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Yield, Antioxidant Activity, and Toxicity of Ethanol Extracts from Air- and Sun-Dried Sikkam Leaves (*Bischofia javanica* Blume) Obtained by Maceration and Soxhlet Extraction

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ABSTRACT

Sikkam (*Bischofia javanica* Blume) is a species of the genus Bischofia in the Euphorbiaceae family that can be used as a traditional medicine. This study aimed to determine the yield, antioxidant activity, and toxicity of sikkam leaf extract. Sikkam leaves were dried at room temperature (air-dried) and under direct sunlight (sun-dried). The extracts were obtained using the maceration and Soxhlet extraction methods with 96% ethanol as the solvent. The yields of air-dried (A-D) and sun-dried (S-D) sikkam leaf extracts isolated by maceration and Soxhlet extraction differed significantly ($p<0.05$), with average values of 20.48±2.30, 22.32±1.19, 24.31±2.01 and 24.71±0.09% for A-D maceration, S-D maceration, A-D Soxhlet extraction, and S-D Soxhlet extraction, respectively. The antioxidant activity (IC_{50}) of sikkam leaf extracts, tested using the DPPH method, showed significantly different mean values ($p<0.05$), with the following averages: 11.13±1.30, 12.86±2.88, 11.36±1.47, and 14.82±1.19 μg/mL for A-D maceration, S-D maceration, A-D Soxhlet extraction and S-D Soxhlet extraction, respectively. The ethanol extracts of air-dried and sun-dried sikkam leaves isolated via maceration and Soxhlet extraction fall into the category of very strong antioxidant activity. The toxicity (LC_{50}) of the sikkam leaf extracts tested using the Brine Shrimp Lethality Test (BSLT) differed significantly ($p<0.05$), namely 941±37, 914±67, 985±72 and 700±39 μg/mL for A-D maceration, S-D maceration, A-D Soxhlet extraction and S-D Soxhlet extraction, respectively. Ethanol extracts of air-dried and sun-dried sikkam leaves, isolated by maceration and Soxhlet extraction, are classified as non-toxic.

Keywords: Sikkam (*Bischofia javanica*), drying method, yield, maceration, Soxhlet extraction, antioxidant, toxicity

1. INTRODUCTION

Indonesia is a tropical country rich in medicinal plants. One such plant that has been used by local communities as a traditional remedy is the sikkam plant. Sikkam is a type of tree belonging to the genus Bischofia in the Euphorbiaceae family. Sikkam is native to North Sumatra and is widely distributed across the Pacific Islands, Malaysia, Southeast Asia, Southern China, as well as in Tonga and Samoa, including Taiwan, Southern Japan, Myanmar, and India (Rajbongshi, 2014).

According to Sonowal (2022), sikkam typically grows in humid forests with high humidity and is also found in evergreen forests, swamps, and teak forests. This plant is a large tree with tall wood; it can reach a height of 35–50 m and has a cylindrical trunk. The leaves are green, approximately 10.16–20.31 cm long, pinnately compound, elliptical or oblong in shape, and have serrated edges (serrulate). The plant's flowers are unisexual, regular, pentamerous, small, greenish-yellow, and lack petals. The plant typically bears fruit from August to October. The fruit is round, berry-like, fleshy, and exudes a milky sap when squeezed; it is supported by a thin, small stalk and contains an oblong seed approximately 5 mm long. The seed is smooth and glossy, with a flat, egg-white fleshy cotyledon. The wood of this plant is reddish, somewhat hard, and has a small, darker heartwood.

Figure 1. Sikkam (*Bischofia javanica* Blume) plant: bark, leave, flower, and fruit

The chemical constituents of sikkam include carbohydrates, proteins, terpenoids, flavonoids, tannins, steroids, glycosides, saponins, and phenolic compounds that exhibit antibacterial activity against Escherichia coli, a bacterium that infects the digestive tract. These chemical compounds possess antibacterial properties (Sarmah et al., 2020). According to Rajbongshi (2014), the triterpene urosolic acid and the steroid β-sitosterol found in Bischofia javanica Blume exhibit anti-inflammatory effects through COX-1 inhibitory activity.

Sikkam leaves contain several triterpenoid and phenolic compounds, such as betulinic acid, ursonic acid, β-amyrin, chrysoeriol, and quercetin (Mai, 2017). In a study by Manurung (2020), phytochemical analysis of sikkam bark revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids. The compound with the highest content is tannin. According to Rajbongshi (2014), sikkam bark contains epi-fiedelanol acetate, friedelin, as well as tannins, alkaloids, betulinic acid as its ester, and β-sitosterol. The roots of this plant contain β-amyrin, urosolic acid, and β-

sitosterol. Meanwhile, Mai (2020) reported that the methanol extract of sikkam leaves (Bischofia javanica Blume) contains five chemical compounds, namely 5'-β -D-glucopyranosyloxyjasmonic acid methyl ester, 2-(4-hydroxy-3-methoxyphenyl) ethyl-O-β-D-glucopyranoside, hexyl-O-β-D-glucopyranoside, friedelan-3-one, and gallic acid.

Sikkam has traditionally been used to treat inflammatory conditions such as tonsillitis and boils throughout Asia, including China, Indonesia, and the Philippines. However, the antioxidant and anti-inflammatory properties of sikkam remain largely unknown (Lee et al., 2021). People in North Sumatra use sikkam bark as a remedy for diarrhea, wounds, stomach ulcers and acid reflux, as well as a mouthwash (Sitohang, 2020). Additionally, communities in Simalungun Regency, North Sumatra, use sikkam bark as a spice and flavor enhancer in local dishes such as Dayok Nabinatur and Nahinasumba. The sikkam bark used in Nahinasumba dishes imparts a red color and flavor to the dish.

In addition to using sikkam bark, the Simalungun people also use sikkam leaves as a traditional anti-diabetic remedy. They boil the sikkam leaves in 3 cups of water and wait until the volume of water is reduced to 1 cup. The resulting decoction is then strained and can be consumed. This sikkam leaf infusion contains flavonoids that can lower blood glucose levels due to their antioxidant properties (Chauhan et al., 2023). Several studies have shown that sikkam leaf extract exhibits antioxidant and anti-inflammatory properties that hold potential for treating inflammatory diseases and redox imbalances (Leet et al., 2021). The antioxidant activity of Bischofia javanica Blume leaves is demonstrated by significant free radical scavenging properties, particularly by the compound β-amyrin, which has the lowest IC50 value (Sutharson et al., 2009).

Natural antioxidants in plants are primarily classified as phenolic compounds, isoflavones, and flavonoids (Sukardi, 2017). According to Berawi (2018), antioxidants play a crucial role in preventing oxidative stress that the body may experience. Antioxidant compounds are electron-donating compounds that can reduce the level or number of free radicals and mitigate the effects of oxidative stress triggered by free radicals. Scientifically, antioxidants are of significant interest due to their numerous benefits, including anti-inflammatory and anti-aging properties (Zehiroglu et al., 2019). Furthermore, both synthetic and natural antioxidant compounds can help regulate blood glucose levels and prevent diabetes complications (Erlidawati et al., 2018). One natural source of antioxidants capable of lowering blood sugar levels is the sikkam plant (Sukardi, 2017).

Rumahorbo (2021) reported that ethanol extract of sikkam leaves demonstrated the ability to lower blood glucose levels and improve the histopathology of the islets of Langerhans in rats. The most effective dose of sikkam leaf ethanol extract for its antidiabetic effects was 900 milligrams per kilogram of body weight. In a study by Lingadurai et al. (2011), methanol extract of sikkam leaves demonstrated significant cytotoxicity ($P<0.001$) in leukemia cell lines. It exhibited a very low IC50 value of 3.5 μg/ml after a 72-hour incubation period in Human Leukemia 60 (HL-60) cells in vitro. These findings suggest that sikkam leaves can be used in ethnomedicine as a cancer treatment via the apoptosis pathway.

Some of the chemical constituents of sikkam leaves that have been reported include alkaloids, flavonoids, tannins, saponins, and terpenoids (Sarmah et al., 2020). These active compounds can be isolated from the plant using various extraction methods. One factor influencing the quality of the extraction yield is the extraction technique and duration used (Mawarda et al., 2020). Generally, the isolation of natural compounds from plants is performed using maceration and Soxhlet extraction methods.

Maceration is a cold extraction method with the advantage that the method and equipment used are simple and no heating is required, so the natural material does not degrade (Mawarda et al., 2020). The disadvantages of the maceration method are that it takes longer for the sample to oxidize, which affects the sample's antioxidant capacity, and the extraction process with a solvent causes the solvent to become saturated, requiring a larger amount of solvent for the extraction process (Zhang, Lin, and Ye, 2018). Meanwhile, Soxhlet extraction is a method of separating

substances from their mixtures through heating. The Soxhlet method offers the advantage of faster extraction times, preventing sample oxidation and preserving antioxidant activity, while ensuring a more complete extraction process as the vaporized solvent prevents solvent saturation. The working principle of the Soxhlet method is continuous extraction using a smaller amount of solvent. Once extraction is complete, the solvent can be evaporated to obtain the extract (Wijaya et al., 2019). A disadvantage of the Soxhlet method is that it can damage heat-sensitive components or chemical compounds due to the heating of the extract.

There are differences between several studies on sikkam leaves that have been conducted in Indonesia and India. In previous studies, the sikkam leaf samples were primarily sourced from North Sumatra, and toxicity testing was conducted using mice. In this study, samples were obtained from the Riau region, and toxicity testing was performed using shrimp larvae. Additionally, this study employed two sample drying methods: air-drying and sun-drying.

Currently, the use of natural materials for medicinal and other purposes is on the rise. Research on various medicinal plants continues, particularly for traditional medicine, which is highly beneficial to the community. According to Sam (2023), the use of medicinal plants in the community is still not optimal and remains limited to empirical experience, lacking scientific knowledge regarding the effectiveness of traditional medicines and proper treatment methods. Many people simply believe that a plant can be used as medicine without knowing the toxicity it contains. Consequently, the use of medicinal plants as traditional medicine remains suboptimal. It is crucial to conduct toxicity tests on medicinal plants. Therefore, the researchers were interested in conducting antioxidant and toxicity tests on sikkam leaves using the maceration and Soxhlet extraction methods.

2. METHOD

Material, Equipment and Instrument

The materials used in this study were sikkam leaves, 96% ethanol, PA-grade methanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Artemia salina eggs, sea water, and distilled water. The equipment used in this study included a blender, an analytical balance, a magnetic hot plate stirrer, a set of glassware, a Soxhlet extractor, sample bottles, a simple distillation apparatus, a shaker, filter paper, and aluminum foil. The instrument used in this study was a UV-Vis spectrophotometer.

Sample Preparation

The samples used in this study were sikkam leaves from Sukaramai Village, Tapung Hulu Subdistrict, Kampar Regency, Pekanbaru. The sikkam leaves were picked while fresh, green, undamaged, and intact. The sikkam leaves were thoroughly washed and then dried. A total of 1,000 g of sikkam leaves were dried indoors at room temperature (25–30°C) for 5 days and in direct sunlight (35–41°C) for 3 days. After drying, the mass of the sikkam leaves was 213 g for air-dried leaves and 200 g for sun-dried leaves. The air-dried and sun-dried sikkam leaves were ground into a powder using a blender and sifted to produce a fine powder. The fine sikkam leaf powder was stored at room temperature before further use.

Maceration

Air-dried sikkam leaf powder (25 g) was placed in a glass bottle, 125 mL of 96% ethanol was added, and the mixture was shaken and macerated for 24 hours. The ethanol solution was separated from the solids by filtration. The residue was macerated again with 96% ethanol for 2 x 24 hours. The filtrates obtained from each maceration were combined and distilled using simple distillation to remove the ethanol. The resulting concentrated extract was then heated to 50°C while being stirred carefully until it dried into a powder. The mass of the resulting powder was determined, and it was stored for further use. The same process was also performed for sun-dried sikkam leaves. The maceration process was repeated three times for both air-dried and sun-dried sikkam leaves.

Soxhletation

Air-dried sikkam leaf powder (25 g) was wrapped in filter paper and placed in the Soxhlet extractor chamber. 250 mL of 96% ethanol was added to the round-bottomed flask of the Soxhlet extractor, and extraction was carried out by heating. During Soxhlet extraction, the ethanol will evaporate, enter the condenser, and then drip onto and saturate the sikkam leaf sample. The hot ethanol will dissolve the compounds present in the sikkam leaves. Once the extraction chamber is full of ethanol, the ethanol will flow into the round-bottom flask through a side tube in the extraction chamber. The extraction process continues until all compounds in the sikkam leaves are dissolved, indicated by the ethanol becoming colorless, which takes 25 cycles. The ethanol solution is then cooled and filtered. The resulting dark green filtrate is distilled using simple distillation to separate the ethanol. The resulting concentrated extract is then heated to 50°C while being stirred carefully until it dries into a powder. The mass of the resulting powder is determined, and it is stored until further use. The same process is also performed for sun-dried sikkam leaves. The extraction process is repeated three times for both air-dried and sun-dried sikkam leaves.

Antioxidant Activity Assay using DPPH Method

The antioxidant activity of sikkam leaf extracts was determined using the DPPH method with a UV-Vis spectrophotometer. Stock solutions with a concentration of 100 μg/mL were prepared from 0.005 g of each sikkam leaf extract dissolved in 50 mL of PA-grade methanol. From these stock solutions, a series of sample solutions was prepared with concentrations of 0, 5, 10, 15, and 20 μg/mL for air-dried and sun-dried Soxhlet extracts, and concentrations of 0, 50, 100, 150, and 200 μg/mL by dilution. Vitamin C was used as a reference in this study. A series of vitamin C solutions with concentrations of 0, 1, 2, 3, 4, 5, and 6 μg/mL was prepared from a 10 μg/mL vitamin C stock solution made by dissolving 0.0010 g of vitamin C in 100 mL of PA-grade methanol. Meanwhile, the DPPH solution used had a concentration of 40 μg/mL, prepared from 0.002 g of DPPH powder in 50 mL of PA-grade methanol.

A total of 2 mL of each concentration in the series of Sikkam leaf extract solutions was added to 2 mL of DPPH solution (40 mg/L) in a dark glass bottle and shaken for 30 minutes before measurement. A color change from purple to yellow indicates the scavenging of DPPH free radicals by the Sikkam leaf extract solution. This change can be measured using a UV-Vis spectrophotometer in the wavelength range of 400–800 nm, with the maximum absorbance determined at a wavelength of 517 nm (Damanis et al., 2020). The inhibition percentage was calculated using the equation $\% I = \left\{\frac{Ao-Ai}{Ao}\right\} x\ 100\%$, where Ao is the absorbance of the DPPH solution without the extract sample solution and Ai is the absorbance of the DPPH solution with the addition of each concentration of the extract sample solution. Antioxidant activity, IC_{50}, was determined by plotting %I against extract concentration using Excel, and IC_{50} was calculated for the Sikkam leaf extract solution concentration at 50% inhibition using the equation $\%I = mC + a$ (Susiloningrum et al., 2021).

Toxicity Assay using BSLT Method

The toxicity of sikkam leaf extract was determined using the Brine Shrimp Lethality Test (BSLT) method. A stock solution of each sikkam leaf extract sample at a concentration of 2000 μg/mL was prepared by dissolving 0.50 g of the extract in 250 mL of seawater. From these stock solutions, a series of sample solutions was prepared with concentrations of 0, 1, 10, 50, 100, 200, 500, 1000, and 2000 μg/mL. A total of 2.5 mL of each concentration of the Sikkam leaf extract solution was added to a container containing 2.5 mL of seawater and 10 shrimp larvae. The shrimp larvae were then incubated for 24 hours. After that, the number of dead shrimp larvae was counted. The percentage of shrimp larval mortality was calculated for each extract concentration using the following equation: $\% M = \left\{\frac{b-c}{a}\right\} x\ 100\%$, where a = number of test larvae, b = number of larvae that died during the test, c = number of larvae that died in the 0 μg/mL extract treatment. Subsequently, the toxicity and LC_{50}

values of the Sikkam leaf extract were determined using probit analysis. The toxicity test was conducted in triplicate for each type of sikkam leaf extract.

Data Analysis

The hypotheses proposed in this study are as follows: (1) H_{a1}: The yields of air-dried and sun-dried sikkam leaf extracts isolated using the maceration and Soxhlet extraction methods differ, (2) H_{a2}: The antioxidant activity (IC_{50}) of air-dried and sun-dried sikkam leaf extracts isolated using the maceration and Soxhlet extraction methods differs. And (3) H_{a3}: The toxicity (LC_{50}) of air-dried and sun-dried sikkam leaf extracts isolated using the maceration and Soxhlet extraction methods differs. To test the hypotheses, the data on isolation yield, antioxidant activity, and toxicity of the sikkam leaf extracts were statistically analyzed using two-way ANOVA. The criteria are as follows: if $F_{calc} > F_{tab}$, then H_0 is rejected; or (b) if significant ($p < 0.05$), then H_0 is rejected.

3. RESULTS AND DISCUSSION

Yield of Extract

Prior to extraction, sikkam leaves were dried indoors at room temperature (air-dried) and in sunlight (sun-dried). The moisture loss from these drying processes was 78.7 and 80.0 % for air-dried leaves and for sun-dried leaves, respectively. In this study, extracts from air-dried and sun-dried sikkam leaves were isolated using maceration and Soxhlet extraction methods with 95% ethanol as the solvent. Ethanol was chosen as the solvent because it is relatively safe and non-toxic compared to other solvents and is capable of dissolving nearly all secondary metabolites present in the plant material (Endah, 2017). Ethanol has polar properties due to the presence of hydroxyl (OH) groups containing oxygen with very high electronegativity. This results in the formation of hydrogen bonds with other molecules. Additionally, ethanol also contains ethyl (C_2H_5) groups, which are nonpolar. Ethanol also has a low boiling point of 79°C, so the concentration process requires less heat and it evaporates easily (Labagu et al., 2022). The yields of air-dried and sun-dried sikkam leaf extracts using the maceration and Soxhlet extraction methods are presented in Table 1.

Table 1. The yields from the isolation of air-dried and sun-dried sikkam leaves using the maceration and Soxhlet extraction methods.

	Maceration			Soxhletation		
	Leave (g)	Extract (g)	Yield (%)	Leave (g)	Extract (g)	Yield (%)
Air-dried (AD)	25	4.99	19.98	25	5.49	21.99
	25	4.62	18.48	25	6.38	25.52
	25	5.76	23.00	25	6.35	25.43
Mean	25	5.12	20.48±2.30	25	6.07	24.31±2.01
	Leave (g)	Extract (g)	Yield (%)	Leave (g)	Extract (g)	Yield (%)
Sun-dried (SD)	25	5.82	23.30	25	6.43	25.75
	25	5.17	21.00	25	6.03	24.14
	25	5.66	22.67	25	6.06	24.24
Mean	25	5.55	22.32±1.19	25	6.17	24.71±0.90

Based on the results of a two-way ANOVA, a significance value of <0.001 was obtained, which is less than 0.05. Thus, H_0, which states that "there is no difference in the extraction yields of air-dried and sun-dried sikkam leaves isolated using the maceration and Soxhletation methods," is rejected. In other words, H_1, which states "there is a difference in the extraction yields of air-dried and sun-dried Sikkam leaves isolated using the maceration and Soxhlet methods," is accepted. The

extraction yields for air-dried maceration (ADM), sun-dried maceration (SDM), air-dried Soxhlet extraction (ADS), and sun-dried Soxhlet extraction (SDS) were 20.48±2.30, 22.32±1.19, 24.31±2.01, and 24.71±0.09%, respectively. This indicates that both the drying technique and the isolation method influence the yield of the extract obtained, with the Soxhletation method of sun-dried sikkam leaves yielding the highest extract yield. This indicates that the drying technique, which affects the dryness level (moisture content) of the Sikkam leaves, significantly influences the extract yield obtained, where crude drugs with a higher dryness level or lower moisture content yield a higher extract yield, as they contain a greater amount of extract. Similarly, the isolation method used also affects the yield obtained. In this case, the Soxhlet extraction method yields better results, as it employs a hot solvent and a continuous, repeated extraction process. In a hot solvent, the secondary metabolites in bandotan leaves dissolve more easily, and the repeated extraction process extracts more of these secondary metabolites, resulting in a higher yield.

These results are consistent with the study conducted by Rudiana et al. (2023), in which the extraction method had a significant influence on the overall yield of the extract in the sample, with a significance value of $0.009 < 0.05$ ($p < \alpha$). The results showed that the Soxhlet extraction method using ethanol as a solvent yielded a higher extract yield for *B. macrophylla* leaves compared to the maceration method, at 19.78% and 43.87%, respectively. According to Zang (2023), Soxhlet extraction offers greater extraction efficiency and uses less solvent than maceration. However, this method is not suitable for heat-sensitive compounds. Although maceration is a simple and effective method for extracting active components, it is time-consuming, uses a large amount of solvent, and has a relatively low yield. The extraction process using the Soxhlet method yielded a higher amount of Vernonia cinerea leaf extract (Alara et al. 2018). The experimental results showed that the highest extract yield (10.01 ± 0.85% w/w) was achieved using an extraction time of 2 hours, a solid-to-solvent ratio of 1:20 g/mL, and an ethanol concentration of 60% v/v.

Antioxidant Activity

Antioxidant activity, IC_{50}, is defined as the concentration of an antioxidant compound that can reduce DPPH free radical activity by 50%. In this study, the antioxidant activity of Sikkam leaf extract was determined using the DPPH method. The DPPH method was chosen because it is simple, easy, and fast, highly sensitive, and requires only a small sample to evaluate the antioxidant activity of natural compounds (Hasan et al., 2022). The DPPH antioxidant assay was conducted by observing color changes in each sample incubated with DPPH. This color change in DPPH occurs when all DPPH electrons pair with electrons in the sample, resulting in a shift in sample color from dark purple to light yellow (Irwinsyah et al., 2019). This test utilized a UV-Vis spectrophotometer at a maximum wavelength of 517 nm with a wavelength range of 400–800 nm. Figure 2 shows the results of the antioxidant activity test for macerated and Soxhlet-extracted air-dried and sun-dried Sikkam leaf extracts. The antioxidant activity (IC_{50}) of the sikkam leaf extracts ADM, ADS, SDM, and SDS were 11.13 ± 1.30; 11.36 ± 1.47; 12.85 ± 2.88; and 14.82 ± 1.19 μg/mL, respectively, classified as very strong. However, these values are still lower than the antioxidant activity of ascorbic acid, IC50 = 5.937 μg/mL. The lower the IC_{50} value, the greater the antioxidant activity of a compound (Molyneux, 2004). Specifically, IC_{50} values < 50, 50–100, 100–150, 150–200 and > 200 μg/mL indicate very strong, strong, moderate, weak and very weak antioxidant activity (Sarfina & Handayani, 2017).

Based on the results of a two-way ANOVA, a significance value of <0.001 was obtained, which is less than 0.05. Thus, H0, which states that "there is no difference in the antioxidant activity of air-dried and sun-dried sikkam leaf extracts isolated using the maceration and Soxhlet extraction methods," is rejected. In other words, H1, which states "there is a difference in the antioxidant activity of air-dried and sun-dried sikkam leaf extracts isolated using the maceration and Soxhlet extraction methods," is accepted. As for antioxidant activity, the IC_{50} values for ADM, ADS, SDM, and SDS were 11.13 ± 1.30; 11.36 ± 1.47; 12.85 ± 2.88; and 14.82 ± 1.19 μg/mL, respectively. This indicates that both the drying technique and the isolation method influence the antioxidant activity

of the obtained extracts, with the maceration method of air-dried sikkam leaves (ADM) yielding the highest antioxidant activity.

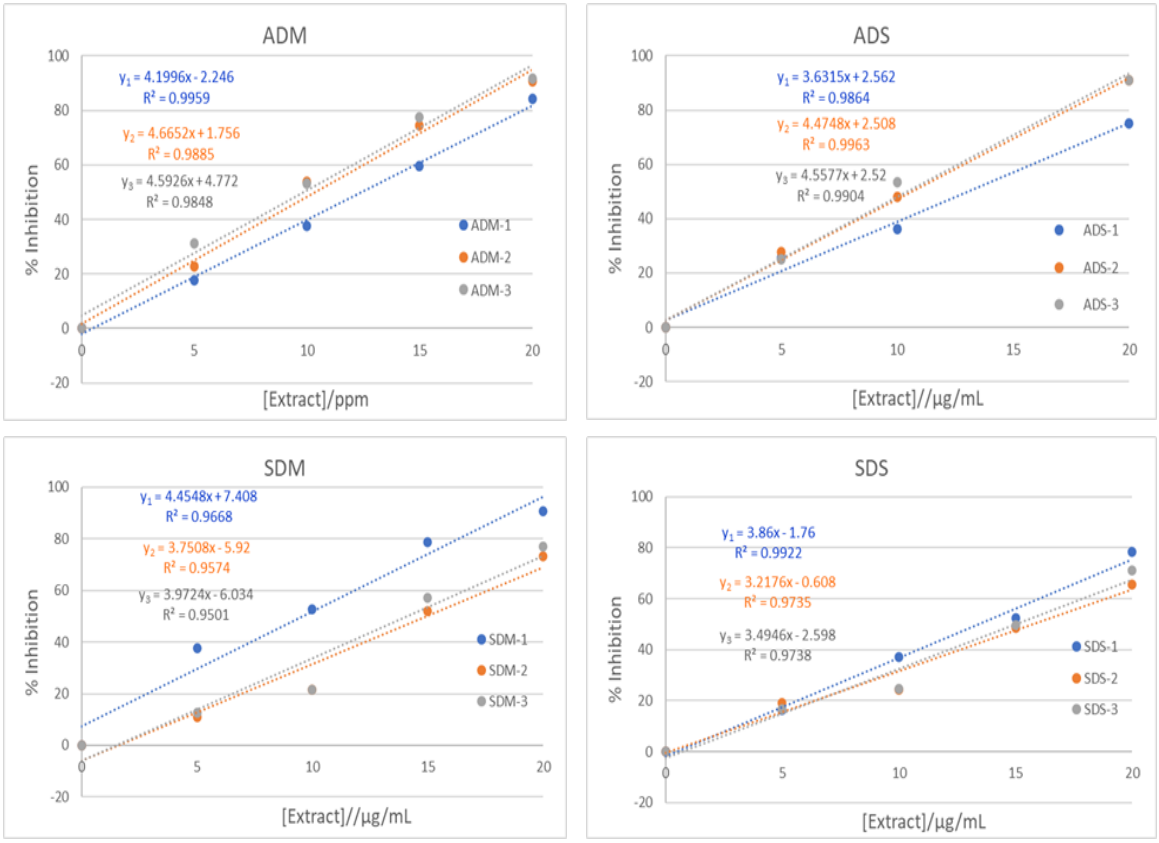

Figure 2. Inhibition against concentration curves of antioxidant activity for each extract from air-dried and sun-dried sikkam leaves isolated by maceration and Soxhlet extraction.

In general, extracts obtained by the maceration method exhibit better antioxidant activity compared to those obtained by Soxhlet extraction. This may be due to the effect of temperature on the extraction process; at higher ethanol solvent temperatures, secondary metabolites degrade, thereby reducing overall antioxidant activity (Setyowati et al., 2014). High temperatures during the isolation process affect the degradation of compounds, particularly unstable ones. According to Luliana et al. (2016), various simple drying methods such as oven drying, direct sunlight (DSL), indirect sunlight (IDSL), and air drying (AD) have a significant impact on antioxidant activity. According to Bernard et al. (2014), the sun-drying process can cause the total degradation of flavonoids contained in the sample. This degradation occurs due to prolonged and intense drying, which subsequently affects phytochemical compounds through enzymatic processes. Chan et al. (2009) reported that total phenols can decrease due to enzymatic reactions occurring during the drying process. Prolonged drying in open air can lead to enzyme damage, particularly an increase in polyphenol oxidase activity. As a result, sunlight can cause the complete degradation of phenols and flavonoids in the samples (Bernard et al., 2014). Furthermore, according to Setyaningrum et al. (2021), the drying method used for the crude drug can affect the resulting flavonoid content and antioxidant activity. The drying process can reduce the yield of sikkam leaves and also affect the bioactive components present in the crude simplicia.

The results of the study indicate that sun-dried sikkam leaves exhibit lower antioxidant activity. This is due to the high temperatures involved in sun drying and the presence of UV rays, which can damage the bioactive compounds in sikkam leaves. Higher drying temperatures result in a greater decrease in antioxidant activity, as ultraviolet radiation can degrade secondary metabolites

such as flavonoids and phenols present in the crude drug (Apsari et al., 2021). As stated by Chan et al. (2009), direct sun-drying leads to a more significant reduction in antioxidant activity. During this sun-drying process, oxidative enzymes such as peroxidase and polyphenol oxidase are activated, contributing to the loss of phenolic compounds (Gümüşay et al., 2015).

Ethanol extracts of air-dried sikkam leaves exhibit greater antioxidant activity or lower IC50 values. In this study, air drying was conducted at room temperature, ensuring that the temperature did not become too high and did not damage the chemical composition of the sikkam leaves, although the drying process took longer. Air, temperature, and humidity have a significant effect on the air-drying process. As air temperature increases, air humidity tends to decrease, resulting in increased water evaporation from the material being dried. Conversely, if the temperature decreases, air humidity tends to increase, and water evaporation from the material decreases (Setyaningrum et al., 2021).

Toxicity of the Leave Sikkam Ectract

The toxicities of sikkam leaf extracts were tested using the Brine Shrimp Lethality Test (BSLT). The BSLT of the ethanol extracts of sikkam leaves on *Artemia salina* Leach larvae were conducted over a 24-hour period, and the results are shown in Figure 3.

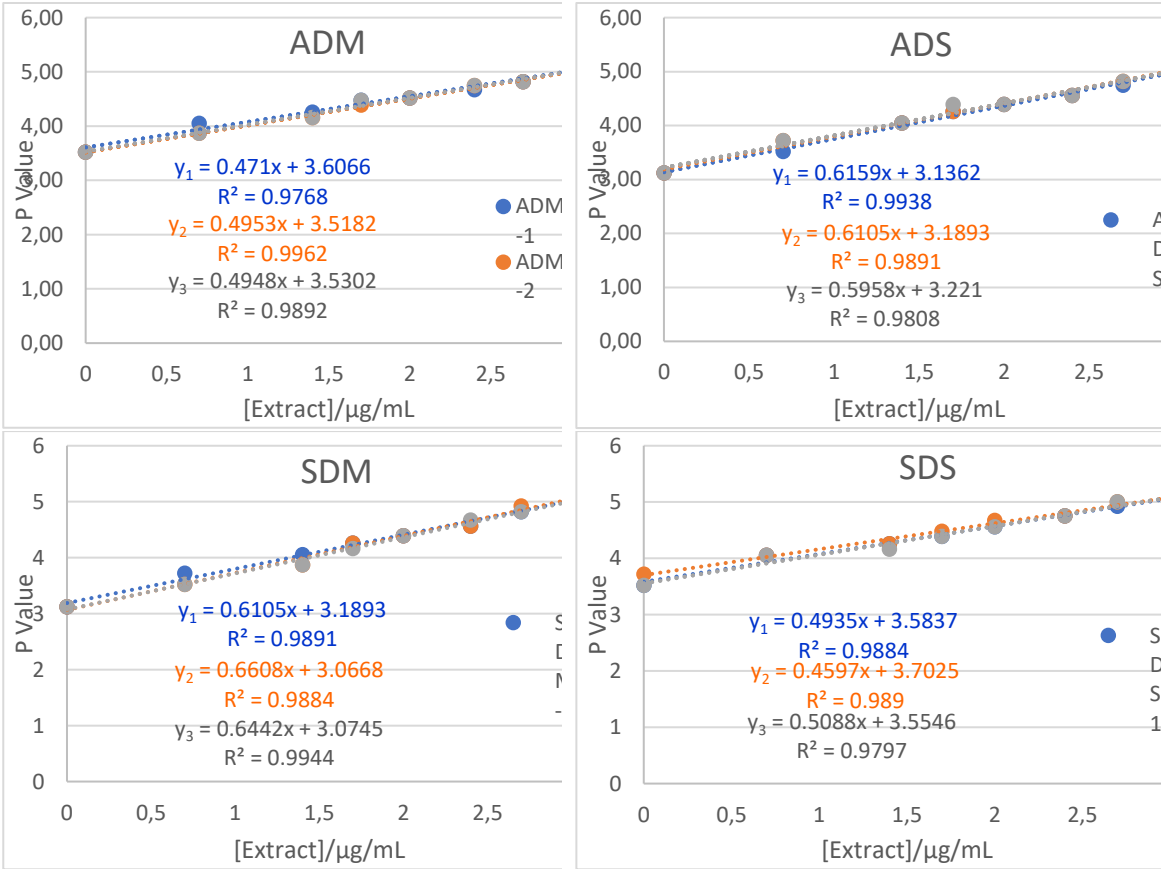

Figure 3. Plot of probit values against the concentration of ethanol extracts from air-dried and sun-dried sikkam leaves isolated by maceration and Soxhlet extraction.

The toxicity of the sikkam leaf extract (LC_{50}) was calculated using probit analysis in SPSS with a 95% confidence level, followed by a linear regression test to examine the effect of different concentrations of the sikkam leaf ethanol extract on the mortality of *Artemia salina* Leach shrimp larvae, Figure 3. From the linear regression test, if the significance value is < 0.05, there is a significant effect between the two variables. Meanwhile, if the significance value is > 0.05, there is no significant

effect between the two variables. The LC_{50} values of air-dried and sun-dried sikkam leaf ethanol extracts isolated using the maceration and Soxhlet extraction methods are presented in Table 2.

Table 2. LC_{50} regression analysis of ethanol extracts obtained by maceration and Soxhlet extraction of air-dried and sun-dried sikkam leaves on the Probit values for the mortality of Artemia salina Leach larvae.

Extract	Run	Linear Regression	R^2	LC_{50} (μg/mL)	Mean of LC_{50} (μg/mL)
ADM	ADM-1	$P_1 = 0.4710C + 3.6066$	0.9768	909	941±37
	ADM-2	$P_2 = 0.4953C + 3.5182$	0.9962	981	
	ADM-3	$P_3 = 0.4948C + 3.5302$	0.9892	934	
ADS	ADS-1	$P_1 = 0.6159C + 3.1362$	0.9938	1060	985±70
	ADS-2	$P_2 = 0.6105C + 3.1893$	0.9891	925	
	ADS-3	$P_3 = 0.5958C + 3.2210$	0.9808	968	
SDM	SDM-3	$P_1 = 0.6105C + 3.1893$	0.9891	925	914±67
	SDM-2	$P_2 = 0.6608C + 3.0668$	0.9884	842	
	SDM-3	$P_3 = 0.6442C + 3.0745$	0.9944	975	
SDS	SDS-1	$P_1 = 0.4935C + 3.5837$	0.9884	741	700±39
	SDS-2	$P_2 = 0.4597C + 3.7025$	0.9890	664	
	SDS-3	$P_3 = 0.5088C + 3.5546$	0.9797	693	

The LC_{50} values of the ethanol extracts of air-dried sikkam leaves prepared by the maceration and Soxhlet methods were 941±37 and 985±72 μg/mL, while those of the ethanol extract of sun-dried sikkam leaves using the maceration and Soxhlet methods were 914±67 and 700±39 μg/mL. Based on the results of a two-way ANOVA test for the toxicity of sikkam leaf extracts, a significance value of <0.001 was obtained. This value is smaller than 0.05, so it can be concluded that H_0 is rejected and H_a is accepted. This implies that there is a difference in toxicity between air-dried and sun-dried sikkam leaf extracts isolated by maceration and Soxhlet extraction.

The toxicity of herbal extracts expressed as LC_{50} values is commonly valorized either by comparison to Meyer's or to Clarkson's toxicity index. According to Meyer's toxicity index, extracts with $LC_{50} < 1000$ μg/ml are considered as toxic, while extracts with $LC_{50} > 1000$ μg/ml are considered as non-toxic (Meyer et al., 1982). Whereas, Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with $LC_{50} > 1000$ μg/ml are non-toxic, LC_{50} of 500 - 1000 μg/ml are low toxic, extracts with LC_{50} of 100 - 500 μg/ml are medium toxic, while extracts with LC_{50} of 0 - 100 μg/ml are highly toxic (Clarkson et al., 2004). This indicates that the sikkam leaf extracts have low toxic and relatively safe for consumption.

The results obtained in this study differ from those reported by Chowdhury et al. (2020), in which the methanol extract of sikkam (*Bischofia javanica* Blume) leaves was shown to exhibit moderate toxicity, with an LC_{50} value of 224.7 μg/mL in brine shrimp lethality assays.

4. CONCLUSION

Sikkam is a tropical plant widely used by the community as a traditional medicine. The results of this study confirm that sikkam leaves, which have long been used traditionally, have a fairly high yield and possess biological activity that supports the plant's use as a traditional medicine. The yields of ethanol extracts from air-dried and sun-dried sikkam leaves isolated using maceration and Soxhlet extraction differed significantly, with ADM, ADS, SDM, and SDS at 20.48±2.30, 24.31±2.01, 22.32±1.19, and 24.71±0.09%, respectively. Ethanol extracts of air-dried and sun-dried Sikkam leaves isolated via maceration and Soxhlet extraction exhibit very strong antioxidant activity, with IC_{50} values for ADM, ADS, SDM, and SDS of 11.13, 11.36, 12.85, and 14.82 μg/mL, respectively. Meanwhile, the toxicity of air-dried and sun-dried sikkam leaf extracts isolated by maceration and

Soxhlet extraction was classified as non-toxic, with LC_{50} values for ADM, ADS, SDM, and SDS of 941±37, 985±72, 914±67, and 700±39 μg/mL, respectively, which are classified as non-toxic. mg/mL. This proves that sikkam leaves are safe for consumption and are considered a potential medicinal agent with strong antioxidant properties.

CONFLICT OF INTEREST

We declare that there are no conflicts of interest regarding this study.

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