The antioxidant properties and α-amylase inhibition activities of polyphyto mixture with honey formulations


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Abstract

Ficus carica, Orthosiphon stamineus, Ficus deltoidea and Trigona honey are known for their high antioxidant amount. In this research, these plants and honey were used to study their interactions in terms of the antioxidant properties and the inhibition of α-amylase enzyme. These plants samples were chosen as they are usually used in traditional medicine to treat diabetes. Therefore, the objectives of this study are to measure the antioxidant activity as well as the α-amylase enzyme inhibition properties of the polyphyto formulations and Trigona honey. Simplex Centroid Mixture Design (SCMD) was used to design the formulations. In this experiment, the highest (p<0.05) antioxidant content for 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was a formulation of 100% Trigona honey (90.09%). Meanwhile, a mixture of 50% F. carica – 50% O. stamineus and 50% O. stamineus – 50% Trigona honey yield the highest (p<0.05) total phenolic content (TPC) value (133.77 and 133.17 μg/mL, respectively), which indicated a synergistic interaction effect. However, for the measurement of ferric reduction antioxidant potential (FRAP), the formulation of 100% O. stamineus and 100% F. deltoidea exhibits the highest (p<0.05) value (148.95 and 148.78 μg/mL, respectively). In terms of enzyme inhibition, F. deltoidea showed the highest inhibition activity for α-amylase (8.826%). In conclusion, different percentages of F. carica, O. stamineus, F. deltoidea and Trigona honey have different interaction effects on the antioxidant activity and α-amylase inhibition depending on the amount of each factor.

1. Introduction

People nowadays tend to find alternative medicine instead of only depending on medication prescribed by the doctors or pharmacists. Therefore, indicates a significant increase in sales of herbal supplements due to its numerous health benefits (Rashidi et al., 2019). By years, a lot of researches has been done on finding the plants that may help to reduce or treat diabetes as diabetes is known as the most common global disease where it affects 200 million individual and around 300 million people are at risk by approximate (McCune and Johns, 2002). The alternatives of using plants as substitutes for chemical drugs is due to the research on the bioavailability presence in each plant. Upon generations, F. carica has been said to have abundant of health benefits to human as mentioned in the Holy Qur’an (Marwat et al., 2011).

One of the causes of diabetes is the reduced plasma antioxidant level (Facchini et al., 2000). Antioxidants are mostly obtained from plants and herbs as they may prevent the development of the disease (Thompson and Godin, 1995; McCune and Johns, 2002). According to Kelble (2005), a decreasing amount of plasma antioxidant level increase the induction of the vascular disease seen in type II diabetes. Inhibition activity of α-amylase enzyme can also stunt glucose uptake by the body as they function in promoting the uptake of glucose in the body.

F. carica, O. stamineus and F. deltoidea are the plants used in this experiment. The previous study reported that F. deltoidea may reduce the blood glucose level due to enhancement of glucose uptake in muscle and reduction of hepatic gluconeogenesis (Adam et al., 2007). Plants and herbs formulation are regularly reckoned to have lesser toxicity and does not contribute to any synthetic medicine’s side effects (Malviya et al., 2010). Trigona honey was chosen to be added as it has strong antioxidant activities (Yazan et al., 2016).
Antioxidant compounds such as organic acids, carotenoids, phenolic and vitamin E may inhibit the oxidative mechanisms that will eventually cause degenerative illnesses (du Toit et al., 2001). The purpose of determining the total phenolic content of the formulation is to understand the relationship of the phenolic content to the antioxidant and anti-diabetic properties of the formulation. Therefore, the objectives of this study were to measure the antioxidant and α-amylase enzyme inhibition properties of polyherbal formulations and Trigona honey.

2. Materials and methods

2.1 Chemicals and instruments

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), methanol, Folin-Ciocalteu reagent, sodium carbonate, acetate buffer, glacial acetic acid and TPTZ (2, 4, 6-tripyridyl-s-triazine) were purchased from Merck, Germany. 3, 5-Dinitrosalicylic acid were bought from QReC, Asia while α-amylase were bought from Sigma-Aldrich, USA. The instrument used was UV-Vis spectrophotometer (T60u, PG Instrument, USA) located in Food Analysis Laboratory, UTHM.

2.2 Preparation of leaves extracts

F. carica dried leaves were bought from Formeniaga Company in Selangor while O. stamineus and F. deltoidea dried leaves were bought from Ethno Resources, Sg. Buloh. All of these plants were ground before being extracted to reduce the size. Each plant has a different extraction method based on the optimum yield of extraction for each plant. F. carica was extracted with maceration of 70% ethanol as applied from Vongsak et al. (2013). A total of 165 g of F. carica dried ground leaves were weighed using the weighing machine model TX3202L, Shimadzu, Japan and macerated with 70% ethanol for 20 mins at room temperature. The blank sample was prepared to contain the exact volume of methanol and DPPH, then its absorbance value was measured. The experiment was done in triplicate. The calculation of the percentage inhibition can be expressed using the equation shown:

\[
\text{% inhibition} = \frac{\text{y} - \text{x}}{\text{y}} \times 100
\]

Where \( x \) = the blank absorption at \( t = 0 \) min; and \( y \) = the extract absorption at the 15th mins.

2.3 Development of plants formulation

The formulations are made up of different percentages of mixture generated by a statistical software program, Design Expert® 6.0.4. (Stat-Ease, United States) and it was standardized at 20 g/100 mL (100% w/v) of the formulation. Four components in the mixtures, including the extracts of F. carica, O. stamineus, F. deltoidea and Trigona honey were selected as causal factors. The interaction effects of phytochemicals in antioxidant and α-amylase inhibitory activity were determined by comparing the experimental result with the predicted value according to the method of Palafoux-Carlos et al. (2012), with slight modification. The predicted value is the addition of the individual values of the samples according to each proportion in the formulation.

2.4 DPPH radical scavenging assay

Radical scavenging activity of this formulation was determined spectrophotometrically by using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The DPPH is basically reduced due to the donation of hydrogen from the antioxidant compound when the DPPH reacts with it. The colour changes were measured on a UV-Vis spectrophotometer at 515 nm. In this method, the DPPH solution was incorporated with 77 μL of extract in cuvettes and kept in dark condition for 15 mins at room temperature. The blank sample was prepared to contain the exact volume of methanol and DPPH, then its absorbance value was measured. The experiment was done in triplicate. The calculation of the percentage inhibition can be expressed using the equation shown:

\[
\text{% inhibition} = \left(\frac{\text{Abs} \text{ of blank} - \text{Abs} \text{ of sample}}{\text{Abs} \text{ of blank}}\right) \times 100
\]

Where \( x \) = the blank absorption at \( t = 0 \) min; and \( y \) = the extract absorption at the 15th mins.

2.5 FRAP assay

Ferric reduction antioxidant potential (FRAP) assay was done conferring to Benzie and Strain (1996) with minor modification. Reagents used were 300 mmol/L acetate buffer, pH 3.6 (3.1 g C₆H₁₂NaO₃H₂O), and 16 mL C₆H₁₂O₆ (glacial acetic acid) in 1 L of buffer solution; 10 mmol/L 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mmol/L HCl; 20 mmol/L FeCl₃·6H₂O. Working FRAP...
reagent was prepared as required by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL FeCl₃·6H₂O solution. Warmed (37°C) FRAP reagent (3 mL) and its absorbance was read at 593 nm. About 100 μL of the sample was then incorporated with 300 μL H₂O. Absorbance reading was measured after 4 mins. The experiment was done in triplicate. The absorbance change between the final value and the blank absorption value was quantified for every single formulation and compared with a standard curve. The standard curve was made by using different concentration of the reagents as shown in Table 1. The absorbance value was read at 593 nm after exposed in the dark for 30 mins.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O (μL)</td>
<td>1000</td>
<td>985</td>
<td>970</td>
<td>940</td>
<td>880</td>
<td>820</td>
<td>760</td>
<td>700</td>
</tr>
<tr>
<td>1mM Std (μL)</td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>120</td>
<td>180</td>
<td>240</td>
<td>300</td>
</tr>
<tr>
<td>FRAP (mL)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Std Conc (μM)</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

2.6 Total phenolic content (TPC)

The total phenolic content (TPC) assay was experimented according to Miliaukas et al. (2004). The reference sample was made by taking 1 mL gallic acid solutions aliquots with the concentration of 10, 20, 40 and 80 μg/mL and incorporated with 5 mL of Folin-Ciocalteu reagent (diluted ten-fold) added with 4 mL (75 g/L) sodium carbonate. Absorbance reading was taken at 20°C at 750 nm after 30 mins to determine its calibration curve. About 100 μL of the sample was added to 2 mL sodium carbonate (2 g in 100 mL distilled water) for 2 mins at room temperature before mixing with Folin-Ciocalteu reagent. It was left for 30 mins before taking the reading at 750 nm. The experiment was done in triplicate and the absorbance value was compared to the standard curve.

2.7 α-Amylase inhibitory activity assay

Firstly, 500 μL of each sample and 500 μL of 0.02 M phosphate buffer (pH 6.9) containing porcine pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated for 10 mins at room temperature. Next, 500 μL of 1% starch solution in 0.02 M phosphate buffer (pH 6.9) was incorporated in the mixture solution. Then, the solution was incubated for 10 mins at room temperature before added with 1.0 mL of dinitrosalicylic acid (DNSA). Thereafter, the reaction in the solution was ceased by incubating it for 5 mins in a 100°C water bath before it was let to cool to room temperature. About 10 mL of distilled water was added to dilute the reaction mixture before measuring the absorbance at 540 nm (Worthington, 1993). All other reagents and the enzyme without the test sample was referred as blank sample. The α-amylase inhibitory activity was referred as percentage inhibition.

\[
\text{% inhibition} = \left( \frac{y - x}{x} \right) \times 100
\]

Where \(x\) = the reference sample absorption value; and \(y\) = the test sample absorption value.

2.8 Statistical analysis

The statistical analyses were done by a one-way ANOVA using IBM SPSS Statistics 20. The results were expressed as mean±SD, to show variations in the various experimental conditions. Differences are considered significant when \(p<0.05\).

3. Results and discussion

3.1 DPPH radical scavenging assay

Table 2 shows the results for the DPPH assay. Formulation with 100% *Trigona* honey exhibits the highest scavenging activity with percentage of 90.09% followed by *F. carica* (88.09%) and *F. deltoidea* (81.93%). The antioxidant activity of the honey is attributed from its phenolic and flavonoids structure (Khalil et al., 2011; Muhammad, 2012). This is because *Trigona* honey has bioactive compounds such as polyphenols, organic acids, vitamins, glutathione peroxidase and catalase that aids in improving the antioxidative property (Aljadi and Kamaruddin, 2004). However, formulations that were combined with *Trigona* honey has an antagonistic interaction. These results correspond to a previous study which shows that interaction between flavonoid compounds can work either synergistically or antagonistically (Hidalgo et al., 2010). *O. stamineus* showed the least DPPH activity (24.29%) as compared to other studied samples (*F. carica*, 86.98%; *F. deltoidea* 82.93%; *Trigona* honey, 90.09%). It might be due to the extraction process which was done under high temperature with prolonged time. The previous study showed extraction at higher temperatures and longer extraction time decreased the free radical-scavenging activity of *O. stamineus* extracts (Chew et al., 2011).

3.2 FRAP assay

Based on Table 3, the highest FRAP value was found from 100% *O. stamineus* and 100% *F. deltoidea* (148.95 and 148.78 μg/mL, respectively) with no significant difference \((p<0.05)\). Although formulation 6, 7 and 9 showed synergistic interaction effect, the FRAP values are still lower significantly \((p<0.05)\) than the individual *O. stamineus* and *F. deltoidea* extract. In contrast to formulation 5, indifferent interaction can be
The ability of interaction between phenolic compounds in each mixture showed synergistic interaction effect for TPC content. FRAP value (µg/mL) compared to other formulations because compounds to the antioxidative capacity of the honey due to the contributions of flavonoids and phenolic compounds are high FRAP content if compared to other types of honey contains in F. carica leaves contain phenolic acids such as oxalic, citric, malic, quinic, shikimic and fumaric acids (Oliveira et al., 2008). Other than that, Trigona honey main compounds are salicylic, p-coumaric acid, aromadendrin and taxifolin (Biluca et al., 2020). These findings also supported by Liu (2003) which reported that the synergistic effects of plant combinations may be due to the increased interaction possibilities which allowing more cooperative effects between compounds in the mixtures (Liu, 2003).

3.3 Total phenolic content (TPC)

The highest TPC content would be a mixture of 50% F. carica – 50% O. stamineus and 50% O. stamineus – 50% Trigona honey (Table 4). Notably, all formulation showed synergistic interaction effect for TPC content. This effect might be contributed from the positive interaction between phenolic compounds in each mixture of plant extract that attributed to their antioxidant activity (Muhammad et al., 2012; Widyawati et al., 2017; Rahim et al., 2019). Such phenolic compounds which are eupatorin, sinensetin, 3’-hydroxy-5,6,7,4’-tetramethoxyflavone and rosmarinic acid, were reported contains in O. stamineus (Abullah, 2012). While F. carica leaves contain phenolic acids such as oxalic, citric, malic, quinic, shikimic and fumaric acids (Oliveira et al., 2008). Other than that, Trigona honey main compounds are salicylic, p-coumaric acid, aromadendrin and taxifolin (Biluca et al., 2020). These findings also supported by Liu (2003) which reported that the synergistic effects of plant combinations may be due to the increased interaction possibilities which allowing more cooperative effects between compounds in the mixtures (Liu, 2003).

3.4 α-Amylase inhibitory activity assay

Based on Table 5, highest α-amylase percentage inhibition is exhibited by F. deltoidea (8.826%). This is might be due to the presence of a more potent compound called vitexin, sitosterol and isovitexin which reported could inhibit α-amylase enzyme (Joseph and Raj, 2011; Bakar et al., 2018). The ability of F. deltoidea in

<table>
<thead>
<tr>
<th>No</th>
<th>Description</th>
<th>Predicted value</th>
<th>DPPH scavenging activity (%)</th>
<th>Type of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100% D</td>
<td>-</td>
<td>90.09±0.51a</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>100% B</td>
<td>-</td>
<td>24.29±1.03i</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>100% A</td>
<td>-</td>
<td>86.98±0.51b</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>100% C</td>
<td>-</td>
<td>82.93±1.02c</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>50% A + 50% B</td>
<td>56.19±0.44</td>
<td>60.90±0.05e</td>
<td>Synergistic</td>
</tr>
<tr>
<td>6</td>
<td>50% B + 50% D</td>
<td>57.19±0.46</td>
<td>46.85±0.10d</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>7</td>
<td>50% C + 50% D</td>
<td>86.01±0.53</td>
<td>79.28±0.09d</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>8</td>
<td>33.33% C + 33.33% B + 33.33% D</td>
<td>65.44±0.48</td>
<td>60.00±1.32e</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>9</td>
<td>33.33% A + 33.33% B + 33.33% D</td>
<td>67.49±0.47</td>
<td>57.09±0.55f</td>
<td>Antagonistic</td>
</tr>
<tr>
<td>10</td>
<td>12.5% A + 12.5% C + 12.5% D + 62.5% B</td>
<td>47.69±0.43</td>
<td>40.01±1.33h</td>
<td>Antagonistic</td>
</tr>
</tbody>
</table>

(A) F. carica, (B) O. stamineus, (C) F. deltoidea, (D) Trigona honey. Different superscript within the column indicates significant differences (p<0.05)

<table>
<thead>
<tr>
<th>No</th>
<th>Description</th>
<th>Predicted value</th>
<th>FRAP value (µg/mL)</th>
<th>Type of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100% D</td>
<td>-</td>
<td>119.55±1.342e</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>100% B</td>
<td>-</td>
<td>148.95±0.801a</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>100% A</td>
<td>-</td>
<td>143.73±2.165e</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>100% C</td>
<td>-</td>
<td>148.78±0.394a</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>50% A + 50% B</td>
<td>143.99±1.40</td>
<td>144.11±0.08bc</td>
<td>Indifferent</td>
</tr>
<tr>
<td>6</td>
<td>50% B + 50% D</td>
<td>132.52±1.26</td>
<td>136.46±0.68e</td>
<td>Synergistic</td>
</tr>
<tr>
<td>7</td>
<td>50% C + 50% D</td>
<td>131.51±1.09</td>
<td>139.46±1.75de</td>
<td>Synergistic</td>
</tr>
<tr>
<td>8</td>
<td>33.33% C + 33.33% B + 33.33% D</td>
<td>137.32±0.99</td>
<td>145.18±1.52abc</td>
<td>Synergistic</td>
</tr>
<tr>
<td>9</td>
<td>33.33% A + 33.33% B + 33.33% D</td>
<td>134.69±1.51</td>
<td>141.23±0.98def</td>
<td>Synergistic</td>
</tr>
<tr>
<td>10</td>
<td>12.5% A + 12.5% C + 12.5% D + 62.5% B</td>
<td>143.35±1.02</td>
<td>148.13±0.51ab</td>
<td>Synergistic</td>
</tr>
</tbody>
</table>

(A) F. carica, (B) O. stamineus, (C) F. deltoidea, (D) Trigona honey. Different superscript within the column indicates significant differences (p<0.05)
inhibiting α-amylase enzyme also reported in another study conducted by Adam and his colleagues (2011). It was also found in this study formulation 5 which contains 50% *F. carica* - 50% *O. Stamineus* gives synergistic interaction effect. However, its value of α-amylase inhibitory activity is still lower than individual inhibition of *F. deltoidea*. Farsi *et al.*, (2011) reported there was a direct correlation between antioxidant activity and α-amylase inhibitory activity. Hence, it is concluded that the highest α-amylase inhibitory activity of *F. deltoidea* may be contributed by its high antioxidant compounds.

4. Conclusion

In conclusion, formulation with 100% *Trigona* honey gives the highest DPPH free radical scavenging activity. While the formulation of 50% *F. carica* – 50% *O. stamineus* and 50% *O. stamineus* – 50% *Trigona* honey exhibits the highest TPC content. Other than that, the best formulation for the FRAP assay is 100% *O. stamineus* and 100% *F. deltoidea*. This indicates that these individual extracts and mixtures can be used to regulate free radicals. The highest percentage inhibition of the α-amylase enzyme is achieved by individual formulation of *F. deltoidea* which exhibit high antioxidant activity hence it can be concluded that α-amylase inhibitory activity of *F. deltoidea* may be contributed by the antioxidant compounds. More in-depth studies are required to isolate, classify and analyze the active ingredients of this plant for the management of diabetes.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgements

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