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Research paper

Analysis of Flavonoid and Toxicity Test of *Calamus scipionum* Lour Leaf Ethanol Extract Using Brine Shrimp Lethality Test (BSLT)

Analisis Flavonoid dan Uji Toksisitas Ekstrak Etanol Daun Calamus scipionum Lour Menggunakan Brine Shrimp Lethality Test (BSLT)

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ABSTRACT

Calamus scipionum Lour is rattan that grows wild in the forest areas of Indonesia, especially in southern Sumatra, Kalimantan, and Sulawesi. This study aims to determine percent yield, phytochemical tests, levels of flavonoids, and toxicity of the ethanol extract of *Calamus scipionum* Lour leaves using the BSLT method using *Artemia salina* leach larvae test. The percent yield of ethanol extract was contained 9.36%. The phytochemical test of the ethanol extract of the *Calamus scipionum* Lour leaves contained secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and steroids. The test results for the levels of flavonoids at 1000 ppm obtained a value of 53.307 µg/ml and the toxicity test of the ethanol extract of *Calamus scipionum* Lour leaves obtained an LC_{50} 130.390 ppm which was toxic.

***Keywords:**: flavonoids, toxicity, sembu rattan, BSLT, Artemia salina leach*

INTRODUCTION

Indonesia is a tropical country crossed by the equator and has a diverse biodiversity of traditional medicinal plants (Arifin et al., 2019). Traditional medicinal plants have secondary metabolite compounds such as flavonoids, alkaloids, tannins, terpenoids, saponins, and others (Fatimah & Santoso, 2020). Secondary metabolites present in plants have diverse potential pharmacological activities. Pharmacological activities produced from these plants such as natural insecticides (Darmadi & Meilasri, 2019), antioxidants (Sakka & Muin, 2022), antibacterials (Mayasari, 2022), anti-inflammatory (Octavian, 2022), and toxicity (Yani et al., 2022).

The rattan plant is commonly used by the community as a material for making handicrafts and household needs such as woven mats, fish traps, baskets, rice sieves, and head protectors (Fendri, 2021). People also utilise rattan tubers as food and traditional medicine (Kalima, 2022). Rattan is a monocotyledonous plant with a flexible stem generally found in humid tropical forests (Saputra et al., 2019). One type of rattan that can be utilized is *Calamus scipionum* Lour. The rattan that grows wild in the forest areas of Indonesia, especially in southern Sumatra, Kalimantan, and Sulawesi (Mayasari, 2022). *Calamus scipionum* Lour is a tuber that is used by the people of South Sumatra Province as a dish that is processed by stir-frying coconut milk. The tuber part of the rattan is also used as a medicine for asthma, mouth ulcers, and stomach pain which is processed by boiling and then eaten. *Calamus scipionum* Lour also has annual fruits that can be consumed. However, the leaves of *Calamus scipionum* Lour have no known benefits.

Leaves are plant parts that are more abundant than stems (Sada & Jumari, n.d.). Old leaves have more secondary metabolites than young leaves because the increasing age of the leaves will affect the secondary metabolite compounds and bioactive compounds produced (Malik & others, 2022). An important thing to do before determining the pharmacological potential of *C. scipionum* Lour leaves is to conduct an initial toxicity study.

Toxicity tests of plant extracts are carried out to determine the safety level of an extract and one of the prerequisites for a plant to be developed as a medicine. One method that can be used for toxicity testing is the BSLT with *Artemia salina* Leach larvae as test animals (Fatimah & Santoso, 2020). The BSLT method is the most rapid, reliable, and inexpensive way, and the results obtained are often related to the cytotoxic activity of the extract. Therefore, in this study, an initial screening test was carried out on the toxicity level of bioactive compounds of old leaves of *C. scipionum* Lour. The initial screening carried out was a toxicity test using the *BSLT* method by looking at the LC_{50} value.

METHODOLOGY

Tools

The tools used are glass jars, plastic jars, blenders, beakers *(pyrex)*, micropipettes, microchips, measuring cups*(py*rex), drop pipettes, measuring pipettes, suction pipettes, spatulas, stirring rods, test tubes (*pyrex*), volumetric flasks *(pyrex)*, vials, labels, plastic wrap, analytical balance type ABJ 320-4NM (kren), rotary evaporator (Buchi), aerators and lights for hatching *Artemia* salina shrimp larvae.

Materials

The sample used was the leaves of the *C. scipionum* Lour plant obtained from Sungai Rotan District, Muara Enim Regency, South Sumatra Province. The materials used in this study are *C. scipionum* Lour leaves, distilled water, ethanol, filter paper, *methylene blue salt*, artemia salina eggs, quercetin, $AlCl_3$, CH_3COONa, iron (III) chloride, hydrochloric acid, magnesium powder, sulfuric acid, chloroform,dimethyl sulfoxide, *wagner* reagent.

Sample Preparation and Extraction

The *C. scipionum* Lour leaves are collected and then cleaned of other impurities and then dried. After the dry ingredients were mashed using a blender then test material was weighed again. The *C. scipionum* Lour leaves ethanol extract was prepared using the maceration method. The *C. scipionum* Lour leaves powder was extracted using 1 L of ethanol solvent and then separation using maceration technique. The extract is filtered using filter paper so that the filtrate is obtained. The extract obtained was evaporated using a rotary vacuum evaporator at 40°C. The extract obtained was then weighed to obtain the percent yield (Marzuki et al., 2019).

Qualitative analysis

Qualitative examination of the compounds found in *Calamus scipionum* Lour leaves, including assays for tannins, steroids, alkaloids, flavonoids, and saponins (Setyawaty, 2020).

- **Flavonoids :** Three drops of strong hydrochloric acid and two grams of magnesium powder were added to a test tube containing the sample extract. After shaking the extract, color changes were noted. Flavonoids that are positive if extract change color to yellow, orange, and red.
- **Alkaloids** : A test tube containing the sample extract was filled with 1-2 milliliters of Dragendorff's reagents. The development of a reddish-brown precipitate indicates the presence of alkaloids in positive extracts.
- **Saponin** : Ten milliliters of hot distilled water were added to a test tube containing two milliliters of the sample extract. The filtrate in the test tube is forcefully shaken for approximately 30 seconds after it has cooled. If foam forms with a height of at least 1 cm, remains for 10 minutes, and does not disappear when 1 drop of diluted hydrochloric acid is added, the saponin test is positive.
- **Tannins** : A test tube containing two milliliters of the sample extract was filled with a 10% iron (III) chloride solution. If the extract turned dark blue, black blue, or greenish black, it indicated the presence of tannin components.

Determination of flavonoid content

The sample extract of 2 ml was put into a test tube and then added with 10% iron (III) chloride solution if dark blue, black blue, or greenish black showed the presence of tannin compounds. Next, determine the maximum wavelength of quercetin by pipetting 0.5 mL of quercetin standard master solution, adding 0.1 mL of $AlCl_3$ and 0.1 mL of CH_3COONa 1 M and 2.8 mL of distilled water, and then incubating for 30 minutes. The solution was measured at a maximum wavelength of 400 nm - 800 nm using a UV-Vis spectrophotometer.

A quercetin calibration curve was prepared using quercetin standard solution at concentrations of 20, 30, 40, 50, and 60 ppm. Quercetin standard solution of 0.5 mL was added 0.1 mL $AlCl_3$ 0.1 mL CH_3COONa 1 M and 2.8 mL distilled water. The mixture was stirred until homogeneous then left for 30 minutes. The absorbance of each concentration of the standard solution was measured by UV-Vis spectrophotometer (400-800 nm) at the maximum wavelength. The flavonoid content of *Calamus scipionum* Lour leaf extract using a UV-Vis spectrophotometer at the maximum wavelength and then calculate the flavonoid content using the quercetin standard curve (Chotimah, 2019).

Toxicity Test

Toxicity testing of ethanol extracts of *C. scipionum* Lour leaves using the BSLT method. Artemia salina Leach larvae were put into 1 L of distilled water plus 30 grams of methylene blue salt into a glass container. The container was aerated using an aerator for 1 hour and *Artemia* salina Leach eggs. The shrimp eggs were given a 40 watt lamp for 1 hour to warm and stimulate the hatching process. (Kurniawan & Ropiqa, 2021).

C. scipionum Lour leaf extract solution was made at a concentration of 2000 ppm. Ethanol extract as much as 5 mg of extract plus 1 ml of DMSO and 50 mg of methylene blue salt and distilled water until the volume of 25 mL. The extract solution was made in concentration variations of 25 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 1000 ppm. Ten shrimp larvae were put into bottles containing 9 ml of methylene blue salt solution and 1 ml of extract solution. The test bottles were then kept in a well-lit place for 24 hours. The number of dead larvae was observed and the number of larval deaths was calculated as percent mortality. Percent larval mortality after 24 hours of treatment using the formula:

$$\% \text{ Mortality} = \frac{number\ of\ larval\ deaths}{number\ of\ test\ larvae}\ x\ 100\ \%$$

RESULTS AND DISCUSSION

Extraction Results

Secondary metabolite compounds obtained in this study are expressed in percent yield. The total percent yield of ethanol extract of Calamus scipionum Lour leaves obtained was 9.36%.

Qualitative test results

The qualitative test results were carried out to determine qualitative secondary metabolite compounds. The classes of secondary metabolites tested were flavonoids, alkaloids, saponins, terpenoids and tannins. The qualitative test results for the secondary metabolites contained in the ethanol extracts of *Calamus scipionum* Lour leaves can be seen in **Table 1**.

Table 1. Qualitative test results for the secondary metabolites contained in the ethanol extracts of Calamus scipionum Lour leaves

Compound	Young areca seed extract	Old areca seed extract	indicating positive test
flavonoids	(+)	(+)	Red, Yellow, and Orange
alkaloids	(+)	(+)	a reddish-brown precipitate
saponins	(+)	(+)	Foam
tannins	(+)	(+)	Dark blue or blackish green

The results of phytochemical screening of the ethanol extracts of *C. scipionum* Lour leaves show that the content of secondary metabolite compounds containing flavonoids, alkaloids, tannins, and saponins.

Analysis of Flavonoid

Analysis of flavonoid content aims to determine the amount of flavonoids contained in the sample, the amount of flavonoids in a sample is determined based on the standard. The standard solution used is the quercetin compound. The quercetin calibration curve obtained can be used to help determine the levels of flavonoid compounds in the sample. The absorbance measurement of quercetin standard solution at λmax = 413 nm is shown in Figure 1.

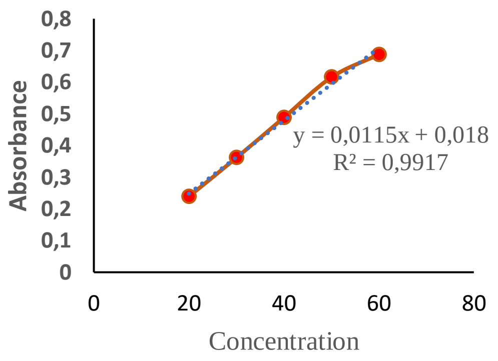


Figure 1. Quercetin Calibration Curve at λ_{max} at 413 nm

The standardized results of quercetin obtained a linear regression equation Y = 0.0115x + 0.018 with an r value obtained of 0.9917. This quercetin calibration curve equation is used as a comparison to determine the concentration of flavonoid compounds in the sample. Based on the research that has been done, the flavonoid content of the ethanol extract is 53.307 µg/mL. *C. scipionum* Lour leaf extract that has been measured is a compound a structural framework similar to the standard used quercetin. Therefore, it can be concluded that the data obtained is a flavonoid compound of the flavonol group.

Secondary metabolite compounds such as the flavonol group have a major role in cytotoxic cancer cells. Flavonoid compounds have an acute toxicity level that causes death in test larvae within 24 hours. One of the flavonoid mechanisms is related to antioxidant activity through of activating the apoptotic pathway of cancer cells. Flavonoids have an effect as pro-oxides that can suppress the proliferation of cancer cells by inhibiting the performance of their enzymes (Amalina et al., 2021). Activation of the apoptotic pathway is the programmed death of cancer cells, which aims to maintain the stability of the cell population. Failure to activate the apoptotic pathway can cause cancer cells to divide uncontrollably.

Toxicity Test

The toxicity test in this study used Artemia salina leach larvae that were 48 hours old. The selection of 48-hour-old larvae is because the larvae have a fully formed digestive tract that is sensitive to a substance that will be inserted . The hatching process in shrimp eggs that will become larvae requires aeration using an aerator as a source of oxygen for Artemia salina Leach larvae. In addition, lighting is given using a 40-watt lamp in the hatching process which aims to warm and stimulate the hatching process for 24 hours and make the larvae move towards the bright room because the larvae are phototaxis (Yani et al., 2022). The results of cytotoxic testing of *Calamus scipionum* Lour leaves can be seen in **Table 2**.

Table 2. Toxicity test results table of *C. scipionum* Lour leaves

No	Concentration (ppm)	Number of test larvae	Number of dead larvae			Average mortality	Percent mortality (%)	Probit value
1	0	10	0	0	0	0	0	0
2	25	10	1	2	1	4	13	3,88
3	50	10	2	3	3	8	26	4,37
4	100	10	4	5	5	14	46	4,91
5	250	10	7	7	7	21	70	5,52
6	500	10	8	8	9	25	83	5,59
7	1000	10	10	10	9	29	96	6,82
8	1500	10	10	10	10	30	100	7,33

Based on **Table 1**, the percentage of mortality of Artemia salina Leach larvae increased as the concentration of the sample extract increased. This shows that the higher the concentration, the greater the toxicity of the sample to the death of Artemia sal ina Leach larvae (Yani et al., 2022). The increase in mortality in the test larvae can be seen in the graph of the linear regression value of ethanol extract of *Calamus scipionum* Lour leaves seen in **Figure 2**.

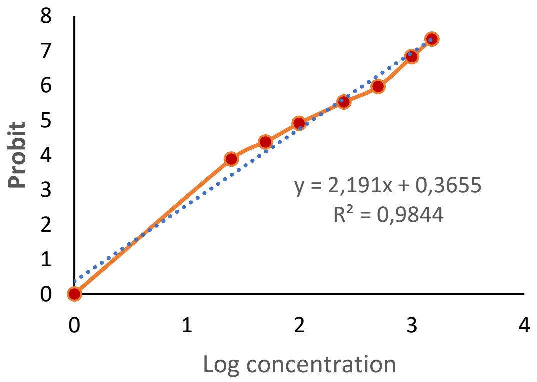
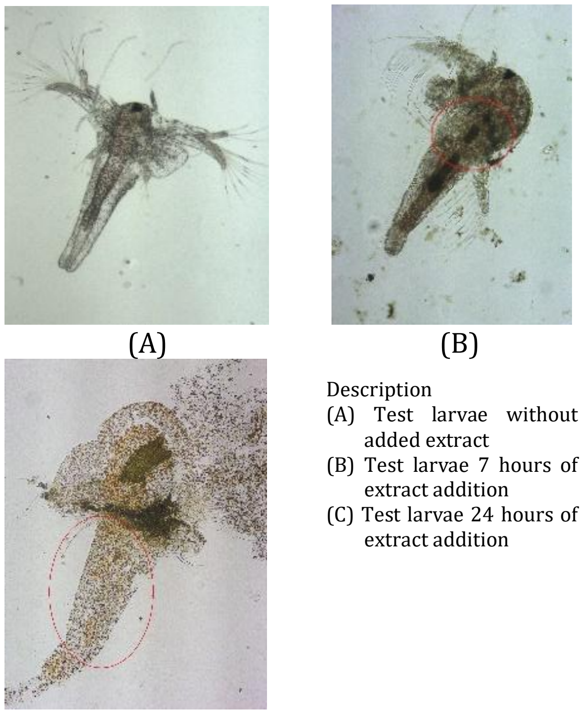


Figure 2. Linear regression of ethanol extract of *Calamus scipionum* Lour leaves

Based on **Figure 2**, the addition of ethanol extract of *Calamus scipionum* Lour leaves to the sample solution will cause an increase in the death of test larvae. The probit value is an analysis of the presumption of the amount of effective dose through the determination of the death concentration. The solution with a concentration of 0 ppm showed no larval mortality. This indicates that there was no toxic effect of the larval transfer during the larval mortality test. The mortality of the test larvae was only influenced by the bioactive compounds (secondary metabolites) in the *Calamus scipionum* Lour leaf extract.

The toxicity activity of *Calamus scipionum* Lour leaf extract can be expressed in the LC_{50} value. The LC_{50} value is the percent mortality of 50% of the test larvae caused by the sample of ethanol extract of *Calamus scipionum* Lour leaves. Based on the toxicity test of ethanol extract of *Calamus scipionum* Lour leaves, the LC_{50} value was 130.390 ppm. This indicates that the ethanol extract tested on Artemia Salina leach larvae has toxic properties. The LC_{50} value is said to be very toxic when ≤ 30 ppm, the LC_{50} value between 30-100 ppm is categorized as strongly toxic and the LC_{50} value between 100-1000 ppm is toxic (Yani et al., 2022). The requirement of a compound can be used as an anti-cancer drug if the LC_{50} value is <1000 ppm. Based on the results obtained, ethanol extract of *Calamus scipionum* Lour leaves has the potential to be developed into anti-cancer drugs.

The toxic properties of cancer cells of *Calamus scipionum* Lour leaf extract can be influenced by the amount of flavonoids contained in the extract. One type of flavonoid that has a large role in cytotoxic cancer cells is flavonol type because the standard quercetin used is flavonol type. The mechanism of flavonols in the extract is related to antioxidant activity, namely through activating the apoptotic pathway of cancer cells (Khoirunnisa & Sumiwi, 2019). Cell damage in the test larvae was observed using a microscope to compare differences in damage to the test larvae. The difference in cell structure in the test larvae in microscopy can be seen in **Figure 3**.



(A) (B) (C)

Description
(A) Test larvae without added extract
(B) Test larvae 7 hours of extract addition
(C) Test larvae 24 hours of extract addition

Figure 3. Microscopic Test of Artemia Salina leach larvae test.

Microscopic observations in Figure 3A show that 48-hour-old larvae still have a complete and perfect metabolic system without damage. Figure 3B shows the digestive system in the test larvae starting to swell after 7 hours of extract addition. Figure 3C shows damage to the cells of the test larvae after 24 hours of extract addition. This shows that Calamus scipionum Lour leaf extract affects cell damage in the test larvae, causing larval death. Secondary metabolite compounds can attack cells in the digestive system of the test larvae resulting in damage and death (Khoirunnisa & Sumiwi, 2019).

CONCLUSIONS

Based on the research that has been done, it can be concluded that the ethanol extract of *Calamus scipionum* Lour leaves contains secondary metabolite compounds in the form of flavonoids, alkaloids, tannins, saponins, and steroids. The ethanol extract of *Calamus scipionum* Lour leaves contains a yield of 9.36%, with flavonoid levels obtained at a wavelength of 413 nm as much as 53.307 μg/mL. The results of the toxicity test of the ethanol extract of *Calamus scipionum* Lour leaves were expressed with an LC_{50} value of 130,390 ppm with a toxic category.

REFERENCES

Amalina, N. D., Mursiti, S., & Marianti, A. (2021). Mengungkap Potensi Aktivitas Antikanker Senyawa Citrus Flavonoid (Citrus sp.). *Pemanfaatan Sumber Daya Alam Indonesia: Ketahanan Pangan, Energi Dan Material Maju*, 1–39.

Arifin, H., Oktavia, S., & Chania, S. (2019). Efek Toksisitas Sub Akut Fraksinasi Air Ekstrak Etanol Daun Bandotan (Ageratum Conyzoides (L.) L.) Terhadap Beberapa Parameter Darah Mencit Putih Jantan. *Jurnal Farmasi Higea*, *11*(2), 166–174.

Chotimah, C. (2019). *Uji total flavonoid dan aktivitas antioksidan ekstrak daun dan kulit batang Dadap Serep (Erythrina subumbrans (Hassk.) Merr.) menggunakan pelarut yang berbeda*. Universitas Islam Negeri Maulana Malik Ibrahim.

Darmadi, D., & Meilasri, S. (2019). Senyawa Metabolit Sekunder Kulit Duku (lansium domesticum corr) Sebagai Penghambat Pematangan Telur ascaris lumbriccoides. *Klinikal Sains: Jurnal Analis Kesehatan*, *7*(2), 68–75.

Fatimah, R., & Santoso, B. S. A. (2020). Toksisitas Akut Dekok Daun Kersen (Muntingia calabura) Menggunakan Metode BSLT (Brine Shrimp Lethality Test). *Jurnal Farmasi Medica/Pharmacy Medical Journal (PMJ)*, *3*(2), 47–52.

Fendri, S. T. J. (2021). Uji Aktivitas Antioksidan Ekstrak Buah Rotan (Calamus Sp) Dengan Mengunakan Metode Dpph. *Jurnal Katalisator*, *6*(2), 223–232.

Kalima, T. (2022). Identifikasi dan Klasifikasi Spesies Rotan di Indonesia. *Prosiding SNPBS (Seminar Nasional Pendidikan Biologi Dan Saintek)*, 33–40.

Khoirunnisa, I., & Sumiwi, S. A. (2019). Peran flavonoid pada berbagai aktivitas farmakologi. *Farmaka*, *17*(2), 131–142.

Kurniawan, H., & Ropiqa, M. (2021). Uji toksisitas ekstrak etanol daun ekor kucing (Acalypha hispida Burm. f.) dengan metode Brine Shrimp Lethality Test (BSLT). *Journal Syifa Sciences and Clinical Research*, *3*(2), 52–62.

Malik, N., & others. (2022). Analisis Metabolit Sekunder Dan Antibakteridaun Sintrong (Crassocephalum crepidioides (Benth.) S. Moore) TerhadapEscherichia coli. *Meditory: The Journal of Medical Laboratory*, *10*(2).

Mayasari, U. (2022). Uji Aktivitas Antibakteri ekstrak Batang Muda Rotan Manau (Calamus manan) terhadap pertumbuhan bakteri Klebsiella pneumonia. *KLOROFIL: Jurnal Ilmu Biologi Dan Terapan*, *6*(1), 9–12.

Octavian, I. P. Y. (2022). Aktivitas Antiinflamasi Ekstrak Etanol Tanaman Patah Tulang (Euphorbia Tirucalli L.). *Humantech: Jurnal Ilmiah Multidisiplin Indonesia*, *1*(7), 902–908.

Sakka, L., & Muin, R. (2022). Identifikasi kandungan senyawa antioksidan ekstrak daun bidara (Ziziphus mauritiana Lamk.) dengan menggunakan metode DPPH. *Journal Syifa Sciences and Clinical Research*, *4*(1).

Saputra, N. G., Idham, M., & Yani, A. (2019). Identifikasi Jenis Rotan Di Kawasan Hutan Adat Dusun Ensibau Desa Semirau Kecamatan Jangkang Kabupaten Sanggau. *Jurnal Hutan Lestari*, *7*(2).

Setyawaty, R. (2020). Preliminary Studies on the Content of Phytochemical Compounds On Skin of Salak Fruit (Salacca zalacca). *Pharmaceutical Journal of Indonesia*, *6*(1), 1–6.

Simamora, A. C. Y., Yusasrini, N. L. A., & Putra, I. N. K. (2021). Pengaruh jenis pelarut terhadap aktivitas antioksidan ekstrak daun tenggulun (Protium javanicum Burm. F) menggunakan metode maserasi. *Jurnal Ilmu Dan Teknologi Pangan*, *10*(4), 681.

Yani, D. F., Wijayanti, F., & others. (2022). Uji Toksisitas Ekstrak Dan Fraksi Kulit Biji Kebiul (Caesalpinia Bonduc L.) Dengan Metode Brine Shrimp Lethality Test. *Al Ulum: Jurnal Sains Dan Teknologi*, *7*(1).