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Parang Romang (*Boehmeria virgata* (Frost.) Guill.): Correlation of Phytochemistry with Antioxidant and Xanthine Oxidase Inhibitory Activities

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ABSTRACT

Secondary metabolites such as phenolic and flavonoid from the plant are essential in their activity, especially activities related to the prevention of oxidation and the inhibition of xanthine oxidase. One plant that is believed to have prevention of oxidation and XOI and correlates with secondary metabolites is parang romang. The purpose of this study is to assess the antioxidant capacity and xanthine oxidase inhibitory activities of parang romang, and to examine their relationship with the TPC and TFC. Total phenolic and flavonoid were measured for all parts, and the DPPH, FRAP, and CUPRAC methods determined antioxidant capacity. The activity of xanthine oxidase inhibition was also assessed. Flavonoids, alkaloids, and coumarin were found in parang romang. The steroid/triterpenoid was found in the stem, leaves, and flower; saponin was in the roots and flower; quinone and tannin were only in the roots. The root gave the highest of TPC, while the flower had the highest TFC. The highest antioxidant capacity was found in roots, leaves, flowers, and stems. The highest xanthine oxidase inhibitory was given by leaves (9.74±0.14 µg/ml), followed by flower, steam, and rood. The correlation was shown between the phenolic and flavonoid quantities and the actions of antioxidants and xanthine oxidase inhibitors.

1. Introduction

The significance of free radicals and other oxidants in biology has increased due to their pivotal involvement in numerous physiological states and their association with multiple illnesses. reactive oxygen species (ROS) and reactive nitrogen species (RNS), types of free radicals, can be generated both within endogenous (mitochondria, etc.) and from external factors like pollution, alcohol, smoke, heavy metals, certain medications such as halothane, and radiation (Phaniendra *et al.* 2015).

The natural cellular metabolism produces free radicals. One of them is the formation of uric acid, which will produce superoxide and hydrogen peroxide free radicals catalyzed by the xanthine oxidase enzyme. Both radicals will produce hydroxyl radicals. These radicals have lipid peroxidation, which causes oxidative stress and damages cell membranes. To inhibit xanthine oxidase activity during the reoxidation process, an antioxidant and xanthine oxidase inhibitor are required (Lobo *et al.* 2010; Kostić *et al.* 2015; Phaniendra *et al.* 2015).

Antioxidants can be naturally produced in plants, animals, and microbes and can also synthetically produced. Higher plants (Spermatophyta) and their component contain are strong in plant-based antioxidants such as phenolic and tocopherols. These antioxidants were found in large quantities in spices, herbs, fruits, and vegetables. Antioxidants derived from marine sources, such as algae, have also been explored (Shahidi and Ambigaipalan 2015; Xu *et al.* 2017; Ghosh *et al.* 2022). One of the potential plants for antioxidant and xanthine oxidase inhibition is parang romang (*Boehmeria virgata*). Parang romang was reported to contain phenolic and flavonoids on the roots (Rusdi 2014). As we know, the compounds have the potential as an antioxidant and xanthine oxidation inhibitory. Parang romang has strong to very strong antioxidant activity in each part, and the

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isolate from leaves can decrease the growth of HeLa cells (Lukman *et al.* 2014; Arsul *et al.* 2022).

Parang romang was primarily used in folk Makassar medication to cure cancer (Lukman *et al.* 2014). This plant grows in mountainous areas such as Sinjai, Malino, Maros, and Enrekang, South Sulawesi (Rusdi 2014). The activity of parang romang is still limited and not widely known. The current study is different from previous ones in that it determines antioxidant capacity and xanthine oxidase activity, which has never been done before. This research objective is to investigate the antioxidant and xanthine oxidase inhibiting effects of parang romang and the extent to which they are correlated with the phytochemical content.

2. Materials and Methods

2.1. Material

The specimens included in this investigation consisted of roots, stem, leaves, and flower of parang romang obtained from Malino district, South Sulawesi, Indonesia, as determined by the Department of Biology, Makassar State University. Xanthine, xanthine oxidase, neocuproine, Trolox, DPPH, TPTZ, gallic acid, and quercetin, all sourced from Sigma-Aldrich Chemicals. Additionally, ascorbic acid and phosphate buffer solution were obtained from Merck (Darmstadt, Germany), and allopurinol was procured from TCI (Tokyo, Japan). All other reagents used met the standards for analytical quality.

2.2. Extraction

The plant was dried and subsequently pulverized into powder. The powder was extracted with ethanol (96%) using reflux and subsequently evaporated through a rotavapor, ensuring a constant temperature of 45°C and a rotating speed ranging from 45 to 50 rpm (Juanda *et al.* 2021).

2.3. Phytochemical Screening

The phytochemical screening was conducted on extracts from various parts of parang romang. The purpose was to identify the existence of biochemical, including alkaloids, flavonoids, saponins, steroid/triterpenoids, tannins, quinones, coumarins, and phenols (Farnsworth 1966).

2.4. Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent adapted to McDonald's technique (Mc.Donald *et al.* 2001) was used to assess the TPC. As much as 0.5 ml of each extract was transferred into a 5 ml solution of mixture consists of Folin-Ciocalteu reagent (10%) and 4 ml of NaOH (1 M). The solutions were then incubated for 15 min. The absorbance measurements were carried out at λ 765 nm. The results were presented in the form of milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract), using the standard calibration curve of gallic acid around from 20 to 140 micrograms per milliliter (µg/ml), which was performed in the same way on extract.

2.5. Total Flavonoid Content (TFC)

The quantification of the overall flavonoid content was conducted followed Chang's modified procedure (Chang *et al.* 2002). As much as 0.5 ml of each extract was transferred in 1.5 ml ethanol, 2.8 ml of distilled water, 0.1 ml of 10% aluminum chloride, and 0.1 ml of 1 M sodium acetate. The solution was left to incubate for a duration of 15 min. The measurement of absorbance was taken at a wavelength of 415 nm. The findings were reported in terms of milligram quercetin equivalents per gram of extract (mg QE/g extract), based on the quercetin standard calibration curve, which ranges from 10 to 75 µg/ml, performed the same way on extract.

2.6. DPPH Assay

The method proposed by Celep *et al.* (2015) was barely adapted using antioxidant capacity against DPPH radicals. As much as 0.5 ml of samples were added with 3 ml of a 0.1 mM DPPH solution that was produced using methanol. The test solution was placed in a dark environment at the temperature of the room for a duration of 30 min. The absorbance of the combination was measurements were carried out at λ 517 nm. The Trolox (60-100 µg) and ascorbic acid (100-500 µg) were employed as reference substances. The outcomes were expressed as milligrams of Trolox equivalent (TEAC) and ascorbic acid equivalent (AEAC) per gram of extract.

2.7. CUPRAC Assay

The CUPRAC ion reduction capacities of the extract were assessed using the Özyürek technique (Özyürek *et al.* 2008). CUPRAC solution contained copper chloride ($CuCl_2$) at a concentration of 0.01 M, neocuproine at a concentration of 0.0075 M in ethanol, and an ammonium acetate buffer with a pH of 7.0 (in a ratio of 1:1:1). Extract 250 µl was added by CUPRAC solution 750 µl. The measurement of absorbance was taken at λ 450 nm, relative to a reagent blank. The outcomes

were expressed as TEAC and AEAC per gram of extract. Every antioxidant was synthesized using Trolox and ascorbic acid as standards.

2.8. FRAP Assay

The FRAP ion reducing capacities of the extract were assessed followed the Özyürek technique (Özyürek *et al.* 2008). A total of 3 ml of the FRAP reagent was applied in 1 ml consisting of 0.05 ml of the sample and 0.95 ml of distilled water. The measurement of absorbance at a wavelength of 595 nm was taken after 30 min using a reagent blank as a reference. Trolox and ascorbic acid served as reference standards. The overall antioxidant capacity of parang romang was quantified as TEAC and AEAC per gram of extract.

2.9. Xanthine Oxidase Inhibitory Activity

The measurement of the inhibitory activity of xanthine oxidase (XOI) was conducted using 96-well plates using an ELISA reader, following Noro's, Owen's, and Duong's method with slight modifications (Noro *et al.* 1983; Owen and Johns 1999; Duong *et al.* 2017). The extracts were dissolved in 1.5% (w/v) Tween 80 and 3% (v/v) ethanol and then diluted with phosphate buffer (pH 7.5). As a control, allopurinol was used. The mix was made by combining 50 µl of extract, 69 µl of phosphate buffer (pH 7.5), and 15 µl of freshly prepared enzyme solution (0.2 unit/ml xanthine oxidase in phosphate buffer pH 7.5) then preincubated for 15 min at 24°C. Subsequently, the reaction was initiated by adding 66 µl of substrate solution (0.15 mM of xanthine in phosphate buffer pH 7.5). The test solution was incubated for 15 min at 24°C. A blank was prepared in the same way without a sample. The measurement of absorbance was taken at λ 290 nm.

The XOI activity was quantified as the inhibitory percentage (I) using the following formula:

$$\text{Inhibitory percentage (I)} = (\Delta OD_{blank} - \Delta OD_{sample}) / \Delta OD_{blank} \times 100$$

Where ΔOD_{blank} is determined by subtracting the change in absorbance without the enzyme with the enzyme (Δabs. with enzyme − Δabs. without enzyme), and ΔOD_{sample} is determined by subtracting the change in absorbance without the enzyme with the plant extract (Δabs. with enzyme − Δabs. without enzyme).

2.10. Statistical Analysis

The results were shown as the average values ± standard deviation (SD) obtained from at least three separate tests. The IC_{50} value of XOI was determined using MS Excel Software. The statistical analysis was conducted using SPSS 23. The Pearson's method was employed to examine the association between the overall TPC and TFC with the activities of antioxidant and XOI.

3. Results

A Phytochemical screening was performed to identify and analyze secondary metabolites on the root, stems, leaves, and flower extracts of parang romang. The findings of the phytochemical analysis are presented in Table 1. Flavonoids, coumarin, and alkaloids were detected in all parts of parang romang; saponins and quinones were detected in roots and flowers; steroids/triterpenoids in stem, leaves, and flowers, meanwhile tannins were only seen in the roots.

The extract's total phenol content was determined by converting it to gallic acid equivalent using the calibration curve calculation (y = 0.0075x - 0.0213; R^2 = 0.9992). Total phenol levels for each part of parang romang differed from 98.61 to 350.33 mg GAE/g extract (Table 2). A significant disparity ($p<0.05$) was detected between all parts of parang romang, as seen in Table 2. TPC of the extract was obtained by calculating it as quercetin equivalent, utilizing a calibration curve equation y = 0.0113x - 0.0196 (R^2 = 0.9997). Each extract's flavonoid content varied from 22.44 to 118.61 mg QE/g extract (Table 2). A significant difference ($p<0.05$) was found between all parts of prang romang, as shown in Table 2. The root extract had the highest TPC and TFC in the flower.

The parang romang possesses the capacity to scavenge free radicals was conducted by DPPH method. The scavenge radical activities of each sample varied from 223.69±1.40 to 387.92±0.24 mg AEAC/g extract and 33.93±0.16 to 52.93 ± 0.02 mg TEAC/g extract. Roots extract exhibited the highest antioxidant capacity, but there was no significant difference existed among roots and leaf extracts (Table 3).

Table 1. Phytochemical screening of parang romang

Compound	Root	Steam	Leaves	Flower
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	-	+
Quinones	+	-	-	-
Tannins	+	-	-	-
Alkaloids	+	+	+	+
Coumarin	+	+	+	+
Steroids/Triterpenoids	-	+	+	+

Table 2. TPC and TFC of parang romang

Sample	Yield (%)	Density of extract	TPC (mg GAE/g extract)	TFC (mg GAE/g extract)
Root	6.5	0.82	350.33±0.76[a]	27.99±0.41[a]
Stem	8.1	0.82	98.61±0.27[b]	22.44±0.94[b]
Leaves	19.2	0.81	162.96±0.93[c]	101.97±0.20[c]
Flower	6.5	0.82	330.50±0.72[d]	118.61±1.82[d]

The given value is the mean ± SD (n = 3). When comparing values represented by the same letter (a–d) in one column, there is no significant difference at p<0.05

Table 3. Antioxidant and XOI of parang romang

Sample	Antioxidant capacity						XOI IC_{50} (µg/ml)
	mg AEAC/g extract			mg TEAC/g extract			
	DPPH	FRAP	CUPRAC	DPPH	FRAP	CUPRAC	
Root	387.92±0.24[a]	23.21±0.56[a]	105.78±0.17[a]	52.93±0.03[a]	23.28±0.31[a]	135.71±0.17[a]	54.83±0.75[a]
Stem	223.69±1.40[b]	4.96±0.48[b]	62.54±2.79[b]	33.93±0.16[b]	13.29±0.26[b]	92.84±2.77[b]	13.32±0.14[b]
Leaves	378.36±3.17[a]	24.15±1.09[a]	100.95±0.62[c]	51.76±0.37[a]	23.80±0.60[a]	130.92±0.62[c]	9.74±0.14[c]
Flower	265.81±8.75[c]	4.64±1.67[b]	72.08±1.83[d]	38.85±1.01[c]	13.11±0.92[b]	102.29±1.81[d]	12.29±0.18[d]
Allopurinol	-	-	-	-	-	-	4.09±0.01[e]

The given value is the mean ± SD (n = 3). When comparing values represented by the same letter (a–d) in one column, there is no significant difference at p<0.05

The FRAP approach yielded a range of antioxidant capacities for each sample varied from 4.63±1.67 to 24.15±1.09 mg AEAC/g extract and 13.11±0.92 to 23.80±0.60 mg TEAC/g extract. The leaf extract exhibited higher antioxidant capacity compare the other extracts. Still, there was no significant difference between roots and leaves, as well as stems and flowers (Table 3) in this method.

Antioxidant capacity by CUPRAC method of each sample varied from 62.54±2.80 to 105.78±0.18 AEAC/g extract and 92.84±2.77 to 135.71±0.16 mg TEAC/g extract. CUPRAC value of root extract gave higher antioxidant capacity than the other extracts. A significant disparity (p<0.05) was detected between each part extract (Table 3).

The results demonstrated that parang romang leaves extract possessed high XOI activity with IC_{50} of 9.74±0.14 µg/ml, followed by flower, stems, and roots, respectively (Table 3). A significant disparity (p<0.05) was detected between all parts of parang romang, as shown in Table 3. Meanwhile, the specific XOI, allopurinol, gave an IC_{50} of 4.09±0.01 µg/ml higher than each part of the extract.

4. Discussion

The antioxidative effects of phenolic compounds stem from their strong reactivity as donors of hydrogen or electrons, the ability of radical polyphenol-derived to neutralize and stabilize the unpaired electron (acting as chain-breakers), and their capacity to form complexes with transition metal ions through chelation. The antioxidant capacity of flavonoids is contingent upon their structure, which determines the relative efficacy of the compounds in neutralizing free radicals. The highest antioxidant activity is achieved through specific structural arrangements. These include the B ring contains an ortho 3',4'-dihydroxy moiety, the A ring has metha 5,7-dihydroxy groupings; and the C ring combines a 2,3-double bond with both a 4-keto group and a 3-hydroxyl group. These arrangements allow for electron delocalization. It is crucial to acknowledge that the existence of the o-dihydroxy structure in the B ring is also required (Rice-Evans *et al.* 1997). This is the relationship among phenolic and flavonoid total with the antioxidant capacity of parang romang in reducing metal in the FRAP and CUPRAC method and as an electron donor in the DPPH method.

The elevated antioxidant capacity is probably significant abundance of phenolic and flavonoid compounds responsible for this, which have exhibited robust antioxidant activities through several mechanisms (Kähkönen *et al.* 1999; Hakkinen *et al.* 1999; Halvorsen *et al.* 2002). The previous research stated that the same genus (*Boehmeria*) had the highest total phenolic and flavonoid compound and indicated the highest antioxidant capacity (Chen *et al.* 2014). All parts of parang romang had a perfect correlation between antioxidant capacity with TPC and TFC (Tables 4 and 5) with positive and negative correlations (Akoglu 2018). TPC of leaf extract had

Table 4. Pearson's correlation coefficient of TPC, TFC, and AEAC

Antioxidant parameter		Pearson's coefficient correlation (r)		Interpretation (Akoglu 2018)	
		TPC	TFC	TPC	TFC
Root	DPPH	0.757**	1.000**	Moderate	Perfect
	FRAP	0.995**	0.688*	Very strong	Moderate
	CUPRAC	0.845**	0.989**	Very strong	Very strong
Stem	DPPH	0.994**	0.968**	Very strong	Very strong
	FRAP	0.851**	0.764**	Very strong	Moderate
	CUPRAC	0.959**	0.907**	Very strong	Very strong
Leaves	DPPH	0.894**	0.555ns	Very strong	Fair
	FRAP	0.994**	0.917**	Very strong	Very strong
	CUPRAC	1.000**	0.866**	Perfect	Very strong
Flower	DPPH	0.800**	0.994**	Very strong	Very strong
	FRAP	0.872**	0.972**	Very strong	Very strong
	CUPRAC	0.992**	0.811**	Very strong	Very strong

**Significant correlation with a value of 0.01 (two-tailed)
*Significant correlation with a value of 0.05 (two-tailed)
nsnot significant

Table 5. Pearson's correlation coefficient of TPC, TFC, and TEAC

Antioxidant parameter		Pearson's coefficient correlation (r)		Interpretation (Akoglu 2018)	
		TPC	TFC	TPC	TFC
Root	DPPH	0.757**	1.000**	Moderate	Perfect
	FRAP	0.995**	0.688*	Very strong	Moderate
	CUPRAC	0.847**	0.989**	Very strong	Very strong
Stem	DPPH	-0.589ns	-0.701ns	Fair	Fair
	FRAP	0.850**	0.764**	Very strong	Moderate
	CUPRAC	0.959**	0.907**	Very strong	Very strong
Leaves	DPPH	-0.835ns	-0.998ns	Fair	Fair
	FRAP	0.994**	0.917**	Very strong	Very strong
	CUPRAC	1.000**	0.866**	Perfect	Very strong
Flower	DPPH	0.800**	0.994**	Very strong	Very strong
	FRAP	0.872**	0.972**	Very strong	Very strong
	CUPRAC	0.992**	0.812**	Very strong	Very strong

**Significant correlation with a value of 0.01 (two-tailed)
*Significant correlation with a value of 0.05 (two-tailed)
nsnot significant

a perfect correlation with antioxidant capacity (r = 1.000, p<0.01) in the CUPRAC method; TFC of root extract also showed perfect (r = 1.000, p<0.01) with antioxidant capacity in the DPPH method. A positive correlation means antioxidant capacity may increase with increasing TPC and TFC. However, TEAC (Table 5) negatively correlated with TPC and TFC for stem and leaf extracts. The negative sign means the two variables are inversely related (Chan 2003).

The DPPH test is a kinetic study of the relationship among phenols and DPPH with electron transfer (ET) reaction characteristics. This reaction's rate-determining phase is an ET mechanism from the phenoxide anions to DPPH. Because it occurs very slowly in strong hydrogen-bond-accepting solvents like methanol and ethanol, DPPH removal of hydrogen atoms from the neutral ArOH becomes less significant reaction route. Furthermore, exogenous acids or bases present in the solution can significantly impact the equilibrium of ionization in phenols, resulting in a reduction or augmentation of the rate constant reaction (Foti *et al.* 2004). Besides, the present research assumed that the negative values could potentially be attributed to variance in chemical components (as indicated in Table 1) found in the extracts of stems and leaves, but additional investigation is required to validate this.

The FRAP test measures ferric ions (Fe^{3+})-ligand complex is reduced become brilliantly blue ferrous complex (Fe^{2+}) through antioxidants in acidic conditions. It is commonly used to determine antioxidant capacity with a typical single ET-based approach. Electron donation, one of the key pathways of antioxidant activity, includes Fe^{3+} reduction. The

antioxidant activity of the resulting compound is determined in molarity equivalents of Fe^{2+} or compared to a standard antioxidant. Acidic conditions induce a change in the main reaction mechanism by reducing the ionization potential, facilitating drives ET, and elevating redox potential (Hagerman *et al.* 1998; Antolovich, *et al.* 2002; Celep *et al.* 2013). Cu^{2+}, like Fe^{3+}, contributes to forming free radicals; hence, determining antioxidant activity by converting cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}) indicates antioxidant capability. The ligand used in the CUPRAC assay is neocuproine. The CUPRAC test is a relatively new metal ion reduction method used to evaluate a plant's antioxidant potential, mainly phenolic compounds (Celep *et al.* 2013; Munteanu and Apetrei 2021).

Several studies have found that phenolic and flavonoids can inhibit xanthine oxidase. Flavonoid's structure-activity relationship as XOI and superoxide radical scavengers generated by the enzyme xanthine oxidase is due to the presence of the B ring in flavonoids with hydroxyl groups at C-5 and C-7, as well as the double bond between C-2 and C-3, which demonstrated high inhibitory activity on xanthine oxidase (Cos *et al.* 1998; Boumerfeg *et al.* 2009; Baghiani *et al.* 2010; Kostić *et al.* 2015). Several investigations have found that flavonoid and phenolic compounds decrease xanthine oxidation significantly. According to earlier research, aglycones of flavonoid are effective of XO-catalyzed xanthine oxidation inhibitor (Cos *et al.* 1998; Nagao *et al.* 1999; Nile and Park 2013; Sabahi *et al.* 2018; Mohos *et al.* 2020; Kim *et al.* 2020).

Parang romang had a fairly strong correlation between XOI and TPC and moderated to perfect with TFC (Table 6). Both TPC and TFC had a positive correlation. TFC of stem extract had an excellent and significant correlation ($r = 1.000$, $p<0.01$) with XOI, which means XOI may increase with increasing TFC. Despite previous research indicating that flavonoids have a considerable inhibitory capability against XOI, various studies imply that they have mild (Nagao *et al.* 1999; Lin *et al.* 2015a), similar (Cos *et al.* 1998; Lin *et al.* 2002), stronger (Hoorn *et al.* 2002; Lin *et al.* 2015b), or even negligible (Mohos *et al.* 2020) effects when compared to allopurinol. According to Cos *et al.* (1998), parang romang showed a similar activity impact to allopurinol.

In conclusion, all parts extracts of the parang romang and the analytical method affected the estimation of antioxidant and XOI activities. Furthermore, the current study revealed that the root exhibited the highest phenolic and low flavonoid content, accompanied by a strong antioxidant capacity and low XOI activity. Phenolic and flavonoid compounds in parang romang resulted in antioxidant and XOI effects. Based on the literature, some phenolic compounds such as 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid have potent antioxidant activities. The higher content of the three compounds showed a significant antioxidant potential (Liu *et al.* 2012; Li *et al.* 2018) however, they have low XOI (Nguyen *et al.* 2006; Wan *et al.* 2021). The three compounds have also been confirmed in plants of the same genus, *B. caudata* (Mienhart *et al.* 2017, 2018).

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Table 6. Pearson's correlation coefficient of TPC, TFC, and XOI

XOI parameter	Pearson's coefficient correlation (r)		Interpretation (Akoglu 2018)	
	TPC	TFC	TPC	TFC
IC_{50} root	0.977**	0.879**	Very strong	Very strong
IC_{50} stem	0.989**	1.000**	Very strong	Perfect
IC_{50} leaves	0.969**	0.721*	Very strong	Moderate
IC_{50} flower	0.500ns	0.957**	Fair	Very strong

**Significant correlation with a value of 0.01 (two-tailed)
*Significant correlation with a value of 0.05 (two-tailed)
nsnot significant

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