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To cite this article: Syarifah Rokiah Syd Kamaruzaman *et al* 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* **864** 012211

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## The Effect of Harvesting Time on the Antioxidant and Antidiabetic Activity of *Piper Crocatum* (Sirih Merah) Extract

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**Abstract.** *Piper crocatum* is traditionally used as a treatment against diabetes mellitus, minor wounds and inflammation. This study aimed to evaluate the effect of harvesting time on the antioxidant and antidiabetic activity of *P. crocatum* harvested at 2, 4, 6 and 8 months and identification of the compounds responsible for antidiabetic activity. The antioxidant activity of the extracts is evaluated by DPPH scavenging activity, total phenolic content (TPC) and total flavonoid content (TFC) tests while the antidiabetic activity is evaluated by alpha amylase and alpha glucosidase inhibition assays. The results show that extracts obtained from harvesting time at 8 months have the highest antioxidant activity with the DPPH scavenging activity, TPC and TFC being  $74.90 \pm 0.27\%$ ,  $492.92 \pm 1.40$  mg GAE/g and  $79.58 \pm 1.17$  mg QE/g respectively and the highest antidiabetic activity with the alpha amylase and alpha glucosidase inhibition activity being  $87.05 \pm 0.64\%$  and  $88.70 \pm 0.42\%$  respectively. Principal component analysis (PCA) was also carried out and it is found that principle component 1 and 2 are associated with the harvesting time (81.7%) and extract concentration (12.4%) respectively. Five compounds are identified to be responsible for antidiabetic activity: caffeic acid, p-coumaric acid, cyanidin-3-O-glucoside, tannin and gallic acid.

### 1. Introduction

Diabetes mellitus is a major health problem that is characterized by hyperglycemia with the disruption of lipid, protein and carbohydrate metabolism resulting from deficiency in insulin secretion, insulin action or both [1]. The number of diabetes mellitus adult patients worldwide is expected to increase from 415 million in 2015 to 642 million on 2040 [2]. The number of diabetes mellitus patients in Malaysia is also expected to increase to 2.48 million in 2030 compared to 0.94 million in 2000 [3]. One of remedial to prevent diabetes mellitus is by decreasing post prandial increase in blood glucose level [4]. Various medicinal drugs have been used to treat Type 2 diabetes mellitus including sulfonylureas, biguanide, and thiazolidinediones [5] but they can cause undesirable side effects. Alternatives like medicinal plants are worth consideration because they are typically effective, non-toxic and have little or no side effects [6].

More than 1200 medicinal plants have been reported for the treatment of diabetes mellitus [7]. *P. crocatum* is a medicinal plant that have properties as an antidiabetic, anticancer, antiseptic and anti-inflammatory [8]. Ethanol extract of *P. crocatum* contained phytochemical compounds such as alkaloids, steroids and tannins which is responsible for diabetes mellitus [9]. Decoction of *P. crocatum* have phytochemicals alkaloid, flavonoid and tannin which known to be antioxidant and antidiabetic



compounds [10]. Previous study by Tomson & Kruma [11] found that harvesting time influence antioxidant activity and chemical composition, as well as the content of compounds and properties vary from plant development stages. It has been described in the literature that developmental stage and reproductive parts influence the antioxidant activity in *Hypericum pruinatum* [12]. Green & Mitchell [13] reported that specific harvesting periods effect level of curcumin compound in *Curcuma Longa*. There is little information specifically on the antioxidant and antidiabetic activity of extracts obtained from different harvesting time of *P. crocatum*. Therefore, this study aims to investigate the effects of harvesting time on the antioxidant and antidiabetic activity of *P. crocatum* extract and identify the antidiabetic compounds in the extract.

## 2. Material and methods

### 2.1. Plant preparation and extraction

*P. crocatum* was planted at the Institute of Sustainable Agrotechnology (INSAT), Universiti Malaysia Perlis (UniMAP). Stems cuttings (8-10 cm) were planted in polybags with topsoil as the media. Sprinklers were used to water the plants twice a day. The harvesting of *P. crocatum* leaves was carried out every 2 months (2, 4, 6 and 8 months). The leaves were cut and washed thoroughly under running tap water to remove contaminants and then dried in an oven at 40 °C for 3 days. The leaves were then ground down into small pieces using a heavy-duty blender before the extraction process. The *P. crocatum* extracts are obtained using the pressurised hot water extract (PHWE) method. Briefly, 2 g of the grounded dried sample is placed in a vessel with 50 ml of distilled water. The sample is then heated at 125 °C for 20 minutes. All extracts are kept at 4 °C until analysis. Each extraction process is carried out in triplicate.

### 2.2. Antioxidant Activity

#### 2.2.1. DPPH Scavenging Activity

The free radical scavenging activity of *P. crocatum* extract is measured by DPPH assay [14]. 2 mL of 0.1 mM DPPH in methanol is added into 200 µL of 5, 10 or 20 mg/ml plant extract and 800 µL methanol. The mixture is shaken vigorously and allowed to stand at room temperature for 30 minutes. The absorbance of the sample is measured at 517 nm using a UV-Vis spectrophotometer. Methanol is used to set the absorbance to zero. A blank sample containing the same amount of methanol and DPPH is used as the reference. Each assay is performed in triplicate. The DPPH scavenging activity is determined based on the formula:

$$\text{DPPH scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where 'Ac' is the absorbance of control and 'As' is the absorbance of sample.

#### 2.2.2. Total Phenolic Content (TPC)

The TPC of *P. crocatum* extract is measured using the Folin ciocalteu method [14]. 200 µL of 5, 10 or 20 mg/ml extract is added into 200 µL of Folin reagent and 1.58 mL distilled water. After 4 minutes, 1 mL of 20% sodium carbonate is added and the mixture is stirred thoroughly. The mixture is allowed to stand for 2 hours in the dark at ambient conditions. Then, the absorbance of the sample is measured at 760 nm using the UV-Vis spectrophotometer. Folin reagent without the addition of the extract is used as the control. Gallic acid is used as a standard and the results are expressed as mg gallic acid equivalent (GAE)/g dry weight (DW). A calibration curve is prepared using gallic acid concentrations of 100, 200, 300, 400 and 500 ppm. Each test is carried out in triplicates.

### 2.2.3. Total Flavonoid Content (TFC)

The TFC of *P. crocatum* extract is measured using the aluminium chloride colorimetric method [15]. In brief, 1 ml of 5, 10, or 20 mg/ml extract is added into 4 ml of distilled water. After 5 minutes, 300  $\mu$ l of 5% sodium nitrate and 300  $\mu$ l aluminium chloride are added into the mixture. Then after 6 minutes, 2 ml of 1 M sodium hydroxide is added into the mixture. The mixture is shaken thoroughly and the absorbance of sample is measured at 510 nm using UV-Vis spectrophotometer. Quercetin is used as the standard and the results are expressed as mg quercetin equivalents (QE)/ g dry weight (DW). The calibration curve was prepared using quercetin concentrations of 20, 40, 60, 80, and 100 ppm. Each test is carried out in triplicates.

## 2.3. In vitro Antidiabetic Assay

### 2.3.1. Alpha Amylase Inhibition Assay

The alpha amylase inhibition assay method was adopted from [1]. In brief, 1 mL of 5, 10 or 20 mg/ml extract is added to a test tube followed by 1 mL of 0.1 M sodium phosphate buffer (pH 6.9) and 100  $\mu$ L alpha amylase enzyme. The mixture is then incubated at 37 °C for 10 minutes. A starch solution in 0.1 M sodium phosphate buffer (pH 6.9) is added to the tube every 10 minutes. The reaction is finally terminated by adding 1 mL of dinitrosalicylic acid (DNS) reagent and incubating in boiling water for 5 minutes followed by cooling to room temperature. The content of each test tube is diluted with distilled water and the absorbance measured at 540 nm. A positive control is prepared using metformin instead of the plant extract and enzyme in 0.1 M phosphate buffer. The alpha-amylase inhibitory activity (%) is calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{Ac - As}{Ac} \times 100 \quad (2)$$

where 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample.

### 2.3.2. Alpha Glucosidase Inhibition Assay

The alpha glucosidase inhibitory assay method was adopted from [16] with some modifications. Alpha glucosidase enzyme is dissolved into 0.1 M pH 6.9 phosphate buffer to a concentration of 0.75 units / ml and 2mM p-nitrophenyl- alpha-D-gluco pyranoside (PNPG) substrate is prepared in 0.1 M phosphate buffer (pH 6.9). The reaction mixture consists of 500  $\mu$ l of the substrate solution, 500  $\mu$ l of 0.1 M pH 6.9 phosphate buffer and 500  $\mu$ l of 5, 10, or 20 mg/ml extract. The reaction mixture is incubated at 37 °C for 5 minutes, then 1 ml of enzyme is added and the mixture is further incubated for another 15 minutes. The reaction is then terminated by adding 1 ml of 0.1 M sodium carbonate. The resultant p-nitrophenol content was determined by measuring the absorbance at 405 nm. A blank mixture not containing the enzyme is used to correct background absorbance. Metformin is used as a positive control. Each assay is carried out in triplicate. The percentage inhibition of alpha glucosidase is calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{Ac - As}{Ac} \times 100 \quad (3)$$

where 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample.

### 2.3.3. Correlation between DPPH Scavenging Activity, TPC, TFC, Alpha Amylase Inhibition and Alpha Glucosidase Inhibition

Correlation between the mean results of the DPPH scavenging activity assay, TPC test, TFC test, alpha amylase inhibition assay and alpha glucosidase inhibition assay is measured by carrying out multivariate analysis using JMP 13.0. The loading and score plot is used to evaluate the correlation.

### 2.4. Analysis of antidiabetic compounds

The chromatographic profiles of the extracts are determined using LCMS (micrOTOF II, USA). Separation is carried out using Zorbax SB-C18 column (3.5  $\mu$ m, 150 x 2.1 mm). The column temperature is set to 25 °C. Gradient elution is used with ultrapure water containing 0.25% formic acid and methanol containing 0.25% formic acid. The flow rate used is 0.2 ml/min and the injection volume is 5  $\mu$ l. The diode array detector is set at 210-370 nm. Electrospray ionization technique is used and positive ions are detected by scanning from 50 to 2000 m/z while negative ions, from 50 to 1500 m/z.

### 2.5. Statistical Analysis

All analyses are carried out in triplicate and the results are expressed as mean values with standard error of the means (SEM). The differences among means are calculated by analysis of variance (ANOVA) using JMP 13.0, and the average values are compared using the Tukey test ( $p < 0.05$ ).

## 3. Results and Discussions

### 3.1. Antioxidant Activity

#### 3.1.1. DPPH Radical Scavenging Activity

The DPPH radical is widely used in assessing free radical scavenging activity because of the ease of reaction [17]. The DPPH scavenging activity of extracts at different harvesting time (2, 4, 6 and 8 months) is measured and the results are tabulated in table 1.

**Table 1.** DPPH scavenging activity of *P. crocatum* extracts from different harvesting time

Harvesting time (months)	Sample concentration (mg/ml)		
	5	10	20
	DPPH scavenging activity (%)		
2	35.59 $\pm$ 1.98 <sup>f</sup>	58.79 $\pm$ 3.37 <sup>cd</sup>	67.02 $\pm$ 0.23 <sup>abc</sup>
4	43.47 $\pm$ 2.32 <sup>ef</sup>	61.90 $\pm$ 2.90 <sup>c</sup>	71.34 $\pm$ 0.57 <sup>ab</sup>
6	52.11 $\pm$ 0.48 <sup>de</sup>	66.72 $\pm$ 1.74 <sup>abc</sup>	74.50 $\pm$ 1.49 <sup>a</sup>
8	65.06 $\pm$ 0.61 <sup>bc</sup>	67.17 $\pm$ 1.61 <sup>abc</sup>	74.90 $\pm$ 0.27 <sup>a</sup>

Data are expressed as means  $\pm$  standard error (SE) (n=3). Mean values that do not share a letter are significantly different with  $p < 0.05$ .

The results show that DPPH scavenging activity increases with extract concentration with the values ranging from 35.59 to 74.90%. The most significant difference ( $p < 0.05$ ) in DPPH scavenging activity were obtained from harvesting time on 6 and 8 months to a concentration of 20 mg/ml. Parallels can be drawn from [18] who found that Sabah Snake grass leaves extracts have the best DPPH scavenging activity if the plant was allowed to matured for 1 year prior to extraction. Ghasemzadeh et al. [19] also found antioxidant activity to be the maximum in nectarine leaves extract if the plant was the last harvest of the season. Brahmi et al. [20] reported that the physiological stage of the plant affects the quality of the extracts. Karami et al. [21] found many different factors affect the antioxidant activity of extracts such as the composition of the antioxidant and pro-oxidant molecules, their synergistic effects and the methods used to measure antioxidant activity. Therefore, it can be concluded that the antioxidant capacity of *P. crocatum* leaves is markedly influenced by environmental conditions.

### 3.1.2. Total Phenolic Content (TPC)

In the TPC test, phenolic compounds are oxidised by phosphotungstic and phosphomolybdic acids present in the reagent and the solution develop a blue colour. The intensity of the blue colour can be correlated to the TPC present in the extract [22]. The TPC of *P. crocatum* extracts obtained from different harvesting time (2, 4, 6 and 8 months) is measured and the results are tabulated in table 2.

**Table 2.** TPC of *P. crocatum* extracts from different harvesting time.

Harvesting time (months)	Samples concentration (mg/ml)		
	5	10	20
	TPC (mg GAE/g)		
2	78.43±1.01 <sup>h</sup>	121.18±3.69 <sup>g</sup>	234.44±2.53 <sup>d</sup>
4	77.78±0.96 <sup>h</sup>	139.66±1.93 <sup>f</sup>	318.79±3.66 <sup>c</sup>
6	112.99±1.69 <sup>g</sup>	224.37±0.43 <sup>e</sup>	380.67±0.25 <sup>b</sup>
8	114.51±1.01 <sup>g</sup>	227.85±1.00 <sup>de</sup>	492.92±1.40 <sup>a</sup>

Data are expressed as means ± standard error (SE) (n=3). Mean values that do not share a letter are significantly different with p<0.05.

The measured TPC ranges from 78.43 ± 1.01 to 492.92 ± 1.40 mg GAE/g. The TPC is found to increase significantly (p<0.05) from 2 to 8 months harvesting time. These results reflect those of Mhamdi et al. [23] who found the TPC of *Borago officinalis* seed extracts to be the maximum when the seeds are at the last stages of maturation. Noriham et al. [24] however found the TPC in *Cosmos caudatus* leaves to be the highest when the leaves are young. They stated that this occurs because the phenolic compounds in the plant are increasingly converted into secondary metabolites by enzymes with age. Nobosse et al. [25] on the other hand, found that TPC in *Moringa oleifera* leaves increase with age due to their active biosynthesis and accumulation in the cells during plant growth.

Other works reported that TPC to be dependent on pre- and post-harvest factors such as species (intraspecies and interspecies differences), environmental characteristics (climatic conditions, humidity and brightness), agronomic features (soil, water supply, use of fertilizers or manure), ripeness, harvesting and transportation method, storage, drying process, and extraction method [26]. The increase in TPC is result from their active biosynthesis and accumulation in the cells during plant growth [25]. The results obtained in this study reinforces the notion that synthesis and accumulation of phenolic compounds is influenced by harvesting time.

### 3.1.3. Total Flavonoid Content (TFC)

The flavonoids contained in *P. crocatum* extracts can be analysed using the AlCl<sub>3</sub> colorimetric method. AlCl<sub>3</sub> forms a complex with the flavonoid and turns the solution yellow in colour. The intensity of the yellow colour can be correlated to the TFC present in the extract. table 3 reveals the statistical differences between TFC of extracts from plants of different harvesting time.

**Table 3.** TFC of *P. crocatum* extracts from different harvesting time

Harvesting time (months)	Samples concentration (mg/ml)		
	5	10	20
	TFC (QE/g)		
2	15.87±0.08 <sup>i</sup>	24.79±0.36 <sup>g</sup>	41.49±0.46 <sup>d</sup>
4	16.25±0.23 <sup>i</sup>	24.77±0.28 <sup>g</sup>	49.67±0.13 <sup>c</sup>
6	21.79±0.17 <sup>h</sup>	27.38±0.17 <sup>f</sup>	52.94±0.03 <sup>b</sup>
8	25.99±0.48 <sup>fg</sup>	37.41±0.17 <sup>e</sup>	79.58±1.17 <sup>a</sup>

Data are expressed as means ± standard error (SE) (n=3). Mean values that do not share a letter are significantly different with p<0.05.

The TFC values are found to range from  $15.87 \pm 0.08$  to  $79.58 \pm 1.17$  QE/g. The TFC of the plant extracts increase significantly ( $p < 0.05$ ) with harvesting time and concentration. The highest TFC was found in plant harvested at 8 months at a concentration of 20 mg/ml. This is consistent with the work of Ghasemi et al. [19] which found that nectarine leaf extracts have the maximum TFC if the leaves are from the third harvest of the cycle. This is however in contrast with Dian-Nashiela et al. [22] which found that *C. caudatus* leaves contain the maximum TFC when the leaves are young. Cezarotto et al. [26] reported that phytochemical composition varies according to the stages of the plant growth and the plant maturity status is reflected in the physiological, biochemical and structural processes of the plant tissue. The accumulation of flavonoids in mature plants is due to the fact that during this stage, the plant is largely protected by phenolics which are highly accumulated during this stage [20]. In conclusion, the harvesting time of the plant impacts the accumulation of flavonoids in *P. crocatum* leaves.

### 3.2. Antidiabetic Activity

#### 3.2.1. Inhibition of Alpha Amylase Activity

The antidiabetic activity of *P. crocatum* extract obtained from plants of different harvesting time (2, 4, 6 and 8 months) is analysed using alpha amylase assay. The results are presented in table 4.

**Table 4.** Inhibition of alpha amylase activity in different harvesting time of *P. crocatum*.

Harvesting (months)	time	Samples concentration (mg/ml)		
		5	10	20
Inhibition (%)				
2		26.67±0.21 <sup>i</sup>	40.66±0.24 <sup>g</sup>	51.83±0.44 <sup>f</sup>
4		31.11±1.05 <sup>h</sup>	63.84±0.66 <sup>d</sup>	70.82±0.45 <sup>d</sup>
6		80.41±1.34 <sup>c</sup>	80.43±0.70 <sup>c</sup>	81.37±0.32 <sup>c</sup>
8		82.84±0.97 <sup>bc</sup>	85.73±0.70 <sup>ab</sup>	87.05±0.64 <sup>a</sup>

Data are expressed as means  $\pm$  standard error (SE) (n=3). Mean values that do not share a letter are significantly different with  $p < 0.05$ .

The inhibitory activity against alpha amylase enzyme is found to increase significantly ( $p < 0.05$ ) with concentration. The highest inhibition activity (87.05%) was found in harvested plant at 8 months at a concentration of 20 mg/ml. This result can be explained by the higher polyphenolic content in older leaves being able to bind with more carbohydrate hydrolysing enzymes, therefore inhibiting them [1, 27]. Coman et al. [28] explains that the antioxidant property of the compounds also acts synergistically with its hypoglycaemic property to exert an overall antidiabetic action. It is also possible that the results are due to other factors such as geographical region, maturity stages, climate, and type of soil. Therefore, it is concluded that the harvesting time of the plant extract is obtained from impacts the alpha amylase inhibition of the extract.

#### 3.2.2. Inhibition Alpha Glucosidase Activity

The effect of different harvesting time on the alpha glucosidase inhibition activity of the plant extract is presented in table 5.

The *P. crocatum* extracts show a significant difference in activity against alpha glucosidase enzymes with different harvesting time and concentration. There is also a significant interaction ( $p < 0.05$ ) between the effects of harvesting time and concentration on alpha glucosidase inhibition. Extracts at 20 mg/ml concentration obtained from 8 and 6- months harvested extracts are found to have significantly better or equal inhibition activity against alpha glucosidase. These results agree with an earlier study by Verma et al. [27] which showed that the alpha glucosidase inhibitory activity of *Vigna aconitifolia* increasing with harvesting time. The active substance in the extract responsible for alpha glucosidase inhibition is affected by many factors such as harvest time, cultivation, storage conditions, processing, climate, and

genetic background [11]. In conclusion, there is a statistically significant effect of harvesting time on the alpha glucosidase inhibition activity of the extract.

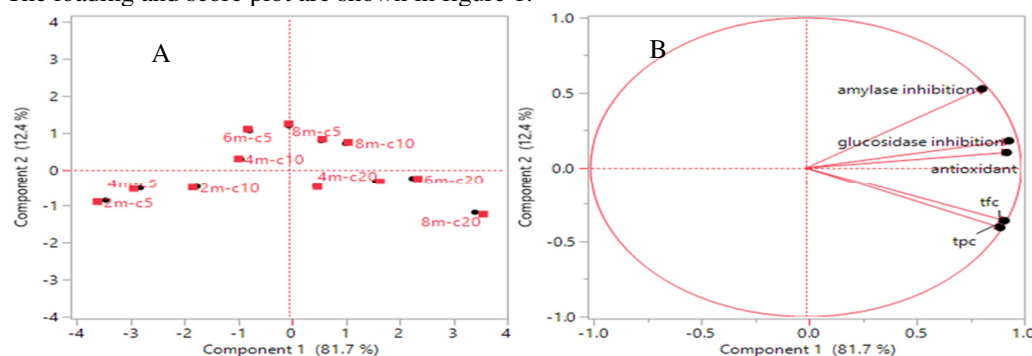
**Table 5.** Inhibition of alpha glucosidase activity in different harvesting time of *P. crocatum*

Harvesting time (months)	Samples concentration (mg/ml)		
	5	10	20
	Inhibition (%)		
2	46.65±0.63 <sup>g</sup>	53.16±1.85 <sup>f</sup>	82.47±1.16 <sup>bc</sup>
4	54.98±0.67 <sup>f</sup>	59.87±0.85 <sup>e</sup>	86.11±0.19 <sup>ab</sup>
6	72.70±0.50 <sup>d</sup>	80.84±0.53 <sup>c</sup>	88.22±0.29 <sup>a</sup>
8	75.57±0.44 <sup>d</sup>	83.81±0.53 <sup>bc</sup>	88.70±0.42 <sup>a</sup>

Data are expressed as means ± standard error (SE) (n=3). Mean values that do not share a letter are significantly different with p<0.05.

### 3.3. Correlation between DPPH Scavenging Activity, TPC, TFC, Alpha Amylase Inhibition and Alpha Glucosidase Inhibition Activities

Principal component analysis is used to better elucidate the relationship among the analysed variables. The loading and score plot are shown in figure 1.



**Figure 1.** (A) Loading plot of *P. crocatum* (B) Score plot of *P. crocatum*

The variables considered are DPPH scavenging activity, TPC, TFC, alpha amylase assay and alpha glucosidase assay in different concentration and harvesting time. The first principal component accounts for 81.7% and the second principal component accounts for 12.4% of the total variance. PC1 is associated with harvesting time while PC2 is associated with concentration. The most influential variables responsible for the clustering are identified using the loading plot. The 8-months extracts and 20 mg/ml extracts are clustered on the positive side of PC1. No correlation was observed between the variables for 2 and 4-months extract at concentrations lower than 20 mg/mL. High values for DPPH scavenging activity, TPC, TFC, and alpha amylase assay are the most important factors for the clustering of the 8-months samples (8m-c5, 8m-c10 and 8m-c20). This finding agrees with several other studies that have shown matured plants to have better antioxidant and antidiabetic activities [29-30]. High values for DPPH scavenging activity, TPC, TFC, alpha amylase inhibition and alpha glucosidase inhibition activity are the most important factors for the clustering of the 20 mg/mL samples. This finding correlates with several other studies that had also found antioxidant and antidiabetic activity to increase with extract concentration [31-32].

### 3.4. Analysis of Antidiabetic Compounds in *P. Crocatum* Leaves Extract

LCMS with electrospray ionisation (ESI) technology has become a mainstay method of analysis in the field of drug development [33]. The 4 and 8 months extracts are further analyzed by LCMS to identify the antidiabetic compounds and the result is presented in Table 6. There are 4 compounds detected in

each extract. The major ions associated with each peak in the spectrum are used to determine the identity of the eluting parent compound. MS analysis reveals the presence of several classes of secondary metabolites. The spectrum for both extracts was found to contain a large peak for a compound that has a retention time of 38.5 mins and a mass/charge ratio of 480.20 m/z and it is attributed to gallic acid [34] in both extracts. Caffeic and p-caumaric acids are identified in the 4 months sample with the mass/charge ratio of 181.16 m/z and 165.06 m/z respectively [35] and cyanidin 3-O-glucoside and tannin are identified in the 8 months sample with a mass/charge ratio of 449.11 m/z and 579.18 m/z respectively [36-37]. Caffeic acid and p-caumaric acid content decreases with *P. crocatum* age as they are consumed by the plant itself. Previous studies have also found caffeic acid in the extracts of *P. nigrum*, *P. longum*, and *P. chaba* [35]. Anthocynins (cyanidin 3-O-glucoside) are secondary metabolites from the flavonoid family [36] which are also the colour pigments found in plants [37]. However, the compound having the mass/charge of 441.19 m/z remains unidentified because there is no prior study for reference. These findings also corroborate previously published works by other researchers who have shown all the compounds identified in this study have antidiabetic properties [38-42].

**Table 6.** Compounds identified by LCMS

Compound	Retention time (min.)	Area		MS (m/z)	Proposed molecular formula	Proposed Compound name	Reference
		4-months	8-months				
1	15.9	294659	-	181.16	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	Caffeic acid	[35]
2	19.9	862968	-	165.06	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	p-Coumaric acid	[35,43]
3	22.6	-	545528	449.11	C <sub>21</sub> H <sub>21</sub> O <sub>11</sub>	Cyanidin 3-O-glucoside	[44]
4	24.0	-	1815010	579.18	C <sub>24</sub> H <sub>22</sub> N <sub>10</sub> O <sub>8</sub>	Tannin	[45]
5	36.0	73379059	64341486	441.19	-	Unidentified	None
6	38.5	74705379	92011531	480.20	C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>	Gallic acid	[34]

#### 4. Conclusions

From this study, it can be concluded that extracts obtained from mature *P. crocatum* that are harvesting time at 8 months have the greatest antioxidant and antidiabetic potential. Five of the six compounds found in the *P. crocatum* extract are identified and are thought to contribute to the antidiabetic property of the plant. Based on these findings, it is recommended that matured leaves of *P. crocatum* be used as a complementary treatment for diabetes mellitus. However, further studies are still necessary to evaluate the potential of *P. crocatum* for clinical applications and make clear any synergistic effect between components as well as their pharmacological mechanism.

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