



## POTENCY OF RADICAL SCAVENGING ACTIVITY FROM ETHYL ACETATE FRACTION OF *Phaseolus vulgaris* L., *N. lappaceum* L., and *Pleurotus ostreatus*

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**Abstract:** Several studies show that some plants and parts of them could protect the human body from oxidation of lipids and free radical damage exposure. Various possibilities can occur as a result of free radical damage exposure, including malfunctioning of cells, damage to cell structures, until the occurrence of cancer. One of the compounds found effective as an antioxidant is flavonoid. This study has been done to examine the ethyl acetate fraction from *Phaseolus vulgaris* L., *N. lappaceum* L., and *Pleurotus ostreatus* which has potency as a radical scavenger. Antiradical activity assay was determined by the DPPH method (1,1-diphenyl-2-picrylhydrazyl). Based on the study, the radical scavenging activity respectively from the lowest to the highest activity are vitamin C (5.4 µg/ml); fraction from *Nephelium lappaceum* L. (21.81 µg/ml); fraction from *Phaseolus vulgaris* L. (50.64 µg/ml) and *Pleurotus ostreatus* (80.51 µg/ml).

Keywords: *Phaseolus vulgaris* L.; *N. lappaceum* L.; *Pleurotus ostreatus*; Antiradical; DPPH

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### 1. Introduction

Free radical is an atomic group which has one or more uncoupled electrons. It's very reactive and can interact with cells or some part of the body (Fessenden and Fessenden, 1986). This condition can cause damage to cells and human tissue. The disease caused by free radicals includes hypertension, Alzheimer's, Parkinson's, cancer, and inflammation (Toda *et al.*, 1991). The main focus of the research is antioxidant activity which is needed in order to inhibit free radical activity, especially from the ethyl acetate fraction of *Phaseolus vulgaris* L., *N. lappaceum* L., and *Pleurotus ostreatus*. Some antioxidant compounds are contained in plants such as ascorbic acid, flavonoids, and phenolic compounds (Teow *et al.*, 2006) and other compounds such as vitamins C and E (Windono *et al.*

al., 2001) that showed some advantage in human body protection as free radical scavenger. Jamur tiram (*Pleurotus ostreatus*) has many compound including protein, fat, phosphor, zinc, thiamin, and ribovlavin (Djamil and Tria 2009). Ethanolic extract from *Pleurotus ostreatus* have higher antioxidant activity than vitamin C with value of inhibition concentration ( $IC_{50}$ ) is 2,75 mg/ml and 6,65 mg/ml (Arbayah and Umi, 2013). *Phaseolus vulgaris* L. has compound including flavonoid, saponin, tannin, steroid, and cumarin. *Nephelium lappaceum* L. contained steroid, phenolic compound, and flavonoid. Ethanolic extract from *Nephelium lappaceum* L has higher antioxidant activity than vitamin C with value  $IC_{50}$  is 0,412  $\mu$ g/ml and 1,77  $\mu$ g/ml. The focus of this study was to observe the antioxidant activity from combination of *Nephelium lappaceum* L, *Phaseolus vulgaris* L and *Pleurotus ostreatus*.

## 2. Material and Method

### 2.1. Sample Preparation

*Nephelium lappaceum* L, *Phaseolus vulgaris* L., and *Pleurotus ostreatus* was obtained from Karanganyar, Central Java, Indonesia. It was extracted with ethanol, evaporated, and partitioned with ethyl acetate by separating funnel. The fractions was evaporated by using rotary evaporator and identified with TLC.

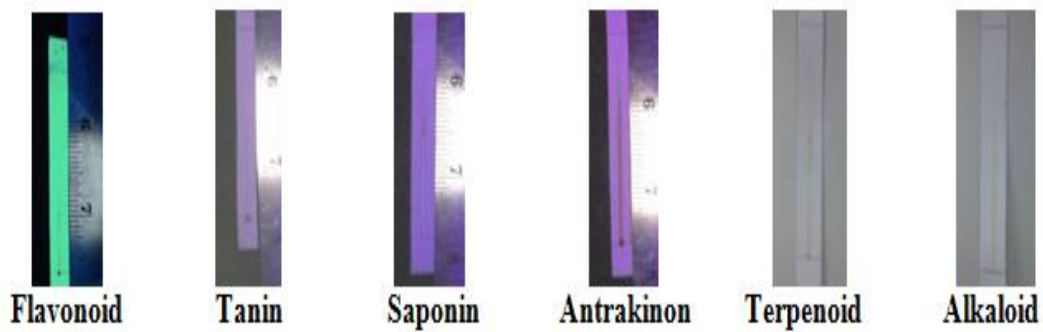
### 2.2. Antioxidant Activity Assay (DPPH method)

Sample from stock solution of ethyl acetate fraction from *Nephelium lappaceum* L, *Phaseolus vulgaris* L., and *Pleurotus ostreatus* and vitamin C were prepared in five different concentration. Sample added by 0,6 mL, DPPH 0,4 mM and ethanol added up to 5.0 mL. This mixture was homogenized by mixing for 30 second and incubated for 30 minutes. The sample absorbance was measured by UV-Vis spectofotometer (Shimadzu) with  $\lambda_{max}$  516 nm. The sample absorbance also compared with the control solution containing 0,6 mL DPPH 0,4 mM diluted in ethanol. The percentage (%) of antiradical activity were measured. The linier regretion between concentration curves versus antiradical activity percentage was obtained. Then, the linier regretion formula and sample concentration at 50% activity were determined.

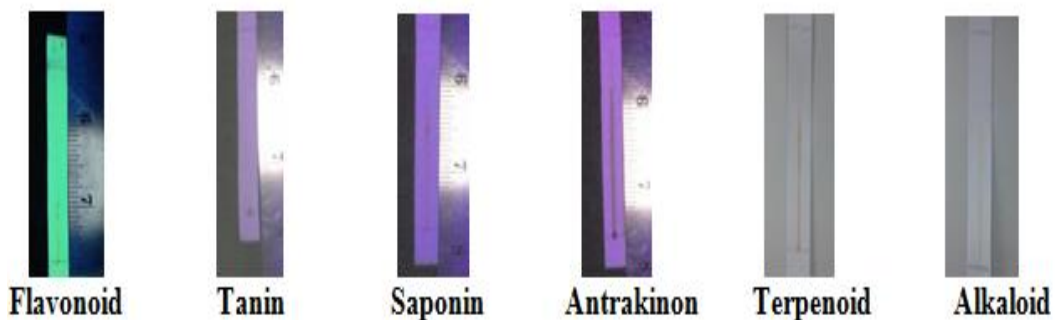
## 3. Result

### 3.1. Phytochemistry Analysis

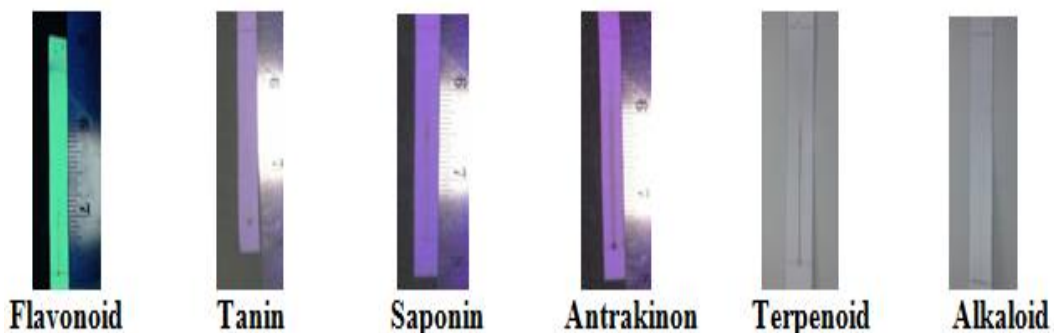
Qualitative method of ethyl acetate fraction from *Nephelium lappaceum* L., *Phaseolus vulgaris* L., and *Pleurotus ostreatus* is identification by Thin Layer Chromatography (TLC) under UV 254 and UV 366. Each sample from TLC process in mobile phase chloroform : etyl acetate : n-haxane (7:2:1).



**Figure 1.** TLC Profile of ethyl acetate from *Phaseolus vulgaris* L. on spot detection under (a) UV 254 nm and (b) UV 366 nm. TLC method was performed using a stationary phase of silica gel 60 F254 and a mobile phase of chloroform: etyl acetate : n-haxane (7:2:1 v/v)



**Figure 2.** TLC Profile of ethyl acetate from *Nephelium lappaceum* L. on spot detection under (a) UV 254 nm and (b) UV 366 nm. TLC method was performed using a stationary phase of silica gel 60 F254 and a mobile phase of chloroform : etyl acetate : n-haxane (7:2:1 v/v)



**Figure 3.** TLC Profile of ethyl acetate from *Pleurotus ostreatus* on spot detection under (a) UV 254 nm and (b) UV 366 nm. TLC method was performed using a stationary phase of silica gel 60 F254 and a mobile phase of chloroform : etyl acetate : n-haxane (7:2:1 v/v)

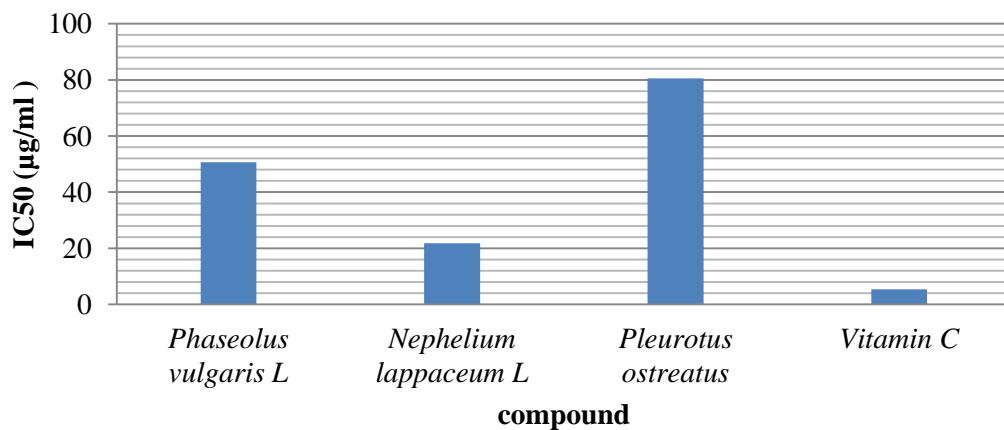
### 3.2. Radical Scavenging Activity

Antiradical activity was determined by the ability of a compound contained in fraction to reduce the purple colour intensity of DPPH radical in maximum wavelenght (Rohman and Riyanto, 2006). The reduction of purple colour intensity of DPPH radical is caused by the decrease of

chromophore and conjugated with double bond in DPPH compound. This caused by the existence of fraction compound which scavenging the radical by donating hydrogen atom to DPPH structure so that become reduced DPPH-H. It have yellow colour (Huang *et al.*, 2005). Based on Reynertson *et al.* (2007) active antioxidant can be determined from plant if its  $IC_{50}$  value is lower than 50  $\mu\text{g}/\text{ml}$ . The medium antioxidant activity is determined if the  $IC_{50}$  value is between 50-100  $\mu\text{g}/\text{ml}$  and less active antioxidant activity is determined if the  $IC_{50}$  value is between 100-200  $\mu\text{g}/\text{ml}$ , meanwhile  $IC_{50}$  value more than 200  $\mu\text{g}/\text{ml}$  showed that the compound is not active as antioxidant.

**Table 1.** The Comparison of The Antiradical Activity of Fraction

Fraction	Concentration ( $\mu\text{g}/\text{ml}$ )	Antiradical activity (%)
<i>Phaseolus vulgaris</i> L	100	50,64 $\pm$ 0,571
<i>Nephelium lappaceum</i> L	100	21,81 $\pm$ 0,387
<i>Pleurotus ostreatus</i>	100	80,51 $\pm$ 0,458



**Figure 4.** The  $IC_{50}$  values of ethanolic fraction of *Phaseolus vulgaris* L., *Nephelium lappaceum* L., *Pleurotus ostreatus* and vitamin C. The sample absorbance were measured by UV-Vis spectrofotometer with  $\lambda_{\text{max}}$  516 nm

The parameter used in the interpretation of antiradical activity is the value of inhibitory concentration 50% ( $IC_{50}$ ). It is the sample concentration having 50% antiradical activity compared to control which obtained by linier regrestion between the concentration versus the percentage of radical activity (Rohman and Riyanto, 2006). The result on radical scavenging activity of ethyl acetate fraction of *Phaseolus vulgaris* L., *Nephelium lappaceum* L., *Pleurotus ostreatus* are compared to on radical scavenging activity of vitamin c. The radical scavenging activity respectively from the lowest to the highest activity are vitamin C (5,4  $\mu\text{g}/\text{ml}$ ); fraction from *Nephelium lappaceum* L (21,81 $\mu\text{g}/\text{ml}$ ); fraction from *Phaseolus vulgaris* L (50,64 $\mu\text{g}/\text{ml}$ ) and *Pleurotus ostreatus* (80,51 $\mu\text{g}/\text{ml}$ ). *Nephelium lappaceum* Lindicate as the most potential concentration which has potential antioxidant effect.

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