Effect of extracting solvents on phytochemicals content, antioxidant activity and metal chelating property of Mak mao (Antidesma velutinosum)

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Keywords: Extracting solvent, Phenolic, Flavonoid, Antioxidant, Antidesma velutinosum

Introduction

Premature skin aging, appearance of wrinkles or uneven skin tone are the signs of damaged skin which caused by aging, stress, UV exposure and oxidation. In order to prevent or delay those signs, many natural and synthetic substances have been researched and used in the cosmetic product. However, the global marketing trend of using natural products significantly increased in the past years. Thus, various species of plants around the world have been reported and investigated for the potent for cosmetic benefits. Polyphenols and flavonoids are the major chemical constituents of plant extracts. Many research reported that those compounds had the potent to scavenge the ROS (reactive oxygen species), chelate metal ions, inhibit the tyrosinase activity and anti-aging¹,².

Various extraction methods have been utilized in order to obtain those phytochemicals. Generally, the extraction was carried out using water-cosolvent system with different types of organic solvents as a cosolvent such as ethyl acetate, methanol and ethanol³. However, some of the organic solvents are toxic and not safe to use in cosmetic. Therefore, propylene glycol was considered as the alternative solvent for plant extraction which known as hydroglycolic extract⁴. Advantages of propylene glycol are odorless, colorless and not easily volatile under the ambient temperature. Moreover, it has humectant and antimicrobial properties and is safe to use in food and cosmetic.

Antidesma Velutinosum Blume (Thai name, Mak mao) which is a plant widely grown in South East Asia region, including Thailand, Malaysia and Indonesia. Mak mao has been traditionally and commercially used as foods, especially for the fruits which have been produced as healthy juice drink or red wine. The ethanolic extract of Mak mao was reported to have high levels of nutrients and some phytochemicals which showed important for health and beauty benefits⁵. In this work, the hydroglycolic extracts from Mak mao (Antidesma velutinosum) fruit were firstly characterized and studied for its total phenolic and flavonoid contents, in vitro antioxidant and metal ion chelation properties.
Methods

Plant extraction
The whole fruits of *Antidesma velutinosum* (Thai name, Mak mao) were purchased from local market, Ubon Ratchathani, Thailand. The fresh fruits were washed, dried in the oven at 50°C and powdered by electric blender. Mak mao fruit dried powder was macerated in various concentrations of propylene glycol (PG) which were 30%, 50%, 70% and 90% v/v in water. The maceration was performed for three days at room temperature. After three days of maceration, the samples were filtered through cheesecloth (500 mesh), centrifuged at 10,000 rpm and further filtered by using a sheet of Whatman No. 6 filter paper. The obtained hydroglycolic (HG) extracts were separately stored in a closed container until used.

Determination of total phenolic content
The analysis of total phenolic content was carried out by Folin-Ciocalteau’s method. Total phenolic contents were determined by using gallic acid calibration curve. The results are expressed as milligrams of gallic acid equivalence (GAE)/g crude extract.

Determination of total flavonoid content
Aluminum chloride colorimetric assay was used to investigate the total flavonoid content. The procedure was done according to the method of D. Marinova et al with slight modification. Epigallocatechin gallate (EGCG) was used as a standard reference for total flavonoid content analysis. The results of total flavonoid content are expressed as milligrams of epigallocatechin gallate equivalence (EGCG)/g crude extract.

Determination of in vitro anti-oxidant activities
The free radical scavenging potential was determined by DPPH assay. Ascorbic acid was used as a positive control in this study. The percentage of DPPH color change versus a control was calculated to determine the antiradical activity. The results are expressed as the concentration of test sample that scavenged 50% of free radicals (IC₅₀).

Determination of metal chelating activity
Ferrous ions chelating potential was carried out by using ferrozine-Fe²⁺ complex. EDTA was used as a positive control. The results are expressed as the concentration of test sample that chelated 50% of metal ions (IC₅₀).

Statistical analysis
Each treatment was performed in triplicate. The results are expressed as mean ± standard deviation (SD). The significant differences between means were calculated by SPSS software using One-way ANOVA followed by LSD test where the significant difference was considered at p < 0.05.

Results
Phenolic and flavonoid are important for antioxidant and metal chelating activities. Total phenolic and flavonoid contents of Mak mao fruit extracts with the hydroglycolic solvent were firstly determined and antioxidant and metal chelating activities were subsequently studied.

The total phenolic and flavonoid contents of Mak mao fruit extracts were shown in Table 1. Total phenolic and flavonoid contents of Mak mao fruit extracts were ranked in the following order: PG50 > PG70 > PG90. Mak mao PG90 was the only extract showed the statistically significant difference from others (i.e., Mak mao PG50 and Mak mao PG70). However, there was no statistically significant difference between Mak mao PG50 and Mak mao PG70.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic content (mg GAE/g crude extract)</th>
<th>Total flavonoid content (mg EGCG/g crude extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mak mao PG50</td>
<td>7.20 ± 0.59*</td>
<td>12.19 ± 1.01</td>
</tr>
<tr>
<td>Mak mao PG70</td>
<td>5.65 ± 0.11</td>
<td>10.51 ± 2.01</td>
</tr>
<tr>
<td>Mak mao PG90</td>
<td>4.45 ± 0.96</td>
<td>5.73 ± 3.04*</td>
</tr>
</tbody>
</table>

* Significant difference (p < 0.05) among the samples

DPPH radical scavenging results are shown in Table 2. DPPH radical scavenging activities of Mak mao extracts inhibited in a concentration-dependent manner. IC₅₀ values of Mak mao fruit extracts were ranked in the following order: PG90 > PG50 > PG70 > Ascorbic acid. The scavenging activities of Mak mao extracts were significantly lower than ascorbic acid.

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Table 2  DPPH radical scavenging activities of Mak mao fruit extracts* and ascorbic acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>0.02 ± 0.00***</td>
</tr>
<tr>
<td>Mak mao PG50</td>
<td>10.80 ± 0.19’***</td>
</tr>
<tr>
<td>Mak mao PG70</td>
<td>10.34 ± 0.48’***</td>
</tr>
<tr>
<td>Mak mao PG90</td>
<td>14.18 ± 0.83’***</td>
</tr>
</tbody>
</table>

*:’’ Significant difference (p < 0.05) between two groups
*** Significant difference (p < 0.05) among the samples

The capability of the extract to chelate the metal ions was demonstrated by the ability of the extract to chelate the ferrous ion in the presence of ferrozine. In this experiment, all extracts showed the ability to chelate metal ions in a dose-dependent manner. Metal ion chelation activities of Mak mao fruit extracts are shown in Table 3 and IC$_{50}$ values were ranked in the following order: PG50 > PG70 > PG90 > EDTA. Mak mao extracts showed some metal ion chelation activities which were significantly lower than EDTA.

Table 3  Metal ion chelation activities of Mak mao fruit extracts and EDTA

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0.01 ± 0.00’’</td>
</tr>
<tr>
<td>Mak mao PG50</td>
<td>814.88 ± 39.46’’</td>
</tr>
<tr>
<td>Mak mao PG70</td>
<td>733.72 ± 137.98’’</td>
</tr>
<tr>
<td>Mak mao PG90</td>
<td>135.02 ± 4.28’’</td>
</tr>
</tbody>
</table>

*:’’ Significant difference (p < 0.05) among the samples

Discussion

Mak mao extracts obtained from different concentrations of PG in water revealed different quantities of polyphenols. Increasing in PG proportion resulted in less polyphenols obtained. These results could be implied that major polyphenols from Mak mao fruit may be more soluble in water than semi-polar solvent like PG. Referring to the study of Judprasong, et al.$^4$, there were several polyphenols found in Mak mao fruit extract including gallic acid, caffeic acid, ferulic acid and quercetin which are mostly soluble in water. Regarding to total phenolic and total flavonoid contents, PG50 and PG70 were chosen as extracting solvents to obtain higher quantities of polyphenols and flavonoids that possibly offer desired benefits.

In vitro antioxidant activity of Mak mao extracts presented a promising potential to scavenge the free radicals. Although much lower IC$_{50}$ values obtained than that of the control, the extracts were prepared in such a way that are ready for use or in hydroglycolic dilutions. From the study of Jorjong, et al.$^{10}$, 14 cultivars of Mao-Luang (Antidesma bunius), another specie of Mak mao, had been studied for their antioxidant capacities by FRAP, ABTS and DPPH assay which the highest antioxidant capacities was found from Kumlai cultivar where the IC$_{50}$ values were 35.35±8.29 mmol Fe(II)/g dried weight (DW), 46.37±5.66 mmol TE/g DW and 103.04±8.15 mmol VCEAC/ g DW, respectively. Kumlai cultivar had the highest amount of phenolic acids, flavonoids and anthocyanins, the antioxidant activities were related to its phytochemical constituents. In DPPH study, there was no statistical difference between the IC$_{50}$ values of the hydroglycolic extracts of Mak mao fruit even though there were significant differences between total phenolic contents of the hydroglycolic extracts. Thus, not all polyphenols presented in Mak mao fruit had the main property as an antioxidant.

Transition metal ion such as Fe$^{2+}$ has an ability to catalyze the generation of highly reactive hydroxyl radicals$^{11}$. In order to avoid the generation of radicals, chelating agents were used to sequester around the metal ions. According to the report of Pak J.$^{12}$, flavonoids were mentioned to be able to scavenge free radicals or chelate the metal ions while phenolic compounds were the free radical terminators. From the obtained results, Mak mao PG90 provided the highest potential to bind metal ions. Nevertheless, the total phenolic and flavonoid contents of Mak mao PG90 were the lowest among three extracts. Metal chelating activity of Mak mao fruit may mainly contribute to neither polyphenols nor flavonoids and further characterization will be required to gain more understanding.
Conclusion
Mak mao fruit was successfully extracted using PG-water or hydroglycolic system. Different hydroglycolic extracts of Mak mao fruit with various percentages of PG in water revealed vary polyphenol and flavonoid contents. Moreover, these hydroglycolic extracts also showed promising potential to scavenge free radicals and chelate metal ions. From these results, hydroglycolic extracts of Mak mao could be further studied and used for health and beauty benefits.

Acknowledgements
The author would like to acknowledge Professor Dr. Malyn Ungerungsie for her valuable advices and Research & Development Divisions, S&J International Enterprises PCL, Bangkok, Thailand for all supports for this work.

References