Borneo Journal of Pharmacy
Vol 2 Issue 2 November 2019
Page 114 – 118

http://journal.umpalangkaraya.ac.id/index.php/bjop/article/view/1070
DOI: https://doi.org/10.33084/bjop.v2i2.1070
e-ISSN: 2621-4814

Antioxidant Activity of Ethanolic Extract from Tandui Leaves (*Mangifera rufocostata* Kosterm.) by DPPH Radical Scavenging Method

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Keywords:
Antioxidant
DPPH
Ethanol extract
Mangifera ruficostata Kosterm
Tandui

Abstract

Tandui (*Mangifera rufocostata* Kosterm.) is a typical plant from South Kalimantan which belongs to the genus of Mangifera. Several species of Mangifera are known to have antioxidant activity. This study aimed to determine the antioxidant activity of ethanol extract of Tandui leaves. Tandui leaves that were obtained from the maceration method used 70% ethanol. Antioxidant activity was conducted quantitative using the DPPH (2,2-diphenyl-1-picrylhydrazil) method. The result of the antioxidant activity of Ethanol extract of Tandui leaves quantitatively obtained IC50 value was 60.7042 µg/mL. The ethanol extract of Tandui leaves has strong antioxidant activity.

Received: October 17th 2019
Accepted: October 31th 2019
Published: November 14th 2019

INTRODUCTION

Free radicals are atoms or molecules that have one or more unpaired electrons on their outer orbital, highly reactive. Increase the formation of free radicals (Phaniendra *et al.*, 2015). Oxidative stress conditions would increase the formation of free radicals (Lobo *et al.*, 2010). Highly reactive free radicals and oxygen species can initiate degenerative diseases such as cancer, diabetes, hypertension, atherosclerosis, and neurodegenerative disorders (Liguori *et al.*, 2018). Antioxidants, molecules with a radical-scavenging capacity, are thought to exert a protective effect against free radical damage (Lee *et al.*, 2017). In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retarded the progress of many chronic diseases (Yashin *et al.*, 2017).

Genus of Mangifera has been reported as an antioxidant activity with the DPPH scavenging method (Ramirez *et al.*, 2014; Sultana *et al.*, 2012). The members of the Mangifera genus are used as food or traditional herbal medicine. Several members of Mangifera plants also have been used as an antioxidant, both traditionally used in the community and separated by the fractionation method to obtain the fraction with the highest antioxidant activity (Lukmandaru *et al.*, 2012; Sutomo *et al.*, 2014).

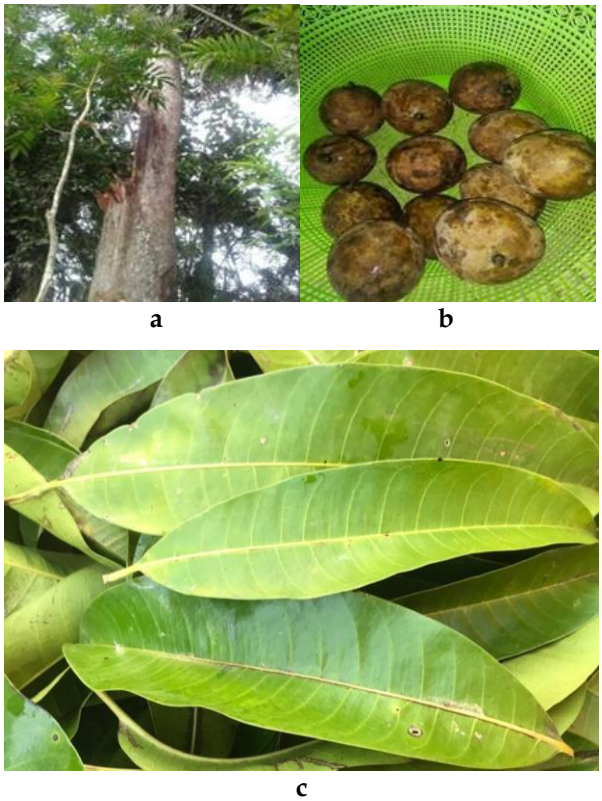
One of the medicinal plants from this genus was tandui (*Mangifera rufocostata* Kosterm.). This plant was grown in South Kalimantan, Indonesia. Preliminary studies show that leaves of tandui contain tannins, phenolics, flavonoids, and steroidal saponins. Flavonoid and phenolic compounds may be useful as antioxidants from natural sources (Tungmunnithum *et al.*, 2018). However, the antioxidant activity of tandui has not yet been

evaluated. Therefore, our study aims to investigate the phytochemical composition, in vitro antioxidant activity of tandui from South Kalimantan.

MATERIALS AND METHODS

Materials

Plant materials are tandui leaves obtained from the Banjarbaru forest area, South Kalimantan. Other ingredients used were 70% ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrochloride acid, ferric chloride reagent, quercetin, aluminum chloride, methanol, ethyl acetate, n-hexane, chloroform, anhydrate acetate acid, magnesium, H_2SO_4, Dragendorff's reagent, quercetin, and thin-layer chromatography (TLC) gel GF_{254}. The figure of the tandui tree, fruits, and leaves is presented in **Figure 1**.



a **b**

c

Figure 1. Tandui tree (**a**), fruits (**b**), and leaves (**c**)

Extraction

The leaves of tandui dried in room temperature and ground into powder. As much as 200 g of leaf powder was extracted with ethanol. The liquid extracts were filtered with filter paper. The filtrates were evaporated with a rotary evaporator to remove the solvent and get crude extracts as much as 35.14 g with a yield of 17.57%.

Phytochemical screening

A small portion of the dry extract was used for the phytochemical tests for compounds, which include tannins, flavonoids, and steroids following the methods (Tiwari *et al.*, 2011; Nugrahani *et al.*, 2016) with little modifications. Exactly 1 g of the plant extract was dissolved in 10 ml of distilled water and filtered using Whatman no.1 filter paper. The extract was then added with a 1% gelatin solution. The formation of white deposits indicates the presence of tannin. Meanwhile, a blue coloration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of phenols in the extract. Exactly 1 g of the plant extract was dissolved in 10 ml of distilled water and filtered using Whatman no.1 filter paper addition gelatin test. About 0.2 g of the extract was dissolved in 2 ml of methanol and heated with the hot plate. A chip of magnesium was added to the mixture, followed by the addition of a few drops of concentrated HCl. The occurrence of red or orange color was indicative of flavonoids (Aiyegoro & Okoh, 2010). As much as 1 ml of the extract and five drops of concentrated H_2SO_4 was added in a test tube. The red coloration is indicative for the presence of triterpenoids, and green coloration as indicative of steroids. Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on the water bath. About 1 ml of the filtrate was treated with a few drops of Dragendorff's reagent. Saponin test with froth test, the foam was indicative of saponin (Auwal *et al.*, 2014).

DPPH radical scavenging assay

The samples with different concentrations of plant extracts (10-50 µg/ml) were reacted with the stable DPPH radical in methanol solution. The reaction mixture

consisted of adding 2 ml of sample and 2 ml of DPPH (0.4 mM) in methanol solution. The mixture was gently homogenized, and after 30 minutes incubation in dark condition at room temperature (Molyneux, 2004). When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color from deep violet to light yellow then measured its absorbance at 515 nm using a spectrophotometer UV-Vis. The ability of plant extracts for scavenging DPPH was estimated using the formula $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample. Quercetin was used as the standard reference. The control solution was prepared by mixing 2 ml methanol and 2 ml DPPH radical solution. The half-maximal inhibitory concentration (IC_{50}) values were calculated using their calibration curve until the linear regression equation is obtained (Bakti *et al*., 2017).

RESULTS AND DISCUSSION

The extracts of tandui leaves were further investigated to determine phytochemical compounds in the extract. The typical phytochemistry content from the ethanol extract of tandui, such as flavonoids, phenols, tannins, and saponins have been identified as presented in **Table I**. Tannins and flavonoids is one of polyphenol that can donate hydrogen and act as an antioxidant. While saponins are a group of compounds commonly found in the genus Mangifera (Shah *et al*., 2010).

Table I. Phytochemical screening of tandui leaves ethanol extract

Phytochemical Compound	Reagent	Result	Conclusion
Tannins	Gelatin 1%	White deposits	+
Phenols	$FeCl_3$	Bluish-green solution	+
Flavonoids	Mg + HCl	Orange color	+
Steroids	H_2SO_4	Brownish green solution	-
Triterpenoids	H_2SO_4	Brownish green solution	-
Alkaloids	Dragen dorff	No deposits	-
	Mayer	No deposits	-
Saponins	Water	Froth formed	+

Antioxidant activity was done by the DPPH scavenging method. The antioxidant will react with DPPH by electron donate mechanism, which stabilized DPPH was demonstrated by decreasing the intensity of DPPH's violet color and slowly turns into yellow. This decrease could be measured by visible spectrophotometry at a wavelength of 515-520 nm (Elkhamlichi *et al*., 2017). In the present study, the percentage of scavenging effect on the DPPH radical was concomitantly increased with the increase in the concentration of leave of ethanolic extracts for 10 to 50 µg/ml. The DPPH radical scavenging activity is given in **Table II**.

Quercetin, as a well known potent antioxidant, was used as a positive control for DPPH scavenging activity. Leaves of tandui showed the highest ability in DPPH scavenging activity, which measured by the lowest IC_{50} value. The IC_{50} value of quercetin was 3.4065 µg/ml. The IC_{50} value of tandui leaves extracts determined by linear regression. From the linear regression equation, obtained the IC_{50} value of ethanol extracts that was 60.7042 µg/ml. These results indicate that the ethanol extract of tandui leaves has strong antioxidant activity (Mustarichie, 2017).

Table II. Phytochemical screening of tandui leaves ethanol extract

Samples	Concentration (µg/mL)	DPPH Scavenging activity (%)	IC_{50} (µg/ml)
Quercetin	1	19.3398 ± 2.18	3.4065
	2	32.5400 ± 1.1861	
	3	44.9558 ± 0.8052	
	4	57.9265 ± 1.0468	
	5	69.6885 ± 1.1696	
Tandui leaves	10	31.5108 ± 0.2694	60.7042
	20	35.9200 ± 1.12	
	30	38.5655 ± 1.1743	
	40	41.6813 ± 2.191	
	50	46.7372 ± 2.7151	

CONCLUSION

The 70% ethanolic extracts from tandui leaves showed antioxidant activity with IC_{50} DPPH scavenging activity of 60.7042 µg/ml, which indicates that tandui leaf extract has strong antioxidant activity.

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