

Kertas Asli/Original Articles

Antioxidant Activities, Total Phenolic Content and Colour Parameters in the Aqueous Extracts of Avocado, Banana and Papaya Leaves (Aktiviti Antioksidasi, Jumlah Kandungan Fenolik dan Parameter Warna dalam Ekstrak Aqueus Daun Avokado, Pisang dan Betik)

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ABSTRACT

Literature has consistently reported that horticultural wastes including leaves, skin, stones and seeds contain substantial amounts of bioactive compounds. Therefore, this study aims to evaluate antioxidant activity, Total Phenolic Content (TPC) and colour parameters in avocado, banana, and papaya leaves. Antioxidant activity of the leaves was determined using Trolox Equivalent Antioxidant Capacity (TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays, TPC was evaluated using Folin-Ciocalteu assay whereas the colour parameters were analysed with a colour picker software. Data analysis was carried out using SPSS version 25.0 of triplicate determinations. Mean differences among the fruit leaves extracts were determined using One-way ANOVA, while the correlations between the studied components were by the Pearson's Correlation Coefficient test. The TEAC values were in the range of 332.30 ± 18.04 $\mu\text{g Trolox/g D.W.}$ (avocado leaves) to 12217.71 ± 18.04 $\mu\text{g Trolox/g D.W.}$ (banana leaves) while the DPPH radical scavenging activity was from $10.07 \pm 3.89\%$ (banana leaves) to $86.70 \pm 0.26\%$ (avocado leaves). Besides, TPC was from 871.33 ± 38.35 $\mu\text{g GAE/g D.W.}$ (papaya leaves) to 1199.08 ± 6.00 $\mu\text{g GAE/g D.W.}$ (avocado leaves). The hue values were from 19° in avocado leaves extract to 37° in banana leaves extract. Results from Pearson's Correlation Coefficient test revealed that there were no significant correlations between the studied assays. Avocado leaves had the highest DPPH radical scavenging activity and TPC among the three extracts. Findings derived from the present study could be exploited in nutraceuticals formulation.

Keywords: Trolox Equivalent Antioxidant Capacity (TEAC); DPPH; Total Phenolic Content (TPC); colour parameters; fruit leaves

ABSTRAK

Sastera telah melaporkan bahawa sisa hortikultur termasuk daun, kulit dan biji benih merupakan sumber kompaun bioaktif. Oleh itu, kajian ini bertujuan untuk menilai aktiviti antioksidasi, jumlah kandungan fenolik (TPC) dan parameter warna di daun avokado, pisang dan betik. Aktiviti antioksidasi dalam daun ditentukan dengan menggunakan Kaedah Kapasiti Antioksidasi Setara Trolox (TEAC) dan Kaedah 2,2-diphenyl-1-picrylhydrazyl (DPPH), TPC dinilai dengan Kaedah Folin-Ciocalteu, manakala parameter warna dianalisis dengan perisian pemilih warna. Analisis data dijalankan dengan perisian SPSS versi 25.0 daripada triplikasi. Perbezaan min di antara ekstrak daun telah ditentukan dengan ANOVA sehala, manakala hubungan antara komponen yang dikaji dinilai dengan korelasi Pearson. Nilai TEAC adalah dalam julat 332.30 ± 18.04 $\mu\text{g Trolox/g D.W.}$ (daun avokado) sehingga 12217.71 ± 18.04 $\mu\text{g Trolox/g D.W.}$ (daun pisang) manakala aktiviti DPPH adalah dari $10.07 \pm 3.89\%$ (daun pisang) sehingga $86.70 \pm 0.26\%$ (daun avokado). Selain itu, TPC dilaporkan dari 871.33 ± 38.35 $\mu\text{g GAE/g D.W.}$ (daun betik) sehingga 1199.08 ± 6.00 $\mu\text{g GAE/g D.W.}$ (daun avokado). Nilai hue dalam julat 19° di ekstrak avokado sehingga 37° di ekstrak daun pisang. Keputusan korelasi Pearson mendedahkan bahawa tidak ada hubungan yang signifikan antara komponen yang dikaji. Daun avokado mempunyai aktiviti DPPH dan TPC yang tertinggi di kalangan tiga ekstrak. Penemuan yang diperolehi daripada kajian boleh dimanfaatkan dalam formulasi nutraseutikal.

Kata kunci: Ujian Kapasiti Antioksidasi Setara Trolox (TEAC); kaedah DPPH; jumlah kandungan fenolik; parameter warna.; daun buah

INTRODUCTION

Avocado, banana and papaya are among the most widely consumed tropical fruits worldwide. Owing to the popularity, these fruits are massively cultivated in countries with tropical climate to cater the global market demand. According to a recent report by Food and Agriculture Organization (FAO) (2019), avocado and papaya are ranked as the most significantly traded tropical fruits worldwide, alongside with pineapple, mango, mangosteen and guava. In the year of 2017 alone, the global production of avocado and papaya have reached the estimation of 5.7 and 13.7 million tonnes, respectively. In contrast to avocado and papaya which are actively traded in the global market, the plantation of banana is mostly served to meet the domestic demand within a country. Nevertheless, it is estimated that the worldwide production of banana in 2017 was 114 million tonnes (FAO 2020).

The mass cultivation and production of fruits worldwide not only supply these commodities to the global market, but it also generated a considerable amount of horticultural wastes including leaves, skin, stones, pulp and seeds (Sagar et al. 2018). Numerous studies have reported that horticultural wastes contain valuable bioactive compounds such as phenolics, carotenoids and flavonoids (Deng et al. 2012). Study by Maisarah et al. (2013), for instance, showed that papaya leaves had a higher DPPH scavenging activity, total phenolic content and total flavonoid content compared to its flesh and seed. Besides, Abdennacer et al. (2015) also demonstrated that the fruit leaves of *Lycium intricatum* Boiss had ample phenolic and flavonoid compounds as opposed to its respective fruit flesh. In addition to the aforementioned, literature also claimed that there is positive correlation between colour parameters and antioxidants, which making it as a good predictor for total phenolic content and antioxidant activity in plants (Cömert et al. 2020). Owing to those previously reported, this study aims to compare the antioxidant activities, total phenolic content and the colour parameters of avocado, banana and papaya fruit leaves. Besides, it is also worth mentioning that this study was the first few studies that examined the antioxidant activities and total phenolic content of fruit leaves in the aqueous extract.

METHODOLOGY

CHEMICALS

Denatured ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulphate, Trolox, Folin-Ciocalteu,

sodium carbonate (Na_2CO_3) and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

SAMPLE PREPARATION AND EXTRACTION

Young avocado (*Persea americana*), banana (*Musa paradisiaca*) and papaya (*Carica papaya*) leaves were from a local fruit farm in Selangor, Malaysia. Leaves without any physical damage on the surface and vibrant green in colour were collected into several pre-labelled black plastic bags and brought to the laboratory for analysis. Upon arrival at the laboratory, all leaves were washed under running tap water for removing dirt and impurities. The leaves were then dried in an oven overnight at 37°C. Stem from all dried leaves was removed before grinding these leaves into a fine powder using a kitchen blender. All samples were stored in air-tight containers at room temperature for at most one week before analysis. Sample extraction was carried out according to Wong & Tan (2020). In brief, 1 g of the sample powder was weighed into 25 ml of distilled water. The mixture was agitated for 120 min in an orbital shaker at 200 rpm and 50°C. The resulting extract was then filtered through Whatman no.1 filter paper and stored in an amber bottle at 4°C before being analysed.

TEAC ASSAY

The TEAC was performed according to 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) decolourisation assay described by Wong & Tan (2020). The ABTS⁺ radical cation was produced by mixing 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate. The reaction mixture was then incubated under a dim-light condition for 16 hours at room temperature. It was diluted to an absorbance reading of 0.700 ± 0.050 with distilled water before use. The decoloration was tested by pipetting 50 µl of sample extract into 1.9 ml of diluted ABTS⁺ solution. This mixture was then stood at room temperature for 6 min before taking absorbance at 734 nm. The standard curve was constructed using Trolox with the concentration ranging between 0–20 µmol/L. The antioxidant activities were expressed as µg Trolox Equivalent/g D.W.

DPPH RADICAL SCAVENGING ACTIVITY ASSAY

The DPPH radical scavenging activity of fruit leaves was conducted according to the method described by Shekhar & Goyal (2014). Briefly, 1.9 ml of 0.1 mM ethanolic DPPH solution was added to 1 ml of fruit leaf extracts. After that, the mixture was incubated in dim-light condition at room temperature for 30 min. Absorbance was measured at 515

nm using a UV-VIS spectrophotometer (DR6000, HACH, US). The ability of fruit leaf extracts to scavenge DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

TOTAL PHENOLIC CONTENT

Total phenolic content was carried out according to the method of Tan et al. (2010). Folin-Ciocalteu reagent was first diluted with distilled water in the ratio of 1: 10. Later, 1.5 ml of the diluted Folin-Ciocalteu reagent was added with 0.2 ml of sample extract. The mixture was stood for 5 min at room temperature before pipetting 1.5 ml of 0.566 M sodium carbonate into it. Absorbance was measured at 725 nm after 90 min incubation at room temperature. Gallic acid was used as standard and results were expressed as μg Gallic Acid Equivalents (GAE)/g D.W.

COLOUR PARAMETERS

The colour parameters including Hue, Saturation and Lightness (HSL) were assessed with a colour picker software available at: <https://htmlcolorcodes.com/color-picker/>. Colour of the extract was determined by comparing the HSL generated from the software with Hue, Saturation, and Light Colour Codes (2020).

STATISTICAL ANALYSIS

Data were analysed using IBM Statistical Package for Social Sciences (SPSS) version 25.0 SPSS Inc, Chicago, IL, USA and expressed as means \pm standard deviation (S.D.) of triplicate determinations. Since data were normally distributed as tested with Shapiro–Wilk, ANOVA with Tukey HSD post- hoc test was used to determine the mean difference of TPC, TEAC and DPPH radical scavenging activity across different fruit leaves. The correlation between TPC and antioxidant activities (as measured with TEAC and DPPH radical scavenging assays) was also determined by Pearson’s correlation

coefficient test. A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Besides, the colour of the extracts was determined by uploading Figure 1 to a colour picker software.

RESULTS

Table 1 depicts the antioxidant activities (as determined by TEAC and DPPH radical scavenging assays) and TPC of fruit leaf extracts (1: 25, w/v). TEAC values of fruit leaves were $332.30 \pm 18.04 \mu\text{g}$ Trolox Equivalent/g D.W. (avocado leaves), $4405.21 \pm 407.85 \mu\text{g}$ Trolox Equivalent/g D.W. (papaya leaves) and $12217.71 \pm 18.04 \mu\text{g}$ Trolox Equivalent/g D.W. (banana leaves). Banana leaves had a significant higher ($p < 0.05$) TEAC value compared to papaya and avocado leaves. Similarly, the TEAC value of papaya leaves was significantly higher ($p < 0.05$) than avocado leaves. On the other hand, the DPPH radical scavenging activities of the fruit leaves were $10.07 \pm 3.89 \%$ (banana leaves), $61.72 \pm 1.73 \%$ (papaya leaves) and $86.70 \pm 0.26 \%$ (avocado leaves). In contrast to TEAC assay, the avocado leaves had a significant higher ($p < 0.05$) DPPH radical scavenging activity compared to the aqueous extracts of banana and papaya leaves. Results also revealed that banana leaves had a significantly lower ($p < 0.05$) DPPH radical scavenging activity than papaya leaves. Moreover, TPC of the fruit leaves was noted as $1199.08 \pm 6.00 \mu\text{g}$ GAE/g D.W. (avocado leaves), $1110.50 \pm 54.22 \mu\text{g}$ GAE/g D.W. (banana leaves) and $871.33 \pm 38.35 \mu\text{g}$ GAE/g D.W. (papaya leaves). However, results from ANOVA analysis indicated that no significant difference ($p > 0.05$) in the TPC of avocado leaves with that of banana leaves. The aqueous extract of avocado leaves had a significantly higher ($p < 0.05$) TPC compared to papaya leaves. A similar observation was also reported between the TPC of banana and papaya leaves, whereby banana leaves had a significantly higher ($p < 0.05$) TPC in comparison with papaya leaves.

Emerging results revealed that no significant correlation was observed between TEAC, DPPH radical scavenging activity and TPC across different extracts (Table 2). Besides, Figure 1 shows the colour of the filtered leaves extracts after underwent 2 hours of aqueous

TABLE 1. Antioxidant activity and total phenolic content of leaf extracts

Leaf extract	Trolox Equivalent Antioxidant Capacity (μg Trolox Equivalent/g DW)	DPPH Scavenging Activity (%)	Total Phenolic Content (μg GAE/g DW)
Avocado	332.30 ± 18.04^a	86.70 ± 0.26^a	1199.08 ± 6.00^a
Banana	12217.71 ± 18.04^b	10.07 ± 3.89^b	1110.50 ± 54.22^a
Papaya	4405.21 ± 407.85^c	61.72 ± 1.73^c	871.33 ± 38.35^b

TABLE 2. Pearson's Correlation Coefficient between the studied components according to leaf extracts.

Studied Components	Avocado		Banana		Papaya	
	TPC	TEAC	TPC	TEAC	TPC	TEAC
TEAC	0.746	-	0.148	-	-0.147	-
DPPH	0.193	0.798	0.602	-0.701	0.619	0.686

TPC= Total Phenolic Content, TEAC= Trolox Equivalent Antioxidant Capacity, DPPH= DPPH Radical Scavenging Activity Assay

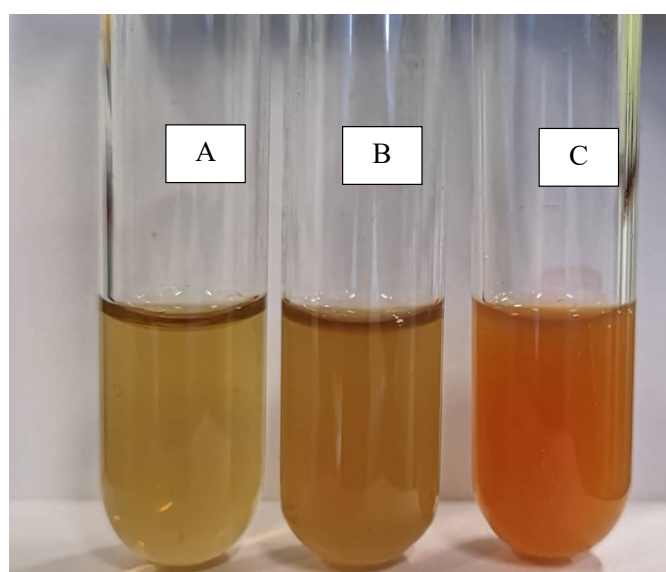


FIGURE 1: Colour of the leaf extracts. A: Banana B: Papaya C: Avocado

extraction. The banana leaves extract was in bronze colour with the hue value of 37°, saturation of 47% and lightness of 42%. On the other hand, the papaya leaves extract was in peru colour with the hue value of 30°, saturation of 59% and lightness of 35%. Avocado leaves extract, on the other hand, had sienna2 colour with the hue value of 19°, saturation of 83% and lightness of 60% (Hue, Saturation and Light Colour Codes, 2020).

DISCUSSION

To date, studies on antioxidant properties of fruit leaves remain scarce and unattained. A study by Matsusaka & Kawabata (2010) demonstrated TEAC values of 545 μmol Trolox Equivalent/g D.W. (136,408 μg Trolox Equivalent/g D.W.) and 462 μmol Trolox Equivalent/g D.W. (115,634 μg Trolox Equivalent/g D.W.) in 50% ethanol extracts of avocado peel and seed, respectively. In contrast to the literature, the aqueous extract of avocado leaves in the present study was approximately 350-fold and 400-fold lower than its respective peel and seed. A recent study by Rafique & Akhtar (2018) reported that the acetone and methanol extract of the avocado pulp was 84% in DPPH

radical scavenging activity. Surprisingly, the DPPH radical scavenging activity of avocado leaves in the present study was in well-agreement with those reported in pulp despite extraction was carried out with different extraction solvents. Yamasaki et al. (2017) revealed that TPC of ethanolic avocado leaves in various extraction temperatures and duration was in the range of 130.0 mg GAE/g D.W. to 233.3 mg GAE/g D.W. In comparison, the aqueous extract of avocado leaves in this study noted a TPC of $1199.08 \pm 6.00 \mu\text{g}$ GAE/g D.W., which was 100-fold and 200-fold lower than the reported range, respectively. Even though this study exhibited a lower TPC compared to the literature, it is worth mentioning that there are overwhelming studies which consistently reported that organic solvents or a combination of different proportion of organic solvent-water are a better extraction solvent when dealing with bioactive compounds extraction (Dailey & Vuong 2015; Do et al. 2014).

Antioxidant properties of banana leaves are the most extensively studied among the three samples. Loganayaki et al. (2010) conducted a study to compare the TEAC and TPC in different parts of the banana including its fruit leaves. The TEAC values of banana leaves in different organic solvents were in the range of 296.67 mg Trolox

Equivalent/g D.W. (methanol extract) to 3605.3 mg Trolox Equivalent/g (acetone extract). In contrast, TPC was from 11 mg GAE/g D.W. (methanol and chloroform extracts) to 21 mg GAE/g D.W. (acetone extract). The TEAC value of the aqueous extract of banana leaves was 24-fold and 295-fold lower compared to methanol and acetone extracts in literature. Fatemeh et al. (2012) carried out a study to determine the DPPH radical scavenging activity and TPC in two varieties of banana peel and pulp flours. The DPPH radical scavenging activities of the 80% methanolic extracts were in the range of 26.55 % to 52.66 %, whereas the TPC was reported from 750.1 µg GAE/g D.W. to 6855.7 µg GAE/g D.W. in the same extracts. The scavenging activity of banana leaves in the present study was 2.6-fold to 5.2-fold lower than those reported, however, the TPC of banana leaves was in well-agreement with the banana pulp and peel flours. Similar with avocado leaves, the vast difference in the antioxidant activities and TPC might be due to difference in the extraction conditions, extraction solvents and studied samples from that of literature.

A recent study by Yap et al. (2020) showed that the TPC of freeze-dried papaya leaves was 21.58 mg GAE/g D.W. whereas TPC in the oven-dried papaya leaves of the present study was approximately 24-fold lower than the previous study. Variation in the methods of sample drying could be the underlying reason for the difference. Another study by Almeida et al. (2011) reported that the pulp of papaya had a TEAC value of 1902.20 µg Trolox Equivalent /g F.W. and a total phenolic content of 532 µg GAE/g F.W., whereby emerging results demonstrated that papaya leaves had higher TEAC and TPC (Table 1). It can be postulated that antioxidant properties are varied according to different parts of the plant. Cosmas Mojulat & Surugau (2018) demonstrated that the DPPH radical scavenging activity in the aqueous extract of papaya leaves was 87.53%. In contrast to the previous study, this study recorded a lower DPPH radical scavenging activity (61.72 ± 1.73%). The difference can be attributed to different extraction conditions, whereby the former study extracted antioxidant compounds in papaya leaves with an extraction temperature of 70°C for 20 min.

Cömert et al. (2020) have recently studied the relationship between colour and the antioxidant activity of fruit and vegetables. Fruits and vegetables with the hue values above 180° and below 20° were reported to have higher antioxidant activity. Coincidentally, the hue value of avocado leaves (19°) fell within the range reported in the previous study. This finding is further supported by a higher TPC and DPPH radical scavenging activity was attained in avocado leaves compared to other fruit leaves. On the other hand, results from Pearson's Correlation test indicated that no significant correlation was obtained between the studied

assays in different fruit leaves. The previous study by Almeida et al. (2011) demonstrated that there were positive and high correlations between antioxidant activities (TEAC and DPPH radical scavenging activity assays) and TPC in the pulp of fresh exotic fruits. However, emerging results failed to observe the correlation as mentioned earlier in the fruit leaves. Therefore, it can be postulated that there might be other bioactive compounds that contributed to the TEAC and DPPH radical scavenging activity.

CONCLUSION

Emerging results suggested the aqueous extracts of fruit leaves contain substantial amounts of bioactive compounds. In comparison to papaya and banana leaves, the avocado leaves had a higher TPC and DPPH radical scavenging activity. High-Performance Liquid Chromatography (HPLC) analysis can be carried out in the future to identify the individual bioactive compound which contributes to the TEAC values and DPPH scavenging activities of fruit leaves.

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