GC-MS Analysis and Antioxidant Activity of Bauhinia nakhonphanomensis Leaf Ethanolic Extract

Wilawan Promprom* and Wannachai Chatan

ABSTRACT

Aims: Leaves of B. nakhonphanomensis were extracted and the extract was used in gas chromatography-mass spectrometry (GC-MS) analysis to evaluate the total phenols, total flavonoids and antioxidant activity. Methods: The extract of B. nakhonphanomensis was analyzed by GC-MS. Quantitative analysis for total phenols was done by the Folin-Ciocalteu method and for total flavonoids by the aluminium chloride method. The antioxidant activity of the ethanolic extract was evaluated by the DPPH method. Results: GC-MS analysis revealed the presence of 19 phytochemical constituents. These compounds were identified by comparing their retention times and peak areas with those from the literature and by interpretation of the mass spectra. The major chemical constituents were inositol (48.55 %), alpha-tocopherol (12.21 %) and phenol (6.61 %). Total phenolic content was 48.69 ± 0.56 mg/100 of Gallic acid equivalent (GE). The total flavonoid content was 10539 ± 6.14 mg/100 of quercetin equivalent (QE). Antioxidant activity was 17.07 ± 0.24 µg/100 of ascorbic acid equivalent antioxidant capacity (AEAC). Conclusion: These findings are the first report and suggest that the rich phytochemical content of B. nakhonphanomensis has good antioxidant activity.

Key words: Bauhinia nakhonphanomensis, GC-MS, Antioxidant activity, Total phenolic content, Total flavonoid content.

INTRODUCTION

Plant secondary metabolites have been referred to as phytochemicals that are naturally occurring and have potential disease inhibiting capabilities.1 Phytochemicals are excellent sources of many bioactive compounds, such as volatile oils, steroids, alkaloids and natural antioxidants, i.e., flavonoids and other phenolic compounds, with beneficial effects on human health. Hence, the screening of active compounds and antioxidant activity determination from plants have led to the discovery and development of novel drugs to be used against diverse diseases. Drugs from plants are easily available, less expensive, safe, and efficient and rarely have side effects.2 The modern methods describing the identification and quantification of active constituents in plant material may be useful for proper standardization of herbal drug formulations. Mass spectrometry coupled with chromatographic separations, such as gas chromatography (GC/MS), is normally used for the direct analysis of the components that exist in both traditional medicines and medicinal plants. In recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oils, fatty acids, lipids and alkaloids.3 Bauhinia is a large genus belonging to the subfamily Caesalpinioideae (Leguminosae). It consists of about 300 species and is distributed in pantropical regions of the world.4 Plants in the genus Bauhinia have characteristic butterfly shaped leaves. Most Bauhinia spp. have applications in traditional medicine, such as the bark of B. tomentosa that is used for the treatment of inflammation, dysentery and skin diseases.5 The leaf ethanolic extracts of B. purpurea exhibited hypoglycemic and antioxidant activity.6,7 Bauhinia is well known for the therapeutic efficacies of its different species. In the last revision of the Flora of Thailand, there were 37 species reported.8 In the year 2013, B. nakhonphanomensis Chatan was collected from the Phulangka National Park, Nakhon Phanom Province and reported as a new and endemic species to Thailand (Figure 1). This liana species is easy to recognize by having tendrils, acuminate or caudate leaf apices, entire leaf margin, oblong or elliptic floral buds, floral bud 25-35 mm long raceme or panical inflorescence, 10-13 mm long hypanthium and anther opening by longitudinal sities.9,10 However, there are no scientific reports on the phytochemicals and antioxidants of B. nakhonphanomensis leaves. Therefore, the present study aims to investigate the presence of phytochemicals in an ethanolic extract of the leaves.

of Bauhinia nakhonphanomensis and to study the antioxidant activity for the first time. This work will help to identify the compounds and antioxidants that might have therapeutic value.

MateriAIALS AND METHODS

Plant collection and preparation of extract

Leaves of Bauhinia nakhonphanomensis were collected from Phulangkha Nation Park, Nakhon Phanom Province, Thailand during April to May, 2013. The voucher specimen (Chatan 1337) was identified and confirmed by the second author and deposited in Natural Medicinal Mushroom Museum (MSUT), Mahasarakham University, Thailand for future reference. The fresh leaves were manually isolated. They were cleaned, air dried, powered and subjected to macerate extraction with ethanol. The extract was filtered through filter paper, an evaporator and dried by air. The crude extracts were analyzed by GC-MS.

Determination of total phenolic content

The phenolic contents were determined by the Folin-Ciocalteau method. Briefly, 200 µl of the Bauhinia nakhonphanomensis extract at appropriated dilutions was mixed with 1 ml of 0.2 M Folin-Ciocalteau reagent. After leaving the solution in the dark at room temperature for 30 min, 800 µl of 7% sodium carbonate was added to it. The absorbance of the resulting blue color was measured at 756 nm. Phenolic contents were expressed as mg of Gallic acid equivalent (GAE)/g dry weight of extract.11

Determination of antioxidant activity

The ability of the extract to scavenge DPPH radicals was determined according to the method described by Braca et al. (2001).12 The plant extract (0.1 mL) was added to 3 mL of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min. The percentage DPPH radical scavenging activity of each extract was determined using the formula % DPPH radical scavenging =[(A0 – A1) / A0] × 100, where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard. The inhibition curves were prepared and the IC50 values were calculated.14

GC-MS analysis

The leaf extract of Bauhinia nakhonphanomensis was analyzed for its chemical constituents by GC-MS (GC 7890A Agilent Technology). The column (DB5) was fused silica 30 m x 0.25 mm ID × 0.25 µm film thickness. The oven temperature was programmed from 80°C @10°C/min to 200 °C @12°C/min to 260°C (30 min). Helium gas (99.999 %) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 1 µl was employed (split ratio of 10:1) at an injector temperature of 250°C; the ion-source temperature was set at 280°C. The compounds were detected in the range 50-550 amu. The molecular weight and structure of the compounds of the test materials were ascertained by interpretation of the mass spectrum of the GC-MS using the database of the National Institute of Standards and Technology (NIST).

Results

GC-MS analysis

The components present in the ethanol extract of the leaf of Bauhinia nakhonphanomensis identified by the GC-MS chromatogram are shown in Figure 2. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area as a percentage are presented in Table 1. The GC-MS analysis showed the presence of mainly three compounds at retention times of 8.12 (6.61%), 13.70 (48.55%) and 46.29 (12.21%), which were phenol, inositol and alpha–tocopherol, respectively.

The major phytochemicals and their biological activities obtained through the GC-MS analysis of the leaf of Bauhinia nakhonphanomensis have been tabulated Table 2.

Total phenolics and flavonoid content

The total phenolic contents of Bauhinia nakhonphanomensis determined by the Folin-Ciocalteau method of the ethanolic extract showed 48.69 ± 0.56 mg/100 of Gallic acid equivalent (GE), while the total flavonoid contents determined by the Folin-Ciocalteau method of the ethanolic extract showed 48.69 ± 0.56 mg/100 of quercetin equivalent (QE). The results of the phenolic contents and flavonoids contents are in Table 4.

Antioxidant activity

The extract showed potent DPPH radical scavenging activity. The ethanolic extract of the leaves was found to have an IC50 value of 17.07 ± 0.24 µg/ml. The IC50 value of the standard ascorbic acid was 7.88 ± 0.1 µg/ml (Table 3).
DISCUSSION

The phytochemical analysis conducted on the extract of B. nakhonphanomensis revealed the presence of constituents that are known to exhibit medicinal as well as physiological activities. In the last few years, GC/MS has been the best technique used for screening, identification and quantification of many susceptible compound in plant extracts.\textsuperscript{15-17} GC/MS data revealed that the ethanoic extract of B. nakhonphanomensis contained three major chemical constituents, i.e., inositol, phenol and alpha-tocopherol. Inositol is used for the treatment of Polycystic Ovary Syndrome (PCOS), as an anti-diabetic and metabolic syndrome in postmenopausal women.\textsuperscript{18-20} Phenol and alpha-tocopherol have antioxidant uses in humans.\textsuperscript{21} Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites.\textsuperscript{22} Several studies have described the antioxidant properties of medicinal plants that had rich phenolic compounds.\textsuperscript{23,24} Natural antioxidants, which mainly come from plants, are in the form of phenolic compounds, such as flavonoids,

![Figure 2: GC-MS chromatogram of Bauhinia nakhonphanomensis ethanolic leaf extract.](image-url)
phenolic acids and tocopherols. In this study, we demonstrated that the antioxidant activity of B. nakhonphanomensis was a very efficient free radical scavenger due to the lowest IC50 value. The activity of the reference antioxidant (vitamin-c) was much higher when compared with the ethanolic extract. The GC-MS analysis of the ethanolic extract of B. nakhonphanomensis revealed the presence of phenol and alpha-tocopherol. Therefore, this study can conclude that the leaf ethanolic extract of this plant is a good source of antioxidants.

CONCLUSION

The presence of antioxidant activity and GC-MS analysis were the first steps towards understanding the nature of the active principles in this medicinal plant and phytochemically will be helpful for further detailed study. The importance of the study was to identify some of the biological activities of these compounds. The present study, which revealed the presence of components in B. nakhonphanomensis leaves, suggests a contribution from these compounds to pharmacological activity in the future.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

None.

Table 2: Major phytocompounds and biological activities of B.nakhonphanomensis.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of compound</th>
<th>Nature of compound</th>
<th>* Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenol</td>
<td>Phenolic compound</td>
<td>Analgesic, Anesthetic, Antibacterial, Antihemorrhoidal, Anti-inflammatory, Antioxidant, Antiprostatic, Anti-tussive, Antiviral Antispastic, Cancer-Preventive, Carcinogenic</td>
</tr>
<tr>
<td>2</td>
<td>Inositol</td>
<td>Vitamin B</td>
<td>Antialopecia, Anticirrhotic, Antiischemic, Cholesterolytic, Antineuropathic</td>
</tr>
<tr>
<td>3</td>
<td>Alpha-Tocopherol</td>
<td>Vitamin E</td>
<td>Antiageing, Analgesic, Antidiabetic, Antioxidant</td>
</tr>
</tbody>
</table>

Table 3: DPPH free radical scavenging activity of B. nakhonphanomensis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. nakhonphanomensis</td>
<td>17.07±0.24</td>
</tr>
<tr>
<td>Vitamin-C</td>
<td>7.88±0.1</td>
</tr>
</tbody>
</table>

The values are means of three replicates. * Gallic acid equivalent, ** Quercetin equivalent

Table 4: Total phenol and flavonoid contents of B. nakhonphanomensis.

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter analyzed</th>
<th>Values obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total phenols (mg/100g) GE*</td>
<td>48.69 ± 0.56</td>
</tr>
<tr>
<td>2</td>
<td>Total flavonoids (mg/100 g) QE**</td>
<td>10539 ± 6.14</td>
</tr>
</tbody>
</table>

ABBREVIATIONS USED

GC-MS: Gas chromatography mass spectrometry; GE: Gallic acid equivalent; QE: Quercetin equivalent; AAEAc: Ascorbic acid equivalent antioxidant capacity.

REFERENCES


GRAPHICAL ABSTRACT

HIGHLIGHTS OF PAPER

- **Bauhinia nakhonphanomensis** Chatan., a new species that is endemic to Thailand. It is found in Phulangka National Park, Nakhon Phanom Province.
- GC-MS analysis revealed the presence of 19 phytochemical constituents. The major chemical constituents were inositol (48.55 %), alpha-tocopherol (12.21 %) and phenol (6.61 %).
- Total phenolic content was 48.69±0.56 mg/100 of Gallic acid equivalent (GE).
- The total flavonoid content was 10539± 6.14 mg/100 of quercetin equivalent (QE).
- Antioxidant activity was 17.07±0.24 µg/100 of ascorbic acid equivalent antioxidant capacity (AEAC).

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