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Antioxidant properties of *Carallia brachiata* stem bark and leaf

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Abstract. *Carallia brachiata* (Lour.) Merr. (Rhizophoraceae) had been used to treat various human disorders. This study reported antioxidant activity of *C. brachiata* of stem bark and leaf in various solvents. Each sample was macerated in various solvent separately. By using DPPH, ABTS, and FRAP assays, antioxidant activity was determined. The TLC method was created using dichloromethane and ethyl acetate mobile phase. The methanol extract of stem bark contained the highest TPC value (262.73 ± 1.58 mg GAE/ g extract), while the methanol extract of leaf contained the highest TFC value (45.46 ± 0.32 mg QE/ g extract). The total phenolic content of stem bark extract was higher than that of leaf extract, but the flavonoid content of the leaf extract was marginally higher. The methanol extract exhibited the highest antioxidant activity for DPPH (13.06 ± 0.08 $\mu\text{g/mL}$), ABTS (7.19 ± 0.08 $\mu\text{g/mL}$), and FRAP assay (155.19 ± 0.34 $\mu\text{M Fe}^{2+}/\text{g}$). In this research, stem bark has greater antioxidant activity than the leaf. In addition, the results of TLC profile were detailed. The TLC chromatogram indicates that *C. brachiata* stem bark contains more components than leaf. This study indicates that *C. brachiata* has a rich source of prospective antioxidants.

Abstract. *Carallia brachiata* (Lour.), antioxidant, DPPH, ABTS, FRAP, TPC, TFC

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1 Introduction

Due to the protective effect of pharmaceuticals and diet against oxidative deterioration in the human body, the human consumption of antioxidants has been rising [1]. On the basis of their mode of action, antioxidants are classified as primary through the HAT and SET mechanisms and secondary through the neutralization of prooxidant catalysts [2]. Antioxidants are classified as either natural or synthetic. People believe natural antioxidants are safer and healthier than their synthetic origin. Most of natural antioxidants widely used are phenolic compounds [3].

Carallia brachiata (Lour.) Merr. (Rhizophoraceae) commonly known as freshwater mangrove or corky bark (nggalsal in Marori tribes in Papua Indonesia). *C. brachiata* is a small to medium-sized tree which grows up to 20 m in height with pink-to-red-coloured fruit. Marori tribe used this plant as a traditional medicine. Document of ethnomedicinal survey of Marori tribes showed that leaves or stem bark of *C. brachiata* (nggalsal) was used to treat dysentery or hematuria [4]. In traditional Thai medicine, *C. brachiata* stem and bark are used for antipyretic treatment [5]. In addition, various parts of *C. brachiata* had been used to treat various human ailments in India [6].

Some scientific reports on this plant extract have also been published. Polar extract of *C. brachiata* stem bark was found to have wound healing capacity [7]. Ethanol extract of the stem of *C. brachiata* has good anti-adipogenesis effect [8]. In addition, hydro-alcoholic extract of leaves exhibited good hypoglycemic activity in diabetic rats [9]. Besides, antioxidant properties of *C. brachiata* extract have also been evaluated. Hydro-alcoholic extract (HAE) showed significant antioxidant activity as compared to the standard [9]. The ability of *C. brachiata* to scavenge DPPH and ABTS radicals and reduce ferric ions is undocumented in the scientific literature of the twenty-first century. Chemical investigation showed that this plant contains numerous compounds such as sesquiterpenoids, (-)-syringaresinol, (+)-diploindonesin D, (+)-parviflorol, proanthocyanidin, vanillic acid, protocatechuic acid, syringaldehyde [4]. This study, therefore, aimed to evaluate the antioxidant properties of *C. brachiata*, including DPPH, ABTS, and FRAP.

2 Material and Procedure

2.1 Plant material

The stem bark and leaves of *C. brachiata* were collected from Wasur National Park, Merauke Papua, Indonesia. The plant was identified by Mr. La Hisa, a botanist from Balai Taman Nasional Wasur.

2.1 Preparation of stem bark and leaf extracts

Stem barks and leaves of *C. brachiata* were cut into small sizes, sun-dried, and ground into powder. Then, 10 g of the powder were macerated separately using *n*-hexane (HX), dichloromethane (DCM), ethyl acetate

(EA), and methanol (MeOH) (1:10, w/v), for 24 h at ambient temperature.

2.2 Total phenolic content (TPC) and total flavonoid content (TFC) determination

The TPC value of various polarity extracts of *C. brachiata* stem bark and leaf was determined by using a previously reported method with minor modifications as usually done in our research group [10]. The TFC of the extracts was calculated using the reported method [11], with slight modification.

2.3 Determination of antioxidant activities

2.3.1 DPPH Test

DPPH value of extracts was determined by using Brand Williams method with slight modification [12, 13].

2.3.2 ABTS Test

ABTS value of extracts was evaluated by using the previously reported method [14].

2.3.3 FRAP Test

In our research group, the FRAP assay was determined using the previously reported method [10].

2.4 TLC Analysis

Each extract solution of *C. brachiata* was directly spotted onto the TLC plates. The TLC plates then were eluted in a chamber containing 100% dichloromethane and 100% ethyl acetate as mobile phases until the solvent reached 0.5 cm from the top of plates. The TLC plates were then observed under UV light at 254 and 366 nm. Then, the TLC plates were sprayed with CeSO_4 solution and heated [15].

3 Results and Discussions

3.1 TPC and TFC

The level of phenolic of *C. brachiata* extract has calculated from the regression formula of gallic acid standard curve of $y = 0.0116x + 0.036$ ($R = 0.998$), as shown in Fig. 1. Flavonoid content has determined using the regression formula of quercetin standard curve of $y = 0.022x$ ($R = 0.998$), as shown in Fig. 2.

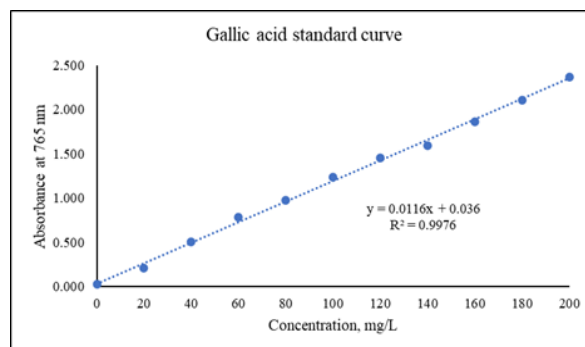


Fig. 1. TPC standar curve.

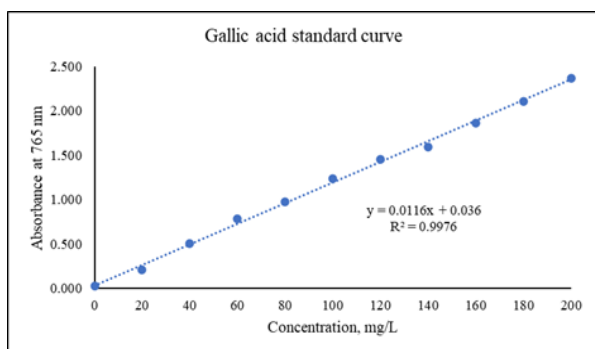


Fig. 2. TPC standar curve.

TPC and TFC value of *C. brachiata* were shown in Table 1 and Table 2. The stem bark extract contained more phenolic compounds than the leaf extract, but the leaf extract contained marginally more flavonoids. This result is supported by Chularojmontri et al, who reported the comparison of TPC value of stem and leaf extract of *C. brachiata* collected from Thailand [8]. Based on the polarity of the solvent, the highest TPC value was exhibited by methanol extract. This fact is appropriate to the polarity of the hydroxyl group in the polar compounds extracted to the polar solvent.

Table 1. The TPC of stem bark and leaf extract

Extract	Leaf (mg GAE/ grams extract)	Stem bark (mg GAE/ grams extract)
HX	1.55 ± 0.21	13.94 ± 0.25
DCM	25.92 ± 0.36	23.85 ± 0.39
EA	41.78 ± 0.56	124.17 ± 0.50
MeOH	45.46 ± 0.32	262.73 ± 1.58

Table 2. The TFC of stem bark and leaf extract

Table 3. Antioxidant activities of stem bark and leaf of *C. brachiata*.

Extracts	DPPH IC ₅₀ , µg/mL		ABTS IC ₅₀ , µg/mL		FRAP µM Fe ²⁺ /g	
	Leaf	Stem bark	Leaf	Stem bark	Leaf	Stem bark
HX	>10,000	>10,000	>10,000	>10,000	11.397±0.26	4.63±0.16
DCM	>10,000	>1,000	>10,000	92.09±0.94	16.757±0.14	30.11±0.30
EA	210.62±2.89	91.15±0.81	>1,000	23.51±0.29	34.371±0.16	34.24±0.29
MeOH	104.11±0.72	13.06±0.08	105.95±0.98	7.19±0.08	35.06±0.08	155.19±0.34
Positive control gallic acid	0.92±0.02		0.37±0.01		309.23±0.166635	
Positive control quercetin	0.73±0.02		0.57±0.02		324.79±0.76	

3.4 TLC Analysis

Analysis of TLC chromatogram was performed for chloroform and ethyl acetat extracts of *C. brachiata* stem bark and leaves. Numbers and type of secondary metabolites group compounds were predicted by the

Extract	Leaf (mg QE/ grams extract)	Stem bark (mg QE/ grams extract)
HX	0.53 ± 0.02	1.10 ± 0.15
DCM	18.12 ± 0.09	2.49 ± 0.08
EA	23.12 ± 0.09	6.43 ± 0.08
MeOH	30.53 ± 0.09	28.14 ± 0.30

3.2 Antioxidant activity

Antioxidant activity of *C. brachiata* was assessed using the FRAP, ABTS, dan DPPH assay. Table 3 shows the comparison of antioxidant potential of stem bark and the leaf, with the highest antioxidant activity exhibited by the methanol extract for DPPH (13.06±0.08 µg/mL), ABTS (7.19±0.08 µg/mL), and FRAP assay (155.19±0.34 µM Fe²⁺/g). In this research, stem bark of *C. brachiata* has greater antioxidant activity than the leaf. The antioxidant power of the extract was linear with the TPC value. This is likely due to the presence of electron-donating hydroxyl groups in the phenolic compound, which neutralize free radical [2].

3.3 TLC Analysis

Analysis of TLC chromatogram was performed for chloroform and ethyl acetat extracts of *C. brachiata* stem bark and leaves. Numbers and type of secondary metabolites group compounds were predicted by the presence of the spots in TLC chromatogram. TLC profiles of extracts are visualized under UV 254 nm and 366 nm, sprayed with CeSO₄ and heated. The chromatogram of dichloromethane elution is shown at Fig 3, while ethyl acetate elution is shown at Fig 4.

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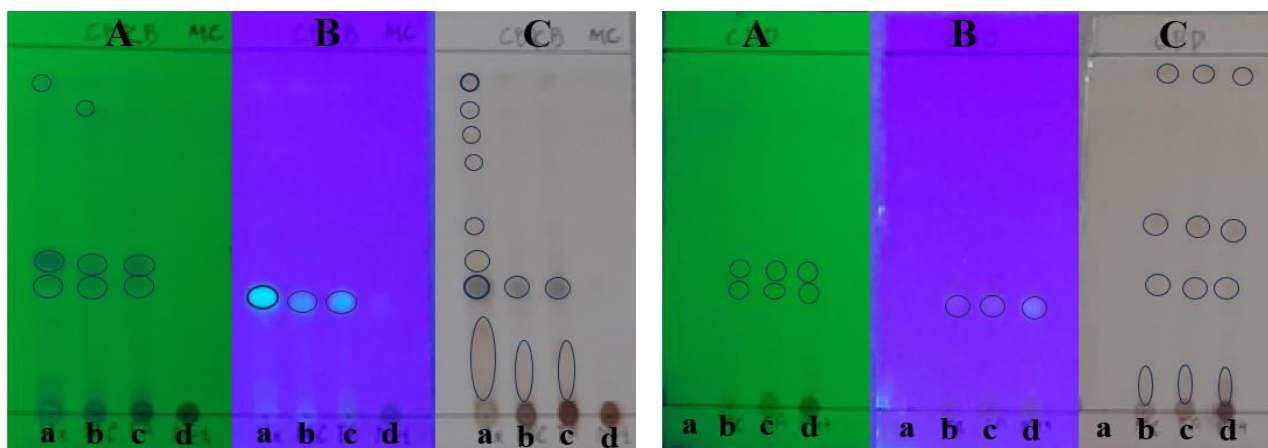


Fig 3. TLC chromatograms at λ UV 254 nm (A), λ UV 366 nm (B), and $CeSO_4$ + heat (C) of HX (a), DCM, (b), EA (c), and MeOH extracts (d) of *C. brachiata* stem bark (left side) and leaf (right side) eluted using 100% dichloromethane mobile phase.

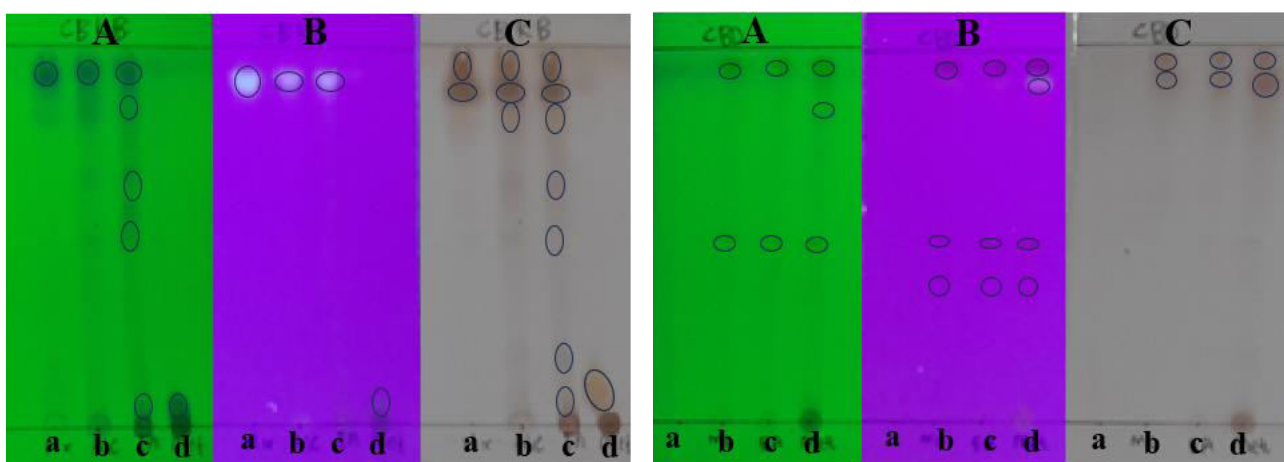


Fig 4. TLC chromatograms at λ UV 254 nm (A), λ UV 366 nm (B), and $CeSO_4$ + heat (C) of HX (a), DCM (b), EA (c), and MeOH extract (d) of *C. brachiata* stem bark (left side) and leaf (right side) eluted using 100% ethyl acetate mobile phase.

According to the results of the TLC chromatograms in Fig 3, we noticed the presence of 9 spots represent at least 9 nonpolar to semi polar secondary metabolite compounds in the stem bark and 4 spots represent at least 5 nonpolar to semi polar compounds in the leaves.

In addition, Fig 4 displayed 9 spots in the stem bark and 5 spots in the leaves TLC chromatogram. It indicates that *C.brachiata* stem bark contains more components than leaf. Retention factor (Rf) of the spots were listed in Table 4.

Table 4. Spot number and Rf at UV light and $CeSO_4$ of stem bark and leaf extract.

Mobile phase (Eluent)	Stem bark extract				Leaves extract			
	Spot number	Rf at UV 254	Rf at UV 366	Rf at $CeSO_4$ + heat	Spot number	Rf at UV 254	Rf at UV 366	Rf at $CeSO_4$ + heat
Dichloromethane	1			0.15	1			0.11
	2		0.32		2		0.32	
	3	0.36		0.36	3	0.36		0.36
	4	0.44		0.44	4			0.52
	5			0.52	5			0.9
	6			0.68				
	7			0.77				
	8			0.84				
	9			0.9				
Ethyl acetate	1	0.04		0.04	1		0.38	
	2			0.1	2	0.48	0.48	
	3	0.17		0.17	3	0.86		0.86
	4	0.56		0.56	4		0.91	
	5	0.66		0.66	5	0.94	0.94	0.94
	6			0.85				
	7	0.86		0.86				
	8		0.91					

	9	0.94		0.94				
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4 Conclusion

The results of the exploration assert the use of *Carallia brachiata* leaf and stem bark in ethnomedicine of Marori Tribes in Papua Indonesia for treatment various diseases. The mehanolic extract of *C.brachiata* stem bark possess the highest antioxidant activity because of the phenolic compounds contained in this extract. TLC profiles showed numerous compounds contained in the stem bark of this plant. This findings imply the value of *Carallia brachiata* as a natural potential source of antioxidants.

Acknowledgements

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