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DETERMINATION OF ANTIOXIDANT ACTIVITIES OF BUAS-BUAS LEAVES (*Premna serratifolia* L.) USING DPPH (2,2-diphenyl-1-picrylhydrazyl) METHOD

PENENTUAN AKTIVITAS ANTIOKSIDAN DARI DAUN BUAS-BUAS (*Premna serratifolia* L.) MENGGUNAKAN METODE DPPH (2,2-diphenyl-1-picrylhydrazyl)

Isnindar[1)], Subagus Wahyuono[2)], Sitarina Widyarini[3)], Yuswanto[2)]
[1.]Faculty of Medicine, Study Program of Pharmacy, University of Tanjungpura
[2.]Faculty of Pharmacy, Universitas Gadjah Mada
[3.]Faculty of Animal Medicine, Universitas Gadjah Mada

ABSTRACT

Antioxidant is a substance that in small concentrations can significantly inhibit or prevent oxidation on the substrate. Buas-buas (Premna serratifolia L.*) is one of the plants that have antioxidant effect. The antioxidant activities of the wasbenzen and ethanol extracts of buas-buas leaves were determined by DPPH method using spectrophotometer UV-Vis. Data resulted from the analysis of antioxidant activity (IC_{50}) is 532.24 µg/mL of wasbenzen extract, 24.40 µg/mL of ethanol extract, and 1.83 µg/mL of vitamin C.*
***Key words**: Buas-buas leaves (Premna serratifolia* L*), wasbenzen extract, ethanol extract, antioxidant activity, DPPH method*

ABSTRAK

Antioksidan adalah senyawa yang dalam konsentrasi kecil dapat secara signifikan menghambat atau mencegah terjadinya proses oksidasi. Buas-buas (Premnaserratifolia L) merupakan salah satu tanaman yang memiliki efek antioksidan. Aktifitas antioksidan dari ekstraksi wasbenzen dan etanol daun Buas-buas ditentukan dengan metode DPPH menggunakan spektrofotometer UV-Vis. Data yang dihasilkan dari analisis aktifitas antioksidan ekstrak wasbenzen adalah 532.24 µg/mL, ekstrak etanol adalah 24.40 µg/mL, dan vitamin C adalah 1.83 µg/mL.
***Kata kunci:** daun Buas-buas (Premnaserratifolia L), ekstrak wasbenzen, ekstrak etanol, aktivitas antioksidan, metode DPPH*

INTRODUCTION

Buas-buas is a tropical plant. This plant, which belongs to the family of Verbenaceae, is originally from Southeast Asia, often cultivated in tropical and subtropical areas. In this study, buas-buas leaves were derived from the village of Rasau Jaya 2, District Rasau Jaya, Kubu Raya, West Kalimantan Province, Indonesia. In addition, young buas-buas leaves can stimulate the production of breast milk (Ali, 2008). Locals generally use buas-buas leaves as fresh vegetables, or as an addition to food as flavor. Chemical content of buas-buas leaves are namely polyphenols, alkaloids, triterpenoids, and flavonoids (vadivu *et al*., 2008). The ethanol extract of wood *Premnaserratifolia* Linn. has anti-inflammatory activity (Rajendran and Krishnakumar, 2010), lignans in the bark *Premnaintegrifolia* has antioxidant activity DPPH and NO Scavenging (Yadav *et al*.,2012). In the early identification, *Premnalatifolia* contains triterpenoids, alkaloids, and flavonoids (Asharani *et al*., 2013).

Related research that had been conducted by Ali (2008) on the antioxidant activity of essential oils of buas-buas leaves showed *Premnacordifolia* hadantioxidant activity by 38 ± 9.0%. Study done by Bakar *et al*. (2010) also showed that the antioxidant activity of the *Premnacordifolia* extract with DPPH method is highly influenced by the group of flavonoids and phenolic compounds which have IC_{50} value of 31.91 ± 0.43 pg/mL. According to Kaur *et al*. (2012) phytochemical screening results indicated that *Premnacorymbosa* leaves Rottl. contained alkaloids, glycosides, flavonoids, steroids, and triterpenoids. The root is adstringen, bitter, has a pungent smell, anti-inflammatory, cardiotonic, expectorant, carminative, laxative, antibacterial

Corresponding Author : Isnindar
Email : isnindar@yahoo.com

and, tonic. The leaves are efficacious for carminative, dyspepsia, flatulence colic, cough, fever, rheumatism, neuralgia, haemorrhoid, and antitumors. In vivo testing showed capability of suppressing arthritis. *Premna serratifolia* Linn. also contains alkaloids, flavonoids, tannins, glycosides, steroid, and phenolic compounds, which are used as pain relievers, arthritis, constipation. Ethanol extract can inhibit the swelling of arthritis in mice. Bose *et al*., (2013) have isolated acteoside from the root wood extract of *Premnaserratifolia* (verbacoside) and had antioxidant activity of 73.8±2.4 pg/mL using DPPH method.

DPPH informs reactivity of compounds tested with something radically unstable. DPPH showed a strong absorption at wavelength 517 nm with dark violet color. Catcher of free radicals causes the electrons into pairs and then causes the disappearance of the color comparable to electrons captured (Oke dan Hamburger, 2002; *Yagi et al.*, 2002). The aim of this study was to determine the antioxidant activity of buas-buas leaves using DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

METHODOLOGY

Materials

The main material used in this study was the buas-buas leaves originated from Kubu Raya, West Kalimantan. Other materials used were solvent wasbenzen and ethanol (technical grade). DPPH, vitamin C, ethanol, and wasbenzen were applied to test the antioxidant. All chemicals used were pro analysis grade (Merck), and DPPH (from Sigma, Chem.Co.).

Procedures

Determination of plants

Buas-buas plants studied in the laboratory was determined by Laboratory of Biology, Faculty of Mathematics and Natural Sciences, University of Tanjungpura, Pontianak.

Preparation of the main material

Buas-buas leaves were derived from the village of Rasau Jaya 2, District Rasau Jaya, Kubu Raya, West Kalimantan Province.

Preparation of extract

Buas-buas leaves were dried in drying cabinet for two days and powdered to the desired size. The powder was macerated for 72 h and extracted with grade wazbenzen, then with ethanol five times to the weight of a powder, and then the residual powder was macerated back with ethanol.

Determination of antioxidant activity

A total of 1 mL of 0.4 mM DPPH was put into a test tube, plus sample of the test material at various concentrations. Extract wasbensen (40 µg/mL, 80 µg/mL, 120 µg/mL, 160 µg/mL, 200 µg/mL) and ethanol extract (10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL, 30 µg/mL). Then a solvent was added to a volume of 5.0 mL and waited until the operating time was reached. After that, the absorbance of the solution was read at wavelength of 516 nm (Gulcin, *et al*, 2004; Kwon and Kim, 2003). Similar treatment was conducted to the positive control vitamin C (0.02 µg/mL, 0.1 µg/mL, 0.2 µg/mL, 0.4 µg/mL, 0.6 µg/mL) without the addition of extracts and vitamin C. The read of absorbance is as follows:

$$\text{The antioxidant activity (\%)} = \frac{\text{Abs.kontrol- Abs.sampel}}{\text{Abs.kontrol}} \times 100\%$$

RESULTS AND DISCUSSION

Chemical content of buas-buas leaves are namely polyphenols, alkaloids, triterpenoids, and flavonoids (Vadivu *et al*., 2008). Phytoconstituents in the ethanol extract of wood *Premna serratifolia* Linn. are irridiod glycosides, alkaloids, phenolic compounds, and flavonoids (Rajendran, and Krishnakumar, 2010), lignans in the bark *Premnaintegrifolia* has antioxidant activity DPPH and NO Scavenging (Yadav *et al*.,2012). Bakar *et al*. (2010) also showed that the antioxidant activity of the extract *Premnacordifolia* with DPPH method is highly influenced by the group of flavonoids and phenolic compounds which have IC_{50} value of 31.91±0.43 pg/mL. According to Kaur *et al*., (2012) phytochemical screening results indicated that *Premnacorymbosa* leaves Rottl. contains alkaloids, glycosides, flavonoids, steroids, and triterpenoids. Bose *et al*., (2013) have isolated acteoside from the root wood extract of *Premnaserratifolia* (verbacoside) and had antioxidant activit of 73.8±2.4 pg/mL using DPPH method. Based on some references that have been outlined, *Premna serratifolia* contains many compounds such as alkaloids, flavonoids, phenols, and steroids. Plant polyphenolic compounds showed potent antioxidant effects in vitro, which is able to inhibit lipid peroxidation by peroxyl radical catcher activity thus breaking the chain reaction. Phenol with 2-OH group or with a chelating structure. Phenol also catches reactive oxygen species such as hydroxyl radical (HO•) directly (Halliwell and Gutteridge, 1999). DPPH is a stable free radical in a solution of water or alcohol and is capable of accepting electrons or hydrogen radical to become a stable diamagnetic molecule (Gulcin, *et al*, 2004; Halliwell and Gutteridge, 1999).

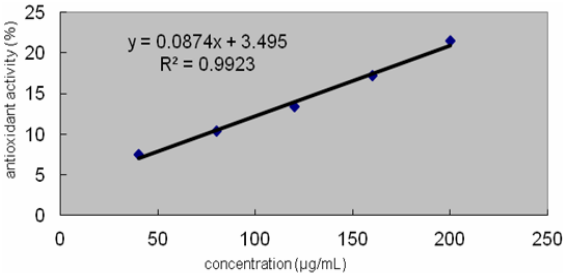

Figure 1. Linear regression curve between the concentration of the extract wasbenzen and percent of antioxidant activity using DPPH method

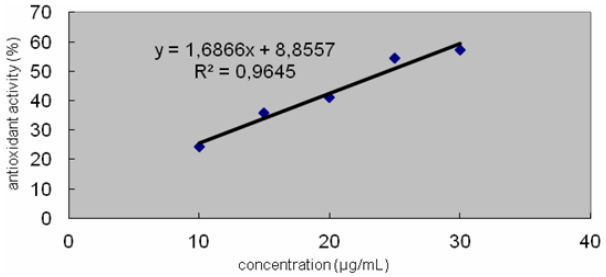

Figure 2. Linear regression curve between concentration of ethanol extract and percent of antioxidant activity using DPPH method

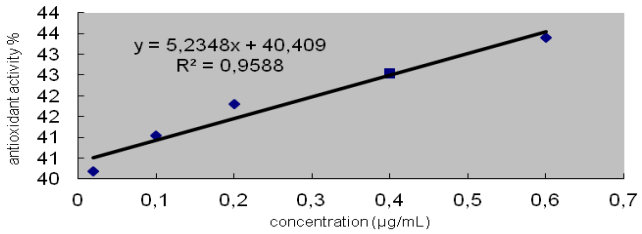

Figure 3. Linear regression curve of vitamin C as a positive control and the pepercent antioxidant activity using DPPH method

Testing radical trapping antioxidant activity of the extract

Determination of antioxidant activity was conducted by radical scavenging DPPH (Jang *et al.*, 2010; Gulcin, *et al.*, 2004; Kwon and Kim, 2003) with a slight modification that 10 mg (Shimadzu analytical balances ATX/ATY Series) of extract dissolved in 10 mL of solvent with various concentrations added 1 mL of 0.4 mM DPPH vortex. Then the mixture was left for 30 min and absorbance was measured at 516 nm wavelength (Milton Roy spectronic 3000 Array). Determination of the maximum wavelength and the determination of operating time were taken to minimize errors while reading the absorbance. Operating time was needed to allow time for a radical catcher compounds react to DPPH completely. Absorbance readings on the operating time aims for a steady reading. IC_{50} values was obtained from the linear regression equation of concentration of the sample against the percentage of antioxidant activity. The smaller the IC_{50} value, the more potent antioxidant activity of these compounds. Linear regression curve wasbenzen extract, ethanol extract, and vitamin C using DPPH method can be seen in figure 1, 2, and 3. From the regression curve data of wasbenzen extract, ethanol extract, and vitamin C, it is shown that there was a close relationship between the concentrations of the antioxidant strength (% inhibition). This was shown by the value of R^2 (correlation coefficient) above 0.9. The R^2 states that the correlation between the concentrations of the sample with the % inhibition was observed with degree of closeness to extract wasbenzen at 0.9923, ethanol extract at 0.9645, and vitamin C as a positive control at 0.9588. This shows that more than 99% degree of inhibition is affected by the concentration of materials, while less than 1 % is influenced by other factors. The test results showed that the higher the concentration of the solvent, the higher the percentage of inhibition. This is due to the more samples used, the higher the antioxidant content, so that the impact is also on the level of inhibition of free radicals made by antioxidant compounds. The spectroscopic data above shows that IC_{50} of vitamin C, wasbenzen, and ethanol extracts of buas-buas leaves (*Premna serratifolia* L.) were 1.83 µg/mL, 532.24 µg/mL, and 24.40 µg/mL, respectively. The data shows that in order to capture a 50 % DPPH radical required Vitamin C, wasbenzen, and ethanol extracts of buas-buas leaves (*Premna serratifolia* L.) that are 1.83 µg/mL, 532.24 µg/mL, and 24.40 µg/mL, respectively. Vitamin C in this study was used as a positive control to prove that the method used to test the antioxidant activity was correct. Vitamin C was used because it belongs to vitamin antioxidants that can counteract free radicals extracellular.

Table I. Data extract concentration wasbenzen extract and percent antioxidant activity using DPPH method

Extract concentration (µg/mL)	Absorbance			Antioxidant activity (%)			Average	SD
	I	II	III	I	II	III		
Control	0.804	0.804	0.804					
40	0.744	0.735	0.753	7.462687	8.58209	6.343284	7.462687	1.119403
80	0.721	0.715	0.727	10.32338	11.06965	9.577114	10.32338	0.746269
120	0.696	0.680	0.712	13.43284	15.42289	11.44279	13.43284	1.99005
160	0.666	0.654	0.678	17.16418	18.65672	15.67164	17.16418	1.492537
200	0.631	0.638	0.624	21.51741	20.64677	22.38806	21.51741	0.870647

Table II. Data percent concentration of ethanol extract and percent antioxidant activity using DPPH method

Extract concentration (µg/mL)	Absorbance			Antioxidant activity (%)			Average	SD
	I	II	III	I	II	III		
Control	0.804	0.804	0.804					
10	0.609	0.598	0.620	24.25373	25.62189	22.88557	24.25373	1.368159
15	0.515	0.500	0.530	35.94527	37.81095	34.07960	35.94527	1.865672
20	0.474	0.490	0.458	41.04478	39.05473	43.03483	41.04478	1.99005
25	0.365	0.355	0.375	54.60199	55.84577	53.35821	54.60199	1.243781
30	0.345	0.350	0.340	57.08955	56.46766	57.71144	57.08955	0.621891

Table III. Data concentrations of vitamin C as a positive control and percent of antioxidant activity using DPPH method

Extract concentration (µg/mL)	Absorbance			Antioxidant activity (%)			Average	SD
	I	II	III	I	II	III		
Control	0.804	0.804	0.804					
0.02	0.481	0.475	0.487	40.17413	40.92040	39.42786	40.17413	0.746269
0.1	0.474	0.464	0.484	41.04478	42.28856	39.80100	41.04478	1.243781
0.2	0.468	0.46	0.476	41.79104	42.78607	40.79602	41.79104	0.995025
0.4	0.462	0.453	0.471	42.53731	43.65672	41.41791	42.53731	1.119403
0.6	0.455	0.441	0.469	43.40796	45.14925	41.66667	43.40796	1.741294

That is because the vitamin C which has hydroxy groups is free to act as free radical catchers, and if having a polyhydroxy group will increase the antioxidant activity (Maslarova, 2001).

CONCLUSION

IC_{50} of wasbenzenand ethanol extracts of buas-buas leaves (*Premna serratifolia* L.) were 532.24 µg/mL and 24.40 µg/mL, respectively.

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