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Original Article

Pharmacognostic and Antimicrobial Studies of *Garcinia latissima* Miq. Leaves (Clusiaceae)

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ABSTRACT

Introduction: *Garcinia latissima* Miq known as Dolo magota (Maluku), is a medicinal plant belonging to the family Clusiaceae. The purpose of the research was to explore the phytoconstituents present, pharmacognostic details, and their antimicrobial efficacy. **Methods:** The preliminary phytochemical components were qualitatively examined using the standard method systems. The antimicrobial screening was carried out using the good diffusion method and the minimum inhibitory concentration (MIC) using dilution method. **Results:** The phytochemical screening of different extract of *G. latissima* Miq leaves revealed the presence of tannins, saponins, and alkaloids and the results were tabulated. The ethyl acetate and methanolic extracts from its leaves showed antimicrobial activity especially for *Bacillus subtilis,* a positive bacteria; the hexane extract did not show any activity against the selected microba. **Conclusion:** The results of the phytochemical and bio-efficacy study revealed most valuable information and also support the continued sustainable use of this leaves in the traditional system of medicine.

Key words: *Garcinia Latissima*, Antimicrobial, Phytoconstituent, Pharmacognostical.

INTRODUCTION

Garcinia latissima is one species of Guttiferae familia and also called Clusiaceae.[1,2] Their fruit like *G. mangostana* or mangosteen, *G. parvifolia* or kandis, *G. dulcis* or mundu, and *G. xanthoxymus* are edible, sweet, with no acid.[3] In addition to widely spread in Indonesia, this plant also spread in sub-tropical regions such as Japan, Korea, and China, tropical Asian, African, and Polynesian country[2-4]. This family is known to contain the yellow sap, which is a source of camboge paint and varnish, like *G. mangostana*, *G. dulcis* (Thailand, India, Sri Lanka), *G. hanburyi* (Thailand), *G. morrella* (India).[5,6] The contains of yellow sap are generally contains resins, oils, and sometimes have black or red glands, that contains hypericin or pseudohiperisin.[7]

Previous research in Papua New Guinea showed that ethanol extract of it dried stem bark has a zone of inhibition against the bacteria *Bacillus subtilis* and *Staphylococcus aureu.*[8] It also has been found that *G. latissima* Miq. stem bark from the center of the province of Papua New Guinea has four new pyranoxanthones, which are latisxanthone-A, latisxanthone-B, latisxanthone-C, and latisxanthone-D.[9] The biological activity of latisxanthone-C showed that it significantly inhibit the activity of viral antigen, which is the causes of tumors.[10] The data of secondary metabolite of it leaves is limited.

This plant, which is called Dolo magota by the local (Maluku), is found in Seram Maluku and Papua but has been cultivated in the Garden.[11] By the local community in Papua, it has been used as itchy medicine. This research used the one that came from Bogor.[7]

This research has a purpose which is to explore the phytoconstituents present, pharmacognostical details, the biological activities of *G. latissima* Miq. leaves as antimicrobial, making it useful in subsequent drug development.

MATERIALS AND METHODS

Plant material: *G. latissima* Miq. leaves were collected and identified from Plant Conservation, Bogor Botanical Gardens, Indonesian Institute of Sciences. After that it was washed, cut into small pieces, and dried in the oven. Dried material is stored in a sealed container in a cool, dry place.[12]

Macroscopic characteristics

For morphological observations, 15-30 cm long fresh leaves were used. The magnifying lens was used to observed the macromorphological features of the leaf.[13]

Microscopic characteristics

Fresh and dry leaf are examined with microscopy was taken using Nikon Coolpix 4500 camera (4.0 megapixel).

Extraction processes

This study uses multilevel maceration extraction methods.[12] Powdered leaves material was extracted by repeated maceration at room temperature using

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various solvents: hexane, ethyl acetate, and methanol in a row. After extraction, the filtrate was evaporated using rotary evaporator.[14] The residue (crude extract) was collected and stored at 4°C before used.[15]

Phytochemical analysis

The qualitative phytochemical tests of hexane extract, ethyl acetate extract, methanol extract were carried out to identify different phyto-constituents.[13]

Antimicrobial activity

This research has conducted two kinds of examination which are, inhibition zone assay, minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) assay. The inhibition zone assay using the well diffusion method. Four bacterial strains that were used are, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853, and *Bacillus subtilis* ATCC 6633, and the two fungal species that were used are, *Candida albicans* and *Trichophyton mentagrophytes*. Microbial stock cultures were cultured in nutrient agar for incubation.[16] The first inhibition zone assay was using 100% extract of *G. latissima* Miq. leaves. From the positive results of the first inhibition, the second inhibition zone assay was conducted using the 2% extract in DMSO (dimethyl sulfoxide) of the leaves. The MIC assay was determined using the broth dilution method.[17] The MBC was determined by plating out onto each appropriate agar plate.[18]

RESULTS

Macroscopic characteristics

Macroscopically, the leaf had a simple composition, it had ovalis shape, margins integer, and the venation patterns of leaves were parallel. It had obtusus apex and base, and thick. The leaves were 15-30 cm in length and 10-20 cm in width. The upper surface was laevis, nitidus, and had dark green color. The lower surface had light or pale green color (Figure. 1).

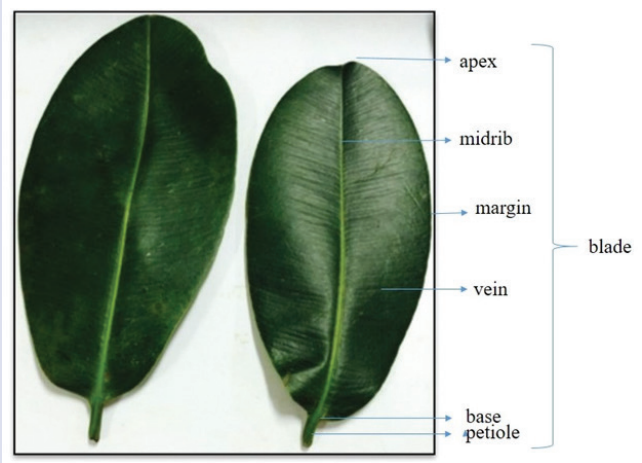


Figure 1: Macroscopic characteristics of *G. latissima* Miq. Leaves

Microscopic characteristics

The transverse section of *G. latissima* Miq. leaf showed the presence of upper and lower epidermis that was covered with a single layer of cuticle. The sklerenkim ured red because it react to floroglusin in chloride acid (Figure. 2).

There are diacytis stomata on longitudinal section was analyzed and photomicrographed (Figure. 3).

Powder study: the crude powder of the leaves were pale brown in colour. The diagnostic features of powder were tetragonal type of crystals of calcium oxalate (Figure. 4).

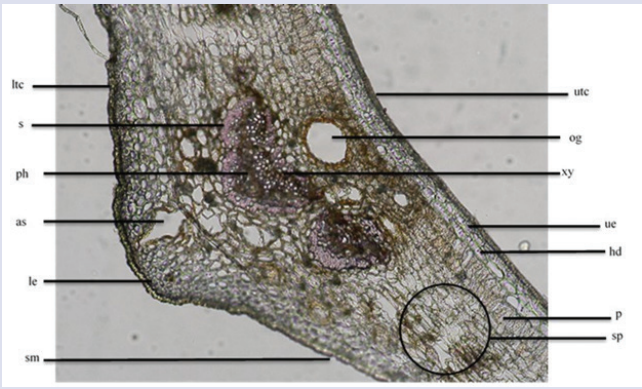


Figure 2: The Photomicrographs of a microscopic characteristic of a transverse section of *G. latissima* Miq. Were: as-air spaces, hd-hypodermis, le-lower epidermis, ltc-lower thick cuticle, og-oil gland, p-palisade, ph-phloem, s-sklerenkim (tured red because it react to floroglusin in chloride acid), sm-stomata, sp-spongy parenchyma, ue-upper epidermis, utc-upper thick cuticle, xy-xylem

Phytochemical analysis

The average of extracts rendemen from the result of multilevel maceration extraction from *G. latissima* Miq. leaves powder with different solvent are shown in Table 1.

The results of phytochemical tests are in the Table 2. Alkaloids were present in the n-hexane extracts and ethyl acetate extracts.

Antimicrobial activity

The results of the antimicrobial activities of n-hexane, ethyl acetate and methanolic extracts of *G. latissima* Miq. leaves are tabulated in Table 3.

All of the extracts showed the inhibition against the selected pathogens. The zone of inhibition of various extracts of *G. latissima* Miq. was compared with available standard antibiotic disc. The 2% ethyl acetate extract and the 2% methanol extract showed that it only active against *B. subtilis* with the diameter of ethyl acetat extract inhibition zone was 7.68 ± 0.076 mm and the diameter of methanolic extract inhibition zone was 9.9 ± 0.786 mm (Table 4).

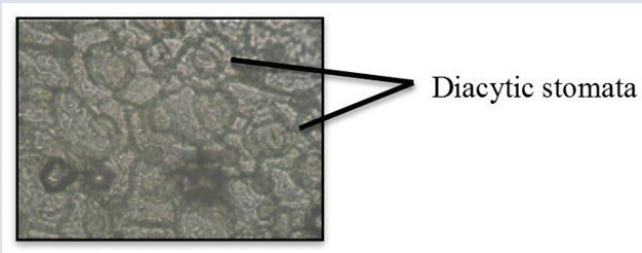


Figure 3: Photomicrographs of a microscopic characteristic of longitudinal section *G. latissima* Miq. leaf

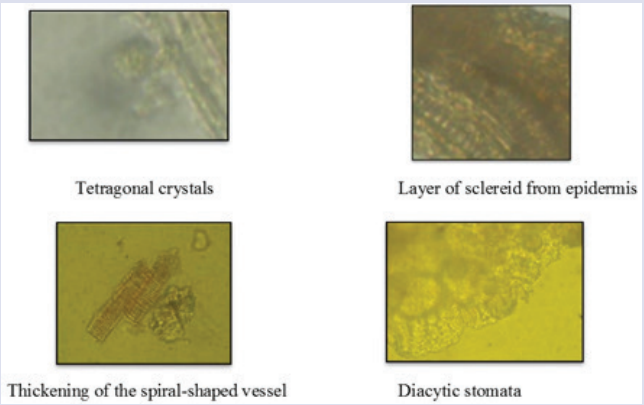


Figure 4: Photomicrographs of a microscopic characteristic of powder of *G. latissima* Miq. leaf

Table 1: The average of extracts rendemen from the result of multilevel maceration extraction from *G. latissima* Miq. leaves

Solvents	Rendemen (%)	Average (%)
n-Hexane	2.918	2.7753 ± 0.2266
	2.894	
	2.514	
Ethyl acetate	2.938	3.3800 ± 0.3930
	3.690	
	3.512	
Methanol	24.744	18.9713 ± 4.9993
	16.094	
	16.076	

Table 2: Phytochemical screening of *G. latissima* Miq. leaves

Tests	Reagents used	n-hexane extractives	Ethyl acetate extractives	Methanolic extractives
Tannins	Acidic $FeCl_3$	-	+	+
	Gelatin	-	+	+
Saponins	Frothing test	-	-	+
Flavonoides	HCl + Mg turnings	-	-	-
Anthraquinones		-	-	-
Terpenoids	Borntragers's	-	-	-
Alkaloids	H_2SO_4	+	+	-
	Dragendorff's	-	+	-
	Mayer's	+	+	-
	Bouchardat's			

Phytochemical screening : +: intensity reaction, -: non detected

The activity of ethyl acetate extract exhibited against *B. subtilis* (MIC 5,000 ppm, MBC 10,000 ppm) (Table 5). The results of the antibacterial activity showed that the activity of methanol extract exhibited against *B. subtilis* (MIC 10,000 ppm, MBC 20,000 ppm) (Table 6).

DISCUSSION

In the present investigation, the detailed pharmacognostic account of *G. latissima* Miq. leaf will be helpful for botanical identification of the drug.[19]

Table 3: Antibacterial activities of 100% extracts of *G. latissima* Miq. leaves

Organisms	Zone of inhibition (mm)		
	n-Hexane	Ethyl acetate	Methanol
B. subtilis	-	++	++
C. albicans	-	-	-
S. aureus	-	+	+
E. coli	-	-	-
P. aeruginosa	-	-	+
T. mentagrophytes	-	-	-

Noted: -: no inhibition zone; +: diameter of inhibition zone < 10 mm; ++: diameter of inhibition zone ≥ 10 mm.

Table 4: Antibacterial activities from 2% *G. latissima* Miq. leaves extracts in DMSO used agar diffusion method

Bacteria	Diameter of inhibition zone (mm)		
	Ethyl acetate	Metahanol	Antibiotic standard
B. subtilis	7.68 ± 0.076	9.9 ± 0.786	21.08 ± 1.928
S. aureus	0	0	23.70 ± 1.928
P. aeuginosa	0	0	21.88 ± 0.511

The average of results with triplo ± SD; Antibiotic standard: Erythromycin 15 µg for *B. subtilis,* Gentamycin 10 µg for *S. aureus,* Ciprofloxacin 5 µg for *P. aeruginosa.*

Maceration method was used because it is suitable for first extraction and for extraction in large number. The solvents that were used in this research were non-flammable, not explosive, and non-toxic. The polarity of it were also increase, so the secondary metabolite can dissolved in the three solvents.[20]

Phytochemical analysis: The plant, which utilize a physiological effect, are a biosynthetic laboratory for a multitude of compounds. The compounds that are responsible for imparting therapeutic effects are the secondary metabolites. The preliminary phytochemical analysis will give an idea about the chemical nature of the drug.[21] The information obtained will be useful in the further structural characterization of the nature of constituents present in this plant. It will be helpful to extract out particular constituents by a particular solvent.[22]

Saponins are a special class of glycoside which have soapy characteristics. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. The presence of tannins suggests the ability of this plant to play a major role for the treatment of some disease.[19]

The leaves have to be avoided from direct sunlight to minimize chemical reactions that can occur as a result of ultraviolet rays. It also has to be dried in the oven to prevent microbial fermentation and degradation of metabolites. Dried material is stored in a sealed container in a cool, dry place. Avoid too much time storage, as it can decipher some of the compounds. Milling is done to increase the yield of the extract, the surface area of the sample and solvent penetration into cells.[23]

Medicinal plants that contain thousands of substances are a precursor for the synthesis of useful drugs and are safe to human health.[15] The concern growing population about health problems has recently led to the development of natural antimicrobials to control the microbial disease. The antimicrobial activity found in the plant extracts have been attributed to some of the secondary metabolites.[15]

The antibacterial activity of plant extracts was not only due to one main active chemical but also due to combined action of other compounds.[16]

Table 5: The result of MBC against *B. subtilis* from ethyl acetate extract

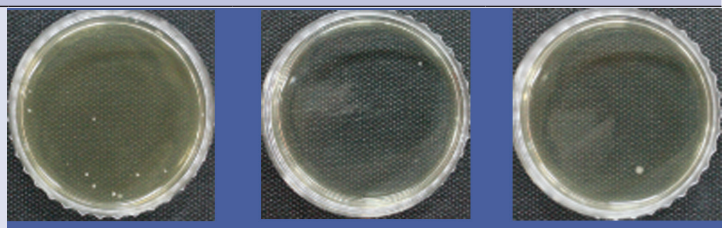
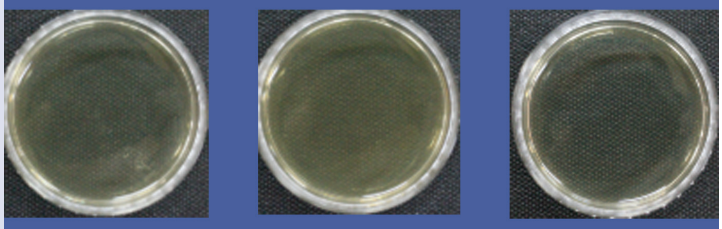
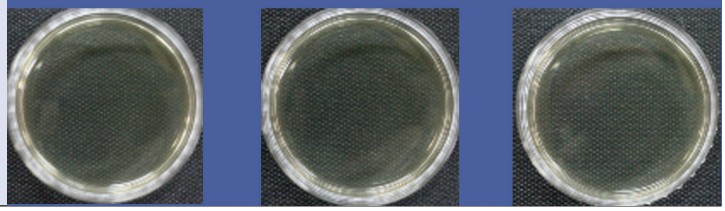
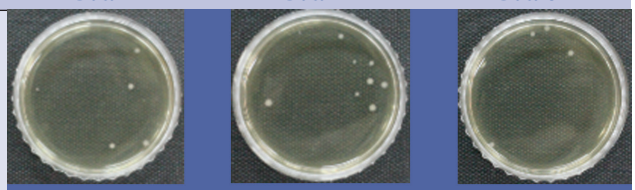
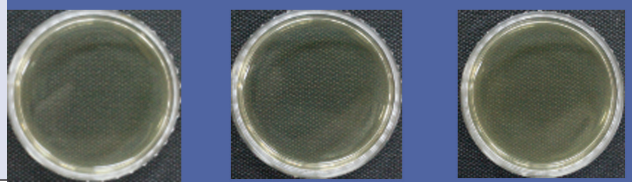
concentration ppm	Data 1	Data 2	Data 3
5,000			
10,000			
20,000			

Table 6: The result of MBC against *B. subtilis* from methanol extract

Concentration (ppm)	Data 1	Data 2	Data 3
10.000			
20.000			

The examples of other compounds are Phenolic acids, alkaloids, flavonoids, terpenes, terpenoids and naphthoquinone.[16] It is clear that the chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plant secondary metabolites.[16]

Maceration method is suitable for both initial and bulk extraction. The main disadvantage of maceration is that the process can be quite time-consuming, and also consume large volumes of solvents and can lead to potential loss of metabolites. Some compounds may not be extracted efficiently if they are poorly soluble at room temperature. On the other hand, maceration is less likely to lead to the degradation of thermolabile metabolites.[23] The physicochemical properties of some common solvents used in natural products extraction: polarity index of n-hexane 0.0, polarity index of ethyl acetate 4.4, polarity index of methanol 5.1. The initial choice of the most appropriate solvent is based on its selectivity for the substances to be extracted. A selective extraction can also be performed sequentially with solvents of increasing polarity.[23] It has done extensive research on the extracts of different types of solvents affect antimicrobial activity inhibition zone.[24] Methods to detect antimicrobial activity can be classified into three groups: diffusion, dilution, and bioautography. The advantage of the diffusion method is high suitability for pure screening substances.

Microbial used in this study is a gram-positive bacteria (*B. subtilis* and *S. aureus*), gram-negative bacteria (*E. coli* and *P. aeruginosa*) and fungi (*C. albicans* and *T. mentagrophytes*).[25]

CONCLUSION

The results of the present study revealed most valuable information and also support the sustainable use of Garcinia latissima Miq. leaves in traditional system of medicine. Moreover, a continuous and progressing research is to be conducted to prove the biological ingredients and test

the safety, efficiency and to determine the types of compounds responsible for the antimicrobial effect of *Garcinia latissima* Miq.[15]

Garcinia latissima Miq. showed a good effects *in vitro* antibacterial. The result presented here may explain the traditional use of this plant.[16]

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CONFLICT OF INTEREST

None

ABBREVIATION USED

MIC: The minimum inhibitory concentration; MBC: The minimum bactericidal concentration; ATCC: The American Type Culture Collection; DMSO: dimethyl sulfoxide; SD: standard deviation.

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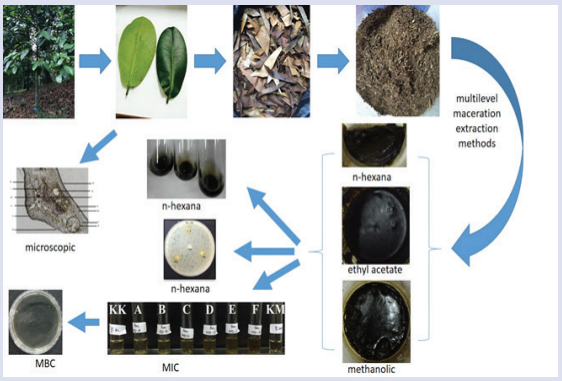
GRAPHICAL ABSTRACT



SUMMARY

- The phytochemical screening of different extract of *G. latissima* Miq leaves revealed the presence of tannins, saponins, and alkaloids and the results were tabulated.
- The ethyl acetate extracts from its leaves showed antimicrobial activity especially for *Bacillus subtilis,* a positive bacteria (MIC = 5,000 ppm).
- The methanol of *G. latissima* Miq leaves has activity against *Bacillus subtilis* (MIC = 10,000 ppm).
- The hexane extract did not show any activity against the selected microba.

ABOUT AUTHORS

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Prof. Dr. Muhammad Hanafi, Professor and Head of natural products, foods, and pharmaceuticals of Research Centre for Chemistry Indonesian Institute of Sciences, Serpong, Banten, Indonesia. He is expert in active compounds isolation and identification, and structure activity relationship.

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