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THE PHYTOCHEMICAL PROFILE OF MANGIFERA RUFOCOSTATA KOSTERM AND ITS ANTIOXIDANT ACTIVITY

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The tandui plant (Mangifera rufocostata Kosterm.) is one of the typical plants of South Kalimantan which belongs to the genus Mangifera. It has the potential as medicine. The local people use boiled water from bark of M. rufocostata to treat diabetes and minor stoke. This study aims to identify compounds and determine the antioxidant activity of leaves and bark ethanol extract M. rufocostata qualitatively and quantitatively. The results of the identification show that ethanol extract of M. rufocostata contains tannins, phenols, flavonoids and saponins. The result of the qualitative antioxidant activity test shows that the ethanol extract of M. rufocostata has an antioksidan activity. The result of quantitative antioxidant acivity test reveals that the leave and bark have IC50 value of 7,614 ppm and 8,254 ppm. Based on this research, it can be concluded that ethanol extract of M. rufocostata from Hulu Sungai Tengah District contains various secondary metabolites such as tannins, phenols, flavonoids, and saponins and have a very strong antioxidant activity.

Keywords: *Antioxidant; Mangifera rufocostata Kosterm; Phytochemical; Antioxidant*

INTRODUCTION

Free radicals produced by metabolism regulate signal transmission between cells and cell growth, and inhibit viruses and bacteria. Excess free radicals will leak intracellularly, which can denature proteins and nucleic acids thereby damaging cells[1]. These events are associated with several diseases, both carcinogenic and atherogenesis. A decrease in natural antioxidant levels or an increase in *reactive oxygen species* (ROS) triggers oxidative stress[2]. Due to the dangers of ROS and free radicals, it is necessary to find natural antioxidants that can prevent oxidative stress[3].

Antioxidants are substances that can react and neutralize free radicals. The impact of antioxidants in the body can prevent or reduce the harmful effects. Antioxidants have been known since ancient times to have pharmacological effects and are found in plants or in synthetic products[4]. Antioxidants act as reducing agents and will experience oxidation in their structure. Synthetic and natural antioxidants have hydroxyl groups which take part in redox reactions. Antioxidants can be in the form of vitamins, flavonoids, some minerals and synthetic phenolic compounds[5].

Secondary metabolites in plants generally consist of several active substances that work synergistically or singly in overcoming various diseases, especially degenerative diseases[6]. One of the plants that has the potential as medicine is Tandui (*Mangifera rufocostata* Kosterm.). The *M. rufocostata* plant is one of the endemic plants of Kalimantan which is rarely found and has medicinal properties. The parts of the plant which are often used by the community is the fruit, leaves and bark. The *M.*

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rufocostata plant has empiric efficacy in treating diabetes and stroke. Diabetes Mellitus and stroke are included in degenerative diseases. Compounds that can prevent cell damage caused by free radicals are antioxidants[7]. This study aims to explore the leaves and bark phytochemical profiles and evaluate their antioxidant properties.

MATERIALS AND METHODS

Collection and preparation of extract

Samples were taken from the village of Tanjung Hanntak, Hanntak District, Hulu Sungai Tengah Regency, South Kalimantan. The determination was carried out at the Banua Botanical Gardens, South Kalimantan. The bark and leave of *M. rufocostata* obtained were immediately wet-sorted, then washed thoroughly with running water. Then, the bark and leave of *M. rufocostata* were chopped to make the drying process more efficient. Furthermore, the drying process was carried out in a drying cabinet at a temperature of 55^{o}C. The dry simplicia was then sorted dry and then crushed using a blender to form coarse powder. The simplicia powder obtained was stored in a tightly closed container.

Extraction

Preparation of *M. rufocostata* stem bark extract was done by placing 500 g of simplicia powder into a maceration container, soaking with 96% ethanol until the simplicia powder was submerged. The maceration container was closed and stored for 6 days (remereration) in a place protected from sunlight and stirred every 8 hours. The solvent was replaced every 1x24 hours. Liquid extract and extraction dregs were separated by filtering using Whatman filter paper. The liquid extract obtained was then evaporated using a water bath to obtain a thick extract with a fixed weight.

Phytochemical screening

Phytochemical screening was done following standart test tube methods[8]. Extract of samples was used to screen some compounds like alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids.

Qualitative Test of Sample Extract

The ethanol extract of *M. rufocostata* was suspended with methanol:chloroform in a ratio of 1:1 then spotted on a silica Gel254 TLC plate (stationary phase). Then, it was eluted using several ratios of the mobile phase (eluent) n-hexane and ethyl acetate (1:9, 2:8, 3: 7 and 5:5) v/v. The TLC plate was sprayed with 0.02% DPPH solution and left to dry. The active compound as an antioxidant formed a yellow stain with a purple TLC plate background [9].

Quantitative Test of Sample Extract

Liquid extract was prepared with a concentration of 500 ppm. Next, a series of concentration solutions with concentrations of 2.5, 5, 7.5, 10, 12.5, and 15 ppm were prepared using a 10 mL volumetric flask. 0.4 mM DPPH solution was added as much as 1 mL to each sample concentration of 4 mL. Furthermore, the solution was allowed to stand for a span of operating time in a dark place. Absorbance readings were carried out using a UV-Vis spectrophotometer in the maximum wavelength range that had been obtained previously[10].

Quercetin was used as a comparison of antioxidant activity with 100 ppm as an innitial concentration. Next, a series of levels was made with concentrations of 0.5, 1, 2, and 4 ppm in a 10 mL volumetric flask. The series of levels that had been made were then taken as much as 4 mL each and then 1 mL of 0.4 mM DPPH solution was added. The grade series solution was left in a dark place for a predetermined operating time. The absorbance was read with a UV-Vis spectrophotometer at the maximum wavelength that has been obtained.

RESULTS AND DISCUSSION

Phytochemical screening

The extraction results obtained were in the form of filtrate which was then evaporated with a water bath at 50^{o}C to obtain a thick extract with a fixed weight. The amount of yield from the leaves was 2 times more than the bark. This can be caused by the amount of chlorophyll that was still in the yield.

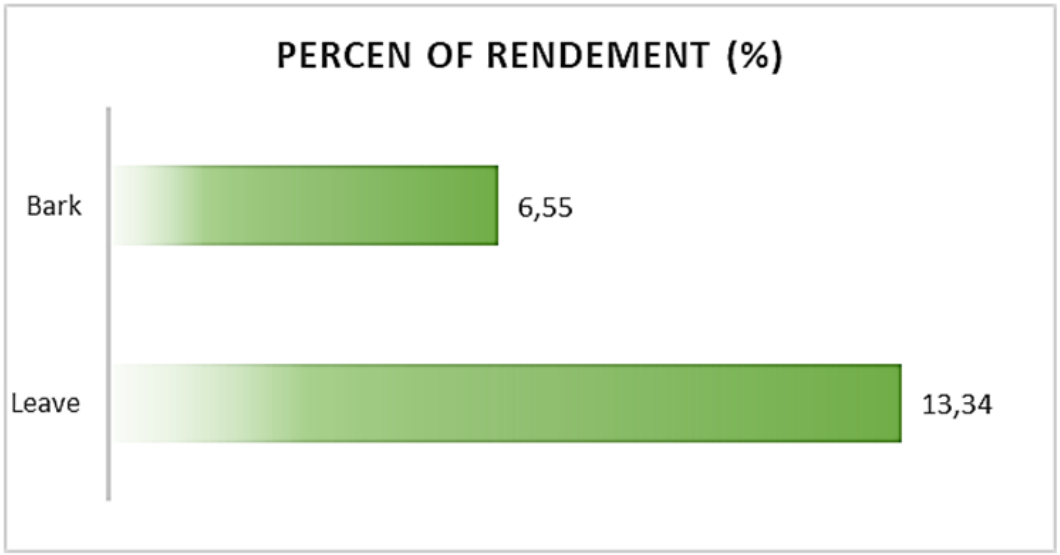


Fig. 1: Percentage of crude extrac.

Table 1: Phytochemical compound of bark and leave extrac.

Phytochemical	Result	
	Leave	**Bark**
Tanin	+	+
Phenol	+	+
Flavonoid	+	+
Saponin	+	+
Steroid	-	-
Terpenoid	-	-
Alkaloid	-	+

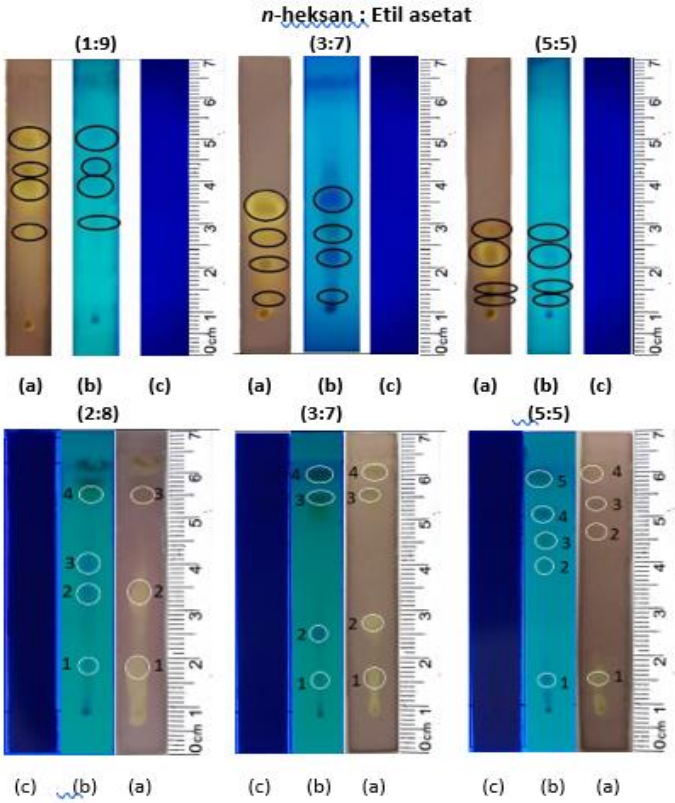
(+) Positive: There is compound, (-) Negative: There is no compound.

Bark and leaves contain tannins, phenols, flavonoids, and saponins. The leaves did not detect any alkaloids. Steroids and terpenoids also produced negative results. Based on their nature, steroids and terpenoids tend to be more non-polar, which means they are difficult to dissolve in polar solvents such as ethanol.

DPPH free radical scavenging activity.

Based on the calculation of the polarity index, it can be seen that with a greater ratio of ethyl acetate, the polarity index value is higher, which means it is more polar. The chromatogram with a semi-polar eluent ratio (5:5) v/v has the most spots with stable separation between spots. This eluent ratio has the lowest polarity compared to the others. Eluent chromatograms with v/v comparisons (3:7, 2:8 and 1:9) have almost the same spot separation. There are several spots that are separated quite well.

Non-polar compounds will move up more asily following the eluent. A compound which has a higher Rf value has low polarity, while a compound with a lower Rf value has higher polarity. The chromatogram that was sprayed with the DPPH spotter changed the color of the stain to yellow with a purple background, indicating the presence of antioxidant activity in the sample[11].



Eluent	Bark Rf Value	
	After Speayer with DPPH	Before Sprayer with DPPH on UV 254 nm
(1:9)	1. 0,58	1. 0,54
	2. 0,72	2. 0,72
	3. 0,8	3. 0,79
	4. 0,93	4. 0,92
(3:7)	1. 0,25	1. 0,23
	2. 0,42	2. 0,38
	3. 0,52	3. 0,54
	4. 0,67	4. 0,65
(5:5)	1. 0,24	1. 0,27
	2. 0,31	2. 0,32
	3. 0,43	3. 0,45
	4. 0,52	4. 0,54

Eluent	Leave Rf Value	
	After Speayer with DPPH	Before Sprayer with DPPH on UV 254 nm
(2:8)	1. 0,14	1. 0,14
	2. 0,43	2. 0,43
	3. 0,82	3. 0,54
		4. 0,82
(3:7)	1. 0,1	1. 0,1
	2. 0,32	2. 0,27
	3. 0,82	3. 0,8
	4. 0,9	4. 0,9
(5:5)	1. 0,1	1. 0,1
	2. 0,65	2. 0,52
	3. 0,78	3. 0,63
	4. 0,9	4. 0,72
		5. 0,89

Fig. 2: Analysis of Rf value with TLC methods, (a) after sprayer with DPPH on UV 254 nm, (b) Before sprayer with DPPH on 254 nm, (c) before sprayer with DPPH on 366 nm.

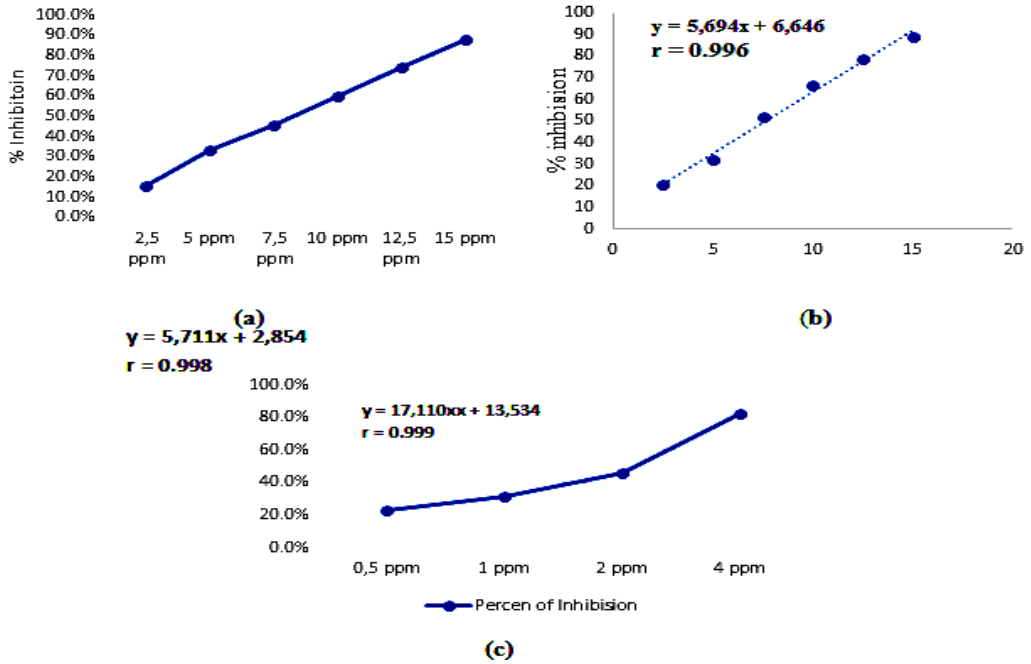


Fig. 3: (a) percent of inhibition bark extrac, (b) percent of inhibition leave extract, (c) percent og inhibition Quersetin**.**

The results of calculating the IC50 value of the sample using linear regression were obtained at 7.614 ppm. These results indicate that the *M. rufocostata* extract sample has very strong antioxidant activity because the IC50 obtained is <50 ppm[12]. IC50 value was calculated using probit analysis. The concentration significance value obtained using SPSS is 0.000, which means it is lower than 0.05. This shows that the concentration of *M. rufocostata* extract samples has a significant effect on the inhibition percentage. An independent t-test was used to compare whether there was a significant difference between the IC50 value of the positive control quercetin comparator and the ethanol extract samples of *M. rufocostata*. The normality test and homogeneity test were carried out first as a condition before carrying out the independent t-test[13]. The normality test obtained data that were normally distributed while the homogeneity test obtained data that were not homogenously distributed[14]. The independent t-test was carried out with the significance value used in the equal variances not assumed section because the homogeneity test of the data is not homogeneous. The results of the independent t-test obtained a significance value of <0.05. So, it can be concluded that there is a significant difference between the average IC50 value of the positive control quercetin comparator and the ethanol extract samples of *M. rufocostata*[15].

Strong natural antioxidants can minimize the performance of ROS. Leaves and bark of *M rufocostata* are strong sources of antioxidants[16]. Phenols and flavonoids are active components that play a role in reducing and preventing damage caused by free radicals. Flavonoids are responsible for protecting the body from oxidative stress[17]. Phenotypic natural phenolic acids work by sending hydrogen to free radicals and breaking chain bonds[18]. In addition, flavonoids have other benefits such as prevention of cardiovascular and liver disease and anti-pain[19]. The difference in the antioxidant strength of the two parts of the plant is influenced by several factors, including plant morphology and harvest time[20]. Differences in the component of flavonoids in the two parts of the plant are caused by genotype[21].

Conclusions

It can be concluded that the ethanol extract of the leaves and bark of *M. rufocostata* from Hulu Sungai Tengah District contains tannins, phenols, flavonoids, and saponins and it has a very strong antioxidant activity.

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نشرة العلوم الصيدلية
جامعة أسيوط

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دراسة فيتوكيميائية لنبات مانجيفيرا روفوكوستاتا كوستيرم ونشاطه المضاد للأكسدة

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[١]مركز دراسة الطب القائم على المكونات الطبيعية ، جامعة لامبونج مانجكورات ، بانجارماسين ، جنوب كاليمانتان، إندونيسيا

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(نبات التاندوي مانجيفيرا روفوكوستاتا كوستيرم) هو أحد النباتات النموذجية من جنوب كاليمانتان والتي تنتمي إلى جنس مانجيفيرا . يمكن استخدامه كدواء. يستخدم السكان المحليون الماء المغليللحاء لعلاج مرض السكري والسكتات الدماغية البسيطة. تهدف هذه الدراسة إلى التعرف على المركبات وتحديد النشاط المضاد للأكسدة لمستخلص الايثانول للأوراق و لحاء نبات مانجيفيرا روفوكوستاتا نوعيا و كميا. اظهرت نتائج التعرف على ان مستخلص الايثانول لمانجيفيرا روفوكوستاتا يحتوي على التانينات والفينولات والفلافونويد والصابونين. أظهرت نتيجة اختبار الفعالية المضادة للأكسدة النوعي أن مستخلص الإيثانول له نشاط مضاد للأكسدة. . أظهرت نتائج اختبارات نشاط مضادات الأكسدة الكمية أن الأوراق واللحاء لها قيم ٧,٦١٤جزء في المليونو ٨,٢٥٤ جزء في المليون. بناءً على هذا البحث ، يمكن استنتاج أن مستخلص الإيثانول لمانجيفيرا روفوكوستاتا من منطقة هولو سونجاي تنجا يحتوي على نواتج ثانوية مختلفة مثل التانينات والفينولات والفلافونويدات والصابونين وله نشاط قوي جدًا كمضاد للأكسدة .