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Comparative Study on the Antioxidant Activity of Leaf Extract and Carotenoids Extract from *Ipomoea batatas* var. Oren (Sweetpotato) Leaves

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Abstract**—***Ipomoea batatas* (Sweetpotato) is currently ranked sixth in the total world food production and are planted mainly for their storage roots. The present study was undertaken to evaluate and compare the antioxidant properties of the leaf and carotenoids extract from the *Ipomoea batatas* var. Oren leaves. Total flavonoids in the leaf extract was 144.6 ± 40.5 µg/g compared to 114.86 ± 4.35 µg/g catechin equivalent in the carotenoids extract. Total polyphenols in the leaf extracts (3.470 ± 0.024 GAE g/100g DW) was slightly higher compared to carotenoids extract (2.994 ± 0.078 GAE g/100g DW). The carotenoids extract marked a higher radical scavenging capacity with the IC_{50}= 491.86 µg/ml compared to leaf extract (IC_{50}= 545.39 µg/ml). Concentration-dependent reducing activity was observed for both extracts. Thus, the carotenoids extraction process retained most of the antioxidant capacity from the leaves and can be made into potential natural yellow dye with antioxidant property.

Keywords**—**antioxidants, carotenoids extract, *Ipomoea batatas*, sweetpotato leaves

I. Introduction

IPOMOEA Batatas, or sweet potato was originated from the Northwest of South America and has been dispersed world-wide because of its high yield potential and wide adaptability. The nutritional value of *Ipomoea batatas* leaves is gaining recognition, as the understanding between diet and health increases. It was observed that all the *Ipomoea batatas* storage roots (regardless of flesh colour) had shown antioxidative and radical scavenging activities [1], [2]. However, their leaves were made into animal feeds or discarded after the storage roots are harvested. These leaves can be a potential source of carotenoids and natural source of antioxidants. Reference [3] firstly discovered the high level of lutein content in the leaves of the *Ipomoea batatas* plant compared to the other leaves. It was also reported that the *Ipomoea batatas* leaves from the different varieties in Malaysia had lutein level comparable to the level found in spinach [4]. Besides, the *Ipomoea batatas* leaves were reported to be an excellent source of antioxidants and contain high level of polyphenolic compounds compared to other commercial vegetables [5].

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Free radicals and reactive oxygen species are thought to cause oxidative damage in the body and contribute to several degenerative diseases such as Alzheimer's, cancer and cirrhosis. Some examples of reactive oxygen species are superoxide radical, hydroxyl radical and nitric oxide radical which attack biological molecules in the body [6]. Natural antioxidants have been isolated and studied from various plants including cereal crops, vegetables, leaves, roots, herbs and spices [7], [8]. Antioxidants have the ability to scavenge these free radicals and protecting the body from potential damage from these free radicals. Natural phenolic compounds which include flavonoids and tannins have been extensively studied due to their role in preventing cell damage. The antioxidant properties in vegetables are contributed by numerous groups of phytochemicals which includes carotenoids, ascorbic acid, α-tocopherol and polyphenols [9]-[11]. Antioxidants that occur in crude mixtures add complexity in attempts to explain and understand the antioxidant capacities of certain extract. Therefore, assays are often used instead to measure the antioxidant activities of these extracts.

Ipomoea batatas plant can be further divided into different varieties which contain different level of pigments in their leaves. The production of pigments in plant is related to the physiological changes that occur in the plants with relation to soil condition, variety, stage of development and condition of growth [12]. The leaves of *Ipomoea batatas* var. Oren with orange fleshed storage roots were found to contain the highest concentration on carotenoids when compared to the different varieties that are commonly found in Malaysia [4]. However, the extraction of carotenoids as natural dye from the leaves may have an effect on the antioxidant properties of these leaves. Hence, the objective of this study is to determine and compare the antioxidant properties of the leaf extract and carotenoids extract from *I. batatas* var. Oren leaves.

II. Materials and Methods

Extraction of leaf extract- Ipomoea batatas var. Oren leaves were ground into fine powder using a mortar and pestle with liquid nitrogen. Methanol was used to extract the powdered leaves sample. The mixture was filtered and the supernatant were used for antioxidant analyses.

Extraction of carotenoids extract- Ipomoea batatas var. Oren leaves powder was extracted with 50ml of acetone and shook overnight at room temperature. The mixture was filtered using Whatman No.1 filter paper and was added with 0.1g of butylated hydroxytoluene (BHT) to prevent carotenoids degradation. Acetone in the filtrate was evaporated to dryness using rotary evaporator (Butchi). Then, 40% of aqueous potassium hydroxide (KOH) was added to the extract and the mixture was left to saponify at 45ºC. Acetone was added to the saponified sample and the mixture was placed in the separating funnel. The bottom layer was discarded and the upper layer was collected in an evaporatory flask. The extract was evaporated using rotary evaporator leaving a layer of oleoresin. The oleoresin was dissolved in methanol.

Total phenols determination- Total phenols contents of the leaf extract were determined using [13] method with modifications. Diluted gallic acid was added with Folin-Ciocalteau reagent (Sigma) and the mixture was incubated for 5 minutes. Sodium carbonate solution (750 µl) was then added, mixed and incubated for 2 hours at room temperatures. The absorbance was recorded at 765nm using the UV-200 spectrophotometer (MRC). A standard curve was drawn using gallic acid of different concentrations (100 mg/L, 200 mg/L, 300 mg/L, 400mg/L and 500 mg/L). The steps above were repeated by substituting gallic acid with sample extracts. The concentration of the extracts used was optimised to ensure the total phenol value is within the excepted standards' range. The concentration of total phenols in the leaf extract was calculated based on the equation from the standard curve and expressed in terms of gallic acid equivalent (mg/ 100g GAE).

DPPH Radical Scavenging Assay- 1,1-diphenyl-2-picryl hydrazyl (DPPH) was used to determine the free-radical scavenging activity of the sample extracts. The method used in experiment is according to [14] and [15]. Different concentrations of Vitamin C (L-ascorbic acid) (500µl) were added with 1ml of 0.1mM DPPH solution as standards. The mixture was vortex for 15 seconds and incubated in the water bath at 37ºC for 30 mins. The absorbance was read at 517nm. The steps above were repeated by replacing Vitamin C with the different concentration of leaf extracts. The IC_{50} values were calculated from the graph which represents the concentration of the sample required to scavenge 50% of the DPPH free radicals.

Reducing Power Assay- The method used was based on [14] with modifications. Leaf extracts (50µl) of different concentrations were added to 200µl of 0.2M Phosphate buffer and 200µl of 1% Potassium Ferricyanide. The mixture was incubated in the water bath for 20 minutes at 50ºC. Trichloroacetic acid (250µl) was added to the mixture and was centrifuged at 1000rpm for 10 minutes at room temperature. The supernatant (500 µl) was added with 500µl of deionised water and 100µl of 0.1% ferric chloride. The mixture was incubated in the oven at 37ºC for 10 minutes. Absorbance was recorded at 700nm.

Total flavonoids determination- The Vanillin-HCl assay [16] was used to determine the amount of condensed tannins in the leaf extract. Diluted catechin standards (250µl) were added with Vanillin-HCl (1ml) reagent in a 2ml microcentrifuge tube. Then, the reaction mixture was incubated in a water bath for 20 minutes at 30ºC. The absorbance was measure at 500nm and blanked with 80% methanol. The steps above were repeated by substituting catechin with the leaf extracts. The calculation for the total flavonoids was based on [16].

Statistical Analysis- The antioxidant values for both the extracts were evaluated with the one-way ANOVA using SPSS software (SPSS 19, IBM). P values less than 0.05 were considered to be statistically significant.

III. RESULTS AND DISCUSSION

A. Flavonoids and Total Phenol Contents of the Extracts

The antioxidant property of flavonoids which contributes to good health in human has been studied intensively. Flavonoids are the major polyphenolic component present in food and include anthocyanins, proanthocyanidins, flavonols and catechins [17]. Flavonoids generally work by scavenging or chelating process [18]. Condensed tannins or proanthocyanidins are flavonoids that consist of two or more flavan-3-ol such as catechin, epicatechin or gallocatechin. The total flavonoids amount in the extracts were calculated based on catechin equivalent (standard curve equation y= 0.0037x – 0.0092, r2= 0.9965). The value of total flavonoids in the leaf extract was found to be 144.6 ± 40.5 µg/g catechin equivalents compared to 114.86 ± 4.35 µg/g catechin equivalents in the carotenoids extract. The difference in total flavonoids between the extracts was found to be significantly ($P \leq 0.05$) different through the one-way ANOVA analysis. Leaf extracts was found to contain higher flavonoids value compared to the carotenoids extract.

On the other hand, the phenolics are the well-known group of secondary metabolites and comprise a large group of biologically active compounds [19]. Polyphenols are phenolics that contain at least two phenol rings. The study of phenolics compound in food has been intensive after the research on red wine showed that moderate consumption of red wine which contains polyphenols could help in the prevention of cardiovascular diseases [20]. Besides, phenolics componds also contain other biochemical activities such as antimutagenic, anticarcinogenic and the ability to modify expression of genes [21]. The antioxidant properties of the phenolics compounds are due to their phenolic hydroxyl groups that have the ability to scavenge radicals. Detection of phenols therefore strongly suggests the potential of antioxidant activity in these extracts [22].

Total polyphenols in both the leaf and carotenoids sample was expressed in terms of gallic acid equivalents (GAE) with the standard curve equation of $y = 0.001x - 0.0072$ ($r^2 = 0.9971$). The value of total polyphenol in the *I. batatas* var. Oren leaf extract was found to be 3.470 ± 0.024 GAE g/100g

DW compared to 2.994 ± 0.078 GAE g/100g DW in the carotenoids extract. The differences between the two amounts were found to be significantly different ($P \leq 0.05$) when analysed using one-way ANOVA. The major criteria that distinguish the carotenoids extract from the leaf extracts is the former does not contain chlorophylls, the main green pigment in plant. However, it was not known whether the differences in the level of polyphenol detected are contributed or affected by presence of chlorophylls in the extract.

Previous studies conducted on olive pulp revealed that the highest level of total polyphenol was detected in the Mishen olive cultivar which contains approximately 2.997±0.361 g GA/100g [23]. The *I. batatas* var. Oren leaves in this study have a higher polyphenol level compared to olive pulp extract whereas the carotenoids extract has almost similar amount of total polyphenol content.

B. Antioxidant Activity

The reducing power assay is used to test the reducing capability of the *Ipomoea batatas* var. Oren leaf extract to reduce the ferricyanide (Fe3+) complex to their ferrous form (Fe2+). The reducing power of the extracts was determined through their absorbance at 700 nm. A concentration-dependent reducing activity was observed for both the leaf and carotenoids extract and was shown in Fig. 1. Both extracts showed increased reducing power with the increased in the extract's concentration. The leaf extract however contain overall higher reducing activity compared to the carotenoids extract. The differences in the reducing power might be contributed by the carotenoids extraction process.

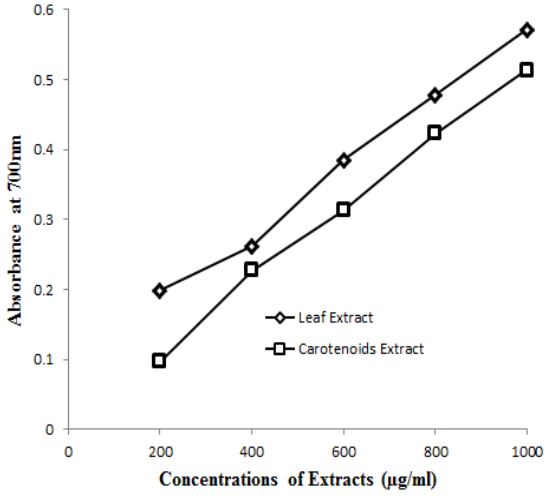

Fig. 1 The Reducing Power of the Ipomoea batatas var. Oren Leaf extract and Carotenoids Extract

One of the known free radical scavenging that occurs exogenously in human body is the mechanism of inhibition of lipid oxidation by antioxidants [24]. The DPPH radical scavenging assay depends on the decoloration of the purple coloured methanolic DPPH solution to yellow by the radical scavengers present in the sample extracts [25]. The radical scavenger acts by reducing the stable DPPH radical in the presence of hydrogen-donating antioxidant to diphenylpricrylhydrazine (yellow) [7]. Radical scavenging activity of the extracts is compared using their respective IC_{50} values.

IC_{50} is used to express the amount or concentration of extracts needed to scavenge 50% of the free radicals. The value of IC_{50} is inversely proportional to the scavenging activity of the leaf extract. The scavenging activity between the three extracts was determined by comparing their scavenging activity. The Vitamin C used has highest scavenging activity (IC50= 471.6 µg/ml), followed by carotenoids extract (IC50= 491.86 µg/ml) and leaf extract (IC50= 545.39 µg/ml). The Vitamin C (Ascorbic acid) used in this study is a well-known antioxidant and thus can be used as a good indicator to compare scavenging activity between the extracts. As shown in Fig. 2, at the lower concentration of 200 and 400µg/ml, vitamin C has higher percentage of inhibition compared to the leaf and carotenoids extract. However, carotenoids content at 600µg/ml was found to have higher inhibition compared to the vitamin C standards and leaf extract. In addition to that, a study conducted on vegetables consumption in Taiwan also shown that sweetpotato leaf indeed have the greatest antioxidant activity compared to other vegetables by both the ABTS and DPPH methods [26].

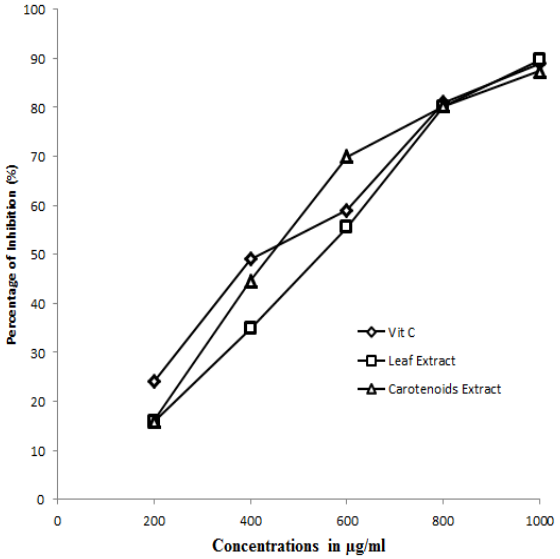

Fig. 2 The DPPH Radical Scavenging Activity for Vitamin C, Leaf Extract and Carotenoids Extract at different concentrations

IV. Conclusion

As a conclusion, the mechanism of antioxidant activity between the leaf extract and carotenoids extract can be stipulated by the above findings through the presence of polyphenols and flavonoids compounds as well as the reduction of free radicals and scavenging activity on the free radicals. The carotenoids extract was able to retain most of the antioxidant capacity when compared to the leaf extracts. Besides, the *I. batatas* leaves are also considered as a cheap source due to their low economic value. Hence, the

carotenoids extract from the *Ipomoea batatas* var. Oren does not only function as a cheap natural yellow dye but with additional feature of antioxidant property that can be beneficial to human health compared to the artificial colouring dye that are currently being used in the market.

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