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Phytochemical Profile and Acute Toxicity of *Meistera aculeata* (Roxb). Skornick. and M.F. Newman Fruits (*Zingiberaceae*)

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Abstract. *Meistera aculeata* (Roxb). Skornick. and M.F. Newman belongs to the ginger family (*Zingiberaceae*) and locally known as 'Susube' by natives of Konawe district of Southeast Sulawesi. Susube is an endemic plant and its fruit has been consumed by the locals. Until today, study on chemistry and biological activity aspects of susube has yet investigated. Hence, this research aimed to identify phytochemical contents and acute toxicity of susube fruits extracted using ethanol as the solvent. Phytochemical screening showed the accumulation of alkaloids, tannins, flavonoids, terpenoids, and saponins in the ethanol extract of susube fruits. Meanwhile, the Brine Shrimp Lethality Test (BSLT) revealed that ethanol extract of susube ruits has a weak acute toxicity (LC_{50} 683,9 ppm) when compared with potassium dichromate as the positive control of the assay (LC_{50} 8,3 ppm). The study concluded that *M. aculeata* (Roxb). Skornick. and M.F. Newman fruits could be used in the development of drugs from natural sources.

Introduction

The people of Indonesia have long been used natural medicines or traditional medicines. Traditional medicines are more easily accepted by the community because they are familiar in usages, not to mention the price that is cheaper than drugs and easier to obtain from local healers (Makalalag et.al, 2011). Traditional medicines derived from plants have been extensively studied for their chemical contents and usefulness. However, the toxicity effect of many plants has yet investigated and thus requiring further studies (Hyeronimus, 2008). Medicinal plants are natural ingredients that have been used for generations based on experience and suggested to be safer. Hence, more interests have been acknowledged for medicinal plants products. In addition, the raising of public awareness towards benefits of medicinal plants to maintain health and treat diseases also influenced the development of products from medicinal plants (Nursiyah, 2013).

Southeast Sulawesi is one of the provinces in Indonesia with 1000 kinds of traditional medicinal plants that have been used by various ethnics. This provides confirmation that the plants in Southeast Sulawesi have the potential to be investigated. One of the potential local plants is *Meistera aculeata* (Roxb). Skornick. and MF Newman) from the ginger family (Zingiberaceae) and known by locals as 'susube'. Aswani et al. (2021) stated that this plant was first reported in 2018 in the journal Taxon 67(1): 25 and is synonymous with the plant *Amomum aculeatum* Roxb. This plant is distributed in several countries, namely India, Myanmar, Thailand, Vietnam, Indonesia, the Malay Archipelago, Malaysia, also extending across Wallace's Line to Sulawesi, New Guinea, and Australia. This plant grows at elevations of 500–600m on semi-evergreen forest floors covered with humus. live in small populations with an average of 20–30 mature plants that can be observed within 100m.

The genus *Meistera* is considered as newly identified plants. Some species under this genus have been reported such as *M. chinensis* and *M. aculeata* (Roxb). Skornick. and MF Newman (Susube), which are endemic to Southeast Sulawesi. The species *M. chinensis* has been evaluated for cytotoxicity activity and phytochemical contents

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(Musdalipah et al., 2021), In vivo toxicity to mice (*Mus musculus*) (Musdalifat et al., 2022), antioxidant (Rusli et al., 2023) and antibacterial against *Staphylococcus aureus* ATCC 25023 and *Eschericia coli* ATCC 35218 (Karmilah et al., 2023). Meanwhile, no further information has been reported for *M. aculeata* (Roxb). Skornick. and M.F. Newman. Hence, the present study investigated the phytochemical contents in the susube fruits (*M. aculeata* (Roxb). Skornick. and M.F.) and evaluated its acute toxicity towards brine shrimp *Artemia salina* Leach.

Experimental

Material and Methods

Sample of *M. aculeata* (Roxb). Skornick. and M.F. Newman was collected from Totombe Jaya Village, Konawe regency, Southeast Sulawesi. The morphology of M. aculeata is displayed in Figure 1. The plant sample was identified by the Biology Research Center, Life Sciences Research Organization with a voucher specimen number of B-085/V/DI.05.07/9/2021.

Ethanol (C_2H_5OH, 95%), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), ferric chloride ($FeCl_3$, 0,1%), magnesium (Mg) band, Dragendorff reagent, methanol (CH_3OH), chloroform ($CHCl_3$), potassium dichromate ($K_2Cr_2O_7$), distilled water, and seawater.

Procedures

Sample preparation

The fruits of *M. aculeata* (Roxb). Skornick. and M.F. Newman were washed, drained, cut into small pieces, and dried in an oven at 40 °C. Then, the dried fruits were coarsely powdered using a blender.

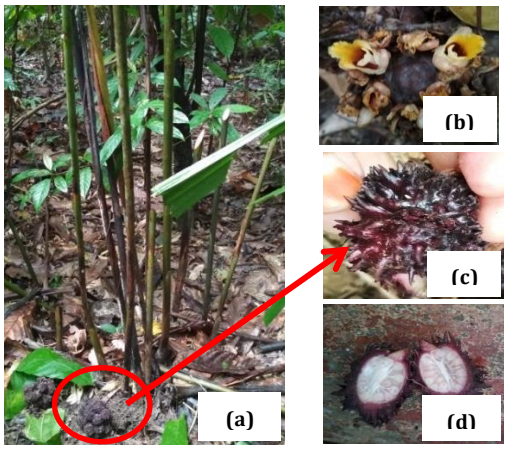

Figure 1. Liquid Morphological characteristic of Meistera aculeate (Roxb). Skornick. & M.F. Newman: whole plant (a), flower (b), fruits (c), and sliced fruit (d).

Sample Extraction

The coarse powder of fruits (22.22 g) was extracted by using a maceration technique. Powders soaked in 95% ethanol for 24 hours at room temperature for three days. Then, the liquid extract filtered manually and the solvent evaporated using a vacuum rotary evaporator to yield a crude extract (1.76 g) The crude extract was stored in a refrigerator until further experiments.

Phytochemical Screening

Phytochemical screening of saponins, alkaloids, tannins, flavonoids, and terpenoids in the extract of susube fruits (*M. aculeata* (Roxb). Skornick. and M.F. Newman) was carried out according to Sabandar et al. (2020) as follows.

Saponins Test. The extract (0.02 g) was put into a test tube and added with H_2O (heated to boiling), then shaken vigorously for 1 minute and observed for foam, if the foam remained and did not disappear for more than one minute, then the sample was positive for saponin.

Alkaloids Test. The extract (0.02 g) was put into a test tube and 2.5 mL of 1% HCl was added and shaken until dissolved. Then 5 drops of Dragendorff reagent were added and the mixture observed. If a reddish orange color is formed in the solution, then the sample is positive for alkaloids.

Tannins Test. The extract (0.02 g) was put into a test tube and adding 5 mL of distilled water and dissolving it. Then 5 drops of 1% $FeCl_3$ solution were added. A positive test is indicated by the presence of brownish green or blackish blue.

Flavonoids Test. The extract (0.02 g) was put into a test tube and dissolved with MeOH. The solution added with 1 mL of concentrated HCl and Mg band (0.5 cm), followed by heating the test tube in a water bath. A positive result is indicated by a reddish change in the mixture.

Terpenoids Test. The extract (0.02 g) was put into a test tube and dissolved using $CHCl_3$. The solution added with 3 mL of H_2SO_4 slowly until layers formed. A positive result is indicated by a reddish-brown color change between layers.

Acute Toxicity Assay

The acute toxicity assay of extract of *M. aculeata* fruits was carried out using a brine shrimp toxicity test (BSLT) method according to Musdalipah et al. (2021).

Hatching Artemia salina Nauplii. One liter of seawater is prepared and filtered to a hatching aquarium. Dried cysts of *A. salina* (1 g) were prepared for hatching. Hatching was carried out by immersing the cysts into 800 mL of seawater with aeration for two days. To separate nauplii from their shells, an incandescent lamp was used to attract the

phototropic nauplii populations, leaving the hatching side to the collection side of the aquarium.

Preparation of Sample Solutions. The extract (0.02 g) of susube fruit is dissolved in 1000 µL of seawater to obtain a stock solution of 20.000 ppm. Two-folds of serial dilutions of extract were made from concentration of 15.6 to 1000 ppm. The concentrations of potassium dichromate as the positive control of the assay also made with similar manner. Meanwhile, seawater used in this assay was inoculated with yeast (6 mg/L) as the food for the nauplii.

Acute Toxicity Test. Serial solutions of extract and potassium dichromate as well as seawater (normal control) were added into a 96-well microplate, each for 100 µL. To each solution in the well, a 100 µL seawater containing 10-12 nauplii was added. Observations of death nauplii were made after 24 hours of incubation under incandescent light and room temperature. The numbers of survivors were counted and the percentage of deaths were calculated. The LC_{50} value was determined by a linear regression analysis using the Minitab 17 application. Mortality is calculated in the following way: Graph is made with log concentration as the x-axis to mortality as the y-axis. The LC_{50} value is the concentration where the substance causes 50% death which is obtained using the linear regression equation. Toxicity was determined by the LC_{50} value obtained, if $LC_{50} \leq 30$ µg/mL is toxic, < 1000 µg/mL is displayed toxicity, and >1000 µg/mL is not toxic (Meyer et al., 1982). Comparison between extract and potassium dichromate was evaluated using ANOVA with Tukey's Test

Result and Discussion

Sample preparation

The sample used in this study was fresh susube fruit (*Meistera aculeata* (ROXB). Skornick. and M.F. Newman). The sample is washed clean to remove dirt in the form of soil on the fruit which can interfere with the extraction process. Then the sample is cut into small pieces to increase the surface area, thereby speeding up the drying and grinding of the sample into powder. Samples were dried in an oven at 40 °C until dry. The purpose of drying is to reduce the water content and prevent mold growth so that samples can be stored for a long time and are not damaged, so that the chemical composition is not easily changed. The dried samples were then mashed using a blender. The aim of the sample to be crushed into powder is so that the separation process produces maximum extract and facilitates extraction. The sample powder that has been obtained is then weighed as much as 22.22 g, then extracted by maceration. Maceration was carried out by immersing the sample in a closed container using 96% ethanol for 3×24 hours. Every 1×24 hours is filtered. The maserate obtained was then evaporated using a vacuum rotary evaporator to obtain a concentrated extract which would be used for phytochemical screening and toxicity tests.

Phytochemical Screening

Saponin Test. Testing for saponins was carried out by foam test, namely by adding distilled water to the extract and heating it to a boil, then shaking it vigorously for 1 minute. In this test the positive saponin extract was due to the presence of foam, in which the foam lasted for more than five minutes. The presence of foam in the saponin test is due to the presence of glycosides which can form foam in water and hydrolyze into glucose and other compounds.

Alkaloid Test. Test for the presence of alkaloid compounds by inserting a small amount of sample extract in a test tube, then adding HCl. The purpose of adding HCl is because alkaloids are alkaline so they are usually extracted with acidic solvents. Qualitative proof of the presence of an alkaloid compound in the sample can be proven by using Dragendorff's reagent. The alkaloid test results in the ethanol extract study were positive for containing alkaloid compounds due to a reddish-orange color change when Dragendorff's reagent was added. The presumptive reactions that occur in the alkaloid test are as follows:

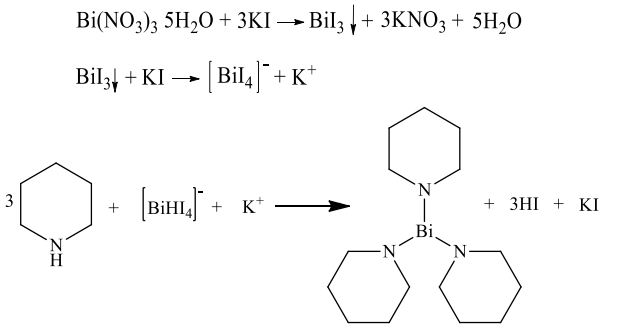

Figure 2. The alleged reaction between alkaloids and Dragendorff reagent.

Tannin Test. The test for tannin compounds in this study was by adding the extract with distilled water and shaking until homogeneous, then adding a 1% $FeCl_3$ solution, the positive results were indicated by a brownish-green color change. This color change is due to the formation of complex compounds between Fe metal and tannins. Complex compounds are formed due to coordination covalent bonds between metal ions/atoms and non-metal atoms (Figured 3).

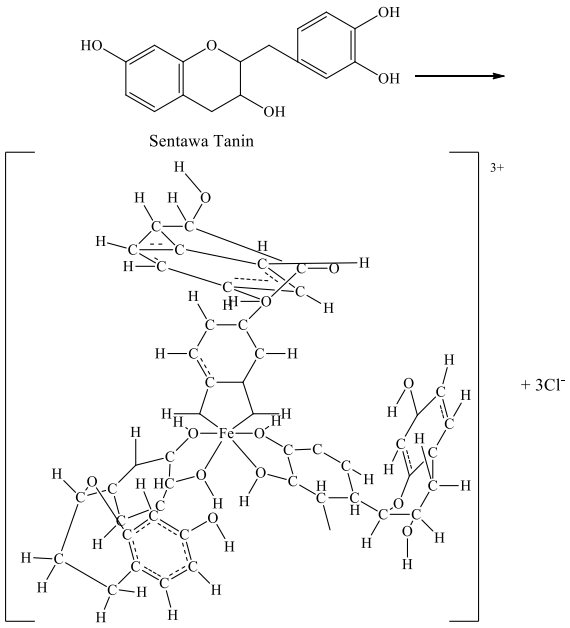

Figure 3. The proposed reaction between tannins and 1% $FeCl_3$.

Flavonoid Test. The flavonoid test was carried out by taking 0.02 g of extract which was dissolved in methanol, concentrated HCl and Mg band, then heated and the positive result was due to a red color change. The addition of concentrated HCl in the flavonoid test was used to hydrolyze the flavonoids into their alglycones by hydrolyzing O-glycosyl (Figured 4).

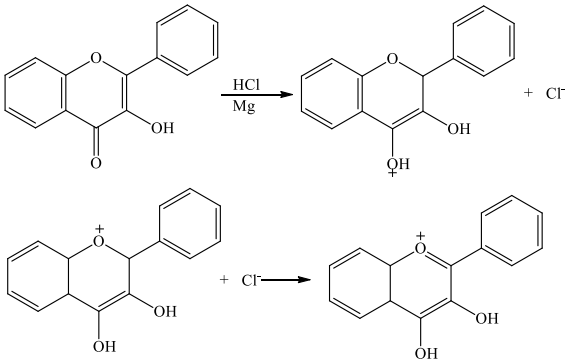

Figure 4. The reaction between flavonoids and Mg and HCl

Terpenoid Test. The terpenoid compound test in this study was by adding the extract which was dissolved with CH_3Cl in a test tube and slowly adding concentrated H_2SO_4 and forming a ring and a reddish-brown color change between layers, this indicated that the extract in the sample was positive for the compound. terpenoids. This is based on the ability of triterpenoid compounds to form color by H_2SO_4 in acetic anhydride solvent. The color differences produced by triterpenoids are due to differences in groups on the C-4 atom (Figure 5).

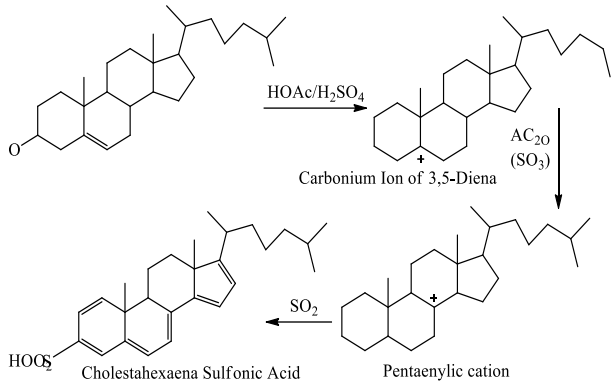

Figure 5. The reaction mechanism for the terpenoid test

Toxicity Test with the BSLT Method

Egg Hatching. The Hatching of eggs is done by placing Artemia salina Leach eggs into sea water while being aerated to contact with air for 48 hours. The process of hatching artemia salina leach eggs has several stages, namely hydration, breaking of the shell, and the paying or expulsion stage. In the hydration stage, water absorption occurs so that the eggs preserved in dry form will become spherical and actively metabolize. The next stage is the rupture of the shell followed by the breaking of the umbrella which occurs a few moments before the naupil (lava) comes out of the shell as shown in Figure 6.

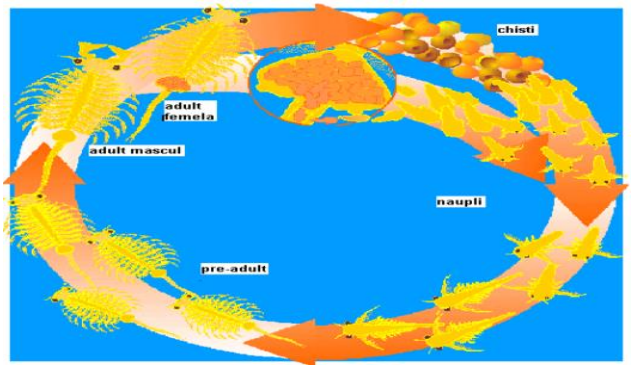

Figure 6. Life cycle of Artemia salina.

Susube fruit extract (*M. aculeata*) and chloroform ($CHCl_3$) solutions were prepared at concentrations of 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.2 ppm, 15.6 ppm, 7.8 ppm. As well as a 0 ppm control solution, namely the solvent without the addition of extracts. A. salina shrimp lava used as test animals, namely 10-12 as toxicity test animals in each concentration. This toxicity test treatment was repeated 3 times to get the accuracy of the data.

Toxicity Test

Toxicity testing was carried out three repetitions on the

sample. The test solution was prepared from a 1000 ppm stock solution of 2 mL by taking 100 µL of the extract into a microplate. Controls (potassium dichromate) were made in the same way, namely by making the same solution without adding extracts. The results of the toxicity test of the susube fruit extract were analyzed using the Minitab 17 program with a 95% confidence level.

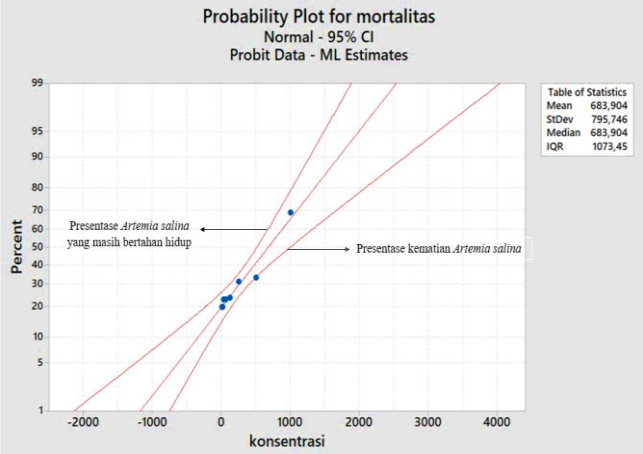

Figure 7. Life Mortality curve of shrimp *Artemia Salina* Leach ethanol extract of susube fruit.

The curve above shows the log concentration (X axis) to the probit value (Y axis) obtained from the mortality of Artemia salina larvae. The LC_{50} value of the susube extract was 683.9 ppm and for potassium dichromate (control) was 8.3 ppm. Based on Figure 7, it shows that the right side of the curve shows the percentage of *A. salina* deaths, while the area to the left of the curve shows the percentage of *A. salina* that are still alive at each concentration of susube fruit extract. The addition of the extract caused the death of *Artemia salina*, which experienced a disorientation of motion (irregular movements). The use of *A. salina* in the BSLT method is because it has the same response or stress response as humans in the form of behavioral and physiological responses to environmental stressors (Nuralifa et al., 2021).

The cause of death of *Artemia salina* larvae is due to a change in the drastic concentration gradient between inside and outside the cell, causing the compound to become toxic and can spread to the body of the shrimp larvae. The damaging effects of metabolism occur quickly and can be detected in a short time for 24 hours, causing up to 50% death (Mokosuli, 2021).

Based on the results of cytotoxic testing on the Susube fruit extract, the LC_{50} was 683.904 ppm and categorized as toxic. According to Meyer et al. (1982), that an extract shows toxic activity in BSLT if the extract can cause the death of 50% of test animals at a concentration of less than 1000 ppm.

Conclusion

Phytochemical tests on the ethanol extract of Susube fruit (*Meistera aculeata* (Roxb). Skornick. and MF Newman) showed the presence of secondary metabolites which were characterized by positive results in the saponin test, alkaloid test, tannin test, flavonoid test, and terpenoid test. Susube fruit ethanol extract (*Meistera aculeata* (Roxb). Skornick. and MF Newman) is toxic with an LC_{50} of 683.904 ppm

Conflict of Interest

The author declares that there is no conflict of interest. The author is fully responsible for the content and writing of this article.

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