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Short Communication

The Potential Antioxidant Activity of N-Hexane Fraction of Mundar Leaves (*Garcinia forbesii* King.)

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ABSTRACT

Mundar (*Garcinia forbesii* King.) is a native plant of South Kalimantan, Indonesia. This study was aimed to identify the compounds and evaluated the antioxidant activity of the n-hexane fraction and sub-fraction of *G. forbesii* leaves. The *G. forbesii* leaves were macerated with methanol. Liquid-liquid extraction of the methanol extract was performed using n-hexane-distilled water. The fraction obtained was then subjected to vacuum liquid chromatography using n-hexane:ethyl acetate gradient system with ratios of 25:1; 20:1; 15:1; 10:1; 8:2; 6:4; 4:6 and 2:8 v/v. Compound identification and qualitative antioxidant tests was done using thin-layer chromatography and then sprayed with specific reagents. The quantitative antioxidant tests were carried out using UV-Vis spectrophotometry. Maceration produced an extract with a yield of 20.4% and an n-hexane fraction of with a yeild of 29.0%. Fractionation with VLC resulted in 8 sub fractions (A-H). The qualitative test using specific reagents revealed that the 8 sub fractions (A-H) contained flavonoids and alkaloids, 2 fractions (B and C) contained terpenoids, 3 fractions (D-F) contained steroids, and 4 fractions (E-H) contained phenols and tannins. The qualitative antioxidant test showed that all sub fractions have potential antioxidant activity. Quantatively, the n-hexane fraction showed a strong antioxidant activity with an IC_{50} value of 26.07 ppm. The findings from this study shows that *G. forbesii* leaves have potential for use as a natural antioxidant agent.

Keywords: *Garcinia fobesii*, Mundar, Extraction, Chromatography, Secondary Metabolites.

Introduction

The immense potential of medicinal plants in Indonesia necessitates the need for the scientific evaluation of the secondary metabolites and the bioactivity of these plants.[1] Secondary metabolites play a role in the self-protection of plants to maintain their existence and these metabolites can be utilized by humans to treat various diseases.[2] These secondary metabolites have different properties and characteristics so that an appropriate extraction method is needed to obtain them. Therefore, it is crucial to identify the content of secondary metabolites so that the efficacy of a plant can be estimated.[1]
The mundar (*Garcinia forbesii* King.) is an endemic plant of Kalimantan that has potential as a medicine.[3] The plants of the genus *Garcinia* have antimicrobial, anticancer, and antioxidant properties.[4] The active substances in the genus Garcinia are xanthones, anthocyanins, tannins, and phenolic compounds.[5] The phytochemical test of the methanol extract of *G. mangostana* leaves has revealed the presence of anthraquinones, carbohydrates, terpenoids, saponins, flavonoids, glycosides, tannins, and phenolic compounds.[6] The phytochemical screening of the ethanol extract of *G. forbesii* leaves has shown the presence of alkaloids, flavonoids, phenols, tannins, and asteroids.[7]

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According to Larasati *et al.*,[8] the n-hexane fraction of *G. forbesii* leaves could inhibit *Escherichia coli* and *Staphylococcus aureus*.
Phytochemical identification of the n-hexane fraction of *G. mangostana* leaves showed that it contains flavonoids, steroids, and saponins.[9]
The present research was carried out to determine the potential antioxidant property of nonpolar fraction of *G. forbesii* leaves using qualitative and quantitative methods.

Materials and Methods

The dry leaves of G. forbesii leaves.
The leaves of *G. forbesii* were collected from Kebun Raya Banua Banjarbaru, South Kalimantan, Indonesia in September, 2019. The plant authentication was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Science, Universitas Lambung Mangkurat, Indonesia. An herbarium specimen number: 068c/LB.LABDASAR/III/2020 was assigned. The plant sample was dried at 50°C following the standard procedure of Farmakope Herbal Indonesia.[10] The dried sample was ground with a grinder, sieved using a mesh size 14, and stored at room temperature in a well-closed container.

Preparation of G. forbesii leaves methanol extract.
A total of 1.5 kg of dried *G. forbesii* leaves powder was extracted by maceration in 3.5 L of methanol (Merck®) for 24 h while stirring every 8 h followed by filtration. Re-maceration of the marc was carried out 5 times. The liquid extract was evaporated using a rotary evaporator at 50°C until a thick extract with a constant weight was obtained.[10]

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Preparation of the n-hexane fraction of G. forbesii leaves methanol extract

The n-hexane fraction was obtained through a liquid-liquid extraction. The methanol extract was weighed (about 10 g) and suspended in 25 mL of distilled water (1:2.5). The suspension was then extracted with n-hexane (Merck®) (1:5).[11] the extraction with n-hexane was carried out seven times so that 350 mL of n-hexane was used. The n-hexane fraction was collected and dried until a thick fraction was obtained. The percentage yield was calculated by comparing the dried fraction with the dried methanol extract of the leaves of *G. forbesii*.[10]

Sub fractionation of the n-hexane fraction using vacuum liquid chromatography

The n-hexane fraction was weighed (10 g) and separated with vacuum liquid chromatography in activated silica gel 60 (Merck®) stationary phase for column chromatography (0.040–0.063 mm). The mobile phase used gradient system of *n*-hexane:ethyl acetate with ratio 25:1, 20:1, 15:1, 10:1, 8:2, 6:4, 4:6 to 2:8 v/v. The sub fractions obtained were dried and weighed.[12]

Thin layer Chromatography (TLC) profile of the n-hexane fraction and sub fractions

The stationary phase used was silica gel GF254 Merck®. The TLC of the n-hexane fraction and the sub fractions were developed with *the n*-hexane: ethylacetate mobile phase in ratio 8:2 v/v. The seperated compounds were observed at UV 254 nm.[13] The secondary metabolites such as flavonoids were identified with ammonia vapour and alkaloids using Dragendorff's reagent.[14] Steroids and terpenoids were identified using Lieberman-Burchard reagent, and phenolics were identified using ferric chloride ($FeCl_3$ 10%).[11,15–17]

Preparation of quercetin comparison solution

Pure quercetin (100 ppm) was prepared by dissolving 5 mg of quercetin with methanol in a 50 mL volumetric flask. The 100 ppm mother liquor was diluted to 10 ppm using a 25 mL volumetric flask. The 10 ppm solution was used to make a series of concentrations of 0.5, 1, 2, 4, 6, and 8 ppm using methanol solvent in a 10 mL volumetric flask.[18,19]

Antioxidant activity screening

The qualitative antioxidant activity of the sub fractions was identified by TLC using *n*-hexane: ethyl acetate mobile phase in ratios 8:2 and 6:4 v/v. The dried TLC plates were sprayed with 0.01% DPPH (2,2-diphenyl-1-picrylhydrazil). The spots have antioxidant activity if they are yellow on a purple background. The quantitative antioxidant activity of the n-hexane fraction was determined spectrophotometrically by the DPPH radical scavenging assay[20,21] using a spectrophotometer (UV-Vis, Thermo Spectronic Genesys 10 instruments). The standard used was quercetin (Merck®). The percentage radical inhibition was calculated by the following equation.[22,23]

$$\% \text{ Inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

The IC_{50} value was calculated by the equation Y = bX + a. The equation was obtained by entering the sample concentration values (x-axis) and % inhibition (y-axis) into the linear regression equation. After the values of a and b were obtained, they were entered into the following equation.[24]

$$IC_{50} = \frac{(50-a)}{b}$$

Where a = intercept and b = slope

Statistical analysis

The concentration and percent inhibition of the antioxidant activity of the n-hexane fraction was analyzed using IBM SPSS® software version 21. The IC_{50} value was analyzed by probit regression using IBM SPSS® software to compare the results of the linear regression method. The IC_{50} value from linear regression was also analyzed by the independent t-test at 95% confidence level ($p<0.05$).

Results and Discussion

Percentage yeilds of the methanol extract and n-hexane fraction

The maceration produced 20.4% (306.1 g) of a viscous extract with dark green colour and a slightly bitter taste. The n-hexane fraction obtained was 29% (2.9 g).

VLC sub fraction of the n-hexane fraction

From the n-hexane fraction, 8 sub fractions were obtained: A, B, C, D, E, F, G and H each with a weight of 1.62, 1.04, 0.70, 0.48, 1.38, 1.60, 0.76 and 0.82 g. The scheme of *G. forbesii* fractionation is presented in Figure 1.

The secondary metabolites of each sub fraction were identified with site-specific TLC reagents (Table 1).

The sub fractions A to H contained flavonoids (Figure 2a) and alkaloids (Figure 2b). Terpenoids were found in sub fractions B and C (white sign in Figure 2c), and steroids in sub fraction D to F (green-blue sign in Figure 2c). Phenolics, including tannin (black sign in Figure 2d) and phenol (blackish-blue sign in Figure 2d), were found in the polar sub fractions (E to H)

Identification of flavonoid compounds with ammonia vapor produced yellow spots on the TLC plate. The TLC identification with Dragendorff's reagent showed positive results for alkaloids indicated by orange or brown spots.[14] The identification of terpenoid and steroid compounds was carried out using the Lieberman-Burchard reagent. The purple spots indicate that the sub fractions contain terpenoids, and the green-blue spots represent steroids.[25] The terpenoid spots appeared on sub fractions B and C with Rf value of 0.56 and 0.57, respectively. The green-blue spots indicate the presence of steroids in sub fraction D with Rf values of 0.29 and 0.36, in sub fraction E with Rf 0.33 and 0.47, then in sub fraction F with Rf 0.38. A dark blue spot indicates the presence of phenolic group after the TLC plate was sprayed with $FeCl_3$ (10%).[15] The spots appeared on sub fraction E with Rf value of 0.18 and 0.4, on sub fraction F with 0.33; sub fraction G with Rf 0.22, and sub fraction H with Rf 0.18. These results are consistent with Sutomo *et al.*,[7] who reported that the ethanol extract of *G. forbesii* leaves contained flavonoids, alkaloids, terpenoids, steroids, and phenolics.

The antioxidant activity

The qualitative antioxidant activity potential of sub fractions has been identified. All sub fractions showed yellow spots on purple background. Circles on the chromatogram indicate the presence of compounds that have potential as antioxidants (Figure 2e-f)). The potential group as an antioxidant was flavonoids based on TLC-specific reagents as presented in Figure 2. The quantitative antioxidant activity of this fraction evaluated by the DPPH radical scavenging method showed that the fraction had high antioxidant activity with IC_{50} value of 26.28 ppm, although the activity was lower compared to that of quercetin (IC_{50} = 3.11 ppm) (Figure 3, Table 2).

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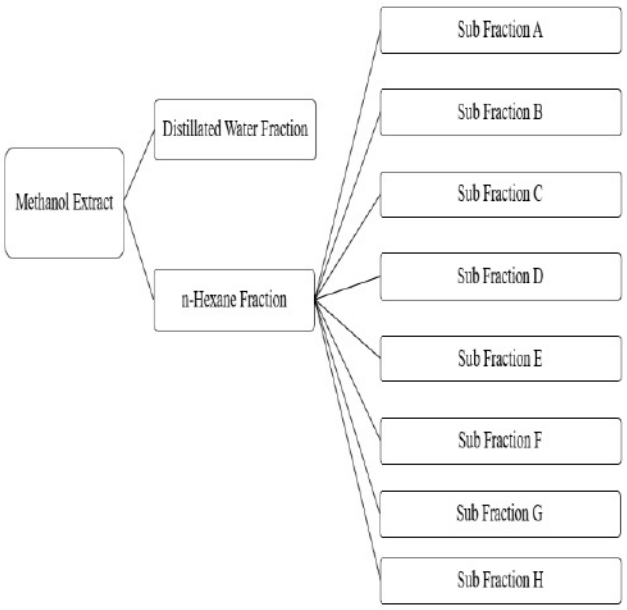

Figure 1: The fractionation of *Garcinia forbesii* leaves

The secondary metabolite profile of the n-hexane fraction and sub fractions

The standard antioxidant compound used for comparison was quercetin (3,3',4',5,7-pentahydroxyflavone), a phenolic compound of the flavonoid group which has been shown to have a strong antioxidant.[26] Quercetin's ability to scavenge free radicals by delocalizing radical electrons into the polyaromatic structure of phenol has been demonstrated to be higher than that of ascorbic acid and gallic acid.[27] In this study, the concentration of quercetin needed to reduce 50% DPPH radicals (IC_{50}) was 3.11 ppm.

Figure 3a shows the plot of the sample concentration versus the inhibition of DPPH. The highest concentration of the sample that caused maximum inhibition of DPPH (85.21±0.07%) was 60 ppm. The n-hexane fraction of *G. forceii* leaves has an IC_{50} value of 26.28 ppm which identifies it as a strong antioxidant. This results indicate that the antioxidant potential of the n-hexane fraction *G. forbesii* leaves is more potent than the ethanol extract of *G. forbesii* leaves (IC_{50} value of 65.7 ppm), the n-hexane fraction of *G. forbesii* fruit peel (IC_{50} value of 534.69 ppm), and the ethyl acetate fraction of *G. forbesii* fruit (IC_{50} of 72.386 ppm).[7,28,29]

The independent t-test at 95% confidence level showed a significant value of 0.001 ($p < 0.05$). It was concluded that there was a significant difference between the two test groups (positive control of quercetin and the n-hexane fraction of *G. forbesii* leaves).

Table 1: The results of TLC- specific reagents

Compound group	Specific reagents	Spot colour	Sub fraction								
			N-F	A	B	C	D	E	F	G	H
Flavonoids	Ammonia vapor	Yellow	+	+	+	+	+	+	+	+	+
Alkaloids	Dragendorff	Orange or brown	+	+	+	+	+	+	+	+	+
Terpenoids	Lieberman-Burchard	Purple	+	-	+	+	-	-	-	-	-
Steroids	Lieberman-Burchard	Green-blue	+	-	-	-	+	+	+	-	-
Phenols	$FeCl_3$ 10%	Blackish-blue	+	-	-	-	-	+	+	+	+
Tannins	$FeCl_3$ 10%	Black	+	-	-	-	-	+	+	+	+

Notes: N-F = n-hexane fraction; (+) = present; (-) = absent

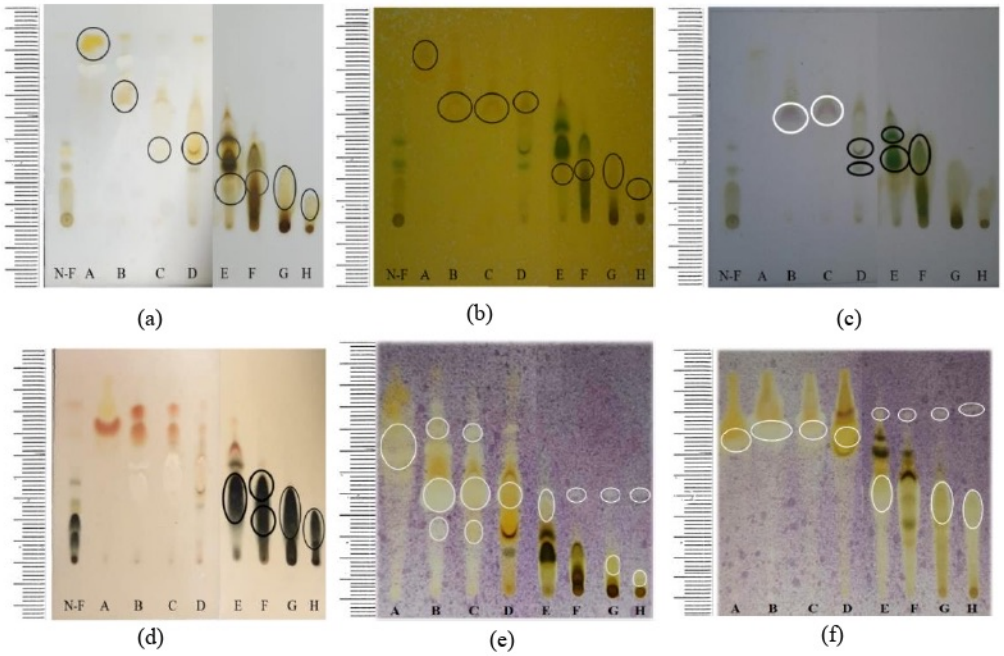

Figure 2: TLC profiles of the n-hexane fraction and sub fractions in mobile phase n-hexane: ethyl acetate (8: 2 v/v) with reagents **(a)** ammonia vapor, **(b)** Dragendorff, **(c)** Lieberman-Burchard, **(d)** $FeCl_3$ 10%, **(e)** DPPH 0.01%, **(f)** in mobile phase n-hexane: ethyl acetate (6: 4 v/v) with DPPH 0.01%.

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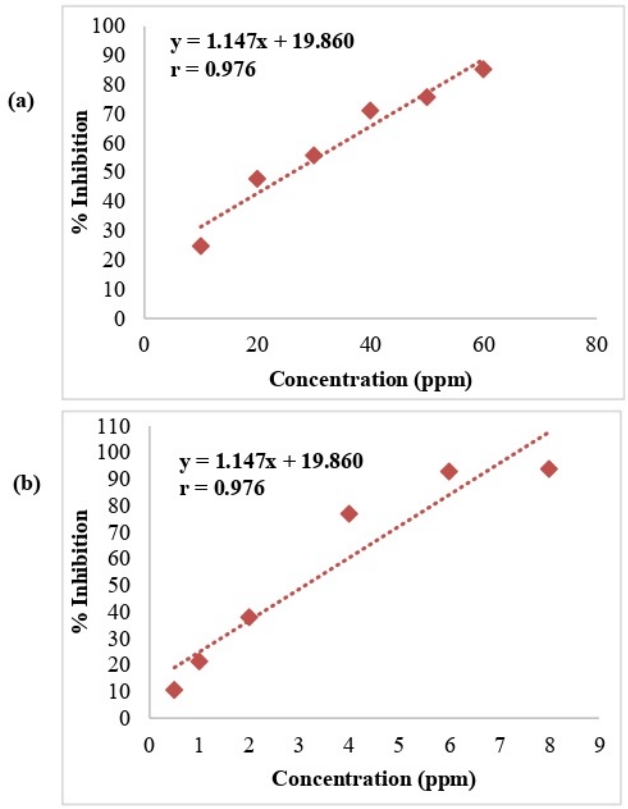

Figure 3: Antioxidant activity showing the percentage DPPH inhibition **(a)** n-hexane fraction of *G. forbesii* leaves with **(a)** quercetin as comparison compound.

Table 2: Quantitative antioxidant activity results from the n-hexane fraction of *G. forbesii* leaves and quercetin in the DPPH method

Sample	Concentration (ppm)	% inhibition ± SD	IC_{50} (ppm)
n-hexane fraction	10	24.73 ± 1.74	26.28
	20	47.73 ± 0.13	
	30	55.70 ± 0.14	
	40	71.07 ± 0.44	
	50	75.64 ± 0.16	
	60	85.21 ± 0.07	
Quercetin	0.5	10.55 ± 0.59	3.11
	1	21.30 ± 0.43	
	2	37.94 ± 0.35	
	4	76.87 ± 0.92	
	6	92.78 ± 0.20	
	8	93.73 ± 0.28	

Conclusion

The sub fractions of *Garcinia forbesii* King leaves from n-hexane fraction in a specific qualitative identification test showed the presence of compound groups such as flavonoids and alkaloids. All sub fractions had the potential as antioxidants based on the TLC-DPPH. The results from this study shows that the n-hexane fraction of *G. forbesii* leaves had potent antioxidant activity.

Conflict of Interest

The authors declare no conflicting interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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