Comparative study of antioxidant activities between ethanolic extract and water extract of *Mesona chinensis*

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**Introduction**

Nowadays, people around the world have become more interested in herbs because they have been identified as sources of various phytochemicals, many of which possess powerful antioxidant activity. Antioxidants play an important role in the prevention of free radical formation in the early stages, or interfere with the propagation reaction of free radical chain reactions, which helps in reducing the risk of various diseases.

*Mesona chinensis* (Labiatae) is commonly known as “Hsian-tsao” in China and “Chao-Kuay” in Thailand. It has been widely consumed as a herbal drink and a jelly-type dessert in the tropical region. In Chinese traditional medicine, it has been used to treat heat-shock, hypertension, diabetes and muscle and joint pains. Various biological activities of *M. chinensis* extract have been reported as antioxidant, antihyperglycemia and hypolipidaemic activities. For its chemical constituents, caffeic acid and other phenolic compounds such as vanillic acid, protocatechuic acid, and syringic acid were investigated. The antioxidant capacity of isolated compounds in the extract was also determined using thiocyanate method and the erythrocyte ghost system. It was found that caffeic acid possessed the highest antioxidant activity. In addition, caffeic acid was a potent antioxidant in several in vitro antioxidant assays such as ferric thiocyanate, reducing power, ABTS*⁺* scavenging, and DPPH scavenging assays when compared to synthetic antioxidants, for example, BHA, BHT, α-tocopherol. However, comparative study of antioxidant profile and bioactive compound content of *M. chinensis* extracts obtained from different extraction methods have not been investigated. Therefore, the aim of this study was to determine the antioxidant activities, total phenolic and total flavonoid contents as well as caffeic acid content in water and 50% ethanolic extracts of *M. chinensis*.

**Methods**

**Plant material:** Dried whole plant of *M. chinensis* was kindly provided by Pattanapoonphol Co., Ltd. (Bangkok, Thailand).

**Plant extraction:** The dried plant of *M. chinensis* was cleaned and dried in a tray dryer at 50 °C for 12 hr. It was pulverized into powder and passed through an 18-mesh sieve.

**Decoction:** Twenty grams of *M. chinensis* dried powder was decocted with 400 ml of boiling water in a slow cooker (Otto, Thailand) for 2 hr to obtain water extract.

**Maceration:** Twenty grams of dried powder was macerated with 400 ml of 50% ethanol using a shaker at room temperature for 24 hr to obtain 50% ethanolic extract. The total extraction steps of decoction and maceration were three cycles. The pooled extracts were filtered through Whatman filter paper No. 4 and 1, then concentrated by a rotary evaporator under vacuo at 45-50 °C. The extracts were dried by lyophilizer and stored in a tight container at -20 °C until used.

**DPPH radical scavenging assay:** Each sample was dissolved in deionized water at varying concentrations. In a 96-well plate, 100 μl of sample solution was mixed with 100 μl of DPPH in methanol solution (152 μM). The mixture was incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a microplate reader (Tecan, USA). Ascorbic acid was used as a positive control.
control and treated under the same condition as sample. The percentage of inhibition was calculated from:

\[
\%\text{inhibition} = \left(\frac{A_c - A_s}{A_c}\right) \times 100,
\]

Where \(A_c\) was the absorbance of control solution at 517 nm and \(A_s\) was the absorbance of sample solution at 517 nm. \(IC_{50}\) values were estimated from linear equation of the plot between \%inhibition and concentration. Each determination was done in triplicate and the data was presented as mean ± SD.\(^{13}\)

**Ferric reducing antioxidant power (FRAP) assay:** The test extract (500 µl) was mixed with 500 µl of 0.2 M sodium phosphate buffer (pH 6.6) and 500 µl of 1% (w/v) potassium ferricyanide solution in test tube. The mixture was incubated in water bath at 50 °C for 20 min and then 2 ml of 10% (w/v) trichloroacetic acid was added. In 96-well plate, 100 µl of the solution was mixed with 100 µl of deionized water before the addition of 0.1% (w/v) ferric chloride solution (20 µl). The absorbance was read at 700 nm using microplate reader. The assay was carried out in triplicate. The FRAP value was calculated as mean ± SD and expressed in mmol ferrous sulfate (FeSO₄) equivalents/100 g extract by using the standard curves constructed by the analysis of six different concentrations of ferrous sulfate.\(^{14}\)

**Determination of total phenolic content:** Twenty microliters of sample solution (1 mg/ml) was mixed with 50 µl of Folin-Ciocalteu reagent (diluted 1:10 with deionized water). The mixture was allowed to react for 3 min and 80 µl of 7.5% (w/v) sodium carbonate solution was added. After 2-hr incubation in darkness at room temperature, the absorbance was determined at 765 nm using microplate reader. The experiment was performed in triplicate. Gallic acid was used for the standard curve. The result was expressed as mg gallic acid equivalent (GAE)/g extract.\(^{14}\)

**Determination of total flavonoid content:** One hundred microliters of the test extract (500 µg/ml) was mixed with 100 µl of 2% (w/v) aluminum chloride solution. The mixture was incubated at room temperature for 10 min. The absorbance was measured at 415 nm using microplate reader. The same procedure was repeated for the standard solution of quercetin. Each solution was analyzed in triplicate. The data was presented as mean ± SD in term of mg quercetin equivalent (QE)/g extract.\(^{14}\)

**Determination of caffeic acid content by HPLC:** \(M.\ chinensis\) extract from both extraction methods (0.1 g) were accurately weighed and dissolved in 10 ml of sterile water. All samples were sonicated for 15 min. Then they were centrifuged at 10,000 rpm for 5 min and filtered through 0.22 µm membrane filter nylon prior to the injection. The analysis was performed on Shimadzu HPLC Class VP series with a LC-10AD VP pump, a UV-Vis detector SPD-10AV VP and a SCL-10A VP system controller. A BDS Hypersil C18 column (5 µm, 4.6 mm internal diameter x 150 mm, Thermo Scientific, USA) was used in chromatographic analysis. The analytical condition was run as isocratic elution with a solvent system of 0.5% acetic acid in water:acetonitrile (85:15, v/v) at a flow rate of 1 ml/min. The detection wavelength was set at 336 nm. The content of caffeic acid in the extracts was determined by using the standard curves constructed by the analysis of five different concentrations of standard caffeic acid.\(^{15}\)

**Statistical analysis:** All experiments were done in triplicate and results were expressed as mean ± standard deviation (SD). Significance differences for comparisons were determined using Independent Sample T-test by SPSS for Mac, version 23.0 (IBM). The results with \(p < 0.05\) were considered to be significant.

**Results**

In the present study, a decoction method was chosen due to its traditional usage. Decoction is a well-established technique used for natural product extractions and offer advantages including rapidity, efficiency and cost effectiveness. The percentages of extraction yield were 18.05 and 7.55% w/w for the water and 50% ethanolic extract, respectively.

Antioxidant activities, total phenolic and total flavonoid contents of the water extract and 50% ethanolic extract of \(M.\ chinensis\) were shown in Table 1.
Table 1  Antioxidant activity, total phenolic content, and total flavonoid content of water extract and 50% ethanolic extract of *M. chinensis*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH radical scavenging activity* (IC50 (µg/mL))</th>
<th>FRAP* (mmol FeSO4/100 g extract)</th>
<th>Total phenolic content* (mg GAE/g extract)</th>
<th>Total flavonoid content* (mg QE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>34.28±4.36a</td>
<td>187.89±14.90a</td>
<td>75.27±3.39a</td>
<td>4.44±0.48a</td>
</tr>
<tr>
<td>50% ethanolic extract</td>
<td>50.47±4.13a</td>
<td>120.87±3.91b</td>
<td>58.63±1.06a</td>
<td>4.19±0.60a</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>3.41±0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

GAE = gallic acid equivalent, QE = quercetin equivalent  
* Data are expressed as mean ± SD (n=3).  
a,b  Mean values within a column with different letters were significantly different at p < 0.05.

HPLC chromatogram of *M. chinensis* extracts showed a prominent peak at a retention time of 5.51±0.03 min which corresponds to caffeic acid reference standard. The calibration curve of standard caffeic acid solutions (0.5 to 8 µg/ml) was expressed as the equation, y = 41147x - 1064.1, with a good correlation (R² = 0.9996). The content of caffeic acid in the water and 50% ethanolic *M. chinensis* extracts were 0.11±0.02 and 0.02±0.00% w/w, respectively.

![HPLC chromatograms](image)

**Figure 1** HPLC chromatograms of the caffeic acid reference standard (A), *M. chinensis* water extract (B), and *M. chinensis* 50% ethanolic extract (C).

**Discussion**  
The antioxidant activities of water and 50% ethanolic extracts of *M. chinensis* were investigated and compared in the present study. The results revealed that both extracts showed strong antioxidant activities, however, water extract has comparatively more effective than 50% ethanolic extract in all assays. For DPPH radical scavenging assay, it was found that the IC50 value of water extract in the present study was lower than the result of Chusak *et al.*, 2014 (140 µg/ml) but higher than in Yen *et al.*, 2003 report (18.7 µg/ml), whereas 50% ethanolic extract was more effective than the study of Le *et al.*, 2017 (69.97 µg/ml). In both extracts, total phenolic content was slightly lower than the report of Jhang *et al.*, 2016 but the amount of flavonoids in our study was 2-fold lower. These differences may be due to variation in extraction method and raw material source. Caffeic acid content in the water extract was higher than that in the dried *M. chinensis*. The result was in accordance with the study of Kreungngern *et al.*, 2013. These findings may suggest that the antioxidant capacity of *M. chinensis* extracts might possibly be related to their phenolic content. Caffeic acid, a major bioactive compound, could be the active component responsible for its antioxidant effect. The antioxidative efficiencies of caffeic acid derive from the presence of two hydroxyl groups which located in meta and para positions because the existence of a second hydroxyl group in the ortho or para position is known to increase antioxidative activity due to an additional resonance stabilization and o-quinone or p-quinone formation and the olefinic double bond is also an essential factor for enhancing antioxidant activity. In addition, the water extract showed higher antioxidant effects may be due to the stronger polarity of the solvent which is optimum for extracting its bioactive compounds and it could be because of the conversion of caffeic acid to other compounds having greater antioxidant activity at higher temperatures.

**Conclusion**  
The present study compared antioxidant activities, total phenolic and total flavonoid contents of water and 50% ethanolic extracts of *M. chinensis*. The results revealed that the water extract significantly exhibited higher antioxidant activity in ferric reducing assay. HPLC analysis exhibited the presence of
caffeic acid which is the major bioactive compound. Based on this study, both extraction methods are favorable but depending on the applications that will be used. Moreover, both *Mesona chinensis* extracts may serve as suitable natural raw materials for developing functional foods. In this regard, other mechanisms or endogenous antioxidant activities such as superoxide dismutase inhibition, the effect of temperature and extraction time on biological activities of the extracts need to be evaluated in the future.

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References