

## 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)

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#### 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  $\mu\text{g/mL}$  of wasbenzen extract, 24.40  $\mu\text{g/mL}$  of ethanol extract, and 1.83  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$ , ekstrak etanol adalah 24.40  $\mu\text{g/mL}$ , dan vitamin C adalah 1.83  $\mu\text{g/mL}$ .*

**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 (vadvu *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* had antioxidant activity by  $38 \pm 9.0\%$ . 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  $31.91 \pm 0.43$   $\mu\text{g/mL}$ . 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, cardiotoxic, expectorant, carminative, laxative, antibacterial

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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  $73.8 \pm 2.4$  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 wasbenzen (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:

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

## 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<sub>50</sub> value of  $31.91 \pm 0.43$  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  $73.8 \pm 2.4$  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 peroxy 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).

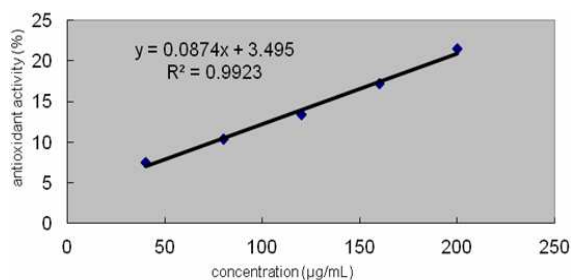


Figure 1. Linear regression curve between the concentration of the extract wasbenzen and percent of antioxidant activity using DPPH method

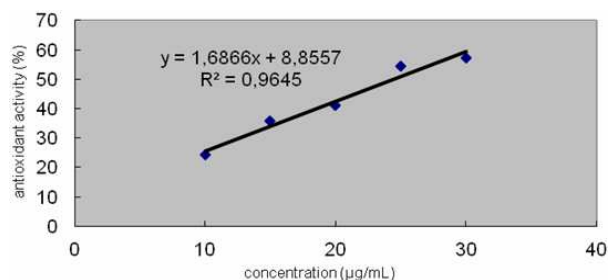


Figure 2. Linear regression curve between concentration of ethanol extract and percent of antioxidant activity using DPPH method

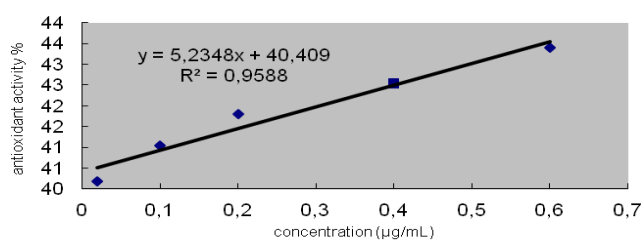


Figure 3. Linear regression curve of vitamin C as a positive control and the percent antioxidant activity using DPPH method

### 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<sub>50</sub> values was obtained from the linear regression equation of concentration of the sample against the percentage of antioxidant activity. The smaller the IC<sub>50</sub> 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<sup>2</sup> (correlation coefficient) above 0.9. The R<sup>2</sup> 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<sub>50</sub> 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<br>( $\mu\text{g/mL}$ ) | 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<br>( $\mu\text{g/mL}$ ) | 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<br>( $\mu\text{g/mL}$ ) | 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  $\mu\text{g/mL}$  and 24.40  $\mu\text{g/mL}$ , respectively.

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