Libo Plant Potential (Ficus variegata, Blume) As Source Of Potential Pharmaceutical Materials

Abstract

Libo (Ficus varieagata) is a wild plant that has not been utilized in any form, including traditional because it has latex plant fruit and bark, and if the latex on the skin causes itching to occur even irritation. The nature of that causes undesirable fruit so that the fruit-eating animals are well maintained. Libo ripe fruit on the tree will fall to the ground and grow into a mature Libo tree. Potential of fruiting and fruit plants continuously disliked fruit-bearing animals that populations of plants Has conducted numerous studies on fruit Libo relations with pharmaceutical potential and proven potential as a source of antioxidants, cytotoxic or anticancer, A. aegypti larval exterminator, and as an antibacterial. These potentials can be used as a preservative, an anticancer drug, and a source of antibiotics if the research is done in detail. Libo fruit also contains a group of secondary metabolites that are highly variable so it still allows for other uses - uses in the pharmaceutical field.

Key Words: *Libo plant (Ficus variegata), a potential pharmaceutical ingredient*

A. INTRODUCTION

The traditional benefits of the Libo plant fruit (F. variegata) are not well known, except for a number of species belonging to the genus Ficus including diarrhea medicine, while the leaves are used to clean the lining of the eyes. The potential of Libo plants (F. variegata) that appears is continuous fruiting, the number of fruits is very much attached to the entire surface of the trunk and branches of trees. Libo fruit that has matured on the tree will fall to the ground and then the fruit will grow to become a mature Libo tree. Libo-eating animals are also very rare so that the survival of Libo plants is very good. The use of Libo trees for timber is also not potential because the structure of the wood trunk does not meet the requirements for building materials, which are dominated by cellulose fiber compared to lignans. Because of that the use of Libo plants is still very minimal with these characteristics, although the potential for sustainability is quite good. The way to utilize Libo plants with this potential must be conducted various studies directed at the potential of the pharmaceutical field especially medicine. However, research on the Libo plant (F. variegata) is also still very minimal so information on its usefulness is also very minimal. Forest fruit-eating animals are reluctant to eat Libo fruit so that it is thrown away.

Utilization of Libo plants especially fruits can be done in the pharmaceutical field because the characteristic potential of pharmaceuticals from natural materials is related to medicine, functional food-drinks, as well as healthy cosmetics. Libo fruit which fruit-eating animals do not like indicates that there are a number of compounds in Libo fruit that are toxic or feel uncomfortable. These properties are important in the pharmaceutical field, for example, potentially toxic properties as cancer drugs with the basic principle of cell killers

(chemotherapy), toxic properties as antimicrobials, pesticides (insecticides, larvicides, bactericides, fungicides, physisides) and others. Libo plants that are easily cultivated, bear fruit continuously are extraordinary potential and must be utilized. Figure 1 is an example of a Libo plant (F. variegata).

Figure 1.1. Examples of Libo plants (F. variegata)

B. METHODOLOGY

The potential of Libo (F. variegata) plants reported in this article are (a) the antioxidant activity of Libo fruit extracts (b) antibacterial activity (c) larvicide (d) cytotoxic or anticancer and (e) the content of secondary metabolites.

1. Intake of Plant Material

Libo fruit is taken from the yard of the Dean of the Faculty of Pharmacy, Mulawarman University, which at any time has fruit, especially in the dry season, which has more fruit than in the rainy season. The Libo that is taken is then chopped after being washed, then dried in direct sunlight. The dried Libo fruit is blended and a dry powder of 5.45 kg is obtained. Libo Fruits are sorted in such a way as to get clean fruit or without contaminants of other particles on the surface of the fruit.

2. Concentrated Ethanol Extraction and Fractionation

5.45 kg of dried powder prepared was extracted by cold maceration technique with ethanol 80 solvent%. Substitution of solvents during extraction is done as much as 6 times to get the maximum extract solution. Extraction activity is stopped after all secondary metabolites present in Libo fruit powder have been extracted into ethanol solvent. Indicators have been extracted from all the secondary metabolites of Libo, the extract solution is clear compared to before, and thin layer chromtographic analysis (TLC) was also performed. The extract solution obtained was immediately evaporated by the solvent using Rotavapor at 30 oC and 246.6 g extract was obtained. Concentrated ethanol extract of Libo fruit that has been obtained, then fractionation using liquid-liquid technique using distilled water. The fractionation is carried out in

a gradient starting with the solvent n-hexane, ethylacetate, n-butanol, and the remaining water fraction. The concentrated ethanol extract used for fractionation was 150 g and the fractions obtained as shown in Table 1.

Table 1. Concentrated ethanol extracts and Libo fruit fractions (F. varieagata)

NO.	Extract	Weight (g)
1	Concentrated ethanol extract	246,6
2	Extracts of n-hexane fraction	38,80
3	Ethylacetate fraction extract	32,45
4	N-butanol fraction extract	34,78
5	Water fraction extract	21,84

3. Cytotoxic Activity Test

Cytotoxic tests were carried out in vitro using the BSLT technique with bioindicator of Artemia salina shrimp larvae. The test results will illustrate the cytotoxic potential if the LC50 value ◎ 30 ppm (Rapael, 1991).

Preparation of Bioindicator Test

About 200 mg of A. salina eggs are hatched in an erlenmeyer container that has been prepared at a temperature suitable to the hatching temperature by heating with a 60 watt incandescent lamp. The hatching lasts 72 hours, and starting 48 hours have obtained larvae that are ready to be tested.

Determination of Test Sample Concentrations

The basic principle of determining the concentration of the test for toxicity or cytotoxic testing is to form a linear line of the relationship between the variation of the test concentration with the number of dead bioindicatory larvae, so that the LC50 value can be determined. Each extract was determined to vary in concentration by making low concentration and high concentration to estimate the concentration series to be made. Table 2 is the concentration of tests that have been found in determining the concentration series.

Table 2. Variation in concentration of test samples for testing the cytotoxic activity of Libo fruit extract (F. varieagata)

Variation Concentration of Libo Extract in ppm						
NO.	Extract	I	II	III	IV	V
1	Concentrated ethanol extract	5	10	15	20	25
2	Extracts of *n* -hexane fraction	10	15	20	25	30
3	Ethylacetate fraction extract	5	10	15	20	25
4	*n* -butanol fraction extract	5	10	15	20	25
5	Water fraction extract	10	15	20	25	30

Testing of Cytotoxic Activity of Libo Plant Fruit Extract

Tests of the ability to kill deadly extracts of A. salina illustrate toxicity and at LC50 values <30 ppm indicate cytotoxic or anticancer potential. All Libo fruit extracts obtained were tested for their ability to kill larvae of A. salina shrimp at the concentration of each extract that was found. The test was carried out in a 50 mL erlenmeyer container with the number of A. salina larvae per 10 treatment larvae. Observations were made every 3 hours up to 12 hours, as was done in determining the concentration of the test extract.

4. Antioxidant Test

Antioxidant activity is a chemical property of a compound that can be utilized in the pharmaceutical field, namely as a drug or preservative of pharmaceutical preparations. All Libo fruit extracts have been tested for antioxidants against DPPH free radical compounds.

DPPH Solution Preparation

The DPPH radical compound is a test indicator that has high reactivity because it has one unpaired electron on the nitrogen atom it contains. The DPPH solution used in this test is 40 ppm which is commonly used in every study of extracts whose compounds are unknown. DPPH solvent used is methanol.

Determination of Test Sample Concentration Series

The basic principle of determining the concentration of the test sample series in each antioxidant test must be obtained a linear relationship between the concentration of the test sample with a decrease in the absorbance value of DPPH solution. The concentration test value of each extract is randomly determined to be tested until found the concentration value that forms a linear line of the relationship between the concentration series with the absorbance value of the treatment. Table 3 is a variation of the concentration of the test sample in antioxidant testing.

Table 3. Variation of sample concentrations for testing the antioxidant activity of Libo fruit extract (F, varieagata)

Variation Concentration of Libo Extract in ppm						
NO.	**Extract**	**I**	**II**	**III**	**IV**	**V**
1	Concentrated ethanol extract	20	35	45	55	65
2	Extracts of *n* -hexane fraction	30	40	50	60	70
3	Ethylacetate fraction extract	10	20	30	40	50
4	*n* -butanol fraction extract	20	30	40	50	60
5	Water fraction extract	40	60	80	100	120

Antioxidant Testing

The actual implementation of the antioxidant test is the same as determining the concentration series of the test using the value of the test concentration that has been found. The extract solvent also uses methanol so that the non-polar fraction

extract is specifically added with DMSO to dissolve it with a maximum size of 1% by 2 mL. Testing has been done well.

5. Antibacterial Test

The test bacteria used were Staphylococcus aureus, Bacillus subtilis, Escherchia coli, and Pseudomonas aeruginosa. The four bacteria have an important role in the occurrence of disease in the community.

Making Bacteria Growing Medium

The medium used was Nutrient Agar (NA) with a composition of 3 g beef extract, 5 g peptone, 15 g agar, and 1000 mL aquades solvent. All ingredients are mixed with a water solvent then heated with a hot plate and sterilized in an autoclave at 121 oC for 15 minutes.

Preparation of Test Bacteria

Pure cultures of the tested bacteria were inoculated in the media and then suspended with a 0.9% sterile NaCl solution and obtained a bacterial suspension. Bacterial suspension is ready to be tested.

Determination of Test Sample Concentrations

The basic principle of determining the antibacterial concentration test series is to determine the maximum killing power of each extract. The concentration value is randomly determined and then tested to get a relationship between the inhibition / kill zone with variations in the concentration of the sample to form a hyperball curve or an inverse parabolic. The value of the concentration of the test taken randomly there are three, namely the lowest concentration that has been able to provide inhibitory / kill power against bacteria, and also determine the value of the concentration of the test that gives the highest inhibitory power and the concentration value that shows the power of killing / inhibition begins to decrease. Tables 4-7 are variations of the test concentrations that have been found in the determination of the antibacterial concentration test series.

Table 4. Variation of antibacterial concentrations of Libo (F. variegata) extracts against Bacillus subtillis bacteria.

Variation Concentration of Libo Extract in ppm						
NO.	**Extract**	**I**	**II**	**III**	**IV**	**V**
1	Concentrated ethanol extract	0,5	1,0	1,5	2,0	2,5
2	Extracts of *n* -hexane fraction	1,0	2,0	3,0	4,0	5,0
3	Ethylacetate fraction extract	1,0	2,0	3,0	4,0	5,0
4	*n* -butanol fraction extract	2,0	4,0	6,0	8,0	10,0
5	Water fraction extract	2,0	4,0	6,0	8,0	10,0

Table 5. Variation of antibacterial concentrations of Libo (F. variegata) extracts against Escherchia coli bacteria.

Variation Concentration of Libo Extract in ppm						
NO.	**Extract**	**I**	**II**	**III**	**IV**	**V**
1	Concentrated ethanol extract	1,0	1,5	2,0	2,5	3,0
2	Extracts of *n* -hexane fraction	5	10	15	20	25
3	Ethylacetate fraction extract	2	4	6	8	10
4	*n* -butanol fraction extract	1	3	5	7	9
5	Water fraction extract	10	15	20	25	30

Table 6. Variation of antibacterial concentrations of Libo (F. variegata) extract against Staphylococcus aureus.

Variation Concentration of Libo Extract in ppm						
NO.	**Extract**	**I**	**II**	**III**	**IV**	**V**
1	Concentrated ethanol extract	1,5	2,5	3,5	4,5	5,5
2	Extracts of *n* -hexane fraction	5	10	15	20	25
3	Ethylacetate fraction extract	3	5	7	9	11
4	*n* -butanol fraction extract	1	2	3	4	5
5	Water fraction extract	5	10	15	20	25

Table 7. Variation in antibacterial concentrations of Libo (F. variegata) extracts against Pseudomonas aeruginosa.

Variation Concentration of Libo Extract in ppm						
NO.	**Extract**	**I**	**II**	**III**	**IV**	**V**
1	Concentrated ethanol extract	1	3	5	7	9
2	Extracts of *n* -hexane fraction	5	10	15	20	25
3	Ethylacetate fraction extract	3	6	9	12	15
4	*n* -butanol fraction extract	1	2	3	4	5
5	Water fraction extract	10	15	20	25	30

6. Extract Activity Test Against Aedes aegypti Larvae

The characteristics of Libo fruit which are not favored by fruit-eating animals are the basis for testing various toxicity, including as eradicating A. aegypti larvae.

Preparation of Aedes aegypti Larvae

Mosquito eggsA. aegyptidi obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University. The age of the mosquito eggs is a maximum of 3 months and must be placed in a place that is not damp. Dropping A. aegypti eggs to become larvae is very easy by adding aquades water to chicken liver extract food, for 48 to 72 hours it will hatch and produce larvae ready to be tested. Testing is exactly

the same as the BSLT method. In this study, 100 mg of eggs were hatched in the container described earlier.

Determination of Test Sample Concentration Series

The basic principle of determining the concentration series of the test is the same as testing in the BSLT method, which is to obtain a straight line curve between the value of the test concentration and the number of dead larvae. Table 8 shows variations in the test concentrations that have been found.

Larvicide Testing

Concentration of sample extracts that have been found to be tested for the death of A. aegypty larvae. The test container used a 50 mL erlenmeyer flask with the number of larvae for each treatment was 10 larvae. Observations made were counting the number of dead larvae in each treatment series of the concentration of the test sample.

Table 8. Variation of larvacide concentration of Libo (F. variegata) extract against Aedes aegypty larvae.

Variation Concentration of Libo Extract in ppm						
NO.	**Extract**	**I**	**II**	**III**	**IV**	**V**
1	Concentrated ethanol extract	10	20	30	40	50
2	Extracts of *n* -hexane fraction	15	30	45	60	75
3	Ethylacetate fraction extract	10	20	30	40	50
4	*n* -butanol fraction extract	5	10	15	20	25
5	Water fraction extract	10	20	30	40	50

7. Secondary Metabolite Group Test

Secondary metabolite test was carried out on concentrated ethanol extract and not carried out on the extract fractions. Test results of concentrated extracts will depict the content of secondary metabolites in their fractions. All groups of secondary metabolites are screened using the typical reagents of each group of secondary metabolites. The secondary metabolite screening test container is a drip plate with the test indicator in general is the appearance of color.

C. RESULT AND DISCUSSION

The potential of Libo (F. variegata) plants reported in this article are (a) the antioxidant activity of Libo fruit extracts (b) antibacterial activity (c) larvaside (d) cytotoxic or anticancer and (e) the content of secondary metabolites**.**

1. **Intake of Plant Material**

Libo fruit is taken from the yard of the Dean of the Faculty of Pharmacy, Mulawarman University, which at any time has fruit, especially in the dry season, which has more fruit than in the rainy season. The Libo that is taken is then chopped after being washed, then dried in direct sunlight. The dried Libo fruit is blended and a dry powder of 5.45 kg is obtained. Libo Fruits are sorted in such a way as to get clean fruit or without contaminants of other particles on the surface of the fruit.

Concentrated Ethanol Extraction and Fractionation

5.45 kg of dried powder prepared was extracted by cold maceration technique with ethanol 80 solvent %. Substitution of solvents during extraction is done as much as 6 times to get the maximum extract solution. Extraction activity is stopped after all secondary metabolites present in Libo fruit powder have been extracted into ethanol solvent. Indicators have been extracted from all the secondary metabolites of Libo, the extract solution is clear compared to before, and thin layer chromtographic analysis (TLC) was also performed. The extract solution obtained was immediately evaporated by the solvent using rotavapor at 30 oC and 246.6 g extract was obtained. Concentrated ethanol extract of Libo fruit that has been obtained, then fractionation using liquid-liquid technique using distilled water. The fractionation is carried out in a gradient starting with the solvent n-hexane, ethylacetate, n-butanol, and the remaining water fraction. The concentrated ethanol extract used for fractionation was 150 g and the fractions obtained as shown in Table 1.

Table 1. Concentrated ethanol extracts and Libo fruit fractions (F. varieagata)

NO.	Extract	LC50 value in ppm
1	Concentrated ethanol extract	15,82
2	Extracts of n-hexane fraction	23,54
3	Ethylacetate fraction extract	11,53
4	N-butanol fraction extract	10,26
5	Water fraction extract	28,62

2. **Potential of Libo Fruit Extract as a Source of Antioxidants**

Antioxidants are molecular chemical properties that can be utilized in the pharmaceutical field, especially as medicines, functional foods, cosmetics, and preservatives of pharmaceutical preparations. The function of antioxidant properties as a drug and functional food and drink is related to the ability to reduce harmful free radicals so that there is no reaction between radical compounds and metabolites in cells. In addition, antioxidants can also help the performance of metabolism so that it

can have an effect on healing certain degenerative diseases. Furthermore, in the field of cosmetics related to the effect of restoring skin fitness so it looks younger than the truth. The relation with preservatives is to prevent oxidation so that the internal reaction of a pharmaceutical preparation can be prevented or slowed. The results of the antioxidant test of Libo fruit extract against DPPH free radical compounds are shown in Table 10. Libo fruit showed a very strong antioxidant potential, all extracts showed IC50 values below 100 ppm, while the level of strength of the ingredients that were included in the antioxidant category had IC50 values <200 ppm (Rapael, 1991). The antioxidant potential of Libo fruit reported in Table 10 cannot be used immediately because Libo fruit is known to cause irritation to the skin so that research on antioxidant detection is still needed associated with processing. However, these data Libo fruit have met the requirements to be considered as a potential source of antioxidants.

Table 10. Antioxidant activity of Libo fruit extract (F. varieagata) against DPPH free radical compounds.

NO.	Extract	LC50 value in ppm
1	Concentrated ethanol extract	47,85
2	Extracts of n-hexane fraction	55,65
3	Ethylacetate fraction extract	28,44
4	N-butanol fraction extract	25,46
5	Water fraction extract	82,46

3. Potential of Libo Fruit as Antibacterial

The activity of a molecule as an antibacterial is an important potential in the pharmaceutical field. Libo fruit has been tested for antibacterial against 4 types of bacteria, namely S. aureus, E. coli, P. aerugunosa, and B. subtillis. The results of antibacterial tests on the four types of bacteria are shown in Tables 11-14. Four test bacteria used to detect the antibacterial ability of Libo fruit extracts all gave a very good antibacterial effect, in terms of the concentration of tests used for each extract and also the killing power produced. The most powerful extract as an antibacterial is the extract fraction of n-butanol against the four types of test bacteria, which are in the> 8 mm zone with the best concentration killing bacteria on average less than 6%. This phenomenon shows that Libo fruit extract is very good as a potential antibacterial source.

4. Potential of Libo Extract As Aedes aegypty exterminator

The nature of Libo fruit which is disliked by insects has chemical properties that are repellent or toxic to insects. Based on these allegations, larvicide testing of A.

aegypti larvae was carried out. Larvicide test results for A. aegypti mosquito larvae are shown in Table 15.

Table 11. Antibacterial test results of Libo fruit extract (F. varieagata) against Bacillus subtillis bacteria.

No	Sample Extract	Kons. Extract in (%)	Kill Zone (mm)	Best concentration (%)
1	Concentrated ethanol extract	1	6,44	
		3	6,48	
		5	6,88	7
		7	7,42	
		9	7,12	
		5	4,22	
		10	4,46	
2	Extracts of *n*--hexane fraction	15	4,86	20
		20	4,98	
		25	4,38	
		3	5,68	
		6	5,89	
3	Ethylacetate fraction extract	9	6,43	12
		12	7,22	
		15	6,24	
		1	8,34	
		2	9,84	
4	*n* -butanol fraction extract	3	11,24	4
		4	13,24	
		5	12,86	
		10	4,21	
		15	4,28	
5	Water fraction extract	20	4,86	35
		25	4,94	
		30	4,64	

Table 15. The larvicidal activity of Libo (F. varieagata) fruit extracts against Aedes aegypti larvae.

NO.	Extract	LC50 value in ppm
1	Concentrated ethanol extract	35,46
2	Extracts of n-hexane fraction	46,24
3	Ethylacetate fraction extract	24,56
4	N-butanol fraction extract	16,78
5	Water fraction extract	34,56

The potential larvaeides of Libo fruit extracts, especially against A. aegypti mosquito larvae, were very strong ie all extracts showed LC50 below 50 ppm. This scientific evidence is a potential that is easily exploited because it is oriented to pesticides, namely insecticides, so it does not require further rigorous testing. Tests that can be carried out by Libo fruit extracts in relation to the potential of mosquito repellent are tested on adult mosquitoes or pupils.

5. Libo Fruit Secondary Metabolite Content

Screening for secondary metabolite content of Libo fruit aims to find out more about the possibility of secondary metabolites that provide cytotoxic, antioxidant, antibacterial, and larvicidal effects in each extract. The secondary metabolites contained in Libo boast are alkaloids (very dominant) and saponins.

D. CONCLUSION

Libo fruit is very potential in the pharmaceutical field as a source of antioxidants, cytotoxic (anticancer), antibacterial, and larvicidal especially as eradicating A. aegypti larvae. Libo fruit also contains a variety of secondary metabolites that are very varied which allows for other potentials. Libo plants that bear fruit continually support these potentials.

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