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Evaluation of the Antioxidant and Anti-diabetic Bioactivities of Natural Phenolics from Mango (*Mangifera indica* Linn) Branches

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Different parts of the mango (*Mangifera indica* Linn) tree are known to contain phenolic compounds that exhibit health-promoting bioactivities. Branches that were cut off during pruning having no significant economic value were utilized as a source for phenolic compounds in this study. Antioxidant and anti-diabetic activities of the extracted phenolics from branches of five mango varieties ("apple mango," "carabao," "pico," "sinaging," and "sipsipin") were evaluated. The bioactivities of the phenolic extracts from mature and young branches of carabao and pico varieties were also compared. Three different methods were used to quantify the antioxidant capacity of the phenolic compounds – namely, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay, ABTS [2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt] cation radical scavenging assay, and CUPRAC (cupric ion reduction antioxidant capacity) test. Anti-diabetic effects were evaluated by quantifying the α-amylase and α-glucosidase inhibitory activities of the phenolic extracts. Findings showed that the bioactivities were variable with respect to the varieties and maturity of the mango branches. The varieties apple mango, pico, and sinaging exhibited the highest antioxidant activity based on their EC_{50} values interpolated from the DPPH scavenging, ABTS scavenging, and CUPRAC assays, respectively. The anti-diabetic properties of the phenolics extracted from mango branches showed better bioactivity than the known anti-diabetic drugs, acarbose, and metformin. The findings of this present study offer a potentially huge economic impact to mango farmers as the extraction of phenolics from mango branches for medical supplement use can be a viable alternative and/or supplementary income source.

Keywords: antioxidant activity, anti-diabetic activity, mango, mango branches, phenolic compounds

INTRODUCTION

Most phytochemicals such as phenolic compounds are known for their antioxidant, antimutagenic, anticarcinogenic, and other health-promoting properties (Khammuang and Sarnthima 2011). The nature of the chemical structure of these compounds consisting of one or more aromatic rings with single or multiple hydroxyl groups enables them to inhibit oxidation reactions by chelating metal ions, scavenging free radicals, and donating hydrogen atoms or electrons. The antioxidant bioactivity of these compounds helps balance the reactive oxygen species in the body, which in large amounts can cause cancer, neurodegenerative disease, and cardiovascular diseases (Kajdžanoska *et al.* 2011).

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Phenolic compounds are also known to exhibit potent anti-diabetic activity as it can slow down glucose absorption in the digestive tract by inhibiting the activities of α-amylase and α-glucosidase – enzymes responsible for carbohydrate digestion (Ali Asgar 2012).

Phenolic compounds, given their health-promoting properties, are being developed and utilized as a food supplement, medicine, and food antioxidants (Martillanes *et al.* 2017). The availability, extraction, and processing of phenolic compounds from agricultural products and by-products can result to the development of phenolic-based products that can bring additional revenues to the farmers. In the Philippines, mango (*Mangifera indica* Linn) is the third most important fruit crop and its industry provides income to 2.5 million farmers (DA-BPI). Different parts of the mango plant are known to contain high amounts of phenolic compounds (Masibo and He 2008). Phenolic compounds derived from mango have been shown to exert potent antioxidative, antilithiatic, hepatoprotective, anti-bacterial, and antimutagenic bioactivities (Khammuang and Sarnthima 2011). Although bioactivities of phenolic compounds from extracts of different mango parts have already been reported, comparative studies on the relationship between the variety and maturity of mango parts on the bioactivity of phenolics are quite scarce. The phenolic compounds found in plants and their bioactivities can be influenced by the differences in cultivar, variety, maturity stage, and other external factors (Ghasemnezhad *et al.* 2011). Studies on the evaluation of bioactivities of phenolic compounds in the extracts of different mango parts may encourage the development of phenolic-based products that can provide mango farmers with additional economic benefits. The current status of our local mango industry is in a dire state as local production continues to decrease at an annual rate of 5.2% (PSA 2019).

In this present study, the antioxidant and antidiabetic properties of the phenolic compounds from mango branches were investigated. The relationship between the bioactivities and the type of mango cultivars were evaluated. The effect of maturity was also analyzed; however, due to limited available samples, only the mature and young branches of carabao and pico varieties were subjected to analysis. Antioxidant capacity was evaluated using DPPH free radical scavenging activity assay, ABTS cation radical scavenging assay, and CUPRAC test. The anti-diabetic potential was evaluated by determining the α-amylase and α-glucosidase inhibitory activities of the phenolic compound extracts.

MATERIALS AND METHODS

Chemical Reagents and Apparatus

Gallic acid, Folin and Ciocalteu's phenol reagent, DPPH, ABTS, 2,9-dimethyl-1,10-phenanthroline (neocuproine), copper (II) chloride, ammonium acetate, acarbose, 3,5-dinitrosalicylic acid, *p*-nitrophenyl-α-D-glucopyranoside (N1337), starch, α-amylase from porcine pancreas (Type VI-B, A3176), and α-glucosidase were obtained from Sigma-Aldrich (St. Louis, MO). Methyl alcohol (methanol) and sodium hydroxide were purchased from J.T. Baker™. Sodium carbonate, ascorbic acid, and sodium chloride were obtained from Loba Chemie Pvt. Ltd., Fluka™, and Univar Solutions, respectively. All reagents were of analytical grades.

Spectrophotometric measurements were performed on a UV-Vis double beam Shimadzu UV-1601 spectrophotometer.

Plant Material and Sample Preparation

Mature branches (Figure 1) of five mango varieties – namely, apple mango, carabao mango, pico, sinaging, and sipsipin – were collected from Santos Mango Farm in San Miguel, Bulacan, Philippines (15.14 N, 121.05 E). Young branches were collected from carabao mango and pico varieties only; hence, comparative analysis relative to the maturity of branches was limited to these two varieties. The mango branch samples were washed using water with approximately 0.01% liquid detergent and rinsed thrice with running water. The branches were then dried in a cabinet type hot air drier (2 × 3 × 5 ft) with a temperature of 60 °C. In full capacity, the 10 kg of branches dried for 48 hr. The dried branches were subsequently ground using Thomas® Wiley Mill Model 4 and Retsch® knife mill GRINDOMIX GM 200 and was sieved using a 60-mesh sieve to obtain a fine homogeneous

Figure 1. Representative branches from the carabao mango variety. Branches are considered young when their barks are still relatively soft, light-green to green-colored, and found at the apex of the lateral branches of the plant (a). Branches are considered matured when their barks are hard and brownish-colored (b).

powder. The collected fresh mango branches yielded almost 40% powdered sample.

Phenolic Extraction from Mango Branch Powder

Phenolics from mango branches were solvent-extracted as described previously (Sapin *et al.* 2020). Briefly, 0.2-g powder of the mango branch was dissolved in 7.5 mL of 60% acetone for 1 h. The mixture was then centrifuged at 13,000 rpm for 20 min to separate the phenolic containing supernatant. The collected phenolic extracts were then stored at 4 °C until use.

Determination of Total Phenolic Content

The amount of total phenols in the mango branch extracts was determined using the Folin-Ciocalteu assay described by Núñez Sellés *et al.* (2002). Absorbances were measured at 720 nm. Gallic acid was used to calculate the standard curve (20–100 µg/mL; $y = 0.0071x - 0.0266$; $R^2 = 0.9994$). Total phenolic content was expressed as milligram of gallic acid equivalents per gram of dried mango branch (mg GAE/g).

Antioxidant Capacity

Many antioxidant capacity assays are needed to fully assess the antioxidant activity of phenolic compounds. The assays available for the evaluation of antioxidant capacities differ in principle and experimental condition since there are different mechanisms in the inhibition of oxidization reactions like scavenging free radicals and donating hydrogen atoms or electrons. DPPH free radical scavenging activity assay and ABTS cation radical scavenging assay were used to assess the radical scavenging ability while the CUPRAC test was used to assess the electron-donating ability of phenolic compounds. The EC_{50} value for each method or the effective concentration that causes radical inhibition or cupric reduction by 50% was calculated by interpolation. Ascorbic acid was used as a reference antioxidant. The antioxidant activity of the extract was expressed as percent inhibition (% inhibition), which was calculated by dividing the difference of the absorbance of the control ($A_{control}$) and the sample (A_{sample}) by that of the control then multiplying it by 100.

DPPH Free Radical Scavenging Activity Assay

The DPPH free radical scavenging ability was determined using a method described by Ribeiro *et al.* (2008) with some modifications. Briefly, mango branch extracts (100 µL; 4-20 µg GAE) were added to 5mL of 0.1 mM DPPH free radical methanol solution. After mixing and 15-min standing at room temperature, the absorbance of the resulting solutions was read at 517nm. DPPH inhibition was expressed as percent DPPH inhibition and EC_{50}.

ABTS Cation Radical Scavenging Assay

A procedure modified from Re *et al.* (1999) was used for this assay. Ascorbic acid or samples (40 µL; 0.5–2.5 µg GAE) was added to 3 mL of ABTS cation radical solution with initial absorbance of 0.72 ± 0.05 at 734 nm. Absorbance reading at 734 nm was taken at exactly 5 min after mixing. ABTS inhibition was expressed as percent ABTS inhibition and EC_{50}.

CUPRAC Test

The cupric reducing ability of mango branch extracts was measured based on the method developed by Alpinar *et al.* (2009) with some modifications. The fresh working solution was prepared by mixing 1.0 mL of 0.01 M $CuCl_2$, 1.0 mL of 1.0 M ammonium acetate, and 1.0 mL of 7.5 mM neocuproine. Mango branch extracts (0.5 mL) were allowed to react with the solution for 15 min. Distilled water (0.6 mL) was added after and readings of the product were taken at 450 nm.

Anti-diabetic Capacity

The anti-diabetic property of the mango branch extracts was evaluated through the *in vitro* α-amylase inhibition assay and *in vitro* α-glucosidase inhibition assay. Different concentrations of the antidiabetic drugs, acarbose and metformin, were used as positive controls. The IC_{50} was used for the measurement of inhibitor potency and was computed by interpolation. The anti-diabetic activities of the extract were expressed as percent inhibition (% inhibition), which were calculated by dividing the difference of the absorbance of the control ($A_{control}$) and the sample (A_{sample}) by that of the control and multiplying it by 100.

***In Vitro* α-amylase Inhibition Assay**

The α-amylase inhibitory activities were evaluated by the modified procedure previously described by Phoboo *et al.* (2015). The inhibition of α-amylase was measured by the amount of released reducing sugars from starch, which can be read at 540 nm after the addition of dinitrosalicylic acid color reagent. The α-amylase inhibition activity of the extract was expressed as percent inhibition and IC_{50}.

***In Vitro* α-glucosidase Inhibition Assay**

The α-glucosidase inhibitory activities of the mango branch phenolic compounds were determined according to the procedure previously described by Nair *et al.* (2013) with slight modifications. Due to limited reagents, the comparative analysis relative to maturity was not carried out.

The inhibition of α-glucosidase activity was determined by measuring the amount of *p*-nitrophenol hydrolyzed from *p*-nitrophenyl-α-D-glucopyranoside that can be read

spectrophotometrically at 410 nm. The α-glucosidase inhibition activity of the extract was expressed as percent inhibition and IC_{50}.

Statistical Analysis

All analysis was done in triplicate and the results were expressed as mean ± standard deviation. The significant differences at 95% confidence level among the EC_{50} and IC_{50} of the mango branches phenolics from different variety and maturity for each method were determined through the one-way analysis of variance and Tukey's honestly significant difference test. The statistical analysis was performed using the SPSS 16.0 statistics software.

RESULTS

Antioxidant Property

DPPH. The free radical-scavenging activity of phenolics from branches was determined by the DPPH method and the results are presented in Table 1. The EC_{50} values of the mango branch extracts from five varieties (10.73–12.80 µg GAE) are 1.5- to 1.8-fold lower than that of ascorbic acid (19.85 µg), making the former a better DPPH radical scavenger than the latter. The phenolic extracts from apple mango and pico exhibited the strongest antioxidant activities. No significant differences in the antioxidant potential were found between the young and mature branches of carabao mango (12.43 *vs.* 12.29 µg GAE), but the phenolic extracts from mature branches of *pico* exhibited stronger antioxidant activity compared to its young branches (10.90 *vs.* 11.96 µg GAE), based on their EC_{50} values shown in Appendix Table I.

ABTS. The EC_{50} values obtained for mature mango branches extracts in the ABTS cation radical scavenging assay ranged from 1.59–2.27 µg GAE (Table 2). Pico and sinaging varieties showed the highest antioxidant potential. The ABTS inhibition of branch phenolics from five varieties was significantly better than that of ascorbic acid, as shown by the latter's EC_{50} value of 5.92 µg.

In terms of maturity, the antioxidant capacity of phenolics from mature branches of pico was also stronger than the young counterpart (Appendix Table II). The phenolics from young branches of pico exhibited higher EC_{50} values of 1.86 µg GAE than mature branches with EC_{50} of 1.59 µg GAE. There are also no significant differences in the antioxidant potential between the young and mature branches of carabao mango (1.90 *vs.* 1.88 µg GAE).

CUPRAC. In the CUPRAC test, the effective concentration of phenolics from mature mango branch extracts to reduce Cu^{2+} to Cu^{+} by 50% ranged from 7.50–8.44 µg GAE, as presented in Table 3. Their EC_{50} values were 2.7- to 3.1-fold lower than that of ascorbic acid with an EC_{50} of 23.15 µg, making the phenolics from branch extracts better cupric ion reducer. The strongest antioxidant activities were observed from sinaging phenolic extract whereas the phenolic extract from pico exhibited the weakest.

The phenolics from young branches of pico had a lower EC_{50} value than that from mature branches (7.73 *vs.* 8.44 µg GAE), resulting from higher CUPRAC shown in Appendix Table III. On the contrary, phenolics from mature carabao mango branches exhibited a lower EC_{50} value than that from young branches (7.75 *vs.* 8.22 µg GAE).

Table 1. Antioxidant activity in terms of DPPH free radical scavenging activity and EC_{50} values of extracts from mature mango branches of different varieties compared to reference antioxidant.

Variety	Phenolic concentration (µg GAE*)					EC_{50}**
	4	8	12	16	20	
	DPPH inhibition (%)***					
Apple mango	22.93 ± 0.53	38.91 ± 0.26	55.21 ± 0.58	68.54 ± 1.00	81.27 ± 0.68	10.73^{a} ug GAE
Carabao mango	20.14 ± 0.16	33.17 ± 0.05	48.99 ± 01.16	63.33 ± 2.05	74.39 ± 0.21	12.29^{b} ug GAE
Pico	22.41 ± 2.42	36.78 ± 1.26	55.03 ± 0.11	66.60 ± 3.84	80.08 ± 1.11	10.90^{a} ug GAE
Sinaging	18.69 ± 0.42	33.02 ± 0.37	47.92 ± 1.42	63.55 ± 0.58	75.99 ± 0.58	12.57^{b} ug GAE
Sipsipin	17.65 ± 0.00	30.94 ± 0.16	46.72 ± 0.58	63.14 ± 0.21	74.57 ± 0.16	12.80^{b} ug GAE
Reference antioxidant	Concentration (µg)					EC_{50}**
	4	8	12	16	20	
	DPPH inhibition (%)***					
Ascorbic acid	11.63 ± 0.37	20.63 ± 0.11	29.10 ± 0.27	37.69 ± 0.16	50.49 ± 0.05	19.85^{c} µg

Values within the same column not sharing the same superscript letter(s) are significantly different ($p < 0.05$).
*Gallic acid equivalent; **effective concentration that causes DPPH free radical inhibition by 50%; *** 100 µL/assay

Table 2. Antioxidant activity in terms of ABTS free radical scavenging activity and EC_{50} values of extracts from mature mango branches of different varieties compared to reference antioxidant.

Variety	Phenolic concentration (µg GAE*)					EC_{50}**
	0.5	1.0	1.5	2.0	2.5	
	ABTS inhibition (%)***					
Apple mango	12.54 ± 1.44	25.00 ± 0.52	37.54 ± 0.52	51.46 ± 0.21	62.61 ± 0.31	1.95^{c} ug GAE
Carabao mango	17.49 ± 0.00	26.60 ± 0.72	43.00 ± 1.44	52.11 ± 0.10	65.60 ± 0.21	1.88^{bc} ug GAE
Pico	16.54 ± 1.06	31.50 ± 0.32	47.59 ± 1.81	61.65 ± 1.28	77.14 ± 0.00	1.59^{a} ug GAE
Sinaging	14.83 ± 0.10	27.57 ± 1.13	44.14 ± 1.23	56.95 ± 0.31	71.27 ± 0.51	1.73^{ab} ug GAE
Sipsipin	13.69 ± 0.20	26.73 ± 1.32	39.63 ± 1.22	50.22 ± 0.31	61.96 ± 1.83	2.27^{c} ug GAE
Reference antioxidant	**Concentration (µg)**					EC_{50}**
	1.5	3.0	4.5	6.0	7.5	
	ABTS inhibition (%)***					
Ascorbic acid	11.70 ± 1.54	24.56 ± 0.62	36.70 ± 0.51	50.87 ± 0.82	64.53 ± 1.64	5.92^{d} µg

Values within the same column not sharing the same superscript letter(s) are significantly different ($p < 0.05$).
*Gallic acid equivalent; **effective concentration that causes ABTS cation radical inhibition by 50%; ***40 µL/assay

Table 3. Antioxidant activity based on CUPRAC test and EC_{50} values of extracts from mature mango branches of different varieties compared to reference antioxidant.

Variety	Phenolic concentration (µg GAE*)					EC_{50}**
	2	4	6	8	10	
	CUPRAC (absorbance at 450 nm)***					
Apple mango	0.138 ± 0.002	0.259 ± 0.001	0.383 ± 0.001	0.505 ± 0.005	0.645 ± 0.000	7.95^{ab} ug GAE
Carabao mango	0.134 ± 0.002	0.253 ± 0.005	0.382 ± 0.002	0.517 ± 0.004	0.648 ± 0.000	7.75^{ab} ug GAE
Pico	0.122 ± 0.003	0.231 ± 0.001	0.359 ± 0.003	0.478 ± 0.006	0.580 ± 0.006	8.44^{b} ug GAE
Sinaging	0.138 ± 0.002	0.273 ± 0.002	0.399 ± 0.002	0.533 ± 0.005	0.659 ± 0.010	7.50^{a} ug GAE
Sipsipin	0.123 ± 0.003	0.254 ± 0.002	0.371 ± 0.002	0.509 ± 0.003	0.616 ± 0.000	7.89^{ab} ug GAE
Reference antioxidant	**Concentration (µg)**					EC_{50}**
	5	10	15	20	25	
	CUPRAC (absorbance at 450 nm)***					
Ascorbic acid	0.112 ± 0.002	0.213 ± 0.007	0.328 ± 0.004	0.429± 0.012	0.542 ± 0.011	23.15^{c} µg

Values within the same column not sharing the same superscript letter(s) are significantly different ($p < 0.05$).
*Gallic acid equivalent; **effective concentration that gives CUPRAC value of 0.5 absorbance reading at 450 nm; ***500 µL/assay

Anti-diabetic Property

α-amylase activity. The inhibitory concentration of phenolics from mature branches of five mango varieties that inhibits enzyme activity by 50% ranged from 2.08–3.58 µg GAE, as presented in Table 4. Mango branch extracts showed stronger inhibitory activity towards α-amylase compared to the reference compound (acarbose) with an IC_{50} value of 4.81 µg. Based on the IC_{50} values, the strongest α-amylase inhibitor was the phenolic extract from the pico variety while the weakest was observed from the phenolic extract of sipsipin variety. The phenolics from young and mature branches of carabao mango (2.86 µg GAE *vs.* 3.14 µg GAE) and pico (2.40 µg GAE *vs.* 2.08 µg GAE) were also stronger α-amylase inhibitor than acarbose (Appendix Table IV). In addition, young branches of carabao mango had stronger enzyme inhibition compared to the mature branches of the same variety. However, surprisingly, the bioactivities of the extracts from the pico variety showed an opposite pattern from the other mango varieties. Mature pico branches exhibited stronger α-amylase inhibitory activities than the young branches.

α-glucosidase activity. Table 5 shows the strong inhibitory activity towards α-glycosidase than α-amylase of the phenolics from mature branches of five varieties. The IC_{50} values with α-glycosidase ranged from 1.20–1.47 µg GAE, which is lower than the IC_{50} values with α-amylase, which ranged from 2.08–3.58 µg GAE. Phenolics from mature branches of five varieties were 541- to 663-fold better α-glycosidase

Table 4. Anti-diabetic activity in terms of alpha-amylase inhibition and IC_{50} values of extracts from mature mango branches of different varieties compared to acarbose, an anti-diabetic drug.

Variety	Phenolic concentration (µg GAE*)					IC_{50}**
	1	2	3	4	5	
	Alpha-amylase inhibition (%)***					
Apple mango	19.53 ± 0.71	40.96 ± 0.71	62.10 ± 0.20	70.23 ± 0.10	76.02 ± 0.05	2.45[b] ug GAE
Carabao mango	14.92 ± 1.02	34.77 ± 2.03	48.54 ± 0.76	59.65 ± 2.85	67.78 ± 0.00	3.14[c] ug GAE
Pico	20.72 ± 0.43	48.66 ± 0.00	65.29 ± 3.39	72.77± 2.05	76.54 ± 0.00	2.08[a] ug GAE
Sinaging	19.44 ± 1.61	36.96 ± 2.67	52.03 ± 1.49	73.78 ± 0.10	79.38 ± 0.21	2.86[c] ug GAE
Sipsipin	18.80 ± 0.37	34.75 ± 1.51	42.58 ± 1.41	55.28 ± 0.37	67.13 ± 1.15	3.58[d] ug GAE
Anti-diabetic drug	**Concentration (µg)**					IC_{50}**
	1.5	3.0	4.5	6.0	7.0	
	Alpha-amylase inhibition (%)***					
Acarbose	24.01 ± 0.87	38.85 ± 2.32	47.90 ± 0.93	58.24 ± 0.43	65.43 ± 0.43	4.81[e] µg

Values within the same column not sharing the same superscript letter(s) are significantly different ($p < 0.05$).
*Gallic acid equivalent; **inhibitory concentration that inhibits alpha-amylase activity by 50%; ***25 µL/assay

Table 5. Anti-diabetic activity in terms of alpha-glucosidase inhibition and IC_{50} values of extracts from mature mango branches of different varieties compared to anti-diabetic drugs, acarbose, and metformin.

Variety	Phenolic concentration (µg GAE*)					IC_{50}**
	0.4	0.8	1.2	1.6	2.0	
	Alpha-glucosidase inhibition (%)***					
Apple mango	5.12 ± 0.94	12.42 ± 0.22	39.26 ± 0.17	64.38 ± 0.22	77.30 ± 0.17	1.37[a] ug GAE
Carabao mango	2.73 ± 0.51	16.27 ± 0.23	20.02 ± 0.05	64.00 ± 0.05	68.61 ± 0.23	1.47[a] ug GAE
Pico	4.64 ± 0.98	10.49 ± 0.05	27.74 ± 0.28	66.01 ± 0.46	84.45 ± 0.23	1.44[a] ug GAE
Sinaging	1.84 ± 0.19	11.80 ± 0.05	39.74 ± 0.33	69.40 ± 0.42	83.04 ± 0.19	1.34[a] ug GAE
Sipsipin	22.25 ± 0.46	28.44 ± 0.05	50.07 ± 0.05	72.68 ± 0.23	86.88 ± 0.23	1.20[a] ug GAE
Anti-diabetic drug	**Concentration (mg)**					IC_{50}**
	200	400	600	800	1000	
	Alpha-glucosidase inhibition (%)***					
Acarbose	13.16 ± 0.00	30.07 ± 0.00	41.25 ± 0.28	50.28 ± 0.07	57.22 ± 0.07	796[b] ug
Anti-diabetic drug	**Concentration (mg)**					IC_{50}**
	1	2	3	4	5	
	Alpha-glucosidase inhibition (%)***					
Metformin	15.83 ± 0.51	18.30 ± 0.05	23.30 ± 0.15	29.86 ± 0.10	50.72 ± 0.41	4.9[c] mg

Values within the same column not sharing the same superscript letter(s) are significantly different ($p < 0.05$).
*Gallic acid equivalent; **inhibitory concentration that inhibits alpha-glucosidase activity by 50%; ***100 µL/assay

inhibitor than acarbose with IC_{50} of 796 µg and 3000- to 4000-fold better than metformin with IC_{50} of 4.9 mg. The strongest α-glucosidase inhibition activity was observed from the phenolic extract of sipsipin, while the extract from carabao mango exhibited the weakest inhibitory activity.

DISCUSSION

Phenolic compounds are commonly utilized as health supplements as these compounds are known to exhibit numerous health-promoting bioactivities. In this study, the bioactivities of the phenolic compounds extracted from mango branches were assessed. Mango branches, having no significant economic value as these are usually discarded during pruning, were utilized as a source for phenolics in order to explore its possible economic

potential. The evaluation of the bioactivities of phenolic compounds was performed by antioxidant capacity and *in vitro* enzyme inhibition analyses. In explicating the antioxidant capacity of the extracts, DPPH, ABTS, and CUPRAC assays were selected for their sensitivity, reproducibility, rapidness, and simple equipment requirement. DPPH free radical and ABTS cation radical scavenging assays were utilized to assess the radical scavenging ability. CUPRAC test, on the other hand, was used to assess the electron-donating ability of phenolic compounds. These are some of the mechanisms of the oxidization reaction inhibition; performing more assays will provide a better evaluation of the antioxidant activity of phenolic compounds. The anti-diabetic property of the extracts was evaluated thru α-amylase and α-glucosidase inhibition assays since type 2 diabetes can be regulated by controlling the absorption of glucose through inhibition of pancreatic α-amylase and intestinal α-glucosidase.

Based on the assays performed, young and mature mango branch extracts were found to have good free-radical scavenging capacities, reducing power, and α-amylase and α-glucosidase inhibition activities. The relationship of the variety of mango branches on the bioactivities of phenolic compounds was evaluated. The effect of maturity was also analyzed but was limited to carabao mango and pico varieties only. Significant differences exist among varieties and between stages of maturity in the total antioxidant and anti-diabetic capacities of mango branches. Mature branches of carabao and pico appear to be better antioxidants than the young branches. Similar observations were reported on other plants. Mango fruits, as well as strawberries and apples, were reported to show antioxidant properties that are strongly influenced by genotype (Ma *et al.* 2011; Scalzo *et al.* 2005). Stems of bear garlic showed an increase in DPPH and ABTS antioxidant capacities as the plant matures (Lachowicz *et al.* 2016). Additionally, Rahman *et al.* (2013) reported that old and dried *Solanum tarvum* exhibited higher antioxidant activity compared to young and fresh fruits. Results of Ghasemnezhad *et al.* (2011) also determined that the antioxidant property of *Capsicum annuum* significantly increased with maturation regardless of cultivars.

No clear trend was observed on the effect of maturity of branches on the anti-diabetic activity of the extract. Examining more varieties of mango will provide a reliable trend in the relationship between maturity and anti-diabetic activities. A study on the hypoglycemic potential of extracts from *Citrus medica* L. cv Diamante endocarp revealed that mature fruits had a better α-amylase inhibition than immature fruits (Menichini *et al.* 2011). Araceli *et al.* (2020) were able to determine that 15-d-old *Cucurbita ficifolia* fruit had a better hypoglycemic effect than the anti-diabetic drug glibenclamide.

Antioxidant capacity exhibited from plants can be accounted for by the phytochemicals present like phenolics, anthocyanin, and flavonoids (Khammuang and Sarnthima 2011). The polyphenols gallic acid, 3,4-dihydroxy benzoic acid, benzoic acid, gallic acid methyl ester, gallic acid propyl ester, benzoic acid propyl ester, catechin, epicatechin, and the predominant xanthone mangiferin have been identified in mango stem bark (Masibo and He 2008). These phenolic compounds are affected by the year of harvest, growth period, and storage conditions. The distinctions in the phenolic compounds present among fruits may cause the variances in the antioxidant capacity since each phenolic compound have different molecular structures, particularly different arrangement of the hydroxyl group (Wojdyło *et al.* 2016). This might explain the observed variance in the phenolic bioactivities of the different varieties or different stages of maturity of mango branches.

The antioxidant capacity assays showed that phenolics from mango branch extracts gave higher antioxidant capacities than the reference antioxidant (ascorbic acid). The DPPH radical scavenging activities of all mango branch extracts are significantly higher (lower EC_{50}) than those reported by Mokhtar *et al.* (2014) on "Chok Anan" mango fruit extract, which indicated maximum antioxidant activity of 6.85 mg/mL (IC_{50}). The inhibitory activities of the phenolics from mango branches towards α-amylase and α-glucosidase, albeit variable depending on the variety, all exhibited better enzyme inhibitory activities compared to that of the known anti-diabetic drugs, acarbose, and metformin. These findings possess high practical implication as it highlights the strong economic potential of the utilization of mango branches as a source for phenolic compounds.

The structures of some phenolic compounds present in the extracts allow them to have anti-diabetic effects due to their solubility, stability, and bonding capacity with the digestive enzymes (Wojdyło *et al.* 2016). In an oral glucose tolerance test performed by Tombozara *et al.* (2020), mice that received methanol extract of *Vaccinium secundiflorum* Hook. (Ericaceae) leaf and stem showed a significant blood-glucose-lowering effect. Song *et al.* (2002) also showed that flavonoids, specifically quercetin, decreased hyperglycemia in diabetic rats. This is due to its ability to modulate glucose transport through their intestinal transporter, thus improving glucose metabolism and reducing oxidative stress. With the potent inhibitory activity towards α-amylase and α-glycosidase exhibited by the phenolic compounds from mango branch extracts, it would be interesting to pursue more detailed animal studies in the future.

Interestingly, the α-glycosidase inhibitory activity of the phenolics from mango branches was stronger than

its α-amylase inhibitory activity. This finding is in contrast to the findings of Wojdyło *et al.* (2016), who reported that extracts from fig fruits showed stronger inhibitory activity towards α-amylase while only moderate inhibition to α-glycosidase. These observations might be due to the different composition of the phenolics found in these plants. As mentioned earlier, different phenolic compounds present in the plant extracts can cause variation in the antioxidant properties due to the differences in their molecular structure. The type of phenolic compounds found in mango branches varies from that in fig fruits, which may variably influence the solubility, stability, and bonding capacity with the digestive enzyme. This could also explain the opposite pattern of α-amylase inhibitory activity of extracts from mature pico branches, which deviates from the pattern observed in other mango cultivars.

The α-glucosidase inhibitory activity being greater than the α-amylase inhibitory activity might be beneficial. Pancreatic α-amylase catalyzes the hydrolysis of starch into simple monosaccharides in the digestive system. The α-glucosidases further degrade these simple monosaccharides to glucose, which enters the bloodstream on absorption. Thus, a stronger α-glucosidase inhibitory activity might delay glucose uptake better and, consequently, reduce blood sugar levels (Algahtani *et al.* 2020).

Finally, the findings that showed that the phenolic compounds from the varieties with lesser market value such as apple mango, pico, sinaging, and sipsipin have better bioactivities than that of the more common variety, carabao mango is also a significant highlight of this study. This has a good practical implication as the utilization of mango branches as a source of phenolic compounds would not compete with the utilization of the common carabao mango cultivar. Moreover, it would add the economic potential of the lesser-known varieties as good sources of polyphenols and their conversion into a valuable product. Comparative analysis of the bioactivities relative to the maturity of mango branches was limited to carabao mango and pico varieties. Based on the two varieties analyzed, mature branches have better bioactivities than young branches. While this finding is not highly desirable as most pruned branches are young branches, the better bioactivities of both extracts from young and mature branches compared to the known antioxidant and anti-diabetic drugs sufficiently negate this issue.

CONCLUSION

Assessment on the bioactivities of the phenolic extracts proved that mango branches, a waste of mango production, can promote higher antioxidant and anti-diabetic activities than the reference compounds; thus, they may have economic relevance since it is a potential source of bioactive agents for food and medical supplements. In addition, this is the first study – to the best of our knowledge – evaluating the antioxidant and anti-diabetic capacities of the extracted phenolics from mango branches as influenced by their variety and maturity. The availability and high level of bioactivities of phenolics in apple mango, pico, sinaging, and sipsipin – the varieties with low market value – could lead to the development of phenolic-based products and can provide additional revenues to farmers.

Given the potential of mango branches as a source of bioactive compounds, it is recommended to conduct further studies to identify the specific compounds that are responsible for their bioactivities. The relationship of maturity with the antioxidant and anti-diabetic capacity should also be studied in other varieties to determine the actual influence of this factor. It is also recommended to determine the effect of maturity on the α-glucosidase inhibition activity, which was not carried out in the study due to limited reagents. Toxicity risk associated with the use of phenolics extracted from mango branches in food and medical supplement should also be considered.

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REFERENCES

ALGAHTANI AS, HIDAYATHULLA S, REHMAN MT, ELGAMAL AA, AL-MASSARANI S, RAZMOVSKI-NAUMOVSKI V, ALGAHTANI MS, ELDIB RA, ALAJMI MF. 2020. Alpha-amylase and alpha-glucosidase enzyme inhibition and antioxidant potential of 3-oxolupenal and katanonic acid from *Nuxia oppositifolia*. Biomolecules 10(1): 61.

ALI ASGAR M. 2012. Anti-diabetic potential of phenolic compounds: a review. International Journal of Food Properties 16(1): 91–103.

ALPINAR K, ÖZYÜREK M, KOLAK U, ARAS C, ALTUN M, ÇELIK SE, BERKER KI, BEKTAŞOĞLU B, REŞAT A. 2009. Antioxidant capacities of some food plants wildly grown in Ayvalik of Turkey. Food Sci Technol Res 15(1): 59–64.

ARACELI M-H, ELSA B-M, JOSÉ RAMÓN V-C, GERARDO B-F, GLORIA MARIBEL T-A. 2020. Hypoglycemic effect and bioactive compounds associated with the maturity stages of *Cucurbita ficifolia Bouché* fruit. Journal of the Science of Food and Agriculture. DOI: https://doi.org/10.1002/jsfa.10566

[DA-BPI] Department of Agriculture–Bureau of Plant Industry. n/d. Mango Production Guide. Retrieved on 29 May 2020 from http://bpi.da.gov.ph/bpi/images/Production_guide/pdf/Mango.pdf

GHASEMNEZHAD M, SHERAFATI M, PAYVAST GA. 2011. Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. Journal of Functional Foods 3(1): 44–49.

KAJDŽANOSKA M, PETRESKA J, STEFOVA M. 2011. Comparison of different extraction solvent mixtures for characterization of phenolic compounds in strawberries. Journal of Agricultural and Food Chemistry 59(10): 5272–5278.

KHAMMUANG S, SARNTHIMA R. 2011. Antioxidant and antibacterial activities of selected varieties of Thai mango seed extract. Pak J Pharm Sci 24(1): 37–42.

LACHOWICZ S, KOLNIAK-OSTEK J, OSZMIAŃSKI J, WIŚNIEWSKI R. 2016. Comparison of phenolic content and antioxidant capacity of bear garlic (*Allium ursinum* L.) in different maturity stages. Journal of Food Processing and Preservation 41(1): 1–10.

MARTILLANES S, ROCHA-PIMIENTA J, CABRERA-BAÑEGIL M, MARTÍN-VERTEDOR D, DELGADO-ADÁMEZ J. 2017. Application of phenolic compounds for food preservation: Food additive and active packaging. IntechOpen 3: 39–58.

MASIBO M, HE Q. 2008. Major mango polyphenols and their potential significance to human health. Comprehensive Reviews in Food Science and Food Safety 7(4): 309–319.

MA X, WU H, LIU L, YAO Q, WANG S, ZHAN R, XING S, ZHOU Y. 2011. Polyphenolic compounds and antioxidant properties in mango fruits. Scientia Horticulturae 129(1): 102–107.

MENICHINI F, LOIZZO MR, BONESI M, CONFORTI F, DE LUCA D, STATTI GA, CINDIO BD, MENICHINI F, TUNDIS R. 2011. Phytochemical profile, antioxidant, anti-inflammatory and hypoglycemic potential of hydroalcoholic extracts from *Citrus medica* L. cv Diamante flowers, leaves and fruits at two maturity stages. Food and Chemical Toxicology 49(7): 1549–1555.

MOKHTAR SI, LEONG PC, VEN LE, AZIZ NAA. 2014. Total phenolic contents, antioxidant activities and organic acids composition of three selected fruit extracts at different maturity stages. J Trop Resour Sustain Sci 2: 40–46.

NAIR SS, KAVREKAR V, MISHRA A. 2013. *In vitro* studies on alpha-amylase and alpha-glucosidase inhibitory activities of selected plant extracts. European Journal of Experimental Biology 3(1): 128–132.

NÚÑEZ SELLÉS AJ, VÉLEZ CASTRO HT, AGÜERO-AGÜERO J, GONZÁLEZ-GONZÁLEZ J, NADDEO F, DE SIMONE F, RASTRELLE L. 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from mango (*Mangifera indica* L.) stem bark aqueous dedoction used in Cuba as an nutritional supplement. J Agric Food Chem 50(4): 762–766.

[PSA] Philippine Statistics Authority. 2019. Crops Statistics of the Philippines. Philippine Statistics Authority, Quezon City, Philippines. 10p.

PHOBOO S, SHETTY K, ABDELKHALIG TEE. 2015. *In vitro* assays of anti-diabetic and anti-hypertensive potential of some traditional edible plants of Qatar. Journal of Medicinally Active Plants 4(3–4): 22–29.

RAHMAN N, MARLIYATI SA, DAMANIK MRM, ANWAR F. 2013. Antioxidant activity and total phenol content of ethanol extract takokak fruit (*Solanum torvum*). Pakistan Journal of Nutrition 12(11): 973–977.

RE R, PELLEGRINI N, PROTEGGENTE A, PANNALA A, YANG M, RICE-EVANS C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine 26(9–10): 1231–1237.

RIBEIRO SMR, BARBOSA LCA, QUEIROZ JH, KNÖDLER M, SCHIEBER A. 2008. Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. Food Chemistry 110(3): 620–626.

SAPIN AB, PEREZ RH, TAMBALO FMZ, GAYLON A, ALAON MKN. 2020. Response surface methodology guided optimization of the extraction of natural phenolics from mango (*Mangifera indica* Linn) branches. Philippine Journal of Science 149(3-b): 1151–1160.

SCALZO J, POLITI A, PELLEGRINI N, MEZZETTI B, BATTINO M. 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition 21(2): 207–213.

SONG J, KWON O, CHEN S, DARUWALA R, ECK P, PARK JB, LEVINE M. 2002. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. The Journal of Biological Chemistry 277(18): 15252–15260.

TOMBOZARA N, DONNO D, RAZAFINDRAKOTO ZR, RANDRIAMAMPIONONA D, RAMANITRAHASIMBOLA D, ANDRIANJARA C, RAMILISON-RAZAFIMAHEFA RD, RAKOTONDRAMANANA DA, BECCARO GL. 2020. The first assessment on antioxidant and antidiabetic activities of leaves and stems of *Vaccinium secundiflorum* Hook. (Ericaceae), an endemic plant of Madagascar. South African Journal of Botany 130: 422–429.

WOJDYŁO A, NOWICKA P, CARBONELL-BARRACHINA ÁA, HERNÁNDEZ F. 2016. Phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits. Journal of Functional Foods 25: 421–432.