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Antioxidant and Cytotoxic Activities of Rhizome and Fruit Extracts of Susube (*Meistera aculeata* (Roxb.) Škorničk. & M.F. Newman) from Southeast Sulawesi

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Abstract. Susube (*Meistera aculeata* (Roxb.) Škorničk. & M.F.Newman) is one of the species of the zingiberaceae family that grows in Southeast Sulawesi, especially in Konawe Regency. The fruit of this plant is often consumed and used as a cooking spice by the native. Meanwhile, the compounds content and bioactivity of the rhizome and fruit which is a characteristic of the Zingiberaceae family, has never been reported. Therefore, this study aimed to identify compounds contained in the rhizome along with their antioxidant and cytotoxic properties. The research method used is experimental research, starting with qualitative phytochemical screening in ethanol exctract and then continued with antioxidant (1,1-diphenyl-2-picrylhydrazyl or DPPH) and cytotoxicity assay (Brine Shrimp Lethality Test or BSLT). The results of the phytochemical screening indicate that the ethanol extract of the Susube rhizome and fruit contains alkaloids, flavonoids, tannins, terpenoids, and saponins. Antioxidant activity was determined based on the ability to reduce DPPH radicals and was calculated by determining the IC_{50} value, while toxicity was assessed based on shrimp larvae mortality and calculated by determining the LC_{50} value. The study results show that the IC_{50} values for the ethanol extract of Susube rhizome, fruit and the positive control ascorbic acid are 4.9 µg/mL, 4,5 µg/mL and 2.2 µg/mL, respectively. Meanwhile, the LC_{50} values for the ethanol extract of Susube rhizome, fruit, and the positive control potassium bichromate are 578 µg/mL, 958 µg/mL, and 2.8 µg/mL, respectively. Based on these results, it can be concluded that the ethanol extract of Susube rhizome and fruit (*Meistera aculeata* (Roxb.) Škorničk. & M.F.Newman) exhibits toxic effects on shrimp larvae (≤1,000 mg/L). Additionally, its ethanol extract shows very strong antioxidant effects in scavenging DPPH free radicals. Therefore, this study suggests that the Susube rhizome and fruit extract warrants further investigation as a promising candidate for treatment or prevention of diseases, supporting the potential of Southeast Sulawesi's local natural resources for phytopharmaceutical or herbal medicine.

Introduction

Since the COVID-19 pandemic spread across the world, many institutions and individuals have claimed to have discovered traditional medicines to cure COVID-19. Public interest in using traditional medicine increased significantly during the pandemic (Yimer et. al., 2021). Traditional medicine refers to formulations made from a mixture of ingredients derived from plants or animals, prepared for consumption, and traditionally believed by communities to treat diseases. Traditional medicine is also known as herbal medicine, as the ingredients used come from natural sources. According to the Indonesian Food and Drug Authority (BPOM), traditional medicines are categorized into several groups: *jamu* (Indonesian

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traditional herbal medicine), standardized herbal medicine (Obat Herbal Terstandar), and phytopharmaceuticals. The difference between these three types lies in the level of testing involved (Elfahmi et. al., 2014).

Indonesia's biodiversity is very diverse. Around 28,000 species of plants live in Indonesia. 80 percent of Indonesia's medicinal plants are estimated to be in Indonesia. Of the various types of plants that have been discovered, around 9600 plants have been identified as medicinal plants. However, only around 4410 natural ingredients have been identified as being able to be used as raw materials for drugs registered with BPOM. In Law Number 17 of 2023 concerning Health, it has been stated that phytopharmaceuticals are one type of natural medicine. Phytopharmaca is defined as a natural medicine used for health maintenance, health improvement, disease prevention, treatment, or health recovery. Phytopharmaca has been scientifically proven to be safe and efficacious through preclinical and clinical trials and its raw materials and finished products have been standardized. Currently, there are only 22 phytopharmaca products that have been produced in Indonesia (Arlinta, 2024). Therefore, the potential for developing phytopharmaca drugs is very large in Indonesia.

Zingiberaceae is one of the most prominent plant families in the world, comprising approximately 47 genera and 1,400 species. Several classes of bioactive compounds have been isolated from this family, including terpenoids, mono- and sesquiterpenoids, diarylheptanoids, arylalkanoids, phenylpropanoids, cyclohexane oxides, flavonoids, and flavonoid derivatives. These compounds have been extracted from some of the largest genera within the Zingiberaceae family, such as *Aframomum*, *Alpinia*, *Amomum*, *Boesenbergia*, *Costus*, *Curcuma*, *Hedychium*, *Kaempferia*, and *Zingiber*. Several Zingiberaceae species are generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA). Furthermore, various species are commonly used as traditional medicine, culinary spices, and dietary supplements in several Asian countries. This underlines the non-toxic nature of Zingiberaceae plants, supporting their potential for clinical testing and therapeutic applications (Balaji et al., 2022).

Meistera is a genus within the Zingiberaceae family, comprising 47 identified species. One of its species is *Meistera aculeata* (Roxb.) Škorničk. & M.F. Newman. In Indonesia, this plant is found exclusively in Southeast Sulawesi, where it is locally known as *Susube* by the Tolaki ethnic group. The fruit is traditionally consumed raw and occasionally used as a cooking spice by the local community (Hendrisno et al., 2022). However, scientific information regarding the pharmacological potential of *Meistera aculeata* Škorničk. & M.F. Newman remains very limited. Few studies have explored the bioactive compound content and the comprehensive biological activities of this plant, particularly those originating from Southeast Sulawesi. Therefore, this study aims to evaluate the antioxidant and cytotoxic properties of *Meistera aculeata* Škorničk. & M.F. Newman extracts as a preliminary effort to utilize and conserve local biodiversity resources that hold significant potential in the fields of health and medicine, particularly in the development of phytopharmaceutical raw materials derived from Southeast Sulawesi.

Experimental

Material and Methods

Rhizome and fruit samples of *Susube* were collected from Totombe Jaya Village, Sampara Subdistrict, Konawe Regency, Southeast Sulawesi. The materials used included 96% ethanol (C_2H_5OH), magnesium (Mg), chloroform ($CHCl_3$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), gallic acid, iron(III) chloride ($FeCl_3$), distilled water (H_2O), Dragendorff's reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), and *Artemia salina* larvae eggs.

The equipment used consisted of a rotary evaporator, digital balance, oven, blender, funnel, pipette, spatula, reaction tubes, Erlenmeyer flasks, filter paper, vials, and a UV-Vis spectrophotometer (Thermo Scientific Genesys 10 UV-Vis).

Procedures

Preparation and Extraction

The rhizome and fruit of *Susube* were first thoroughly washed with water to remove any impurities. After cleaning, the samples were chopped into small pieces (approximately 1–2 cm) and dried in an oven at 40°C. The dried samples were then ground using a blender. The resulting sample powder was weighed and subjected to the extraction process. The dried rhizome and fruit powders were extracted using the maceration technique with 96% ethanol as the solvent at room temperature for 3 × 24 hours. The ethanol extracts were then filtered and concentrated using a rotary evaporator to obtain a thick extract. The resulting thick extract was weighed to determine the sample yield. The extract yield was calculated using Equation (1) (Ngamkhae et al., 2022) as follows:

$$\% \text{ rendamen} = \frac{\text{mass of crude extract}}{\text{mass of dry sample}} \times 100\% \qquad (1)$$

Phytochemical Screening

Alkaloid Test. 20 mg of extract was mixed with 2.5 mL of

1% HCl, shaken for 5 minutes, and then filtered. The filtrate was added to 5 drops of Dragendorff's reagent. The presence of alkaloids was indicated by the formation of an orange-reddish color (Sabandar et al., 2020).

Tannin Test. 20 mg of extract was dissolved in 5 mL of distilled water and then filtered. To the filtrate, 5 drops of 0.1% $FeCl_3$ solution were added. The presence of tannins was indicated by a greenish-brown or bluish-black coloration (Sabandar et. al., 2020).

Flavonoid Test. 20 mg of extract was dissolved in 10 mL of methanol (CH_3OH) and then filtered. The filtrate was added to 1 mL of concentrated HCl and a few drops of $FeCl_3$, then heated in a water bath. The presence of flavonoids was indicated by the appearance of a reddish color (Sabandar et al., 2020).

Terpenoid Test. 20 mg of extract was dissolved in 2 mL of chloroform ($CHCl_3$), and the filtrate was carefully layered with 3 mL of concentrated sulfuric acid (H_2SO_4). The presence of terpenoids was indicated by the formation of a reddish-brown coloration at the interface (Sabandar et al., 2020).

Steroid Test. 20 mg of extract was dissolved in 1 mL of methanol (CH_3OH) and filtered. The filtrate was added to 1 mL of chloroform ($CHCl_3$) and 1 mL of concentrated sulfuric acid (H_2SO_4). The presence of steroids was indicated by the appearance of a greenish-yellow color (Sabandar et al., 2020).

Saponin Test. 20 mg of extract was mixed with distilled water, heated to boiling, and then filtered. The filtrate was vigorously shaken. The presence of saponins was indicated by the formation of a stable froth layer on the surface of the solution (Sabandar et al., 2020).

Antioxidant Activity Assay (1,1-diphenyl-2-picrylhydrazyl (DPPH) Method)

The antioxidant activity of rhizome and fruit extract were determined by using DPPH radical scavenging assay according Kamaruddin et al., 2021. Samples stock solutions were prepared in ethanol 96% and serially diluted into eight concentrations ranging from 100 to 0.8 µg/mL (100; 50; 25; 12,5; 6,3; 3,1; 1,6; 0,8 µg/mL). Triplicate volumes (1 mL) of diluted solutions were transferred into vials and incubated, each with 1 mL of DPPH solution (0.20 mM) for 15 minutes in the dark at room temperature. The absorbance was recorded at 517 nm against samples and blank containing ethanol 96%. Sample absorbance (Abssample) was obtained after blank sample correction. The 50% radical inhibitory concentration (IC50) values of ethanol extract and positive controls (ascorbic acid) were calculated using GraphPad Prism 5 software (GraphPad Inc., California, US). The radical scavenging activity (%RSA) was calculated using quation 2 (Itam et al, 2021) as follows:

$$\% \text{ RSA} = \frac{\text{AbsDPPH control} - \text{AbsSample}}{\text{AbsDPPH control}} \times 100\% \quad (2)$$

Toxicity Assay (BSLT (Brine Shrimp Lethality Test) Method)

Toxicity testing was conducted using the Brine Shrimp Lethality Test (BSLT) method as described by Hendrisno *et al.,* (2022). Concentration variations used for the test included 1000 µg/mL, 100 µg/mL, and 10 µg/mL for both the sample and the positive control (potassium dichromate), with seawater serving as the solvent control. The tested sample was a 96% ethanol extract. A volume of 200 µL was taken from the sample stock solution (prepared at 1000 µL) and added to each well of a microplate. *Artemia salina* Leach eggs (50 mg) were hatched in a small aquarium divided into two sections (a larger dark compartment and a smaller illuminated one), which were separated by a partition with 2 mm holes. The larger section was covered with aluminum foil to create darkness, while the smaller section was exposed to light. Seawater was added and aerated within the aquarium. *Artemia salina* eggs (50 mg) were distributed into the dark section and incubated for 48 hours at a warm room temperature (22–29 °C). During incubation, the aerator and the lamp illuminating the smaller compartment were kept on. Upon hatching, the phototropic nauplii moved toward the light through the 2 mm holes, while the egg shells remained in the dark compartment. The hatched larvae were then pipetted and transferred into wells containing seawater, and the total number of larvae per well was counted. The LC_{50} of the 96% ethanol extract was determined through Probit analysis of the relationship between extract concentrations and the number of *A. salina* Leach larvae fatalities using Minitab® software version 17.1.2.

Result and Discussion

Susube (*Meistera aculeata* Škorničk. & M.F. Newman) is a newly identified plant species from the Zingiberaceae family, recently discovered in Indonesia, specifically in Konawe Regency, Southeast Sulawesi. Among the local community, particularly the Tolaki ethnic group, the fruit of this plant is consumed and is known to have a taste ranging from sour to sweet. This study focused on the rhizome and fruit parts of the Susube plant. The rationale for selecting these parts is based on the fact that the fruit is edible, while the rhizome represents a characteristic component of the Zingiberaceae family, which is widely used not only as food but also for medicinal purposes.

Therefore, analyzing these plant parts may provide valuable additional information regarding the diversity of bioactive compounds and biological activities within the Zingiberaceae family.

Table 1. Yield of Rhizome and Fruit Extracts of *Meistera aculeata*.

Sample	Mass of Dry sample (g)	Extract Color	Mass of Crude Extract (g)	Yied (%)
Rhizome	128.5	Dark brown	22,7	17,6
Fruit	61.60	Brown	8,89	14,4

The extraction of the rhizome and fruit of Susube was performed using the maceration method, due to its simplicity, widespread use, relatively low cost, and the advantage of not requiring high temperatures, which could degrade sensitive chemical compounds. Ethanol was chosen as the extraction solvent due to its safety, volatility, and environmentally friendly nature compared to other organic solvents, as well as its ability to extract compounds with a wide range of polarity. The ethanol maceration of the rhizome and fruit yielded different extract percentages (Table 1), with the rhizome yielding 17.63% and the fruit 14.44%. The percentage yield reflects the efficiency of the solvent in extracting chemical components from the original sample. A higher percentage yield indicates a greater amount of chemical constituents being extracted from the sample (Adam et al., 2019).

Phytochemical Screening

Phytochemical screening was conducted qualitatively to identify the major classes of compounds present in the extract, namely the secondary metabolites: tannins, saponins, flavonoids, alkaloids, steroids, and terpenoids (Dhanani et al., 2017). The results of the phytochemical screening of the ethanol extracts of the rhizome and fruit of *Susube* are presented in Table 2 below:

Table 2. Qualitative Phytochemical Screening Results of *Susube* Plant Extracts.

Ethanol Extract	Phytochemical Test					
	Alkaloids	Tanins	Flavonoids	Terpenoids	Steroids	Saponins
Rhizome	+	+	+	+	-	+
Fruit	+	+	+	+	-	+

The results of the phytochemical screening of ethanol extracts from the rhizome and fruit of *Susube* (*Meistera aculeata*) (Table 2) revealed the presence of alkaloids, tannins, flavonoids, terpenoids, and saponins. The presence of these secondary metabolites provides preliminary data for evaluating the plant's potential health benefits and for determining their influence on various biological activities, such as anticancer, antioxidant, antimicrobial, anti-inflammatory, and others (Musdalipah et al., 2021).

Antioxidant Activity

Antioxidant activity refers to the ability of a compound or substance to counteract or neutralize free radicals that can damage body cells (Munteanu & Apetrei, 2021). One of the key indicators of a compound's ability to scavenge or neutralize free radicals is the IC_{50} value. IC_{50} (Inhibitory Concentration 50%) represents the concentration of a substance required to inhibit 50% of free radical activity. The antioxidant activity assay of *Susube* (*Meistera aculeata*) was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The results of the antioxidant activity of the ethanol extracts from the rhizome and fruit of *Susube* in scavenging DPPH free radicals are presented in Table 3 below:

Table 3. Antioxidant Activity (IC_{50}) of Ethanol Extracts from Rhizome and Fruit of *Meistera aculeata*.

Sample	IC_{50} (µg/mL)	Category of antioxidant activity*
Rhizome	4.9	Very Strong
Fruit	4.5	Very Strong
Ascorbat Acid	2.2	Very Strong

*Itam *et al.,* , 2021

Table 3 shows that the ethanol extracts of the rhizome and fruit of *Susube* (*Meistera aculeata*) exhibited very strong antioxidant activity, with IC_{50} values of 4.9 and 4.5

μg/mL, respectively. This is supported by the IC_{50} value of ascorbic acid as the positive control, which was 2.2 μg/mL. Antioxidant activity is considered very strong when the IC_{50} value is less than 50 μg/mL, strong when between 50 and 100 μg/mL, moderate between 101 and 250 μg/mL, weak between 250 and 500 μg/mL, and inactive if greater than 500 μg/mL (Itam et al., 2021; Molyneux, 2004). Natural sources of antioxidants are primarily plants, including vegetables, fruits, spices, and herbs that are rich in vitamins, phenolic compounds, carotenoids, and trace elements (Flieger et al., 2021). Natural antioxidants have the ability to inhibit oxidation processes and the growth of microorganisms, including many pathogens such as *Salmonella* spp. and *Escherichia coli* (Cetin-Karaca & Newman, 2015). The very strong antioxidant activity observed in *Susube* (*Meistera aculeata*) is likely due to the presence of phenolic secondary metabolites, particularly flavonoids. This is further supported by previous studies that demonstrated similarly strong antioxidant activity in ethanol extracts of fruit from another species in the same genus, *Meistera chinensis*, which showed an IC_{50} value of 47.6 μg/mL (Musdalipah et al., 2021). Therefore, the presence of *Susube* (*Meistera aculeata*) may offer significant contributions to the antioxidant potential found in plants of the Zingiberaceae family.

Cytotoxic Activity

Cytotoxic activity refers to the ability of a compound or extract to inhibit the growth or induce the death of living cells, particularly cancer cells or specific target cells. One of the key indicators of a compound's or extract's cytotoxic potential is the LC_{50} value. LC_{50} (Lethal Concentration 50) is a measure of toxicity that indicates the concentration of a compound or extract required to cause the death of 50% of test organisms within a given period.

The results of the toxicity test using the Brine Shrimp Lethality Test (BSLT) method on the rhizome and fruit extracts of *Susube* (*Meistera aculeata*) are presented in Table 4 below:

Table 4. LC_{50} Values from Cytotoxicity Testing of *Susube* (*Meistera aculeata*) Extracts.

Sample	LC_{50} (μg/mL)	Category of Cytotoxicity
Rhizome	958	Displayed toxicity
Fruit	578	Displayed toxicity
Ascorbat Acid	2,8	Toxic (strong)

*If $LC_{50} \leq 30$ μg/mL is toxic, < 1000μg/mL is displayed toxicity, and >1000 μg/mL is not toxic (Meyer *et al.*, , 1982)

The BSLT method is a simple preliminary toxicity assay used to detect the cytotoxic properties of bioactive compounds, employing *Artemia salina* larvae as a model organism (Geethaa et al., 2013). The principle of this assay is to measure the mortality rate of *Artemia salina* larvae following exposure to a compound or extract. The faster and greater the number of dead larvae, the more toxic the compound or extract is considered to be. Based on the cytotoxicity test results, larval mortality caused by the positive control (potassium dichromate) showed an LC_{50} value of 2.8 μg/mL. This result is not substantially different from the ethanol extracts of the rhizome and fruit of *Susube* (*Meistera aculeata*), which had LC_{50} values of 958 μg/mL and 578 μg/mL, respectively. The death of *Artemia salina* larvae was induced by the presence of the ethanol extracts of *Susube* and the secondary metabolites they contain (see Table 2), such as alkaloids, flavonoids, tannins, terpenoids, and saponins.

These findings are also consistent with our previous studies, which showed that the ethanol extract of *Susube* fruit (*Meistera aculeata*) contained alkaloids, flavonoids, tannins, terpenoids, and saponins and exhibited toxicity against *Artemia salina* larvae with an LC_{50} value of 683.9 μg/mL. Therefore, the observed cytotoxic properties of the rhizome and fruit of *Susube* (*Meistera aculeata*) may contribute to the limited data available on the cytotoxic activity of plants from the *Meistera* genus. These findings help expand the biological activity database of the Zingiberaceae family.

Conclusion

Based on the data and findings of this study, it can be concluded that the antioxidant activity of the rhizome and fruit extracts of *Susube* (*Meistera aculeata*) in scavenging DPPH free radicals was classified as very strong, with IC_{50} values of 4.9 μg/mL and 4.5 μg/mL, respectively, compared to the positive control, ascorbic acid, which had an IC_{50} value of 2.2 μg/mL. In addition, cytotoxic activity against *Artemia salina* larvae was also observed, with LC_{50} values of 958 μg/mL for the rhizome extract and 578 μg/mL for the fruit extract, compared to the positive control potassium dichromate ($K_2Cr_2O_7$), which had an LC_{50} value of 2.8 μg/mL. The antioxidant and cytotoxic activities of the rhizome and fruit of *Susube* (*Meistera aculeata*) are influenced by the presence of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and saponins.

Conflict of Interest

The authors declare that there is no conflict of interest.

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