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**EVALUATION OF ANTIOXIDANT ACTIVITY OF PHENOLIC AND FLAVONOID
EXTRACTED FROM LOCAL BANANA PEEL**

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ABSTRACT

The banana peels are widely known as agriculture residue which is underutilized in various countries. The potential of banana peels has been analyzed in various studies, such as attempts in the production of biomass, protein, ethanol, methane, pectin, and enzymes. Besides, it readily available sources, inexpensive, and composed of bioactive molecules. The contribution of banana peels in variety application was prompted to study the potential of local banana peel and functional as a natural antioxidant promising in health benefits. Based on their highly potential, this studies were evaluated the antioxidant capabilities within its phenolic and flavonoid contents. The phenolic and flavonoids compound are the plant constituents that are possesses in antioxidant activity and to prevent the decomposition of hydroperoxides into free radicals. In evaluation of antioxidant activities the phenolic and flavonoid contents were extracted from the local Dream banana peel. Total phenolic is assessed via Folin-ciocalteau method and calculated as gallic acid equivalent (GAE). Total flavonoid content was conducted by Aluminium Chloride Colometric assay and expressed as mg of catechin equivalent (CE). The correlations coefficients (R^2) of DPPH radical scavenging activity with total phenolic and flavonoid contents were 0.903 and 0.969 respectively. Thus, the remarkable of total phenolic was exhibited the greatest antioxidant activity with their % scavenging activity of free radical. In general, the antioxidant activity of phenolics is due principally to their redox properties. Therefore, the local banana peel could be considered as optional sources of base ingredients in the production of variety nutritious products as well as act as natural antioxidant.

Keywords: *banana peel, residue, phenolic, flavonoid, antioxidant*

INTRODUCTION

The oxidation of food components is well known as lipid oxidation process which is the main undesirable reaction in food application. There are many causes of this reaction such as rancidity, polymerization, and off-flavor compounds. If it happens continuously, the value of food will affected on their shelf-life and lowering the nutritive contents. Nowadays, several studies were focused in overcoming the lipid deterioration occurred using the natural antioxidant base such as an agriculture waste. Within this research application, the using of synthetic antioxidant such as BHT, BHA and PG which highly potential causing in health hazards will be minimized. Thus, the safer and alternatives of other antioxidative compounds are desirable [1]. In this respect, various types of agriculture waste with antioxidant properties have been demonstrated. For example, agriculture waste from guava, papaya, and star fruit [2], citrus fruits peel [3], and banana peel [4] have been evaluated as inexpensive sources of antioxidants.

Banana is a tropical fruits that belongs to the family *Musaceae* where it is already known and have been proven for health beneficial within the present of phenolic compound such as phenolic acid and flavonoids acting as anticancer, antiviral and antioxidant activity [5]. Mostly bananas are used fresh or processed into many products such as chips, puree/pulp, powder, jams, juice, biscuits and others. Significant quantities of banana or plantain peels, equivalent to 40% of the total weight of fresh banana, are generated as a waste product in industries producing banana based products [6]. In many areas, banana peels used as ingredients in livestock feed besides thrown it away [7]. Thus, many studies have been done to use this residue due on its high fiber content that works in many applications, especially in the health care food industry [8].

Recent studies demonstrated that banana peel generally include higher phenolic compounds than that banana pulps [9]. Thus, the local banana peel having a potential to become one of the base ingredients in the content of nutritious foods, besides it can provide a better functionality for humans and environments. To realize the importance of banana peel, so this study was focus on local banana peel and evaluate their of antioxidant activity in the presence of phenolic and flavonoid compound.

MATERIALS AND METHODS

Plant material

The study was contribute of Dream banana peels in the yellow-green stage of ripening. The samples consist of representative banana trees from local orchard Kampung Lata Janggut, Jeli Kelantan in similar stage of biological development and ripeness. All samples data are state in the sampling protocol.

Preparation of powdered banana peel

The banana peel sample were soaked and washed with fruit detergent, then it has cut into small pieces and freezed using freeze dryer (Virtis, benchtop K) for 72 hr. The sample was grounded using commercial grinder into 300 μm size and stored at -18°C in airtight containers for a week.

Preparation of the banana peel extract

The extraction method as described by [10] with some modifications. Briefly, the powdered banana peel (2g) was extracted twice with continuous stirring at room temperature for 1 hr respectively using different solvents, first with 100 ml methanol (80:20 v/v) and next with 100 ml acetone (70:30 v/v) with intermittent centrifugation (4,000 x g, 15 min). The supernatants were transferred into volumetric flasks and 80% methanol was added to a total volume of 200 ml.

Chemicals and Instruments

Folin-Ciocalteu's phenol reagent, gallic acid, anhydrous sodium carbonate, 1,1 diphenyl-2-picrylhydrazyl (DPPH), catechin, aluminium chloride were purchased from Sigma-Aldrich USA. UV spectrophotometer (Genesys 20).

Determination of total phenolic contents

The total phenolic contents in banana peel extract were assessed via the Folin-Ciocalteu method. Gallic acid was used as a standard and total phenolics were expressed as mg/g gallic acid acid equivalent (GAE). Standard gallic acid were prepared in methanol at range of concentration is 0.02 to 0.1 mg/ml. The banana peel extracts were also prepared in methanol at different concentration and 0.5 ml of each were introduced into test tubes and mixed with 2.5 ml of a 10 fold dilute folin-ciocalteu reagent and followed with 2 ml of 7.5% sodium carbonate. The tubes were stand for 30 minutes in the dark room and the triplicate absorbance was read at 760 nm spectrometrically.

Determination of total flavonoid contents

Flavonoids content determined via the Aluminium chloride colorimetric assay according the methods of [1] with some modification. Briefly, 1ml of aliquot extracts or standard catechin solution was added in test tubes containing 4 ml of distilled water. Then, a 0.3 ml of 5% sodium nitrate was added into the solution. After 5 min, 0.3 ml of 10% aluminium chloride was added. After 6 min, 2 ml of 1M solution of NaOH was added and the volume was made up to 10 ml with distilled water. The mixture was then mixed thoroughly and the absorbance was measured against a prepared reagent blank at 510 nm. Total flavonoid contents were expressed as mg of catechin equivalent (CE)/g of dry weight.

Determination of antioxidant properties

The 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined as described by [11] with some modifications. In brief, the test solution prepared with added 1 ml 0.002% of DPPH stock solution to 2.5 ml sample in different concentration (0.1, 1 and 10 mg/ml). 1 ml of methanol was added to 2.5 mL of sample solution of different concentration. These are blank solutions. 1 mL DPPH solution plus 2.5 mL of methanol was used as a control. These solutions were allowed to react at dark room temperature for 30 minutes.

The absorbance values were measured at 518 nm and converted into the percentage antioxidant activity using the Equation (1):

$$\text{Scavenging capacity (\%)} = 100 - [(As - Ab) \times 100 / Ac] \quad (1)$$

Where; As - Absorbance of sample
Ab - Absorbance of blank
Ac - Absorbance of control

Statistical Analysis

All tests were conducted in triplicate. The results are expressed as means \pm SD. Analysis of variance and significant differences among the means tested by the one-way ANOVA, using Minitab (version 16.0.2, minitab inc.). Values of $P < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Total phenolic and flavonoids content

The ability of scavenging free radicals on antioxidant activity are depending to association of their bioactive compounds, mainly antioxidant phenolics. The total phenolic was determined using the Folin-Ciocalteu method. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent (GAE) using the standard curve equation ($y = 0.345x - 0.076$ and $R^2 = 0.995$), and total phenolic contents are varied in Table 1 (0.2486 ± 0.002 to 2.0556 ± 0.0059) in different concentration of extracts were expressed in mg GAE/g d.w. The total phenolic content of banana peel have been reported is 3.8 ± 0.24 [12]. The total phenolic content was quite high compared with this study because of different combination of time and temperature in extraction methods and using the dual beam UV-vis spectrophotometer. Apart from that, the different of extract concentration and different procedures adopted for total phenolic analysis. According to [1] the total phenolic content had higher in green stage of ripening than the ripe components. While in this case, the stage of banana ripening is in yellow-green stage, which contributed to the low total phenolic content. Otherwise, many findings was reported about the flavonoids compound has being a strong antioxidants which capable of effectively scavenging the reaction oxygen species because of their phenolic hydroxyl groups. In this study, the total flavonoid contents were expressed as mg/g catechin equivalent (CE) using correlation coefficient $R^2 = 0.969$. The flavonoid contents were determined in range 0.6 mg/ml to 0.14 mg/ml of extracts concentration, which showing the negatively values. Therefore, positive results can be obtained in the future by increasing the concentration of extract. Thus, the climacteric factors such as natural chemical composition, harvest maturity, soil types and post harvest storage are assisted in variation of phenolic and flavonoid contents among different plantain.

Antioxidant activity

Based on Figure 1 showing the antioxidant activity of the extracts was determined using DPPH scavenging assay that is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress. Table 1 showing the variation of total phenolic content and its scavenging activity respectively in different extract concentration. The inhibition of DPPH radical of local banana peel noted the lowest value 29.38 % in 1 mg/ml concentration and the highest value 83.75 % in 10 mg/ml of concentration. This is contrary with those previous reported by [10] which quenched 79 % in 0.4 mg/ml; [13] indicates the Mondhan Banana peel extract inhibited a higher percentage 98.19 at 10 mg/ml. The different percent of scavenging activity due to suitable reducing agents, during which the electrons become paired off and the solution mixture loses color stoichiometrically depending on the number of electrons taken up.

Table 1: The relationship between Total Phenolic Content and % Scavenging activity

Concentration of banana peel extract (mg/ml)	Total phenolic content, (mg GAE/g d.w)	Scavenging activity (%)
0.10	0.2486 ± 0.0020	74.38
1.00	0.7240 ± 0.0112	29.38
10.00	2.0556 ± 0.0059	83.75

Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

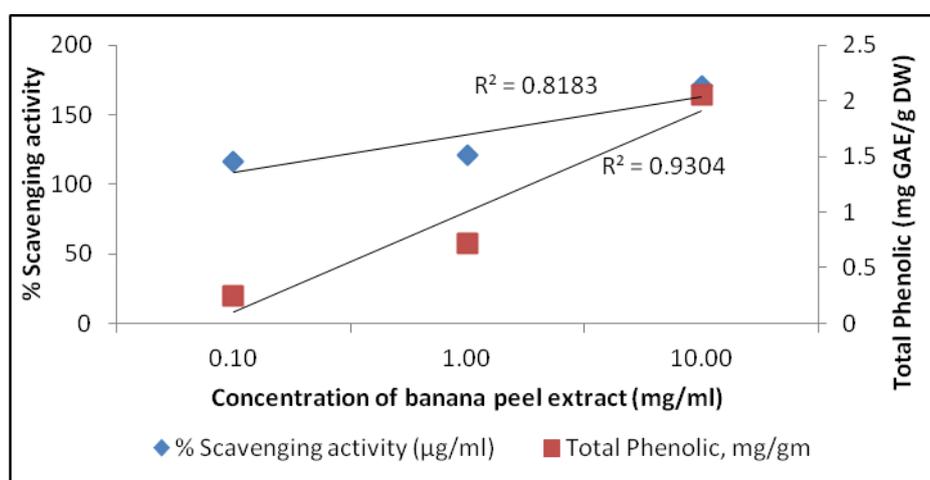


Fig.1: The relationship between total phenolic content and antioxidant activity

CONCLUSION

The total phenolic was determined using the Folin-Ciocalteu method. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalents (GAE) and total flavonoids were expressed as mg/g catechin equivalent (CE). Meanwhile, the antioxidant activity was determined using DPPH scavenging assay that used to evaluate the ability of antioxidants to scavenge free radicals. The result of the present study showed that the concentration of banana peel extract contain highest amount of phenolic compounds exhibited the greatest antioxidant activity. Hence, banana peel powder could be used as an easy accessible source of natural antioxidants.

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