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RESEARCH ARTICLE

Antioxidant Potential of Different Parts of Three Pineapple Varieties N36, Madu and MD2

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ABSTRACT

Pineapple (*Ananas comosus*) is widely consumed and appreciated not only due to its taste and aroma and to its nutritional and antioxidant properties, including its vitamin C and phenolic contents. In an attempt to explore new antioxidant leads, pineapple waste is often neglected in the pineapple industry. Fruit processing has considerably higher ratios of by-products and pineapple by-products are not exceptions as they consist basically of the residual pulp, peels, stem, and leaves. Pineapple waste is a by-product resulting from canning processing of pineapple that produces about 35% of fruit waste and leads to serious environmental pollution. The objective of this study is to determine whether different varieties and parts of pineapple waste (peel, core, crown, and stalk) can affect and give the highest amount of natural antioxidant activity. In this study, the antioxidant activities of different parts of three pineapples (N36, Madu, and MD2 were measured using the DPPH method. Methanol solvent has been used for extraction and various parts of pineapple were used to determine the effect of different plants on antioxidants. The samples were determined by using an ultraviolet (UV) spectrophotometer. The result for scavenging activity (DPPH) indicates Madu variety displayed high scavenging activity compared to MD2 and N36 varieties. Madu varieties demonstrated a significant free radical scavenging ability where their crown has IC_{50} and cores are merely IC_{50} at 175 ppm and 500ppm. The MD2 crown also demonstrates IC50 at 275 ppm. The results suggest that Madu varieties comprised of the crown of pineapple studied may be useful as potential sources of natural antioxidants.

Keywords: Pineapple; antioxidant; N36; Madu; MD2

1. INTRODUCTION

Pineapple (*Ananas comosus*) is a member of the *Bromeliaceae* family, a big and varied family with around 2000 species (Ranjitham et al., 2015). The states of Johor, Sarawak, Sabah, Kedah, Selangor, Negeri Sembilan, Pahang, and Terengganu are the primary planting locations for MD2, Madu and N36 (Suhaimi and Abdul Fatah, 2021). These types of pineapple in Malaysia are as shown as in Figure 1.

N36 **MADU** **MD2**

Figure 1. The pineapple varieties in Malaysia (Ali et al., 2020)

Pineapple is regarded as an economically valuable horticultural crop as it poses a good health benefit that can encourage market potential in the worldwide market (Jaji et al., 2018). Along with calcium, phosphorus, and iron, it is a rich source of vitamins A, B, and C (Yuris and Siow, 2014). The increasing demand of pineapple (*A. comosus*) is expected to grow significantly in recent years as Food and Agriculture Organization of the United Nations (FAO) estimates the world production of pineapple has the ability to grow about 2.3% yearly with 33 million tons in 2029 produced mainly from Asian countries and America (Vald et al., 2021). Pineapple waste grows correspondingly as pineapple output increases (Hikal et al., 2021). Over than 150 000 kg of *A. comosus* waste is generated annually in Malaysia according to the estimations (Selvanathan et al., 2020). The waste produced by the pineapple processing business is enormous, which makes the worldwide economic pollution worse. It is a problem and a chance at the same time to idealize the pineapple waste via additional processing until it is turned into profitable goods utilizing ecologically sustainable methods (Hikal et al., 2021).

Currently, there has been an increasing of interest by researchers and consumers towards natural antioxidants in fruit and vegetables (Xu et al., 2017) since some researches have suggested that consuming synthetic antioxidants may have unfavorable effects (Lourenco et al., 2019). In addition to their biological importance, these natural compounds are also of economic relevance because many of them may be isolated from underutilized plant species and by-products of food production (Lourenco et al., 2019). Free radical is believed to be the major cause of oxidation stress as it can lead to the damage of nucleic acids, lipids, and proteins. All of this can trigger the growth of many dangerous diseases such as cardiovascular and nervous system disorders, cataracts, arthritis, and various types of inflammation (Jovanovic et al., 2018). Meanwhile, the antioxidant will act as an inhibitor or may detain the oxidation substances in chain reactions which can prevent all degenerative diseases (Jovanovic et al., 2018). Phenolic compounds were found in pineapple residues such as myricetin, salicylic acid, tannic acid, *trans*-cinnamic acid, p-coumaric acid, syringic acid and ferulic acid that were reported to be powerful antioxidants (Hikal et al., 2021).

Phenolic compounds are secondary plant metabolites that can lower reactive oxygen species and prevent lipid peroxidation in individuals (Jovanovic et al., 2018). Their antioxidant ability is tightly correlated with the number of hydroxyls. The more the hydroxyl groups a chemical has, the more powerful its ability to break down antioxidant chains. A number of distinct methods, including free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals like superoxide and hydroxides contribute to the antioxidant activity of phenolics (Adhikarimayum et al., 2010).

Hence, this research has been conducted and the goal of this research is to identify the antioxidant activities in different parts of pineapple varieties that are MD2, Madu, and N36 using DPPH assays.

2. MATERIALS AND METHODS

2.1. Sample Collection

The collection of pineapple varieties MD2, N36, and Madu were done in Pekan, Pahang. In essence, Pahang is the second largest pineapple production in Malaysia. Each of the pineapple crown, stalk, peel, and core has been taken for the experiment used to determine the antioxidant activities.

2.2. Chemicals and Apparatus

Methanol from QReC (ASIA) Sdn Bhd, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) from Sign Aldrich Sdn Bhd, ultraviolet-visible spectrophotometer, orbital shaker, oven (memmert), weighing balance, electronic balance, rotary evaporator, measuring cylinder, conical flask, beaker, plastic funnel, glass capillaries, volumetric flask, fume hood cabinet, fume hood chamber, spatula, plastic boat, deionized water, and, distilled water.

2.3. Sample Preparation

The plant parts that have been used are crown, core, peel, and stalk. It was then washed to remove any dust particles and cut into a small piece. The samples were dried in an oven at the temperature of 60°C for 2 weeks. The dried sample was then powdered by using a mechanical grinder. In a prior study, pineapple residues were dried in a fixed-bed drier, and the effect of process variables on the antioxidant capabilities of the residues was examined. It was discovered that after drying, the level of several bioactive chemicals increased (da Silva et al., 2013).

2.4. Preparation of the Plant Extract

Each sample were weighed for 50 g by using a weighing balance and soaked with 200mL methanol each, while the empty fruit bunch used 350mL of methanol. Then, an orbital shaker was used to mix the samples with the solvent where the solutions were shaken for three days. Next, the solutions were extracted by filtration. The extractions were then evaporated to dryness by using a rotary evaporator at 60ºC, 100 rpm, and a pressure of 277 mbr. The result (crude concentrated extract) was weighed. The dried extract was properly stored in the fume hood chamber for further experiment and analysis.

2.5. Antioxidant Activity Determination

DPPH assay was done by the free radical method (Brand-Williams et al., 1995) with slight modification. The antioxidant assay of the sample extracts was carried out by dissolving 100 mL of extract in 2.9 mL of 1.1 mm DPPH This solution should be given vibration by using vortex to ensure all the solution was mixed up and allowed to stand for 30 minutes at room temperature. The standard and absorbance of the extracts were measured against 70% methanol reagent at 517 nm with a UV/Visible spectrophotometer. The determination of antioxidant activity using DPPH assay is based on the ability of 2,2-diphenyl-1-1picrylhydrazyl, a stable free radical to decolourize from purple to yellow colour to show the presence of antioxidants. The DPPH contains an odd electron which responsible for the absorbance at 517 nm and a visible deep purple colour. The DPPH would decolourize when DPPH accepts an electron donated by the antioxidant compound (Bag et al., 2015).

2.6. Preparation of 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

DPPH powder was weighted 39 mg and placed into a 100 mL volumetric flask. Then, methanol was added into the same volumetric flask and the mixture was shaken. The mixture was purple in colour. Prepared DPPH solvent was wrapped by using aluminium foil and stored in a dark cabinet to avoid oxidation.

2.7. Preparation of 500 ppm Stock Solution

Vitamin C and beta hydroxy acid (BHA) were used as a standard solution. Then, 12.5 mg of standard solution and each of the crude samples such as roots, EFB, kernel shell, pressed cake, and chipped trunk were weighed by using an electronic balance and placed into a 25 mL volumetric flask. After that, methanol was added to the same volumetric flask until the mixture gives the total volume of 25 mL.

2.8. Preparation of Standard Solution

In this study, seven standard solutions of different concentrations were used, which are 0 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm and 500 ppm. Solvent methanol was used as negative absorbance or also known as absorbance control. Next, 0.6 mL fraction of standard solution (0, 50,100,150,200,250,500 ppm) was incorporated with 4.5 mL DPPH. Then, the mixture was placed in a dark cabinet for 20 minutes. After that, the colour intensity of the violet solution was measured by using a UV spectrometer with a wavelength of 517 nm. The percentage of scavenging activity was calculated by using the formula shown below.

$$\% \text{ inhibition} = [\ Abs_{control} - Abs_{sample} / Abs_{control}\ ] \times 100$$

The IC_{50} values of DPPH mean how much of a particular substance or what concentrations are needed to scavenge 50% DPPH free radicals. Butylated hydroxyanisole (Bhattacharjee and Dey, 2014) and Vitamin C served as the positive control, while methanol as the negative control.

2.9. Statistical Analysis

All data were expressed as mean ± standard (mean±S.D) deviation from three replicates and averaged. A significant difference was considered at the level of $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Antioxidant Activity Determination

The antioxidant activity aims to determine the presence of protein compounds that act as antioxidant compounds. The principle of this test is the reaction between pineapple peel, core, crown, and stalk extract with 1,1-diphenyl-2-picrylhydrazyl (DPPH). According to (Molyneux, 2014), DPPH is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. DPPH is a stable nitrogen-cantered free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants (Abbas et al., 2009).

Figure 2. The results for antioxidant activity

This test uses positive control and negative controls. The positive control used is DPPH which is reacted routinely to show positive results. The negative control used is DPPH which shows a negative result. Figure 2 shows the reaction between pineapple sample extract with DPPH portraying a positive result with a purple to yellow colour change that can be seen visually with the eye. So, it can be concluded that pineapple peel, core, stalk, and crown extracts have different antioxidant activity assay. Measurement of antioxidant activity in this DPPH method uses IC_{50} parameters. IC_{50} is the concentration of test compounds needed to inhibit free radical compounds by 50%. According to (Zou et al., 2004), this IC_{50} value was obtained from a linear regression equation which states that there is a correlation between the concentration of test compounds and the percent of antioxidant activity caused. The relationship between antioxidant activity and IC_{50} has a direct correlation. The higher the value of the IC_{50} compound, the greater the antioxidant activity of the compound.

3.2. Variety of Pineapple (N36, Madu and MD2)

The previous researches revealed that pineapple contained flavonoid compounds and other phenolic compounds which can act as antioxidants. The previous study presented that the fruit pulp of pineapple (*Ananas comosus*) had antioxidant capacity (Ding and Syazwani, 2014). Parts of the plant may contain similar compounds and have similar effects on antioxidant activity. It was noted that the physical characteristics and chemical make-up of pineapple fruit picked at various ripening stages differs. Total Phenolic Content (TPC) may increase as fruit ripens due to fresh polyphenol production, while TPC may have decreased later in fruit ripening due to polyphenol oxidase-induced polyphenol oxidation (PPO). According to reports, the PPO activity in pineapple fruit differed depending on the stage of ripening, with ripe fruit having the highest activity. Increased PPO activity increases the likelihood that polyphenols may oxidise (Ding and Syazwani, 2014). A study showed that varying concentrations of different solvents were able to extract different amounts of phenolic contents in an unknown variety of pineapple (Alothman et al., 2009). In another study, it was reported that methanol was able to extract a higher number of phenolic compounds compared to water and ethyl acetate in pineapples (Hossain and Rahman, 2011). For testing the antioxidant properties of hydrophilic and lipophilic compounds, methanol is utilised as the solvent. It is an effective and often used solvent to extract phenolics, a type of natural antioxidant component, from plant sources. This might be because the methanol-water mixture is highly polar, which makes it more effective at extracting polar antioxidant chemicals. As a result, choosing the right solvent system is essential for maximising the recovery of TPC and other antioxidants, Ding and Syazwani, 2014). The ability of pineapple extracts to scavenge DPPH, superoxide, and hydroxyl radicals has been reported in several studies (Yuris and Siow, 2014).

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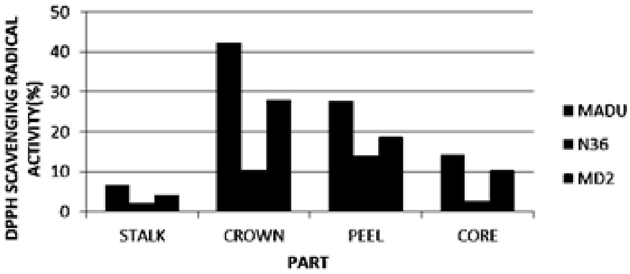

Figure 3. Comparison of DPPH in different varieties of the studied pineapples (Madu, N36 and MD2)

A total of 12 antioxidant activities of three (3) varieties and four (4) parts of pineapple have been identified with BHA and Vitamin C as a positive standard. N36, MD2, and Madu are the pineapple varieties utilized in this study, with stalk, crown, core, and peel being the primary waste components commonly generated by the pineapple canning industry. DPPH radical scavenging activity assay has been applied with absorbance at 517 nm. It shows that pineapple variety which is Madu contained the highest antioxidant activity, while pineapple variety which is N36 contained the lowest antioxidant activity. Madu is a pineapple variety which has high sugar content (16-17°Brix) while N36 average level of sweetness is 12-14 °Brix (Yuris and Siow, 2014). Different variety produces different antioxidant activity levels. Characteristics of pineapple affect the pineapple antioxidant activity. Madu variety produces the highest antioxidant activity as it has the highest level of sweetness compared to other varieties of N36 and MD2. N36 variety contained the lowest antioxidant as the sweetness of the pineapple is the lowest compared to others.

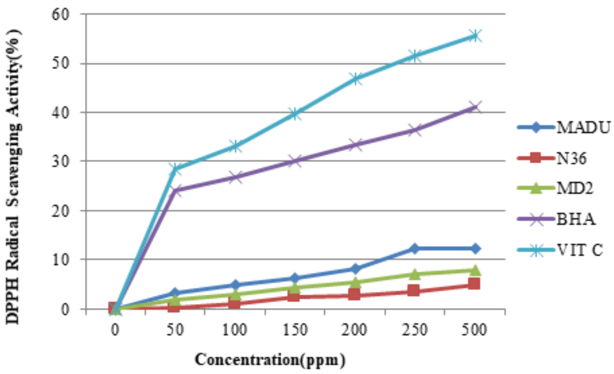

Figure 4. Antioxidant activity of stalk

Figure 4 shows that all three pineapple stalks do not have IC_{50} because the samples taken were having the same defect part. The defect area can affect the nutrient, so this can lead to low activity of antioxidants. According to the study, fertilizer has been examined to influence vitamin C as fertilizers contribute to the effects on antioxidant activity in medicinal plants (Hassan et al., 2012). Comparison of IC_{50} value between all variety stalks with IC_{50} values of vitamin C shows a significant difference in antioxidant activity. This difference can be caused by controlling the variety of stalks used in comparison to vitamin C so that there is no compound in it that can interfere with the process of reducing free radicals. Vitamin C has a remarkable ability to neutralize reactive oxygen and nitrogen species, offering protection against oxidative harm to crucial biological components like DNA, lipids, and proteins. Moreover, it acts as a reducer for redox active transition metal ions within certain enzymatic processes. Nevertheless, when vitamin C interacts with "free" catalytic metal ions, it might potentially lead to oxidative damage due to the formation of hydroxyl and alkoxyl radicals (Carr and Frei, 1999).

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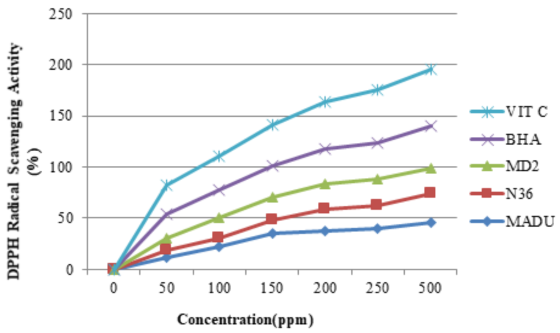

Figure 5. Antioxidant activity of peels

Figure 5 shows that Madu peels are merely having IC_{50} and MD2 contain the lowest antioxidant activity. According to the previous study, the pineapple peel exhibits the most potent antioxidant activity among its components (Li et al., 2014). An example of research is the ethanol peel extract of Bogor pineapple that showed IC_{50} DPPH 0.13 µg/mL and were classified as a very strong antioxidant, methanol peel extract of pineapple waste from Egypt with a concentration of 8 mg/mL had the highest percentage of DPPH scavenging activities (Rashad et al., 2015).

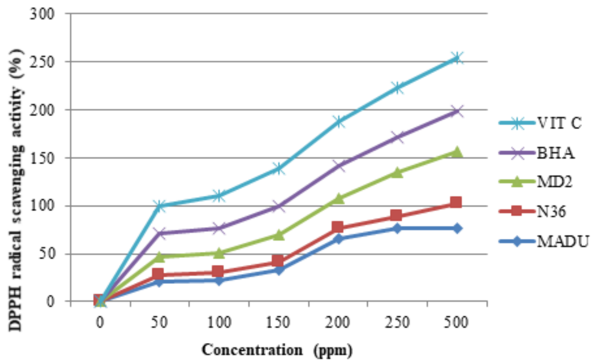

Figure 6. Antioxidant activity of crown

Figure 6 shows that Madu crown and MD2 crown have IC_{50}. Both of this is potential for further study as they are strong antioxidants. According to a study, the crown of the fruit contains significant amounts of antioxidants, possibly even larger amounts than the edible part of the fruit (Jovanovic et al., 2018). Figure 6 shows that the higher the concentration, the higher the absorbance value produced. This is proportional to the magnitude of the percentage of antioxidant activity produced, which is due to the increasing number of atomic donors for DPPH radicals which makes DPPH more stable (Kedare and Singh, 2011).

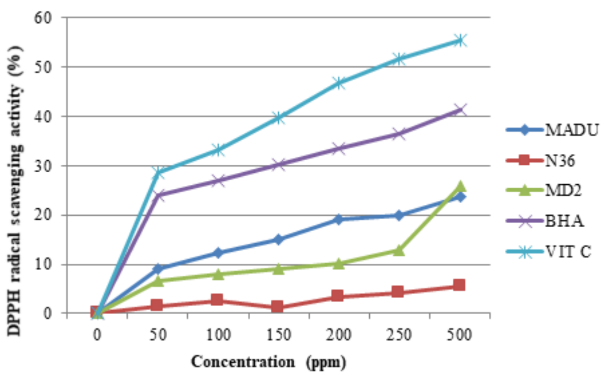

Figure 7. Antioxidant activity of core

Figure 7 shows that all three pineapple cores do not have IC_{50}. Various research data about the antioxidant capacity of fruits and vegetables in the literature clearly show that the methods in many stages of research from sample preparation to antioxidant activity measurement vary highly (Ahmad and Nurhalim, 2012). On the other hand, when the experiments were carried out, no significant variation was recorded in the scavenging activity of the mature fruits of this pineapple variety in successive. Their antioxidant potential is closely related to the number of hydroxyls, the higher the number, the more potent the chain breaking antioxidant action of the compound. The phenolic content and composition of fruits and vegetables depend on genetic and environmental factors as well as postharvest processing conditions (Saraswaty et al., 2017).

4. CONCLUSION

The study of pineapple is important as it shows that different variety and part of pineapple has different antioxidant activity. Pineapple waste can be transformed into a highly valuable product. Madu pineapples contain the highest antioxidant activity as the pineapple characteristic is crunchy in texture and the sweetness level is 13 brix compared to the other pineapple varieties. It should be proceed with further research as it has strong potential of antioxidant activity. All Madu part shows the highest antioxidant activity compared to the other variety which is N36 and MD2. The Madu crown has IC_{50} at a concentration of 40 ppm closely to the synthetic antioxidant (Vitamin C) which shows the concentration of 175 ppm. The confirmation of the antioxidant potential in the crown implies that this part can be used as a source of antioxidants. Thus, further study on this waste product of antioxidant activity is necessary, important, and should be highlighted as the pineapple production in Malaysia is improved through years. Previous research showed many benefits of pineapple, including pineapple waste. This can add the value of pineapple and lead to the increasing number of farmers interested to invest in pineapple plantations. To conclude, different varieties of pineapple and parts produce different antioxidant activities. Thus, further confirmation on antioxidant activity needs to be done so that we can utilize these waste products wisely.

Declaration of Interest

I declare that there is no conflict of interest.

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