

Research Article

Pharmacognostic Study and Antioxidant Activity of Mundar (*Garcinia forbesii* King.) leaves from Banua Botanical Gardens of South Kalimantan

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Abstract

Mundar (*Garcinia forbesii* King.) is a plant from South Kalimantan. This plant has chemical contents that have potential as medicine. The purpose of this study is to provide a pharmacognostic picture of a specific, nonspecific and determine the antioxidant activity of *G. forbesii* leaves. Specific parameters include organoleptic, microscopic testing, thin-layer chromatography profiles, and phytochemical screening. Nonspecific parameters include total ash content, acid insoluble ash content, drying loss, water-soluble extract content, and ethanol-soluble extract content. Antioxidant activity was determined by the DPPH method based on IC₅₀ values. Specific parameter test results are green powder, characteristic odor, and sour taste. Microscopic tests showed stomata, upper epidermis, lower epidermis, cell walls, xylem, phloem, palisade tissue, spongy tissue, and cuticles. *Garcinia forbesii* leaves contain alkaloids, flavonoids, phenols, tannins, and steroids. The TLC profile showed good separation of polar eluents, and yellow spots appeared after spraying DPPH reagents. The non-specific parameter test is the total ash content of 6.52±0.1%; acid insoluble ash content 1.06±0.08%; drying shrinkage 6.43±0.38%; water-soluble extracts 34.3±0.3%; and ethanol-soluble extracts 23.47±0.35%. Ethanol extract of *G. forbesii* leaves has antioxidant activity with IC₅₀ of 65.7 ppm. Pharmacognostic study fulfills the requirements, and *G. forbesii* leaves extract has strong antioxidant activity.

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INTRODUCTION

Indonesia has a diversity of natural materials, most of which have medicinal properties, one of which is the mundar (*Garcinia forbesii* King.) (Figure 1A). *Garcinia forbesii* is an original plant from South Kalimantan. The *G. forbesii* leaves (Figure 1B) are used by the people of Bangun Jaya, South Sumatra, as traditional medicine for constipation treatment. Many studies about *G. forbesii* plant activities from the stem bark and fruit peels. The *G. forbesii* stem bark contains rubraxanthone that has

antimicrobial activities (Alen *et al.*, 2008). The fruit peels were extracted with ethanol 30% and 70% have antioxidant studied on the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC₅₀ value more than 500 ppm (Muthia *et al.*, 2019) and the ethyl acetate fraction of the fruit peels of ethanol 70% extract have antioxidant activity with IC₅₀ value 72.386 ppm (Muthia *et al.*, 2018). Besides that, the fruit peels ethanol extract shows the antioxidant activity on the method of ferric reducing antioxidant power (FRAP) (Dewi, 2018) as well as water

extract with DPPH method (Andarini *et al.*, 2018). However, the part that is often scrutinized is fruit skin that is more difficult to obtain because it is seasonal, and the study of its leaves was reported just from the fraction of *n*-hexane. It has also been investigated as having antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* (Larasati, 2017; Lim, 2012). Our research is the first study of the antioxidant activity of *G. forbesii* leaves.

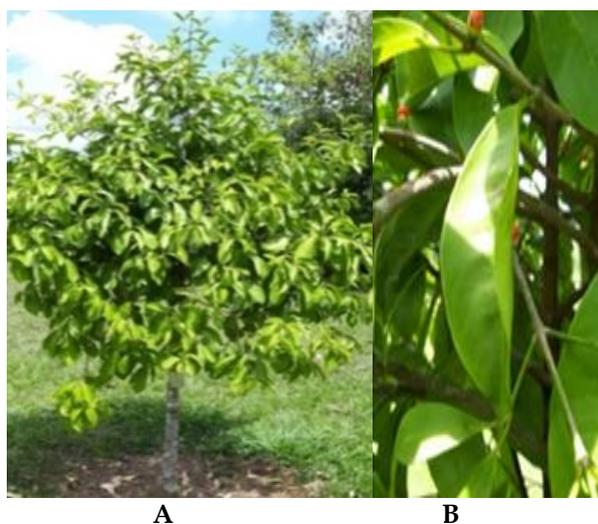


Figure 1. *Garcinia forbesii* whole plant (A) and leaves (B)

All the research was reported not contain the whole of standardization. This causes the need for quality testing and the quality of the materials used to ensure safety and efficacy. The pharmacognostic study is the basis and part of the plant standardization process in a simple way. This test is useful to support identification and determine plant material's efficacy and safety (Sapna *et al.*, 2008). The study of antioxidant activity is useful to provide preliminary data in the development of natural materials to increase the feasibility of using plants as herbal medicines. Antioxidant activity can be determined from the IC_{50} value by the DPPH method (Hadadi *et al.*, 2020). The DPPH method is a simple method through radical reduction with accurate, reliable, and relatively short time (Kedare & Singh, 2011). Based on the description above, researchers are interested in conducting the

pharmacognostic study and antioxidant activity of the *G. forbesii* leaves. The pharmacognostic study can provide an identification and safety guarantee of the *G. forbesii* leaves. The study of antioxidant activity can support the discovery of antioxidant sources in the treatment of various diseases. The results of these studies are expected to provide information on *G. forbesii* leaves as raw material for medicine and can be used as a scientific basis for further research.

MATERIALS AND METHODS

Tools and materials

The tools used in this study were furnaces (Ney-Vulcan D-550), UV lamps 254 and 366 nm, drying cabinet, maceration chamber, microscope, oven (Vinco), UV-Vis spectrophotometer (Genesys 10 v2. 100 2H3K297003), rotary evaporator (Heidolph Laborate 4000 4000 Efficient), analytical scales (Pioneers), and water bath (Memmert). The materials used are distilled water, acetic acid, hydrochloric acid, sulfuric acid, $FeCl_3$, DPPH, ethanol 96%, ethyl acetate (pro analysis), gelatin, potassium hydroxide, chloroform, quercetin, Mg powder, methanol (pro analysis), *n*-hexane (pro analysis), silica gel GF₂₅₄, Dragendorff's reagent, Liebermann-Burchard reagent, and Mayer's reagent.

Material collection, simplicia processing, and extracting

The *G. forbesii* leaves were collected from Kebun Raya Banua Kalimantan Selatan, Banjarbaru, South Kalimantan. The *G. forbesii* leaves powder is made by wet sorting, washing, chopping, dry sorting, and refining. As much as 200 g of *G. forbesii* leaves powder was extracted by maceration method using 96% ethanol solvent. Extraction was carried out for 3 × 24 hours with stirring every eight hours and solvent replacement every 24 hours. The liquid extract obtained was filtered and evaporated with a rotary evaporator and then thickened

on a water bath at a temperature of 50°C until a thick extract with constant weight was obtained.

Pharmacognostic study

The Pharmacognostic study includes specific parameters and nonspecific parameters. Specific parameters consist of organoleptic study, microscopic study, phytochemical screening on simplicia powders and extracts, and TLC profiles. The nonspecific parameters include total ash content, acid insoluble ash content, shrinkage of drying, water-soluble extract, and ethanol-soluble extract.

Determination of antioxidant activity

The *G. forbesii* leaves ethanol extract's antioxidant activity was determined using the DPPH method based on IC₅₀ values. Determination of antioxidant activity is done by determining the maximum wavelength of DPPH, operating time, determining the IC₅₀ value of quercetin as a reference and ethanol extract.

RESULTS AND DISCUSSION

Simplicia processing and extracting

The *G. forbesii* leaves are taken from Kebun Raya Banua Kalimantan Selatan, Banjarbaru, South Kalimantan. Fresh leaves as much as 1.55 kg and after processing obtained dry simplicia (Figure 2A) as much as 458 g. From 200 g of simplicia extracted with 96% ethanol, 47.28 g (23.64%) of thick extract (Figure 2B) was obtained.

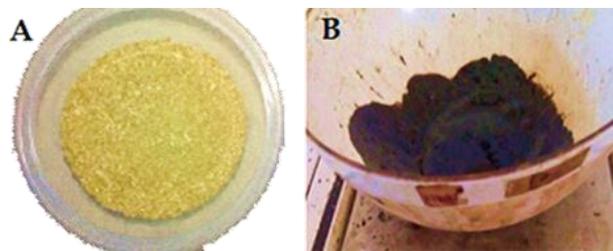


Figure 2. Simplicia (A) and extract (B) of *G. forbesii* leaves

Plant determination

The *G. forbesii* plant's determination was carried out at the Indonesian Institute of Sciences (LIPI), Bogor, West Java.

Determination serves as a confirmation that the plants used in this study are the correct samples so that the test results are specific and right on target (Altemimi *et al.*, 2017). Based on the statement of the results of the determination carried out at LIPI Bogor with letter number B-416/IPH.3/KS/III/2019, family and species data were obtained from the sample. The sample was stated to belong to Clusiaceae's family and was a species of *Garcinia forbesii* King.

Pharmacognostic study

The organoleptic study of the *G. forbesii* leaves simplicia shows green powder, sour taste, and characteristic odor of *G. forbesii* leaves. The green color is faded compared to fresh leaves because it has undergone drying so that the chlorophyll content in this simplicia is reduced. Sour taste is caused by the content of phenolic compounds (Kabera *et al.*, 2014). The pungent smell of simplicia of *G. forbesii* leaves, which is quite strong, is possible from the essential oil content.

The microscopic study was carried out using a light microscope on the longitudinal and transverse cross-section of *G. forbesii* leaves with a magnification of 10 × 10. This examination aims to see the anatomy and characteristics of the sample. The epidermal cells in the *G. forbesii* leaves' longitudinal cross-section are known to have curved and zig-zag cell wall forms. Stomata on the *G. forbesii* leaves are found in adaxial and abaxial with the parasitic type. *Garcinia forbesii* leaves' cross-section showed upper epidermis, cuticles, palisade tissue, spongy tissue, transport bundles such as xylem and phloem, and lower epidermis.

Based on phytochemical screening, the simplicia powder and the *G. forbesii* leaves extract contained the same compounds, such as alkaloids, flavonoids, tannins, steroids, and phenolics. These results indicate that the compound content in the simplex powder did not change after the extraction process. This can show the

consistency of the compounds contained after processing, as presented in [Table I](#).

Table I. Phytochemical screening test results for *G. forbesii* leaves

Compounds	Reagent	Result		Note
		Powder	Extract	
Alkaloid	Dragendorff's	Red sediment	Dark red sediment	+
	Mayer's	Yellowish white sediment	Brownish white sediment	+
Flavonoid	Mg powder + H ₂ SO ₄ concentrated	Reddish orange	Red	+
Tannin	Gelatin 1%	White sediment	White Sediment	+
Saponin	Shake vigorously with water	Unstable foam	Unstable foam	-
Steroid	Lieberman-Burchard	Bluish green	Dark blue	+
Phenol	FeCl ₃ 1%	Black	Black	+

On observing the chromatogram profile, it is known that there is a good separation in the eluent *n*-hexane-ethyl acetate (8 : 2) v/v and *n*-hexane-ethyl acetate (6 : 4) v/v. The chromatograms on the chloroform-methanol (9.5 : 0.5) v/v show one spot on the TLC plate's upper limit. That is because the compounds in the sample have low polarity so that the compound spots are still not separated in the eluent. The spraying TLC by elution of all three types of eluents with DPPH reagents shows that yellow spots on a purple background indicate the compound has antioxidant activity. Yellow spots appeared with R_f values of 0.27 and 0.45 in the eluent *n*-hexane-ethyl acetate (8 : 2), 0.05; 0.12; 0.23; 0.3; 0.81; and 0.96 for the *n*-hexane-ethyl acetate (6 : 4) eluent. Chloroform-methanol (9.5 : 0.5) yellow spots appear at a R_f value of 0.91.

The determination of total ash content aims to determine the internal and external mineral content contained in the sample. The results showed that the total ash of *G. forbesii* leaves simplicia met the requirements. The amount of total ash content of a material indicates the high content of metal elements in the material so that the material should have a small ash content. The determination of

acid-insoluble ash content aims to determine the specific amount of contamination from sand or silicate soil obtained from external factors and carried in the sample of the *G. forbesii* leaves simplicia. Based on the results obtained, the *G. forbesii* leaves simplicia contain external contamination with small levels and still meet the requirements. The contamination of impurities such as soil and sand, silver metal elements, lead, and mercury during processing can cause high insoluble acid ash in a sample ([Handayani et al., 2018](#); [Muchtadi & Ayustaningwarno, 2010](#); [Ratnani et al., 2017](#)).

The determination of drying losses aims to provide a limit or range of the amount of water and volatile compounds lost during the drying process. The drying process's quality is getting better if the value of drying shrinkage is getting smaller. The results of drying losses on the simplicia obtained have met the requirements ([Ministry of Health of the Republic of Indonesia, 2017](#)). The determination of water-soluble extracts aims to determine compounds that can be found in water solvents. The compounds dissolved in water are polar. This data can be used as a reference for herbal medicine in the form of stew (infuse). The compounds dissolved in water are polar compounds such as amino acids, some vitamins, enzymes, sugars, glycosides, inorganic salts, carotenoids, proteins, saponins, tannins, and alkaloid salts ([Ali et al., 2018](#); [Hardiana et al., 2012](#)).

The determination of ethanol-soluble extracts aims to provide an initial picture of the number of compounds found in the ethanol solvents. The ethanol solvents can dissolve the semi-polar and the polar compounds. The compounds dissolved in the ethanol are glycosides, essential oils, alkaloids, small amounts of fatty oils, pigments (chlorophyll and carotene), phenols, terpenoids, steroids, waxes, and resins ([Zhang et al., 2018](#)). The value of ethanol-soluble extracts can be used to make extracts used as a natural medicine and an initial

description of the magnitude of compounds found in organic solvents (Handayani *et al.*, 2018). The determination of non-specific parameters is presented in **Table II**.

Table II. The test results for the non-specific parameters of simplicia of *G. forbesii* leaves

Replication	Total ash content (%)	Acid insoluble ash content (%)	Shrinkage of drying (%)	Water soluble extract (%)	Ethanol soluble extract (%)
1	6.47	0.97	6.6	34.3	23.5
2	6.47	1.1	6	34.6	23.8
3	6.63	1.1	6.7	34	23.1
Average ±	6.52 ±	1.06 ±	6.43 ±	34.3 ±	23.47 ±
SD	0.1	0.08	0.38	0.3	0.35
Requirement ^f	≤ 16.6	≤ 2	≤ 10	≥ 16	≥ 8

^f(Ministry of Health of the Republic of Indonesia, 2017; Ministry of Health of the Republic of Indonesia, 1995)

Determination of antioxidant activity

The maximum wavelength is determined to determine the wavelength of the compound that provides maximum absorbance. A measurement carried out at maximum wavelength will give linear results, high instrument sensitivity, and reduce measurement errors (McBirney *et al.*, 2016). The maximum wavelength obtained in this study is 516 nm.

The time needed for the reaction between a compound and another compound to reach a stable point is called operating time. The operating time in determining antioxidant activity can indicate that DPPH has reacted correctly with antioxidant compounds (Salamah & Widayari, 2015). The operating time obtained in this study is 22 minutes.

The IC₅₀ value determination of the antioxidant activity was performed using positive comparison quercetin. Based on these comparisons, can be seen the amount of antioxidant activity on the sample. The linear regression analysis results between quercetin concentration and the percentage of inhibition obtained using SPSS obtained the equation $y = 9.634x + 12.606$ with a relation coefficient (r) of 0.998. Quercetin has an IC₅₀ value obtained equal to

3.88 ppm and classified as a very strong antioxidant activity.

The IC₅₀ value determination of the *G. forbesii* leaves ethanol extract was obtained from the regression equation between the relationship of the *G. forbesii* leaves ethanol extract concentration with % inhibition that was $y = 0.449x + 20.483$ with relation coefficient (r) of 0.998. The IC₅₀ value of the *G. forbesii* leaves ethanol extract obtained based on the results of linear regression analysis using SPSS was 65.7 ppm and classified as having a strong antioxidant activity. The results of measuring antioxidant activity are presented in **Table III**.

Table III. The antioxidants of quercetin and ethanol extract of *G. forbesii* leaves

Samples	Concentrations (ppm)	Inhibition (%)	IC ₅₀ (ppm)
Quercetin	2	30.93	3.88
	3	43.29	
	4	50.41	
	5	60.67	
	6	70.41	
	7	77.14	
The ethanol extract of <i>G. forbesii</i> leaves	30	33.82	65.7
	50	42.4	
	70	52.49	
	90	61.98	
	110	68.97	

The chemical content responsible for the antioxidant activity of *G. forbesii* leaves from the results of phytochemical screening is alkaloids, flavonoids, tannins, steroids, and phenols. Research conducted by Muthia *et al.* (2019) showed that the extracts of ethanol 30% and 70% for *G. forbesii* rind had IC₅₀ values of 717.01 and 534.69 ppm, respectively. They also reported that the ethyl acetate fraction of *G. forbesii* rind had an IC₅₀ value of 72.386 ppm (Muthia *et al.*, 2018). This result shows that the antioxidant activity of *G. forbesii* leaves is more significant than its rind.

CONCLUSION

The pharmacognostic study results of *G. forbesii* simplicia leaves are green, sour taste, and have a characteristic odor. The structure microscopically of the leaves can be

seen, such as stomata, upper epidermis, lower epidermis, cell walls, xylem, phloem, palisade tissue, spongy tissue, and cuticles. The *G. forbesii* leaves contain positive alkaloids, flavonoids, phenols, tannins, and steroids. The TLC profile showed good separation in non-polar eluents, and yellow spots were appearing after being sprayed with DPPH reagents. The nonspecific parameters performed on *G. forbesii* leaves simplicia fulfill the requirements. The *G. forbesii* leaves ethanol extract has a strong antioxidant activity with an IC₅₀ value of 65.74 ppm.

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