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Manufacturing and Testing the Antibacterial Activity of Cytotoxicity by Lethality Test of Brine Shrimp, Antibacterial Activity of Ripe Fruit Flesh *Mangifera quadrifida* Jack

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Abstract

Isem kembang (Mangifera quadrifida Jack) is part of the Mangifera clan of the Anacardiaceae tribe. Research on the benefits of ripe Mangifera quadrifida Jack fruit as an antibacterial (antimicrobial) and cytotoxic agent with Brine Shrimp of Lethality Test (BSLT) has been successfully conducted.This research was conducted in two stages, namely the extraction of the ripe fruit flesh of Mangifera quadrifida Jack with the maceration method using n-hexane, ethyl acetate, and methanol, followed by antibacterial testing using the well-diffusion method (disk diffusion assay) and cytotoxicity testing using the Lethality Test of Brine Shrimp. According to the results the extraction of ripe fruit flesh Mangifera quadrifida Jack shows that ethyl acetate is the best solvent for extracting and killing bacteria, the methanol extract of ripe fruit flesh from Mangifera quadrifida Jack has the potential as a compound antitumor or anticancer drug as the Brine Shrimp Lethality Test (BSLT) method show acute toxicity to Artemia salina Leach larvae.

Keywords: Mangifera quadrifida Jack fruit; Brine Shrimp Lethality Test (BSLT); antibacterial (antimicrobial); cytotoxic agent.

1. Introduction

The use of different plants for therapeutic purposes dates back to the beginning of human civilization. Plants are like a storehouse filled with a million different benefits, including the ability to treat a variety of diseases. In light of recent advances of this kind in this scenario, every nation needs to investigate, acknowledge, and cultivate the indigenous remedies that are unique to their population. Ingredients used traditionally in medical preparations are nearly all made using natural components that are sourced from various plants [1, 2]. It is estimated that there are between 100 and 150 different plant families in Indonesia. The majority of these plant families have the potential to be utilized as industrial plants, fruit trees, spice plants, or medicinal plants There are 30,000 different plant species found in the tropical forests of Indonesia. Only about 200 of these species have ever been used as raw materials in the traditional medicine industry, despite the fact that approximately 9,600 of these species are recognized to have therapeutic characteristics [3]. Opportunities for developing the cultivation of medicinal plants are still very wide open, in line with the growing development of the herbal medicine industry, phyto-pharmaceuticals, and traditional cosmetics [4-6]. One of the fruit plants that have the potential to become herbal medicine is Isem Kembang (*Mangifera quadrifida* Jack). This fruit plant comes from Lampung, Indonesia, and its existence is very rare.

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The use of different plants for therapeutic purposes dates back to the beginning of human civilization. Plants are like a storehouse filled with a million different benefits, including the ability to treat a variety of diseases. In light of recent advances of this kind in this scenario, every nation needs to investigate, acknowledge, and cultivate the indigenous remedies that are unique to their population. Ingredients used traditionally in medical preparations are nearly all made using natural components that are sourced from various plants [1, 2]. It is estimated that there are between 100 and 150 different plant families in Indonesia. The majority of these plant families have the potential to be utilized as industrial plants, fruit trees, spice plants, or medicinal plants There are 30,000 different plant species found in the tropical forests of Indonesia. Only about 200 of these species have ever been used as raw materials in the traditional medicine industry, despite the fact that approximately 9,600 of these species are recognized to have therapeutic characteristics [3]. Opportunities for developing the cultivation of medicinal plants are still very wide open, in line with the growing development of the herbal medicine industry, phytopharmaceuticals, and traditional cosmetics [4-6]. One of the fruit plants that has the potential to become herbal medicine is Isem Kembang (*Mangifera quadrifida* Jack). This fruit plant comes from Lampung, Indonesia, and its existence is very rare.

Isem kembang (*Mangifera quadrifida* Jack) is part of the Mangifera clan of the Anacardiaceae tribe. The results of the revision that was carried out by Ding Hou on Anacardiaceae noted that the Anacardiaceae tribe, which is spread across the Indonesian region, has as many as 20 genera, with the highest distribution in the regions of Sumatra and Kalimantan [7]. Mangifera is the genus that contains the greatest number of species; there are 19 different species in this genus [8]. Mangifera species generally have a tree habit that can reach a height of up to 30 m or more. The distribution of Mangifera species is very dependent on natural factors that affect its growth. There are three limiting factors: soil, climate, and altitude. There are many types of *Mangifera quadrifida* Jacks in Sumatra and Kalimantan, and they live well in the lowlands and sometimes on the highlands at an altitude of 900 m above sea level. The type of *Mangifera quadrifida* Jack grows well in areas with extreme conditions with nutrient-poor soil conditions and poor drainage, such as stagnant areas, river banks, and calcareous soils, so it will affect the number of species that are able to grow in these conditions [9].

Isem kembang (*Mangifera quadrifida* Jack) is commonly used as a traditional medicine to improve eye, stomach and throat health as well as help the immune system. Research on the benefits of ripe Mangifera quadrifida Jack fruit as an antibacterial (antimicrobial) and cytotoxic agent with Brine Shrimp of Lethality Test (BSLT) has never been reported. The purpose of this study was to examine the potential of Mangifera quadrifida Jack fruit as an antibacterial agent and cytotoxic agent. The BSLT is a preliminary test to see how well a substance fights tumors and cancer [10]. Multiple pieces of research have found evidence that some strains of bacteria may play a role in the development of cancer [11]. Current research shows that traditional remedies are good for your health and are being used more because they are cheaper and easier to get [12-13]. According to some studies, traditional medicine does not produce an excessive amount of negative side effects because it is still able to be digested by the body. This is one of the primary reasons that it is so extensively utilized today [5, 14]. Parts of medicinal plants commonly used are roots, bark, wood, leaves, flowers, or seeds [15-16].

2. Experimental

This research was conducted in two stages, namely the extraction of the ripe fruit flesh of *Mangifera quadrifida* Jack with the maceration method using n-hexane, ethyl acetate, and methanol, followed by antibacterial testing using the well-diffusion method (disk diffusion assay) and cytotoxicity testing using the Lethality Test of Brine Shrimp.

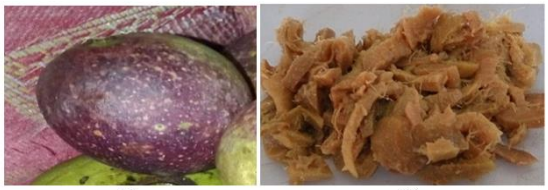

Figure 1. Ripe fruit (a) and ripe fruit flesh (b) of *Mangifera quadrifida* Jack

2. 1. Sample Extraction

Extraction was carried out by soaking or macerating with several organic solvents. *Mangifera quadrifida* Jack fruit was peeled and separated between the skin and fruit, then dried at room temperature. 50 g of dry, ripe fruit (*Mangifera quadrifida* Jack) was macerated for three days in 500 mL of technical organic solvent (n-hexane). The mixture was then filtered, and the filtrate was dried again. The result obtained was a crude extract of n-hexane. The residue from the first soaking was completely soaked again in 500 mL of technical ethyl acetate for 3 days, filtered, and the filtrate was evaporated to obtain crude ethyl acetate extract. The residue from the ethyl acetate soaking was again soaked in 500 mL of technical methanol for 3 days to obtain crude methanol extract. Maceration was

repeated until the extract was clear, and it was expected that all compounds could be dissolved in the solvent.

2. 2. Antibacterial Testing

In a Petri dish containing nutrient agar medium, 200 L of a suspension of one of the test microorganisms (*E. coli* and *B. cereus*) was added along with an ose. Then, prepare sterile tracing paper disks (6 mm in diameter) to be set on the agar in the petri dish. In sterile petri dishes, the discs were immersed in the test solution for 30 minutes. The incubation procedure was then conducted for 24 hours at 37 °C. Observations were made by measuring and recording the inhibition zone formed around the paper disc as a clear area using a ruler. The experiment was performed thrice with repetition.

Zone of Inhibition = Diameter (clear area + disc) - Diameter of disc paper

2. 3. Antibacterial test with agar disc diffusion method

Escherichia coli and *Bacillus subtilis* were tested for antibacterial activity using the agar disc diffusion method with PDA, NA, and NB media. The antibacterial standards used were amoxicillin and the antifungal standards were ketoconazole. The experiment was performed thrice with repetition.

Amount 0.5 mL of test microbial culture was pipetted aseptically into sterile Petri dishes, added NA/PDA medium which was still liquid (45°C-50°C), homogenized, and allowed to freeze. The disc paper was dipped in Alginate/PVP hydrogel, placed on the surface of the NA/PDA medium aseptically, and incubated at 37oC for 24 hours, then the inhibition zone formed was measured.

2. 4. Cytotoxicity test

Artemia salina was used as the test organism in a cytotoxicity test that was conducted using the brine shrimp lethality test (BSLT) method [10]. To the saltwater contained in a container that has been hermetically sealed, 30 mg of brine shrimp eggs derived from *A. salina* are introduced. The eggs are more likely to hatch successfully if one places an air hose at the bottom of the container. The eggs of *A. salina* will begin to hatch and develop into larvae after a period of 24 hours. After that, we picked 10 larvae from each species and placed them in containers with sample solutions of 10, 100, and 1000 mg/L, ethyl acetate and n-hexane, respectively. we also test for solutions of 2, 4, and 8 mg/L methanol. After 24 hours, it was clear that the *A. salina* larvae found in both the samples and the controls had perished. After 24 hours, it was clear that the *A. salina* larvae found in both the samples and the controls had perished. If an *A. salina* larva has not moved for more than a few seconds, then it is possible to assume that it has passed away. After determining the percentage of *A. salina* larvae that were successful in escaping death, the data were checked byanalysis of variance using ANOVA

3.Results and discussion

Extraction of *Mangifera quadrifida* Jack fruit by maceration technique in various solvents for several days yielded a bright yellow solution. After evaporation, the extracts were changed to be dark brown, as shown in Figure 2. The yields of the extraction were listed in Table-1. The highest yields were achieved for ethyl acetate extract, and the lowest yield was obtained for n-hexane extract.

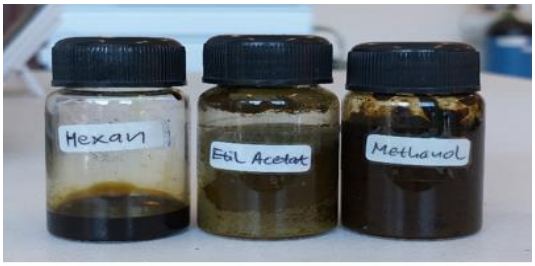

Figure 2. Ripe fruit flesh extract of *Mangifera quadrifida* Jack in n-hexane (a), ethyl acetate (b), and solvent methanol (c)

Extraction by maceration was easier to implement and did not require specific equipment. In addition, the maceration method can be used for both heat-resistant and non-heat-resistant compounds and can be used for compounds that have not been identified [17-19]. Ethyl acetate is a solvent that was included in the semi-polar category and has the ability to effectively bind organic flavonoid compounds. Therefore, the use of ethyl acetate as a sample extractor was able to produce the highest yield, which was 4.89% [20]. According to several reports, the ethyl acetate solvent is excellent for use as a binder for aromatic compounds. In comparison to other solvents, ethyl acetate is superior in all respects, including pollution, availability, and price [21].

Table 1. The Yields of ripe fruit flesh extract *Mangifera quadrifida* Jack) (per 56, 1709 g of samples)

Solvent	Wo (g)	W1 (g)	W1-Wo (g)	% Yield*
n-hexane	**14,9059**	**14,9654**	**0,0595**	**0,11**
Ethyl acetate	**14,5276**	**18,2185**	**3,6909**	**6,57**
Methanol	**14,7418**	**17,4883**	**2,7465**	**4,89**

* % Yield is calculated using the followed equation: ((W1-W0)/56,1709) x 100%

3.1. Antibacterial test results

Based on the data in Table 2, it is known that the best extract solution as an anti-bacterial is ethyl

acetate extract solution, based on 30 mm inhibition zone category data with very strong inhibition for *E. coli* bacteria and 33 mm zone category data for *B. cereus* bacteria with very strong inhibition. It has been reported that the ethyl acetate extractor contains more secondary metabolites compared to the n-hexane extractor, namely flavonoids, terpenoids, steroids, tannins, and saponins [22-23]. This affects the ability of the ethyl acetate extract to inhibit the growth of *E. coli* bacteria. The diameter of the inhibition zone formed around the disc containing ethyl acetate extract increased with increasing concentration. The wider the inhibition zone formed, the higher the level of effectiveness of the extract in inhibiting bacterial growth; this is what causes the ethyl acetate extract solution to act as an inhibitor for bacterial growth.

Table 2: Data on antibacterial and cytotoxicity test results of ripe fruit flesh extract *Mangifera quadrifida* Jack

No	Extract	Zona of Inhibition *E. coli* (mm)	Inhibitory power against E. coli	Zona of Inhibition *B. cereus* (mm)	Inhibitory power against *B.cereus*	LC_{50} (µg/m)	Cytotoxicity Category
1	Hexane	7	currently	15	strong	80,01	Toxic
2	Ethyl acetat	30	very strong	33	very strong	70,03	Toxic
3	Methanol	19	strong	16	strong	29,994	very toxic
Standard*		0	13				

The standard used in the experiment was amoxicillin and ketonazole for bacteria = 0.11 ppm

3.2. Cytotoxicity test

BSLT is a simple, easy acute toxicity test method that gets results quickly and is inexpensive to implement. In addition, BSLT is a bioassay-guided fractionation that can be used to search for bioactive compounds that are toxic in natural ingredients [24]. The BSLT method uses shrimp larvae (*Artemia salina Leach*) as the experimental animals. *Artemia salina Leach*, which is an organism that has high sensitivity to toxins, Toxicity test results with this method have been shown to have a positive correlation with the cytotoxicity of the compounds against cancer. If the toxicity test shows that the LC_{50} is below 1000 ppm, the material has potential as an anticancer agent [25-28]. Advantages of the BSLT method Among other things, the process is fast, only requires 24 hours of observation, is cheap, is a simple method, and only requires a small number of samples. In addition, the implementation does not require special skills [29].

Based on the cytotoxicity test on *Artemia salina*, it was found that the three extracts were toxic, with the highest toxicity value in the methanol extract (LC_{50} value of 29.99 µg/mL). These findings suggest that an extract of the ripe fruit flesh *Mangifera quadrifida* Jack may be capable of inhibiting cancer cell proliferation.

The test was conducted to compare the average zone of inhibition between *E. Coli* and *B. cereus*. Testing is done by paired t test with the following hypotheses:

$$H_0 : \mu_d = 0$$

$$H_1 : \mu_d \neq 0$$

$$\alpha = 5\%$$

The result of t count is -0.84 and pValue is 0.490. Because pValue < α or accept H0, it can be concluded that the average inhibition of *E. coli* and *B. cereus* was not significantly different. Furthermore, tests were carried out to compare the average mortality rate of the 3 solvents. The test was carried out with a one-way ANOVA test with the following hypotheses:

$$H_0 : \tau_1 = \tau_2 = \tau_3 = 0$$

$$H_1 : \exists \tau_i \neq 0$$

$$\alpha = 5\%$$

Based on the ANOVA table (Table 3) the results show that the pValue is < α so that it can be concluded that there is at least one solvent that gives a different average mortality rate for larvae.

Table 3: Results of analysis of variance using ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Solvent	2	62,52	31,259	5,88	0,008
Error	24	127,56	5,315		
Total	26	190,07			

From the further test results shown in table 4, it was found that the death rate with methanol solvent was significantly different compared to the other 2 solvents. Methanol is significantly smaller than the other 2 solvents. Meanwhile, n hexane and ethyl acetate were not significantly different.

Table 4: Grouping Information Using the Tukey Method and 95% Confidence

Solvent	N	Mean	Grouping	
n-Hexane	9	9,333	A	
Etil asetat	9	9,000	A	
Metanol	9	5,67		B

Conclusion

Based on the results of the study, it can be said that the extraction of ripe fruit flesh *Mangifera quadrifida* Jack shows that ethyl acetate is the best

solvent for extracting and killing bacteria. In addition, the methanol extract of ripe fruit flesh from *Mangifera quadrifida* Jack has potential as a compound antitumor or anticancer drug because the results of testing using the Brine Shrimp Lethality Test (BSLT) method show acute toxicity to *Artemia salina* Leach larvae.

Conflict of interest

According to what the authors have stated, they do not have any other interests that could be construed as being in competition with this work

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