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Antioxidant Activity of n-Hexane Subfraction of *Buas-Buas* Leaf (*Premna serratifolia* L.) by DPPH Method

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Abstract

Buas-buas (*Premna serratifolia* L.) is a plant traditionally used as medicine and widely grown in West Kalimantan with various species. There are many benefits of buas-buas plants as medicine, one of which is as an antioxidant. The purpose of this study was to determine the percent inhibition value that describes the antioxidant activity of ethanol extract, n-hexane fraction, and n-hexane subfraction of buas-buas leaves. The n-hexane fraction was obtained by liquid-liquid extraction with separatory funnel from ethanol extract and the fraction was isolated by column chromatography. The antioxidant activity of the ethanol extract, n-hexane fraction, and subfractions C_1, C_2, C_3, D_{123}, and E_3F_{123} was assessed using DPPH (2,2-Diphenyl-1-Picrylhydrazyl). The results showed that ethanol extract, n-hexane fraction, and subfractions C_1, C_2, C_3, D_{123}, and E_3F_{123}, had antioxidant activity with percent inhibition values of 57.946; 53.078; 52.652; 48.706; 49.745; 50.490; and 50.639%, respectively, which indicated that each sample had good antioxidant activity, except for subfractions C_2 and C_3.

Keywords: *Premna serratifolia* L.; n-hexane; subfraction; antioxidant; DPPH.

INTRODUCTION

The demand for herbal medicinal products continues to increase in modern society. This growing interest is largely driven by the perception that herbal medicines are safer and healthier when compared to synthetic drugs. Many consumers believe that products derived from natural sources have fewer side effects and are more compatible with the human body. As a result, herbal medicines are increasingly used both for preventive and therapeutic purposes. However, the rising consumption of herbal products also raises concerns regarding their quality, safety, and efficacy. Variations in raw materials, processing methods, and storage conditions can affect the consistency of herbal medicines. Therefore, proper quality control measures are essential to ensure that herbal products meet acceptable standards. One important approach to quality control is the identification and characterization of marker compounds, which can be used to guarantee consistency and safety of herbal medicines (Do et al., 2019; Yadav et al., 2023).

In line with quality control efforts, the isolation of bioactive compounds from plants is an important step in the development of herbal medicines. Isolating individual compounds allows researchers to better understand their chemical properties and potential medicinal benefits. This process also contributes to the discovery of new natural compounds that may be developed into therapeutic agents. Consequently, continuous exploration of medicinal plants is necessary to support the advancement of herbal medicine research. One plant that has the potential to be developed as a medicinal raw material is buas-buas.

Buas-buas (*Premna serratifolia* L.) is a plant that has been traditionally used as a medicinal plant and is widely grown in West Kalimantan, where it exists in various species (Puspita et al., 2020). The traditional use of this plant indicates its importance in local healthcare practices and suggests the presence of biologically active compounds. Among its various traditional applications, buas-buas is known to be used for its antioxidant properties (Tohomi et al., 2014; Tonius et al., 2016). Antioxidants play a crucial role in counteracting oxidative processes, making plants with antioxidant activity valuable in medicinal research. The medicinal potential of buas-buas is supported by the presence of several groups of secondary metabolites in its leaves, including flavonoids, saponins, polyphenols, terpenoids, and alkaloids (Wulandari, 2019). These compounds are commonly associated with various biological activities and further strengthen the rationale for studying this plant.

To further explore the potential of buas-buas, analytical studies using isolation techniques are required (Yadav et al., 2023). Isolation techniques allow the

separation of complex plant extracts into simpler fractions, making it easier to identify and evaluate individual components. One of the initial and widely used techniques in compound isolation is column chromatography. This method enables the separation of compounds based on differences in their polarity and interactions with the stationary and mobile phases. Despite the existing studies on buas-buas, isolation of the n-hexane fraction of buas-buas leaves is still rarely reported, and its antioxidant activity has not been well documented.

One method commonly used to evaluate antioxidant activity is the DPPH method. This method is widely applied due to its simplicity and effectiveness in measuring the antioxidant capacity of plant extracts or isolated fractions. Therefore, this study focuses on isolating compounds from the n-hexane fraction of buas-buas leaves using column chromatography and evaluating their antioxidant activity using the DPPH method. The results of this study are expected to contribute to the understanding of the antioxidant potential of *buas-buas* and support further research on its medicinal applications.

MATERIALS AND METHODS

Materials

The materials used in this study were *buas-buas* leaves (*Premna serratifolia* L.) taken from Swadaya Market (Jalan Sungai Raya Dalam, Pontianak City, West Kalimantan), thin layer chromatography plate (silica gel F_{254} Sigma Aldrich®), distilled water, technical 96% ethanol, ethanol p.a., methanol p.a., technical n-hexane, DPPH (Merck®), concentrated H_2SO_4, vanillin, Lieberman-Burchard reagent, silica gel (G 60 Merck®) and the tools used in the study were rotary evaporator (Buchi®), UV-Vis spectrophotometer instrument (Shimadzu®), a set of chromatography column (60 cm long, 3.2 cm diameter), desiccator, food dehydrator (Kris®), analytical balance (Radwag®), blender (Miyako®), buchner device (Rocket®), separatory funnel, glassware (Iwaki®, Pyrex®).

Methods

Simplisia Preparation

Buas-buas leaves obtained from Pasar Swadaya (Jalan Sungai Raya Dalam, Pontianak City, West Kalimantan) were determined at the Biology Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA), Tanjungpura University. Samples were cleaned using clean flowing water. Next, the leaves were chopped and dried with a food dehydrator at 60ºC, then ground using a blender and stored in a glass container (Agusti et al., 2022; Kartiko & Fanani, 2021).

Extraction

The maceration jar was filled with 1,361 grams of leaf simplisia *buas-buas* in total. The sample was then submerged in 96% ethanol solvent. Maceration was carried out for 3x24 hours with a 1x24 hour solvent change and occasional stirring. The solvent was worked through until it was nearly clear. The macerate was then filtered using a vacuum Buchner. The resulting filtrate was collected and thickened using a rotary evaporator at 50ºC. (Isnindar & Luliana, 2020; Mawarda et al., 2020; Taufik Hidayat & Luliana, 2021).

Fractionation

497.1 grams of ethanol extract of buas-buas leaves was added to warm distilled water and stirred, then decanted overnight. The results of decantation were taken and then fractionated with n-hexane, then concentrated with a rotary evaporator at 50ºC (Mayda Mahera & Salsabila Firdausia, 2023; Purwaningrum et al., 2022; Yundu et al., 2020).

Chromatography Column Isolation

Column preparation is done by the wet method. The stationary phase was introduced through the column wall slowly while stirring so that there were no air voids in the center of the column and left for 1x24 hours (Mabruroh et al., 2019). The n-hexane fraction was crushed together with silica gel and eluted using the mobile phases n-hexane : ethyl acetate (100:0 (A); 90:10 (B); 80:20 (C); 70:30 (D); 50: 50 (E); 40:60 (F); 30:70 (G); 20:80 (H); 0:100% (I)) and ethyl acetate : methanol (90:10 (J); 80:20 (K); 70:30 (L); 60:40 (M); 50:50 (N); 40:60 (O); 30:70 (P); 0:100% (Q)). Each eluent was made to 300 mL. After that, the separation from column chromatography was collected in a 100 mL vial.

KLT Identification

The ethanol extract, n-hexane fraction and subfractionation were photographed on a silica F_{254} plate, then eluted using the appropriate mobile phase, and then the mobile phase was allowed to waited until it reached the upper limit of the plate. The result plate was then sprayed with vanillin-sulfuric acid, Lierberman-Burchard, and DPPH spots (Candra et al., 2023; Oktaviani et al., 2015). Subfractions with similar profiles were combined and identified by spotting.

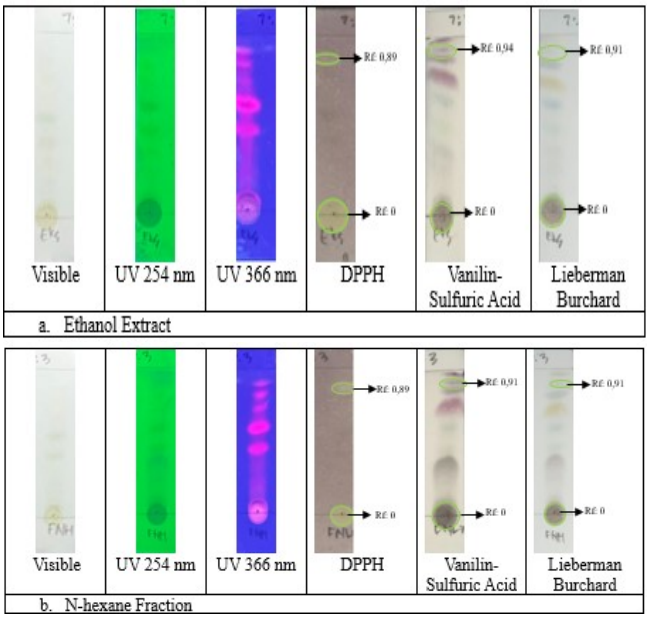
RESULTS AND DISCUSSION

The leaves of *buas-buas* (*Premna serratifolia* L.) as much as 1,361 grams, that have been collected, washed with flowing water and sorted to be clean from impurities. The drying process is carried out to reduce the water content and this process is assisted by a food dehydrator at a temperature of 60ºC. Simplisia was then macerated with ethanol 96% and obtained a yield of 38.560%.

The fractionation process of the ethanol extract produced soluble and insoluble n-hexane fractions. The yield of soluble n-hexane fraction was obtained as much as 4.4 grams with a yield of 0.885%. The fractions were further isolated by the gradient method, resulting in subfractions A_{123}; B_{123}; C_{123}; D_{123}; E_{123}; F_{123}; G_{123}; H_{123}; I_{123}; J_{123}; K_{123}; L_{123} which will be identified by KLT.

Ethanol extract and n-hexane fraction of *buas-buas* leaves were eluted using n-hexane: ethyl acetate (7:3) eluent (Fig. 1). There were 2 pale yellow spots that showed a positive sample of DPPH. The Rf value of the spot was 0.89 and 0. Rf value 0 can occur allegedly because the compound to be separated is still retained on the stationary phase and can be influenced by the polarity of both the compound, the stationary phase, and the mobile phase (Soetjipto et al., 2018). DPPH positive spot at Rf value 0.89 was thought to be terpenoid because there were spots with close Rf values after spraying vanillin-sulfuric acid and Liberman-Burchard, namely at Rf values 0.91 and 0.94. Meanwhile, the profile of the n-hexane fraction showed results that were not much different from the extract (Fig. 1). This was the basis for isolating the n-hexane fraction using column chromatography because the fraction was shown to have antioxidant activity.

Isolated subfractions with similar profiles can be combined and re-identified by KLT with DPPH, Liebermen-Burchard, and vanillin-sulfuric acid sprayers. The test continued with subfractions with consideration of the amount and potential antioxidant activity. The selected subfractions were C_1; C_2; C_3; D_{123}; and E_3F_{123} with weights of 0.1267; 0.2399; 0.2205; 0.2817; and 0.1587 grams, respectively. The identification results show that the compounds that play a role in each subfraction are terpenoids characterized by purple spots on the plate (Fig. 1).



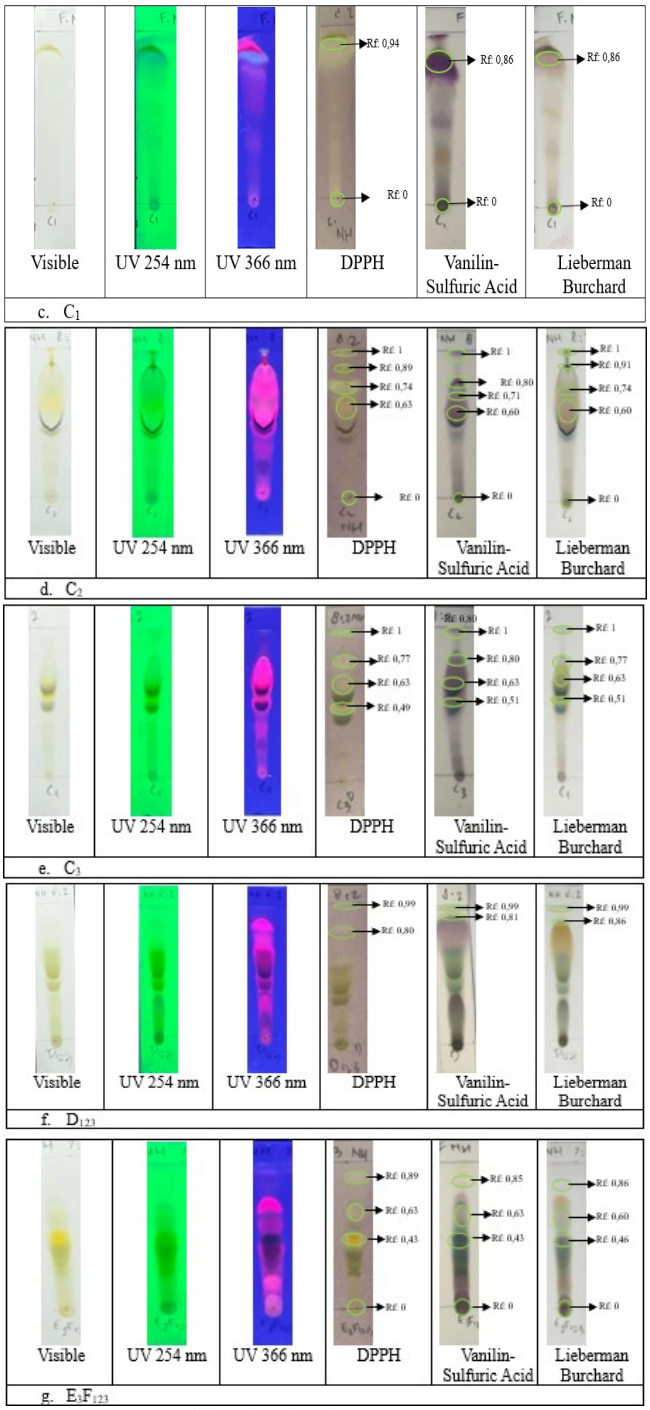


Figure 1. KLT Profile of Ethanol Extract, n-Hexane Fraction, and Subfraction of Buas-Buas Leaf Isolation Results.

Subfractions C_1; C_2; C_3; D_{123}; and E_2F_{123} were then measured for antioxidant activity with the DPPH method using a spectrophotometer. The results of operating time on extracts, fractions, subfractions C_1; C_2; C_3; D_{123}; and E_3F_{123} are 15; 35; 30; 5; and 15 minutes, respectively. Each sample was mixed with DPPH solution for the DPPH silencing test, and the absorbance was then measured at 516.4 nm. The percentage inhibition value will be computed using this absorbance (Table I). The percentage inhibition value results between the ethanol extract and the n-hexane fraction match the findings of

the identification that was previously done using KLT. In the identification of KLT sprayed with DPPH solution, the ethanol extract gives a clearer yellow spot compared to the fraction and subfraction of the isolation results, which shows that the n-hexane fraction has greater silencing. This is evidenced by the greater %inhibition value of the extract, which is 57.946%. This is because of the compounds, like phenolics, flavonoids, tannins, terpenoids, and saponins found in the ethanol extract of *buas-buas* leaves (Riduana et al., 2021).

Table 1. Percent Inhibition of Ethanol Extract, n-Hexane Fraction, and Subfraction of Buas-Buas Leaf.

Sample (20 ppm)	Repetition	Absorbannce	%Inhibition	Average (n=3)	SD	%RSD
Ethanol Extract	1	0,364	57,674	57,946	0,242	0,420
	2	0,361	58,023			0,417
	3	0,360	58,140			0,416
N-hexane fraction	1	0,405	52,962	53,078	0,201	0,380
	2	0,405	52,962			0,380
	3	0,402	53,310			0,377
C_1 Subfraction	1	0,410	52,381	52,652	0,681	1,299
	2	0,412	52,149			1,305
	3	0,401	53,426			1,274
C_2 Subfraction	1	0,432	49,176	48,706	0,471	0,957
	2	0,440	48,235			0,976
	3	0,436	48,706			0,966
C_3 Subfraction	1	0,424	50,176	49,745	0,378	0,753
	2	0,429	49,589			0,762
	3	0,430	49,471			0,764
D_{123} Subfraction	1	0,419	50,764	50,490	0,800	1,576
	2	0,429	49,589			1,613
	3	0,416	51,116			1,565
E_3F_{123} Subfraction	1	0,425	50,639	50,639	0,232	0,459
	2	0,427	50,407			0,461
	3	0,423	50,871			0,457

Discussion

The isolation results of the n-hexane fraction of *buas-buas* leaves turned out to have a lot of terpenoid and phenolic content which is thought to provide antioxidant activity. This is demonstrated in a study by Candra et al. (2023), who isolated and successfully identified the terpenoid compound group using column chromatography and GC-MS. They used the same mobile phase, n-hexane: ethyl acetate, with varying ratios (100% n-hexane; 95:5; 90:10; 85:15; 80:20; 75:25; 70:30; 65; 35; 60:40; 55:45; and 50:50). The isolates of identified terpenoid compounds reported include 3,7,11,15-tetramethyl-2-hexadecen-1-ol and 2 hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl) octahydronaphthalen 1(2H)-one. In addition, the ethanol extract of buas-buas leaves has an IC_{50} value of 22.1 µg/ml (Isnindar & Luliana, 2020). This value is not much different from the IC_{50} value by Puspita et al (2020) and Isnindar, et al (2016) who obtained IC_{50} values of ethanol extracts of *buas-buas* leaves of 20.55 and 24.40 µg/ml, respectively (Isnindar & Luliana, 2020; Puspita et al., 2020). Meanwhile, research conducted by Oktaviani, et al (2015) states that the IC_{50} value in the chloroform, methanol, and n-hexane fractions of *buas-buas* leaves which are partitioned produce very strong antioxidants of 8.7729; 15.2240; and 20.3418 µg/ml. The ethanol extract, n-hexane fraction, and subfractions from chromatographic column isolation have the potential to function as antioxidants, according to the study's findings.

CONCLUSIONS

Ethanol extract, n-hexane fraction, and subfractions C_1, C_2, C_3, D_{123}, and E_3F_{123} of *buas-buas* leaves had antioxidant activity with percent inhibition values of 57.946; 53.078; 52.652; 48.706; 49.745; 50.490; and 50.639%.

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Authors' Contributions: Isnindar and Sri Luliana designed the study, analyzed the data, and supervised research quality control. Darin Iftinan conducted the laboratory experiments and performed data cleaning.

Competing Interests: The authors declare that there are no competing interests.

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