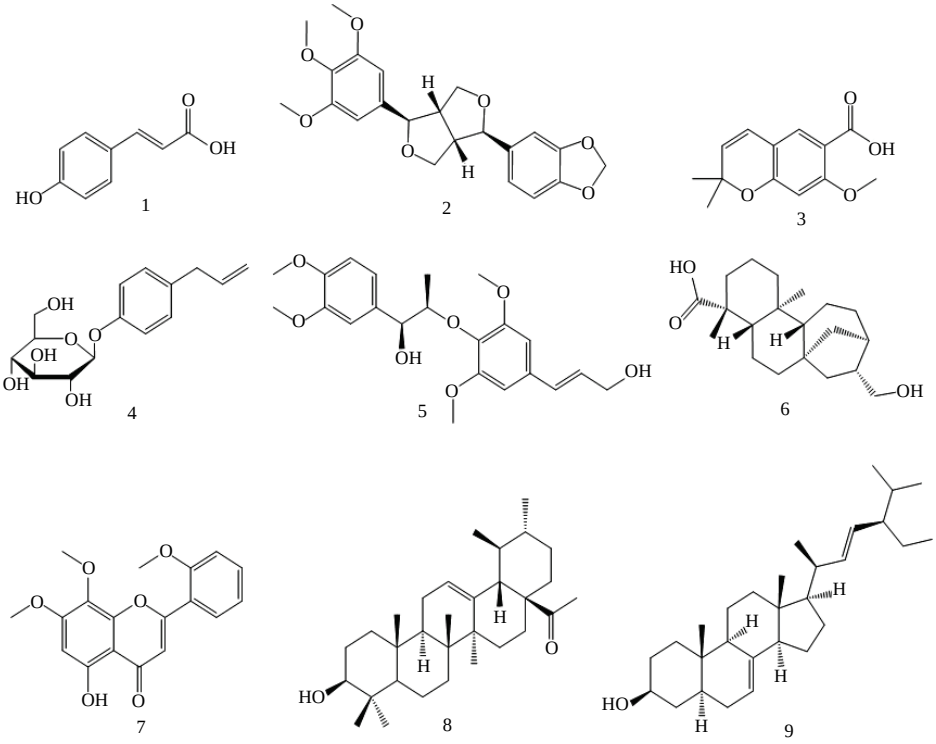

Fig. 3: Molecular structure of identified compounds from *E. alba* rhizome ethanol extract

1: E-p-Coumaric acid, 2: Aschantin, 3: 2-Methoxyanofinic acid, 4: Chavicol-β-D-glucoside, 5: Myristicanol B, 6: ent-16α,17-Hydroxy-19-kaurenoic acid, 7: 5-Hydroy-7,8,2'-trimethoxyflavone, 8: Methyl ursolate and 9: Spinasterol

DISCUSSION

Etlingera alba (Blume) A.D. Poulsen is one of the *Etlingera* plants commonly found in Southeast Sulawesi. The closest relation of *E. alba* is *Etlingera elatior* (Jack) R.M. Smith is one of the plants that belong to *Etlingera* plants that potentially beneficial as an antioxidant. Flower, rhizome and leaves parts of *E. elatior* to possess robust antioxidant activity[3,19]. Therefore, we are attracted to investigate the antioxidant, toxicity and chemical components of *the Etlingera alba* rhizome part.

Plants are a source of natural antioxidants that exhibit a wide range of pharmacological effects associated with Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), including anti-inflammatory, antihyperlipidemic, protective effect and anticancer[20]. Our research found that *Etlingera alba* rhizome extract contains phenolic compounds and flavonoids that naturally playing a role as a source for antioxidants (Table 1). Continued by LC-MS-MS profiling (Table 4) found that the extract provides compounds such as phenylpropanoid (such as E-p-coumaric acid and chavicol-β-D-glucoside), terpenoids (ent-16α,17-hydroxy-19-kauranoic acid, 2-methoxyanofinic acid and methyl ursolate), flavonoids (5-hydroxy-7,8,2'-trimethoxyflavone) and lignans (myristicanol B and aschantin) contained in *E. alba* rhizome extract. They are known to possess antioxidant capacity[20-23]. Therefore, the *E. alba* rhizome extract was assayed for radical scavenging and antioxidant activity with DPPH and ABTS assays.

DPPH assay is used to determine the antioxidant activity based on antioxidants' scavenging activity towards DPPH (1,1-diphenyl-2-picrylhydrazyl), a stable free radical. A hydrogen atom in antioxidants reduces the odd electron in DPPH. Thus the DPPH becomes stable[24,25]. On the other hand, ABTS assay is used to determine the total antioxidant capacity (TAC) by using 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS), stable free radicals. The ABTS assay is measured by activating metmyoglobin with hydrogen peroxide in the presence of ABTS, producing radical cation. This method measures antioxidants' relative ability to scavenge the ABTS by reacting with potent oxidizing agents, compared to Trolox. The ABTS method provides more sensitivity in detecting the antioxidant capacity because its response for antioxidants is higher than the DPPH method and it has faster reaction kinetics[26,27]. Both results demonstrated the ability of extract in antagonizing the free radicals. Followed by cytotoxicity activity test, *E. alba* rhizome extract is categorized as medium toxic to human (Table 3). This finding can be a preliminary screening for determining the optimal concentration of extract because the extract itself showed great antioxidant activity. The bioassay

using brine shrimp *A. salina* can be an indicator for antitumor activity, therefore it will be great for testing the extract against cancer cell lines[28].

Between both methods, it showed similar antioxidant capacity. The IC_{50} in both DPPH and ABTS assay exhibited that extract was higher than both controls used, which were ascorbic acid and Trolox. It means that the antioxidant capacity of the extract is lower than the control. The activity of antioxidants in extract might be affected by the phenolics and flavonoids compound contained in *E. alba* rhizome ethanolic extract.

The function hydroxyl group in flavonoids and phenolic compounds act as an antioxidant by scavenging the free radicals and chelating the metal ions, therefore the radicals are being stable. They are also suppressing the ROS formation by inhibits the enzymes, scavenging the ROS and upregulation or protect cells. Therefore, the damages caused by free radicals are evitable[29,30]. The correlation between antioxidant and anti-inflammatory is positively linear. The inflammatory process promotes carcinogenesis, Diabetic Mellitus (DM) and any disease related to free radicals[31,32].

Antioxidant activity of extract ethanol of *E. alba* implicated in finding in novel agent for any disease related to free radicals. However, it needs further study, especially *in vivo* study to provide broad pharmacological data. Isolating the compounds contained in the extract also needs to be performed to find out which compounds have the potential to provide antioxidant activity.

CONCLUSION

Etlingera alba rhizome extract possesses antioxidant capacity with the IC_{50} were 43.61 ± 0.69 mg L^{-1} for DPPH and 50.57 ± 0.83 mg L^{-1} for ABTS. For the toxicological evaluation, the extract was categorized as medium toxic. These activities were influenced by the compounds contains in it, including phenolic, flavonoid, steroid, terpenoid and alkaloid. According to the LCMS-MS profiling, the extract contains phenylpropanoid (such as E-p-coumaric acid and chavicol-β-D-glucoside), terpenoids (ent-16α,17-hydroxy-19-kauranoic acid, 2-methoxyanofinic acid andmethyl ursolate), steroid (spinasterol), flavonoids (5-hydroxy-7,8,2'-trimethoxyflavanone) and lignans (myristicanol B and aschantin).

SIGNIFICANCE STATEMENT

This study discovers the antioxidant capacity and the toxicological evaluation of the *Etlingera alba* rhizome that can be beneficial in the process of discovering novel drugs. This study will be basic for further analysis in discovering new candidates for drugs. Thus, the possible candidates of drug from this extract may be found in the future.

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