Original Research Article

The Preliminary Study of Antimicrobial Activity of *Borassus flabelifer* L. Mesocarp Extract

ABSTRACT

Lontar or *Borassus flabelifer* is a local plant that mostly found in Nusa Tenggara Province of Indonesia. The juice of the ripe mesocarp has been used traditionally for treatment minor oral ulcer. Microorganism is one of the factors that cause the recurrence of oral ulcer. This study was aimed to investigate the antimicrobial potency of the *B. flabelifer* fresh and dried mesocarp extract. The antimicrobial extract was tested in vitro by using the agar well diffusion method against four bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and one fungus, *Candida albicans* with concentration of the extract were 10 mg/mL and 100 mg/mL. The dried and mesocarp extract was resistant to *P. aeruginosa* and *E. coli*, while the dried mesocarp extract showed potential antibacterial activity against *B. subtilis* and *S. aureus* with the diameter zone of inhibition on concentration 10 mg/ml were 12 ± 0.1 mm and 10± 0.1 mm, respectively. Antifungal activity only exhibited by the dried mesocarp extract with diameter zone of inhibition on concentration 100 mg/mL was 9 ± 0.1 mm. The dried mesocarp extract was more potent than fresh mesocarp extract as antimicrobial.

Keywords: Borassus flabelifer, Antimicrobial, Mesocarp, Candida albicans, Lontar

1. INTRODUCTION

The lontar palm tree (*Borassus flabelifer*), belong to Genus Borassus and family Araceae is one of the most important and economical species from Genus Borassus, such as B. flabellifer and *B. aethiopum* that mostly grows in Indonesia, especially in East Nusa Tenggara [1]. Every part of this tree can be useful for human life. For example, the stems and leaves can be used to build a house and the young palm fruit is widely consumed because it has sweet and fresh flesh fruit. Female flowers from palm trees produced white liquid that has sweetish taste and is non-alcoholic. This liquid can be tapped and fermented into an “arrack-like drink”, called nira [2]. On the other hand, the ripe palm fruit is rarely use and tends to be wasted. The ripe palm fruit has an orange aromatic mesocarp (mesocarp) with sweet and slightly bitter taste. The lontar mesocarp finer than coconut mesocarps.

B. flabellifer, one of the species of the Borassus genus, which is widely grown in tropical parts of Asia, especially in India, Thailand, and Malaysia, has been widely studied and known to have antioxidant activity, anti-inflammatory activity, antimicrobial activity, antidiabetic activity and others [3], [4]. *B. flabellifer* is known to have contents such as flavonoids, alkaloids, saponins, steroids, and terpenoids which are responsible for the pharmacological activity [1], [2];[5]

In contrast to *B. flabellifer*, there are not many studies that have conducted research on *B. flabelifer*. Empirically, the ripe mesocarp (mesocarp) juice from *B. flabelifer* believed by the local people can help treat minor oral ulcer. Oral ulcers or known as Recurrent Aphthous Stomatitis (RAS) are a common ulcers conditions that affecting the oral mucosa. Minor RAS is the most common variant that happens in 80% cases of RAS patient. It is painful and can heal within 10-14 days. One of the factors that can cause oral ulcers is the infection of microorganisms, especially bacteria and fungi [6], [7]. Based on the taxonomic approach, it is expected that the extract of *B. flabelifer* mesocarps can have antimicrobial activity, especially in the treatment of oral ulcers caused by microorganisms. This study also investigated the effect of the sample

drying process on the antimicrobial activity of ripe mesocarp (mesocarp) extracted from *B. flabelifer* against some microbial pathogens.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

2.1 Collection of Plant Material

The selected ripe fruit of *B. flabelifer* were collected from Kupang, East Nusa Tenggara. The peel of the fruit was remove using a knife and the mesocarps were use as the sample. In this study, we use the fresh mesocarps and dried mesocarps. For preparation the dried mesocarps, the mesocarps were dried in shade for a week or placed in an oven at 40 ^{o}C. The dried and fresh mesocarps then respectively chopped and grinded with a grinder to get the powder sample of dried and fresh mesocarps of *B. flabelifer*.

2.2 Extraction of Ripe Mesocarp of *B. flabelifer*

1 kg powder of fresh and dried mesocarps of *B. flabelifer* were extracted by maceration method. The powders immersed with 2 L of 96% ethanol for three days and re-macerated with the same solvent for three times. Both of extract then concentrated with rotary vacuum evaporator to obtain the crude extracts. The yield for the extracts were calculated by the following formula [8]:

Yield(%)= (weight of extract after concentrated)/(weight of the plant powder before extraction) x 100%

2.3 Preliminary Phytochemical Screening

The dried and fresh mesocarp extracts were subjected to preliminary phytochemical screening to detect the different groups of chemical compounds of the extracts. This test was done qualitatively guided by standard protocol described by Harbone with slight modification (Harbone, 1991).

2.3.1 Alkaloids

15 mg of each extract was separately mixed on a water bath with 6 ml of 1% HCl and filtered. These filtrates were then tested with Dragendorff's test, Mayer's test, and Wagner's test, respectively [9]

2.3.2 Flavonoids

100 mg of different extract were dissolved in 5 mL distilled water. Add 0.5 mL NaNO3 into the solution and incubated at room temperature for 5 minutes. Then, added 2 mL of 10% AlCl3 and 1 M NaOH into the solution. The presence of flavonoid was indicated by the yellow color formation of the solution [9].

2.3.3 Glycosides

Both of extracts were dissolved with the same amount of glacial acetic acid and FeCl3 and mixed well. Then add 5 drops of concentrated sulfuric acid from the edge of the test tube and let it settle on the bottom. The presence of glycosides indicates by the formation of brown ring on the surface [9].

2.3.4 Saponins

500 mg of each extract was shaken separately with 10 ml distilled water in a test tube. the presence of saponins showed by the formation of persistent frothing on warming in a water bath for 5 minutes [10]

2.3.5 Tannins

1 mL of the extracts were added with a few drops of 0.1% $FeCl_3$. The blue-black or brownish green coloration was observed as the presence of tannin in the extracts [2]

2.3.6 Triterpenoid

1 mL of extract was added with 2 mL of chloroform and 2 mL of concentrated sulfuric acid from the edge of the test tube. The presence of triterpenoids indicated by formation of reddish-brown coloration on the surface [4]

2.3.7 Steroid

2 ml of extract was added with 1 ml chloroform and concentrated H_2SO_4. The presence of steroids was indicated with the appearance of red color layer in the lower chloroform [4]

2.4 Antimicrobial Activity

2.4.1 Preparation of Inoculum

The microbe that used in this study was purchased from Indonesia National Agency of Drug and Food Control. Gram-positive bacteria (*Staphylococcus aureus* (SA), *Bacillus substillis* (BS), Gram-negative bacteria (*Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC)) and one fungus, *Candida albicans* (CA) were tested for the antimicrobial activity of the dried and fresh mesocarps extract of *B. flabelifer*. The Gram-positive and Gram-negative bacteria were precultured in Nutrient Broth (NB) in a rotary shaker at 37 ^{o}C overnight. The cell density of each strain was adjusted using McFarland standard at concentration 107 cells/ml. The fungal inoculum was prepared and grown on Potato Dextrose Agar (PDB) from 6 – 10 day old culture. The petri dish then flooded with 10 ml distilled water and scraped the conidia using sterile spatula. The spore density was adjusted using spectrophotometer (A595 nm) at a final concentration 106 spores/ml [8], [11]

2.4.2 Determination of Zone of Inhibition Method

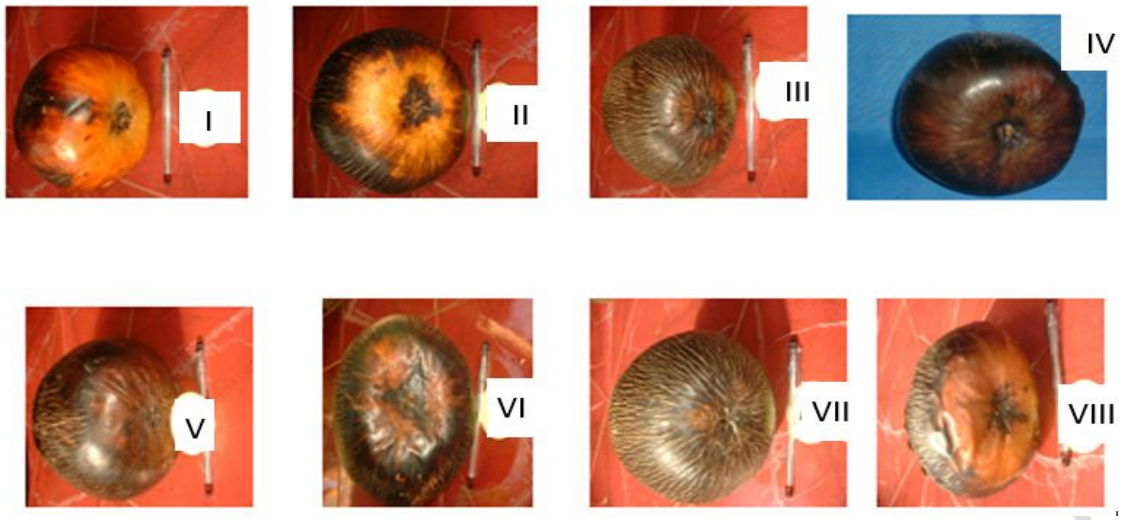
The antibacterial and antifungal activities of the dried and fresh mesocarps extracts were carried out in vitro against four pathogenic bacteria (two Gram negative and two Gram positive) and one pathogenic fungus. The antimicrobial activities were investigated by agar disk diffusion method. 1 g of each purified extracts were dissolved in aquades for the extract stock solution with concentration 1000 mg/mL and stored at 4 ^{o}C. This test is carried out aseptically. 10 mg/mL tetracycline and Nystatine were use as the positive standard antibiotic and the double-distilled water as the control negative. All the extracts were tested for their antibacterial and antifungal activities against bacteria *B. Substillis, S. aureus, E. coli*, *P. aeruginosa* and fungi *C. albicans* [2], [8], [11].

Sterile Nutrient agar plates were seeded with 108 CFU bacterial strains and Potato Dextrose Agar was seeded with the fungal C. albicans. All plates were incubated at 37 ^{o}C for 3 hours. The sets of two dilutions, 100 mg/ml, and 10 mg/ml, of dried and fresh fruit mesocarpextract were prepared in double-distilled water in a tube. 10 µL of both extracts on each concentration were impregnated respectively on a filter paper disk (5 mm in diameter). Each disk then placed on the plate that has been seeded by the bacterial and fungal strains. The zones of growth inhibition around the disk were observed and measured after 24 hours incubation at 37 ^{o}C for bacteria and 28 ^{o}C for 48 hours for fungi. The sensitivity of each extract was determined by the diameter of the bacterial or fungal inhibitory zone around the disk. The measurement of the inhibitory zone was including the diameter of the disk and were done three times [8], [9], [11].

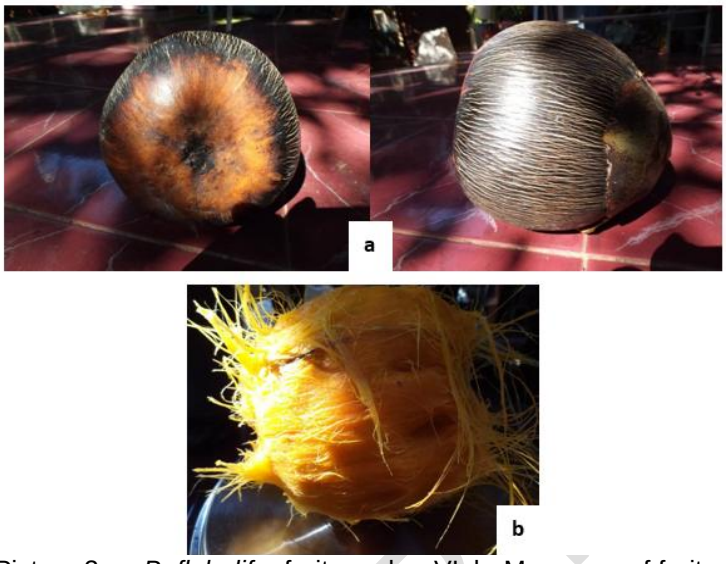
3. RESULTS AND DISCUSSION

3.1 Fruit of *Borassus flabelifer*

There are eight kinds of fruit of *Borassus flabelifer* that mostly found in Kupang-East Nusa Tenggara, Indonesia (Picture 1). This classification is based on the characteristic of the fruit skin and the tasted of the pulp of mesocarp. The fruit that used in this study was fruit number VI that has characteristic thick, chapped, and rough skin fruit. The color is black with 10 to 30% yellow color on the bottom of the fruit and the taste of the fruit was sweeter than the other fruit with a little bitter taste (Picture 2a). The sample that used in this study is the fresh and dried ripe mesocarp or the mesocarp of *B. flabelifer* number VI (Picture 2b). The color of the ripe mesocarp is yellow to orange and gives a specific aroma, probably caused by the yellow pulps that stick on the mesocarps.



Picture 1. Eight kinds of *B. flabelifer* fruit that find in Kupang-East Nusa Tenggara, Indonesia



Picture 2. a. *B. flabelifer* fruit number VI; b. Mesocarp of fruit number VI

3.2 Yield of Extraction

Table 1. Percentage Yield of dried and fresh fruit mesocarp extract

Sample	W_0	W_n	% Extractive value	Appearance
Dried fruit mesocarp	1 kg	176.56 g	17.65 %	Yellowish brown crystal and solid mass
Fresh fruit mesocarp	1 kg	100.89 g	10.01 %	Yellowish brown crystal and solid mass

W0 = initial weight; Wn = final weight

Based on data on Table 1, the yield of dried mesocarp extract was higher than the fresh mesocarp extract. This probably caused by a lot of water content that still existed on the fresh mesocarp that will be affect the number of substances which can be extracted if compared with the dried mesocarp extract.

3.3 Preliminary Phytochemical Screening

The preliminary phytochemical screening was conducted for testing different group of phytochemical compound present in both extracts. This study revealed the presence of saponin, flavonoid, triterpenoid, and glycoside in both dried mesocarp extract and fresh mesocarp extract. Besides that, there is another phenolic compound in dried mesocarp extract. This compound may be responsible for the antimicrobial activities of both extracts.

Table 2. Result of the phytochemical Screening of mesocarp extract of *B. flabelifer*

Phytochemical compound	Dried Extract	Fresh Extract
Alkaloid	-	-
Saponins	+	+
Tannins	-	-
Phenolic	+	-
Flavonoid	+	+
Triterpenoid	+	+
Steroid	-	-
Glycoside	+	+

(+) presence of the phytochemical compound, (-) absence of the phytochemical compound

3.4 Antimicrobial Properties of Mesocarp Extract of B. flabelifer against some human pathogens

The diameter of the zone of inhibition against some microbe pathogens was measured to evaluate the antimicrobial activity of the fresh and dried mesocarp extract. The result was compared with the positive standard, Tetratracycline (10 mg/mL) and Nystatine (10 mg/mL). The result of the antibacterial activity against Gram-negative and Gram-positive bacteria can be showed on Table 3 and Table 4, and for the antifungal activity can be showed on Table 5. The result showed that the fresh and dried mesocarp extract exhibited antibacterial activity against gram positive bacteria, *B. substilis* and *S. aureus* and had no activity against gram negative bacteria, *E. coli* and *P. aeruginosa*.

Table 3. Antibacterial activity of mesocarp extract of *B. flabelifer* against Gram negative bacteria

Concentration Extracts (mg/mL)	Zone of inhibition (mm)			
	E. coli		*P. aeruginosa*	
	DE	FE	DE	FE
100	-	-	-	-
10	-	-	-	-
Tetracycline (10 mg/mL)	22 ± 0.1		19 ± 0.2	

Note: DE: dried mesocarp extract of *B. flabelifer*; FE: fresh mesocarp extract of *B. flabelifer*

Table 4. Antibacterial activity of mesocarp extract of *B. flabelifer* against Gram positif bacteria

Concentration Extracts (mg/mL)	Zone of inhibition (mm)			
	B. subtilis		*S.aureus*	
	DE	FE	DE	FE
100	25 ± 0.2	9 ± 0.1	22 ± 0.1	8 ± 0.1
10	12 ± 0.1	-	10 ± 0.1	-
Tetracycline (10 mg/mL)	36 ± 0.2		37 ± 0.2	

Note: DE: dried mesocarp extract of *B. flabelifer*; FE: fresh mesocarp extract of *B. flabelifer*

Table 5. Antifungal activity of mesocarp extract of *B. flabelifer*

Concentration Extracts (mg/mL)	Zone of inhibition (mm)	
	C. albicans	
	DE	FE
100	9 ± 0.1	-
10	-	-
Nystatin (10 mg/mL)	19 ± 0.2	

Note: DE: dried mesocarp extract of *B. flabelifer*; FE: fresh mesocarp extract of *B. flabelifer*

3.5 Discussion

In this study, both extracts showed activity on inhibited Gram-positive bacteria (*S. aureus* and *B. subtilis*), but none on Gram negative bacteria (*E. coli* and *P. aeruginosa*). Meanwhile, only the dried mesocarp extract posed activity on inhibited the fungus, *C. albicans*. There is also different potency that showed by the fresh mesocarp extract and dried mesocarp extract on inhibiting the microorganism. The dried fruit fibber extract gave higher activity on inhibiting Gram-positive bacteria if compared with the fresh mesocarp extract. At concentration 100 mg/mL, the dried mesocarp extract showed diameter zone of inhibition for *B. subtilis* and *S. aureus* were 25±0.2 mm and 22±0.1 mm respectively, while the diameter zone of inhibition gave by the fresh mesocarp extract were 9±0.1 mm and 8±0.1 mm, respectively. On the other hand, when turn down the concentration of the extract become 10 mg/mL, only the dried mesocarp extract that exhibit diameter zone of inhibition, there are 12 ± 0.1 mm and 10 ± 0.1 mm for *B. subtilis* and *S. aureus*, respectively, while the fresh mesocarp extract indicated no diameter zone of inhibition. This is revealed that antimicrobial activity of the mesocarp extract was depend on the extract concentration.

The dried mesocarp extract of *B. flabelifer* was more effective in inhibit gram positive bacteria than fungi. It can be shown on the higher diameter zone of inhibition given by the extract on Gram positive bacteria than on fungi. The dried mesocarp extract showed small antifungal activity against fungus *C. albicans* at concentration 100 mg/mL, with diameter zone of inhibition 9±0.1 mm and none on concentration 10 mg/m. On the other hand, the antimicrobial activity given by both extracts were smaller, if compared with the positive control, Tetracycline for bacteria and Nystatin for fungi.

View study investigated the antimicrobial activity of *B. flabelifer* seed, but none for the mesocarp. Alamelumangai *et* al (2014) [2], detect that the ethanolic extract of *B. flabelifer* seed coat possess higher antimicrobial activity against some human pathogens, especially on inhibit gram-positive bacteria, *B. subtilis* and *Aspergillus brasiliensis*. In contrast with this study, the study by Banu *et al* [12], found that the aqueous freeze-dried extract of *B. flabelifer* seed, more effective in inhibit gram negative-bacteria, *Klebsiella pneumonia* and *E. coli* if compare with Azithromycin as the positive standard.

The drying process of sample has important role on the activity gave by the extract. By extracted the same amount of sample (1 kg), the dried mesocarp extract showed higher activity than the fresh mesocarp extract. The drying process of the sample helps to reduce the water amount of the sample and can concentrate the secondary metabolite compound which responsible for the antimicrobial activity. There are many studies had proved the role of secondary metabolite, such as Phenols, Terpenoid, Glycoside, and Flavonoid, on the antimicrobial activity with their different mechanism of action. These compounds were responsible and correlated with the antimicrobial activity against some human pathogen i.e *E. coli*, *S. aureus* and *C. albicans* [2], [4], [12].

However, this study was a preliminary study to prove the activity of *B. flabelifer* ripe mesocarp on the treatment of oral ulcers. For further development, the juice of the ripe mesocarp has to formulate into a dosage form or drink product and test for another anti oral ulcers mechanism. We also suggest for further purification to find the bioactive compound that responsible for its antimicrobial activity.

4. CONCLUSION

In conclusion, the dried mesocarp extract of *B. flabelifer* was more potential as antimicrobial compare with fresh mesocarp extract. The extract was more potent on inhibit Gram positive bacteria and fungi *C. albicans*, than on Gram negative bacteria. Preliminary phytochemical screening showed that the dried mesocarp extract contains Saponin, Phenols, Flavonoid, Glycoside and Terpenoid.

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