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RESEARCH ARTICLE

# Antioxidant Activity Assay of Combination of Purified Extract of Banana Peel (*Musa Paradisiaca Sapientum*) and *Andrographis Paniculata* Leaves

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## Abstract

Antioxidant is a compound that able to postpone, slowdown and prevent free radical reaction which able to postpone degenerative disease. Banana peel (*Musa Paradisiaca Sapientum*) and *Andrographis paniculata* contain flavonoid compound which have antioxidant activity. The aim of this study is to measure antioxidant activity of the combination of banana peel extract and *Andrographis paniculata* to DPPH free radical (2,2-difenil-1-pikrilhidrazil).Extraction process of banana peel using methanol, meanwhile *Andrographis paniculata* using ethanol 70%. Extract purification conducted using n-hexan and ethyl acetate. This study using combination of the purified extract of banana peel and *Andrographis paniculata* with the following comparison consecutively (1:10) (1:1) (2:1) (1:2) (0:1) concentration of 20,40, 60, 80 and 100 ppm, measured their antioxidant activity with DPPH method using visible spectrophotometry, thus resulted in $IC_{50}$value. $IC_{50}$value for the combination of purified extract of banana peel and *Andrographis paniculata* (0:1) as many as 256.76 ppm, the combination of purified extract of banana peel and *Andrographis paniculata* (1:1) as many as 140.33 ppm, the combination of purified extract of banana peel and *Andrographis paniculata* leaves (1:2) as many as 110.05 ppm, the combination of purified extract of banana peel and *Andrographis paniculata* (2:1) as many as ppm, the combination of purified extract of banana peel and *Andrographis paniculata* (1:0) as many as 184.13 ppm. The result can be concluded that combination of purified extract of banana peel and *Andrographis paniculata* have an antioxidant activity even though in moderate category included purified extract of combination of banana peel and *Andrographis paniculata* with comparison (0:1) (1:2), (2:1), (1:1) and (1:0) have medium antioxidant activity.

**Keywords**: *Purified extract of banana peel, purified extract of Andrographis paniculata, DPPH.*

## Introduction

Free radicals are chemical compound that do not have electron pairs so they can break down molecules and become very reactive. Reactive free radicals form in cells that can oxidize bio-molecules and cell death and tissue injury [1].Antioxidants are compounds that are able to delay, slow down and prevent lipid oxidation processes or substances that can delay or prevent free radical reactions in lipid oxidation [2].Natural antioxidants such as ascorbic acid, tocopherol, phenol compounds and flavonoids become safe alternative of antioxidants [3].The country of Indonesia has its own wealth in traditional medicine from 30,000 species of plants that exist, 7000 of which are plants that can be used as medicines that are spread throughout the region [4]. Banana plants (*Musa*, sp.), are plants that are widely found in Indonesia. In Southeast Sulawesi, especially the people of Bombana Regency they conduct banana cultivation from various types of varieties including plantain, Ambon banana, Kepok banana and others. So far, the use of bananas is still limited to the fruit, while the skin is discarded as organic waste

or animal feed (20-30%), as well as manure and compost (60-70%) [5]. Banana peels (*Musa paradisiaca Sapientum*) have antioxidant activity [6]. Researchers have found many benefits of banana peels such as antidiabetic, anti-ulcer, inhibit the development of cancer, and have anti-inflammatory and antioxidant effects [7, 8, 9, 10].

*Andrographis paniculata* leaves is a plant that has many properties including anti-inflammatory, anti-carcinogenic, anti-bacterial, anti-fertility, anti-malaria, anti-HIV, anti-diarrhea, anti-diabetes, and has hepatoprotective and cardiovascular activities [11]. *Andrographis paniculata* also has antioxidant activity [12].

The combination of banana peel and *Andrographis paniculata* leaf extract can provide a synergistic effect. Purification extract aims to eliminate certain chemical compounds and eliminate components that are considered as a nuisance such as fat, chlorophyll and other impurities with the aim of getting pure natural component free from other chemical components that are not needed so that it will be more efficiently given when assaying antioxidants. This study aims to measure the antioxidant activity of a combination of purified extracts of Banana peel and *Andrographis paniculata*.

## Materials and Method

### Time and Location of the Research

This study was conducted at Pharmacognosy - Phytochemistry Laboratory of Pharmacy Study Program of STIKES Mandala Waluya Kendari in June – August 2019.

### Tools and Materials

The tools used are a stirring rod, glass beaker, separating funnel, Erlenmeyer, measuring cup, filter paper, spatula, dropper, aluminum foil, oven, analytical balance, 20D spectrometer, rotary evaporator R-144 Buchi with the Buchi B-169 vacuum system , burner funnel.

The ingredients applied are banana peel, methanol, aquades, ethanol, DPPH, ethyl acetate, and n-hexane, and vitamin C.

Samples of ripe plantains were collected from banana farmers in Poleang, Bombana Regency, Southeast Sulawesi. Banana peels are taken as much as 300 grams and cleaned from impurities. *Andrographis paniculata* are obtained from Yogyakarta. *Andrographis paniculata* are collected 300 grams and cleaned from impurities.

### Research Procedure

### Extraction of banana Peel and *Andrographis paniculata*

Each of 300 grams of banana peels and *Andrographis paniculata* are blended with a blender to form powder. Each of the banana peel powder was put in a container and macerated with methanol (3x200 mL) solvent for banana peel and 70% ethanol solvent for the bitter leaf and soaked for 4 days, stirring and remaking occasionally. It is extracted into a new container until a liquid extract is obtained and the extract results are evaporated using a rotary evaporator under boiling point until a thick extract is obtained [13].

### Purification of the Extract of Banana Peel and *Andrographis paniculata*

The thick extracts of the *Andrographis paniculata* and banana peel were purified by n-hexane and ethyl acetate solvent respectively, and put in a separate funnel flask, shaken, and allowed to stand, and filtered. The ethyl acetate layer is filtered and concentrated using a rotary evaporator to obtain a pure extract.

### Preparation of DPPH Radical Reagent

Four mg of DPPH radical was dissolved in ethanol until all were dissolved, then put into a 100 ml measuring flask and diluted to the mark, so that the DPPH solution was obtained with a concentration of 40 ppm.

### Preparation of Main Solution the Purified Extract of Banana Peel and Andrographis Paniculata

Purified extract solution of banana peel and *Andrographis paniculata* is made by weighing 10 mg for each sample, then dissolved in ethanol until all is dissolved, then put into a 100 ml measuring flask and diluted to the mark to get a 100 ppm main solution.

### Preparation of Purified Combination Extract of Banana Peel and *Andrographis paniculata*

Purified extract of banana peel and *Andrographis paniculata* were dissolved in 5

combination, they are combination of purified extract of banana peel and *Andrographis paniculata*, consecutively (1:0), (1:1), (2:1), (1:2), (0:1) were taken from stock solution with the total volume in each combination is 30 ml.

### Preparation of Comparative Solution

The comparative solution was made by weighing 10 mg of vitamin C dissolved in ethanol, then placed in a 100 ml volumetric volume of sufficient volume until an impression was obtained until the main solution was 100 ppm. Comparison solutions were made in 5 concentrations of 2, 3, 4, 5, and 6 ppm.

### Preparation of Blank Solution

A blank solution is made by taking as much as 2 ml of the DPPH stock solution, then put into a test tube, and adding 2 ml of ethanol p.a, stirring until homogeneous. The absorption is measured for use in the calculation of% inhibition.

### Measurement of Antioxidant Activity of Blank Solution

Measurement is conducted by piping 4 ml DPPH. Divortex and incubated at $37^0$C in a dark room. The absorbance is measured at a wavelength of 517 nm [14].

### Antioxidant Activity Assay

Weighed a number of pure extracts of banana peel and *Andrographis paniculata* with ratios (1:10) (1: 1) (2: 1) (1: 2) (0: 1). The extract combination is dissolved with ethanol concentration series made of 20, 40, 60, 80 and 100 ppm. Measured with 4 ml of DPPH 0.0001 M solution and 50 µL of the test solution, then vortex for 1 minute and allowed to stand for 10 minutes. The absorbance is read at a wavelength of 517 nm. The absorbance readings of the DPPH 0.0001 M. control solution were also calculated. The percentage of DPPH radical attenuation was calculated and plotted on the linear regression curve. Then the $IC_{50}$ value is calculated.

### Data analysis

Data from the antioxidant activity test using the DPPH method is the absorbance value read on the UV-Vis spectrophotometer. The absorbance value of the sample or standard comparison of vitamin C is compared with the absorbance value of the control (absorbance of DPPH solution). The percentage of antioxidant activity was calculated using the formula:

$$\%IC_{50} = \frac{Control\ absorbance - treatment\ absorbance}{Control\ absorbance} \times 100\%$$

Explanation

Abs control : Absorbance of DPPH 0,1 mM solution

Abs treatment : Absorbance of concentration series of a combination of purified extract of banana peel and *Andrographis paniculata* or vitamin C standard comparison

The antioxidant potency of a combination of purified extract of banana peel and *Andrographis paniculata* or a standard comparison of vitamin C expressed as $IC_{50}$ values was obtained using probity analysis at a 95% confidence level. The logarithm of the concentration of the test solution (a combination of purified extract of banana peel and *Andrographis paniculata*) was positioned as an independent variable (X) and the percentage of antioxidants as a dependent variable (Y).

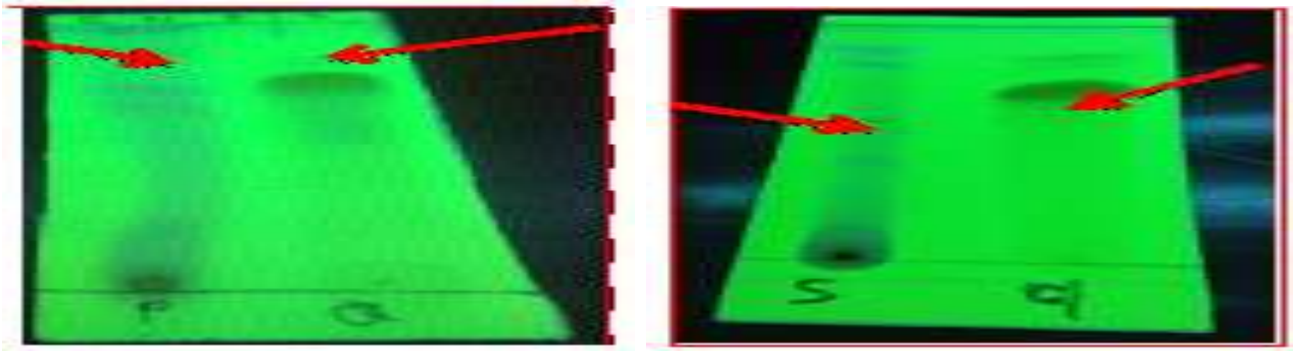
## Results

**Table 1: The results of the phytochemical analysis extract banana Peel and *Andrographis paniculata***

| No | Phytochemical constituents | Extract of banana peel | Extract of Andrographis Paniculata |
|---|---|---|---|
| 1 | Saponin | + | - |
| 2 | Polyphenol | + | - |
| 3 | Flavonoids | + | + |
| 4 | Terpenoids | + | - |
| 5 | Tanins | + | - |

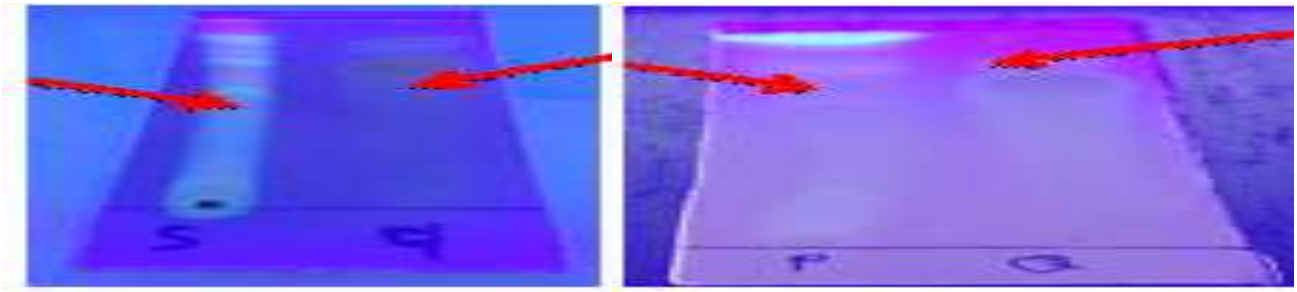
(Present = +, Absent = -)




**(a)** **(b)**

**Figure 1: (a) TLC Profile of purified extract of *Andrographis Paniculata*; elution with chlorophorm : methanol (9:1); (b) Profile KLT of purified extract of *Andrographis Paniculata*; elution with Ethyl acetate : *n*-hexane (7:3); Silent phase used TLC Plate; Observation with $UV_{254}$ nm**




**(a)** **(b)**

**Figure 2: (a) TLC Profile of purified extract of *Andrographis Paniculata*; elution with chlorophorm : methanol (9:1); (b) Profile KLT of purified extract of *Andrographis Paniculata*; elution with Ethyl acetate : *n*-hexane (7:3); Silent phase used TLC Plate; Observation with $UV_{366}$ nm**

**Table 2: Absorbance Score of purified extract of Banana peel and *Andrographis paniculata***

| Concentration (ppm) | Absorbance | | | | | | |
|---|---|---|---|---|---|---|---|
| | Banana peel and *Andrographis paniculata* (0:1) | Banana peel and *Andrographis paniculata* (1:1) | Banana peel and *Andrographis paniculata* (1:2) | Banana peel and *Andrographis paniculata* (2:1) | Banana peel and *Andrographis paniculata* (1:0) | Comparative (Vitamin C) | Blank o |
| 20 | 0.389 | 0,405 | 0,406 | 0,399 | 0.406 | 0.376 | 0,769 |
| 40 | 0.381 | 0,401 | 0,402 | 0,395 | 0.385 | 0.373 | 0,769 |
| 60 | 0.368 | 0,399 | 0,398 | 0,39 | 0.377 | 0.370 | 0,769 |
| 80 | 0.358 | 0,395 | 0,397 | 0,389 | 0.338 | 0.368 | 0,769 |
| 100 | 0.321 | 0,391 | 0,394 | 0,387 | 0.325 | 0.365 | 0,769 |

**Table 3: The results of the regression equation and $IC_{50}$ values from single antioxidant extract measurement and a combination of Banana peel and *Andrographis paniculata***

| Sample | Regression equation | $IC_{50}$ (ppm) |
|---|---|---|
| Comparative (Vitamin C) | y = 0.6522x + 7.9227 | 64.53 |
| banana peel and *Andrographis paniculata* (0:1) | y = 0.192x + 0.7005 | 256.76 |
| banana peel and *Andrographis paniculata* (1:1) | y = 0.0221x + 46.892 | 140.63 |
| banana peel and *Andrographis paniculata* (1:2) | y = 0.0189x + 46.931 | 110.053 |
| banana peel and *Andrographis paniculata* (2:1) | y = 0.0189x + 46.93 | 162.38 |
| banana peel and *Andrographis paniculata* (1:0) | y = 0.2524x - 3.599 | 184.13 |

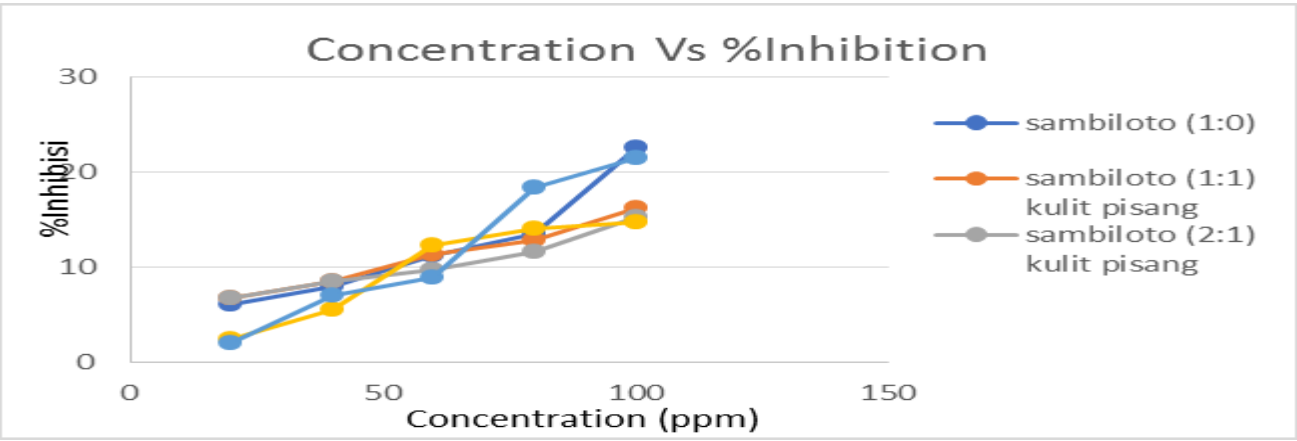



**Figure 3: Sample Absorbance Graphic**

## Discussion

The extraction process with organic solvents produces crude extracts (crude extract) so that in some traces of the active compound it is necessary to purify it to remove components that are considered to be a nuisance such as fat, chlorophyll, etc. Purification process is a method to free pure natural material components from other chemical components that are not needed.

For the purity of a particular compound structure, the purity of the material must be 95-100%. While purified extracts must be explained that purified extracts from chemical components in extracts that are not needed such as lipids, pigments (chlorophyll), tannins, plasticizers, and lubricants that can come from tools [15]. Purified extract is applied as an alternative to minimize the mass of an extract in the practical purpose of making pharmaceutical preparations because some of the components contained can be reduced by the process.

Purification also aims to maintain several chemical extracts that have a synergistic effect to maximize the treatment process because in some cases, chemical components that have been isolated reveal decreasing effect. Antioxidant activity assay in the study was carried out using the DPPH method. DPPH is a synthetic purple free radical that is widely used in antioxidant activity assay.

Determination of the maximum wavelength (λ) is conducted to measure the absorbance of DPPH compounds in the visible region thus maximum absorption is obtained. The blank solution used is ethanol which aims to be a correction factor. DPPH color intensity can be measured at λ 515-520 nm [16].

The maximum wavelength (λ) obtained in this study is 517 nm. DPPH method is a quantitative test to find out how many the combined activities of purified extract of Banana peel and *Andrographis paniculata* extract as antioxidants. The DPPH antioxidant activity assay is based on the DPPH radical capture reaction by antioxidant compounds through the mechanism of hydrogen atom donation so that non-radical DPPH will be generated and causes a decrease in the intensity of the purple color of DPPH [18].The intensity of purple reduction from DPPH can be seen from the absorbance value using a spectrophotometer. To find out the degree of color reduction as a result of the presence of antioxidant compounds that can reduce the intensity of the purple color of DPPH, the color reaction measurements were carried out at different extract concentrations. The higher the concentration of the extract the greater the damping which is indicated by the formation of a yellow color.

The decrease in color intensity is proportional to the amount of DPPH compounds that can be suppressed by antioxidant compounds; this will make the absorbance value smaller. The smaller the absorbance value indicates the greater antioxidant capacity [16]. Vitamin C is used as a positive control in this study because it is one source of antioxidants that is easily obtained, widely consumed by the community, high antioxidant activity and very strong (Sandhiutami and Dwi, 2010).

$IC_{50}$ values are obtained from the linear regression equation obtained from the log concentration of the test solution (x) with the probit value (y). $IC_{50}$ value is inversely proportional to the antioxidant ability of a compound contained in the test material. The smaller the $IC_{50}$ value the greater the ability of its antioxidant activity. The value of % inhibition can be seen in Figure 1. The absorbance of control obtained was 0,769 and calculated with the absorbance of the sample.

The results of the calculation of the percentage of antioxidant activity from purified extracts of banana peel and *Andrographis paniculata* can be seen in table 1. It means at that concentration the test solution can reduce DPPH by 50%. According to Blois (1958) the antioxidant activity of all series of combination combinations is very strong because it has an $IC_{50}$ of less than 50 µg / mL.

In table 2, the smallest $IC_{50}$ value is shown in vitamin C which is 64.53ppm. While the $IC_{50}$ value for the combination of purified extracts of Banana peel and *Andrographis paniculata* (0:1) was 256.76 ppm, the combination of purified extracts of Banana peel and *Andrographis paniculata* (1:1) amounted to 140,33 ppm, the combination of purified extracts of banana peel and *Andrographis paniculata* (1:2) at 162,38 ppm, the

combination of purified extract of banana peel and *Andrographis paniculata* (2:1) at 110.05 ppm, the combination of purified extract of banana peel and *Andrographis paniculata* (1:0) at 184.13ppm. The lowest $IC_{50}$ value was in the combination of purified extract of banana peel and *Andrographis paniculata* (1:0), (1:2), (2:1), and (1:1) included in the category of moderate antioxidants ($IC_{50}$=100-200 ppm), caused by because they contain flavonoid compounds.

## Conclusion

Combination of purified extract of included in the category of moderate antioxidants has an antioxidant activity.

## Acknowladment

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