

## Antioxidant Activity of *n*-Hexane, Ethyl Acetate and Ethanol Extract from Lakoocha Leaves (*Artocarpus lacucha* Buch.-Ham) using DPPH Method

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**Abstract.** The aim of this study was to investigate phytochemical constituents and antioxidant activity of *n*-hexane, ethyl acetate and ethanol extract of *Artocarpus lacucha* leaves. The powdered simplicia was macerated with *n*-hexane, ethyl acetate and ethanol 96% sequentially, filtered, and concentrated using rotary evaporator to obtain *n*-hexane extract, ethyl acetate extract and ethanol extract. Phytochemical screening and antioxidant activities were performed against these extracts. Antioxidant activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method using ultraviolet-visible spectrophotometer at wavelength of 516 nm after incubated for 60 minutes in dark place. Quercetin was used as positive control. The result of phytochemical screening showed that *n*-hexane extract contained steroid, ethyl acetate extract contained steroid, tannin, glycoside, flavonoid and saponin, whereas ethanol extract contained tannin, glycoside, flavonoid and saponin. The IC<sub>50</sub> values of *n*-hexane, ethyl acetate and ethanol extract were 1062.03±1.42 ppm, 323.18±0.02 ppm and 99.23±0.07 ppm respectively, whereas for quercetin was 2.32±0.01 ppm. This study showed that ethanol extract of *A. lacucha* leaves has high antioxidant activity whereas *n*-hexane and ethyl acetate extract of *A. lacucha* leaves possess very low antioxidant activity.

**Keyword:** Antioxidant Activity, DPPH, Lakoocha leaf

**Abstrak.** Penelitian ini bertujuan untuk meneliti skrining fitokimia dan aktivitas antioksidan dari ekstrak *n*-heksan, etilasetat dan etanol dari daun mobe. Serbuk simplisia dimaserasi dengan pelarut *n*-heksan, etilasetat dan etanol secara berturut-turut, disaring, kemudian dipekatkan menggunakan alat penguap berputar untuk mendapatkan ekstrak *n*-heksan, ekstrak etilasetat dan ekstrak etanol. Skrining fitokimia dan aktivitas antioksidan dilakukan terhadap ekstrak-ekstrak ini. Aktivitas antioksidan ditentukan dengan metode pemerangkapan radikal DPPH (1,1-diphenyl-2-picrylhydrazyl) menggunakan spektrofotometer ultraviolet-visibel pada panjang gelombang 516 nm setelah diinkubasi selama 60 menit di tempat gelap. Kuersetin digunakan sebagai kontrol positif. Hasil skrining fitokimia menunjukkan ekstrak *n*-heksan mengandung steroid, ekstrak etilasetat mengandung steroid, tannin, glikosida, flavonoid dan saponin, sedangkan ekstrak etanol mengandung tannin, glikosida, flavonoid dan saponin. Nilai IC<sub>50</sub> masing-masing ekstrak *n*-heksan, etilasetat dan etanol sebesar 1062,03±1,42 ppm, 323,18±0,02 ppm dan 99,23±0,07 ppm sedangkan untuk kuersetin sebesar 2,32±0,01 ppm. Penelitian ini menunjukkan bahwa ekstrak etanol memiliki aktivitas antioksidan dengan kategori kuat sedangkan ekstrak *n*-heksan dan etilasetat tidak memiliki aktivitas antioksidan dengan kategori sangat lemah.

**Kata kunci:** Aktivitas Antioksidan, DPPH, Daun Mobe

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## 1. Introduction

Nowadays, the free radicals are known as one of risk factor of degenerative disease increase in the society. Most events occur because most of diseases are induced by an excessive oxidation reaction in the body representing both free radical and non-radical substances that can destroy the structure and function of cells[1]. However, almost all organisms are protected from the harmful effect of radicals by defense mechanisms such as a preventive antioxidant system and a chain-breaking antioxidant system. However, when the amount of antioxidant in the body is less than the production of free radicals, it leads to an oxidative stress condition. Oxidative stress may risk for chronic disease such as atherosclerosis, ischemic heart disease, ageing, inflammation, diabetes, immunosuppression, neurodegenerative diseases, cancer and others. Hence, antioxidants with free radical scavenging activities may have great correlation in the prevention and therapeutics of free radical mediated disease[2].

*Artocarpus lacucha* Buch.-Ham belongs to the family of Moraceae, popularly regarded as a medicinal plant, commonly called as monkey jack and in Indonesia, it is called mobe. This plant is widely distributed in the tropical regions of south and south-east Asia, mainly Nepal, Srilanka, India, Myanmar, Indonesia, Vietnam and Thailand[3]. It has many pharmacological activities such as anti-inflammatory, antiviral, anticancer, antibacterial and anti-HIV[4]. In Thailand, the dried aqueous extract of its heartwood has been used as a traditional anthelmintic agent[3]. The fruits are generally sweet-sour pulp eaten fresh but mostly made into curries and also used as a liver tonic. Especially in Indonesia, the fruits are made into arch fish curries by Samosir people. The leaves are used in treating dropsy[5].

The previous study showed that hydromethanol extract of lakoocha leaves has an antioxidant activity with the  $IC_{50}$  value of 4.74 ppm[2]. However, the antioxidant activity of *n*-hexane extract, ethyl acetate extract and ethanol extract of lakoocha leaves have not been reported yet. Therefore, the aim of this study was to investigate phytochemical constituents and antioxidant activity of all extracts.

## 2. Methods

### 2.1 Plant material

Leaves of *Artocarpus lacucha* Buch.-Ham were collected from Hutatinggi village, Laguboti sub-district, Toba Samosir district, North Sumatera. Then it was determined in *Herbarium Bogoriense*, Research Centre for Biology, Indonesian Institute of Sciences (LIPI), Bogor. The leaves were thoroughly washed with tap water, continued by wet sortation, then dried until fragile in a drying cabinet and blended into powder.

## 2.2 Extraction

Maceration was carried out in this study using increasing gradient polarity solvents. An amount of 70 g of powdered leaves were macerated using 500 ml of *n*-hexane in a container, left for 5 days, stirred occasionally and stored in dark place. The mixture was filtered. The residue was added by *n*-hexane until the volume of 700 ml was obtained. All macerates were collected and concentrated using rotary evaporator[6]. The remaining residue was then dried overnight and macerated with ethyl acetate and ethanol 96% successively with the steps were similar to process like *n*-hexane extract. So, there were *n*-hexane extract, ethyl acetate extract and ethanol extract.

## 2.3 Phytochemical Screening

Each extract was screened on TLC plate and using appropriate mobile phases. Phytochemical screening aimed to identify the secondary metabolite content of each extract[7][8].

## 2.4 DPPH radical scavenging activity

Antioxidant activity was carried out based on modification of DPPH method[9][10][11]. The *n*-hexane and ethyl acetate extracts were prepared at a concentration of 50, 100, 200 and 400 ppm, and ethanol extract were prepared at a concentration of 25, 50, 100 and 200 ppm. Quercetin as a positive control was prepared made at a concentration of 0.625, 1.25, 2.5 and 5 ppm. Briefly, each concentration was mixed with 1 ml of 0.5 mM DPPH (1,1-diphenyl-2-picrylhydrazyl), then added with methanol until 5 ml of total volume. The mixture was shaken and kept for 60 minutes at room temperature in the dark. The absorbance was measured at a wavelength of 516 nm using ultraviolet-visible spectrophotometer. The radical scavenging activity (RSA) of the extracts and quercetin was calculated using the formula:

$$\text{RSA (\%)} = \frac{A - B}{A} \times 100$$

Where A is absorbance of DPPH and B is absorbance of DPPH and extract or quercetin combination. From the absorbance obtained, % RSA and regression linear equation were made and applied to calculate the IC<sub>50</sub> values (the concentration of extract required to scavenge 50% of DPPH free radicals)

## 3. Results and Discussion

### 3.1 Extraction

An amount of 70 g of lakoocha leaves simplicia were macerated using *n*-hexane, ethyl acetate and 96 ethanol solvents and were obtained concentrated extracts of 1.273 g, 2.72 g and 6.033 g, respectively with a yield of 1.82%, 3.87% and 8.62%, respectively.

### 3.2 Phytochemical screening

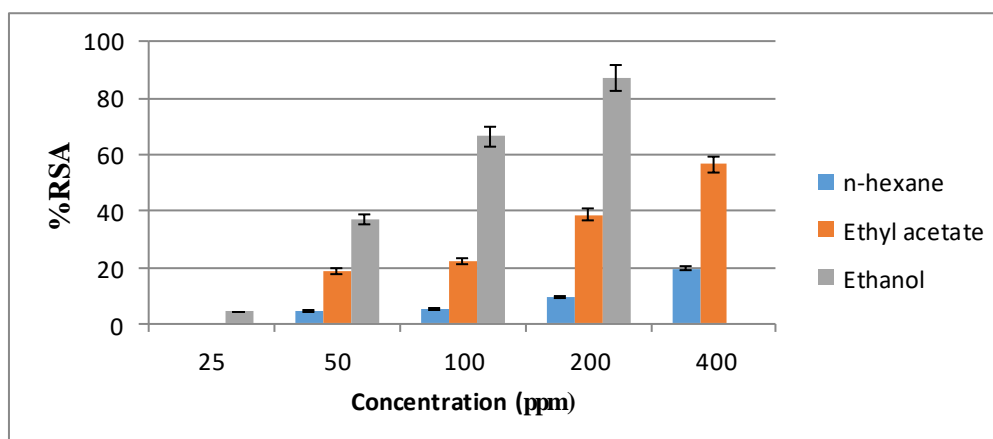
According to Rao (2012), among the secondary metabolites such alkaloids, tannins, glycosides, flavonoids, saponins and steroids/triterpenoids, only flavanoids has potent antioxidant activities. The antioxidant properties of flavonoids are the first mechanism of action studied with regard to their protective effect against cardiovascular disease[12]. The Phytochemical screening result are shown in Table 1. The result showed ethyl acetate and ethanol extract of lakoocha leaves contain flavonoid compounds.

**Table 1.** Phytochemical Screening of Each Extracts

Secondary Metabolites	Extract		
	<i>n</i> -hexane	Ethyl acetate	Ethanol
Alkaloid	-	-	-
Steroid/triterpenoid	+	+	-
Tannin	-	+	+
Glycoside	-	+	+
Flavonoid	-	+	+
Saponin	-	+	+

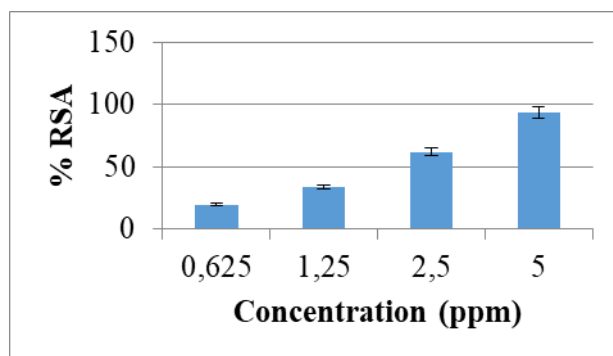
### 3.3 DPPH radical scavenging activity

Antioxidant activity of the extracts and quercetin were performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical method. The result of antioxidant activity in extracts and quercetin were shown in Figure 1 and Figure 2.



\*Data are mean±SD, n= 3

**Figure 1.** Percentage of Scavenging of Extracts



\*Data are mean±SD, n= 3

**Figure 2.** Percentage of Scavenging of Quercetin

The Figure 1 and Figure 2 show the scavenging activity which increased as the concentration sample increases. The reason increase in percentage of scavenging indicate that the greater of antioxidant activity which related the ability of compound in the test sample to act as a free radical scavenger or hydrogen donor. This capability were used to evaluate antioxidant activity. Interaction of antioxidant compound with DPPH was based on transfer hydrogen atom to DPPH radical (DPPH●) and convert it to DPPH non-radical (DPPH-H). The result of interaction which leads to discoloration from purple color to yellow pale color as an indicator of the scavenging activity occurred[13].

The IC<sub>50</sub> value for lakoocha extracts and quercetin were determined using linear regression equation by plotting the serials concentrations of lakoocha leaves extract (25, 50, 100, 200 and 400 ppm) and quercetin (0.625, 1.25, 2.5 and 5 ppm) as X-axis and the percent scavenging of lakoocha leaves extract and quercetin as Y-axis[10]. The IC<sub>50</sub> value was shown in Table 2

**Table 2** Linear Regression and IC<sub>50</sub> Mean±SD of Extracts and Quercetin

Sample	Regression Equation	Mean±SD (ppm)
<i>n</i> -hexane extract	$Y = 0.04633X + 0.8045$	1062.03±1.42
Ethyl acetate extract	$Y = 0.131595X + 7.46875$	323.18±0.02
Ethanol extract	$Y = 0.45698 + 4.6545$	99.23±0.07
Quercetin	$Y = 18.2416X + 7.603$	2.32±0.006

The IC<sub>50</sub> value for *n*-hexane extract was 1062.03 ppm, ethyl acetate extract was 323.18 ppm, ethanol extract was 99.23 ppm and for the comparator which quercetin was 2.32 ppm. Based on the result, ethanol extract showed the highest antioxidant activities achieved compared with other extracts required around 99.23 ppm to inhibit 50% of DPPH radical activity and it is still much weaker than quercetin. The extract should be in certain value to asses in active antioxidant if the condition extracts level in IC<sub>50</sub> value of extract < 200 ppm and inactive antioxidant when IC<sub>50</sub> value >200 ppm [14]. The compound categorized as an antioxidant activity with the presence of phenolic compound such as flavonoid. Flavonoid is a polar compound, thus it could dissolve in semi-polar and polar solvent[10].

In this study revealed that ethanol extract of lakoocha leaves showed the highest antioxidant activity. The greater antioxidant activity could be affected by the amount and position of hydroxyl and methyl groups on ring. Compound having many hydroxyl groups would be stronger to capture free radical, because the capable of donating hydrogen atom increases[15]. That statement explained why ethyl acetate had inactive antioxidant although it contained flavonoid in phytochemical screening results. Flavonoid contained in ethyl acetate might be having many methyl groups than hydroxyl groups, so it made flavonoid tend to semi-polar.

#### 4. Conclusions

Ethanol extract of lakoocha leaves showed the highest antioxidant activity among all extract tested but still lower than quercetin. However, the ability of the lakoocha leaves to protect body from oxidative damage remains to be investigated.

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