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Original Article

# 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-Ciocatteu 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 contentwas 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 .

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## INTRODUCTION

Plant secondary metabolites have been referred to as phytochemicals that are naturally occurring and have potential disease inhibiting capabilities.[1] Phytochemicalsare 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.[4,5]Plants in the genus *Bauhinia* have characteristic butterfly shaped leaves. Most *Bauhinia* spp. have applications in traditional medicine[6], such as the bark of *B. tomentosa* that is used for the treatment of inflammation, dysentery and skin diseases.[7] The leaf ethanolic extracts of *B. purpurea* exhibited hypoglycemic and antioxidant activity.[8,9] *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.[4] In the year 2013, *B. nakhonphanomensis* Chatan was collected from the Phulangkha 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 sits.[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

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**Figure 1:** Flowers and leaves of *Bauhinia nakhonphanomensis*.

of *B. 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 *B. 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, Thailandfor 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 a lyophilizer. 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 *B. nakhonphanomensis* extract at appropriated dilutions was mixed with 1 ml of 0.2 M Folin-Ciocalteu 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 total flavonoid content*

Total flavonoid contents were measured using an aluminum chloride colorimetric assay.[12] The extract (concentration 1 mg/ml) was mixed with 200 µl of distilled water and 100 µl of 5 % $NaNO_2$ solution. After 6 min, 200 µl of 10 % $AlCl_3$ solution was added. After 5 min, 500 µl of 1M NaOH and 275 µl of distilled water were added to prepare the mixture. The absorbance at 510 nm was recorded after 15 min of incubation. The total flavonoid content of the extracts was expressed as a percentage of quercetin equivalent per 100 g dry weight of sample.

#### *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).[13] 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 $=[(A_0 - A_1) / A_0] \times 100$, where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the extract/standard. The inhibition curves were prepared and the $IC_{50}$ values were calculated.[14]

#### *GC-MS analysis*

The leaf extract of *B. 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 1ml/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 *B. 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 phytocompounds and their biological activities obtained through the GC-MS analysis of the leaf of *B. nakhonphanomensis* have been tabulated Table 2.

#### *Total phenolics and flavonoid content*

The total phenolic contents of *B. nakhonphanomensis* determined by the Folin-Ciocalteu 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 aluminium chloride method of the extract showed 10539 ± 6.14 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 $IC_{50}$ value of 17.07 ± 0.24 µg/ml. The $IC_{50}$ value of the standard ascorbic acid was 7.88 ± 0.1 µg/ml (Table 3).

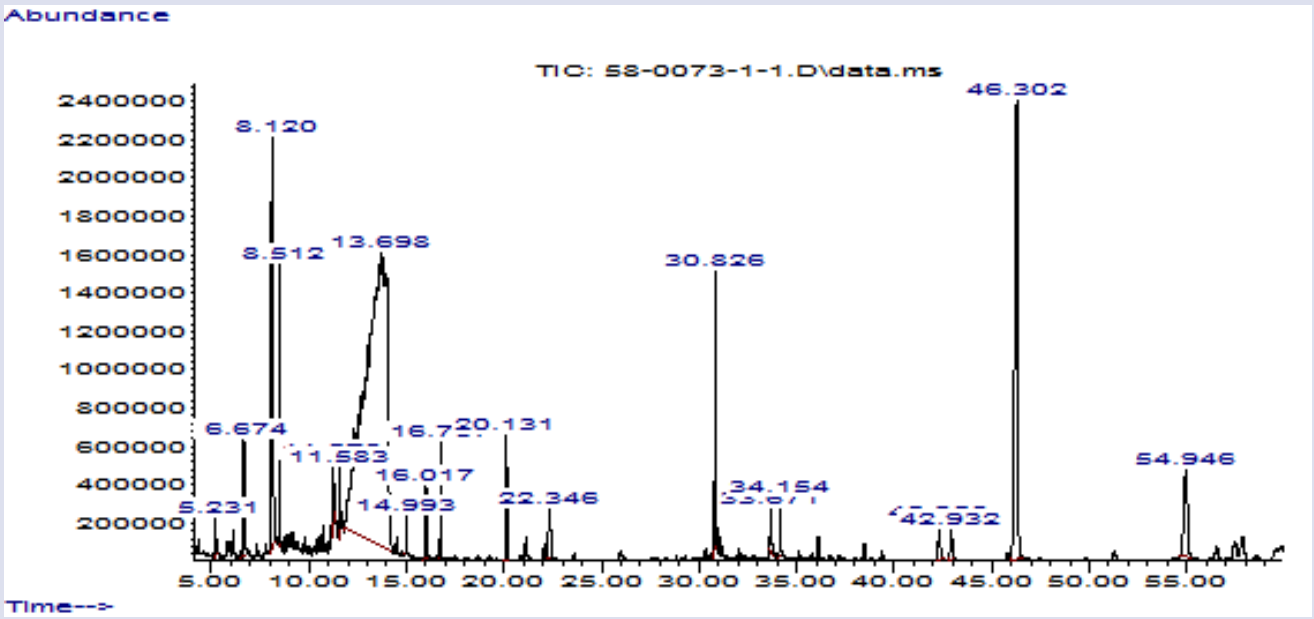


**Figure 2:** GC-MS chromatogram of *Bauhinia nakhonphanomensis* ethanolic leaf extract.

**Table 1: Phyto-components identified in *Bauhinia nakhonphanomensis* leaf ethanolic extract.**

| No. | RT (min) | Name of compound | Molecular formula | Molecular weight | Peak area (%) |
|---|---|---|---|---|---|
| 1. | 5.23 | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | $C_6H_8O_4$ | 144.12 | 0.40 |
| 2. | 6.67 | 3-Methoxycatechol | $C_7H_8O_3$ | 140.13 | 1.52 |
| 3. | 8.12 | Phenol | $C_6H_6O_3$ | 126.11 | 6.61 |
| 4. | 8.52 | Androst-2,16-diene | $C_{19}H_{28}$ | 256.43 | 1.95 |
| 5. | 11.28 | Unidentified | - | - | 1.78 |
| 6. | 11.58 | Unidentified | - | - | 1.41 |
| 7. | 13.70 | Inositol | $C_6H_{12}O_6$ | 180.16 | 48.55 |
| 8. | 14.99 | 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione | $C_{17}H_{24}O_3$ | 276.37 | 0.30 |
| 9. | 16.01 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256.42 | 0.76 |
| 10. | 16.76 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284.47 | 0.98 |
| 11. | 20.13 | (2E) (7R,11R)-3,7,11,15-Tetramethylhexadec-2-EN-1-OL | $C_{20}H_{40}O$ | 296.53 | 1.44 |
| 12. | 22.35 | 9-Octadecenoic acid (Z)-,Ethyl ester | $C_{20}H_{38}O_2$ | 310.51 | 1.02 |
| 13. | 30.86 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ester | $C_{19}H_{38}O_4$ | 330.50 | 2.05 |
| 14. | 33.67 | 9-Octadecenoic acid (Z)-,2-hydroxy-l-(hydroxymethyl) ethyl ester | $C_{21}H_{40}O_4$ | 356.54 | 0.98 |
| 15. | 34.15 | Octadecenoic acid, 2-hydroxy-l-(hydroxymethyl) ethyl ester | $C_{21}H_{42}O_4$ | 358.55 | 0.70 |
| 16. | 42.31 | Beta-Tocopherol | $C_{28}H_{48}O_2$ | 416.68 | 0.65 |
| 17. | 42.93 | Gamma-Tocopherol | $C_{28}H_{48}O_2$ | 416.68 | 0.56 |
| 18. | 46.29 | Alpha-Tocopherol | $C_{29}H_{50}O_2$ | 430.71 | 12.21 |
| 19. | 54.94 | Gamma-Sitosterol | $C_{29}H_{50}O$ | 414.70 | 3.06 |

## 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.[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 antidiabetic and metabolic syndrome in postmenopausal women.[18-20] Phenol and alpha.-tocopherolhave antioxidant uses in humans.[21] Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites.[22] Several studies have described the antioxidant properties of medicinal plants that had rich phenolic compounds.[23,24] Natural antioxidants, which mainly come from plants, are in the form of phenolic compounds, such as flavonoids,

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

| No. | Name of compound | Nature of compound | * Activity |
|---|---|---|---|
| 1 | Phenol | Phenolic compound | Analgesic, Anesthetic, Antibacterial, Antihemorrhoidal, Antiseptic, Antihydrocoele, Antiincontinence, Antionychogryphotic, Antiotitic, Antioxidant, Antiprostatic, Antiisinusitic, Antiviral Antispastic, Cancer-Preventive, Carcinogenic |
| 2 | Inositol | Vitamin B | Antialopecia, Anticirrhotic, Antidiabetic, Lipotropic, Cholesterolytic, Antineuropathic |
| 3 | Alpha-Tocopherol | Vitamin E | Antiageing, Analgesic, Antidiabetic, Antioxidant<br>Anti-inflammatory, Antidermatitic, Antileukemic, Antitumor, Anticancer, Antibronchitic, Vasodilator, Anticoronary, Antiulcerogenic, Antispasmodic, Anticoronary, Antistroke<br>Hypocholesterolemic, Hepatoprotective |

* Activity Source: Dr. Duke's Phytochemical and Ethnobotanical Database

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

| Sample | IC50 Value (µg/ml) |
|---|---|
| B. nakhonphanomensis | 17.07±0.24 |
| Vitamin-C | 7.88±0.1 |

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

| No. | Parameter analyzed | Values obtained |
|---|---|---|
| 1. | Total phenols (mg/100g) GE* | 48.69 ± 0.56 |
| 2. | Total flavonoids (mg/100 g) QE** | 10539 ± 6.14 |

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

phenolic acids and tocopherols.[25] In this study, we demonstrated that the antioxidant activity of *B. nakhonphanomensis* was a very efficient free radical scavenger due to the lowest $IC_{50}$ value. The activity of the reference antioxidant (vitamin-c) was much higher when compare 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 ethanoic extract of this plantis 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.

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## CONFLICT OF INTEREST

None.

## ABBREVIATIONS USED

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

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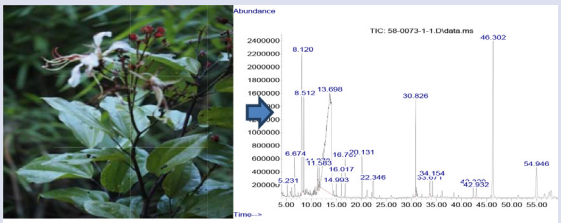
## GRAPHICAL ABSTRACT




Bauhinia nakhonphanomensis GC-MS chromatogram

## HIGHLIGHTS OF PAPER

- *Bauhinia nakhonphanomensis* Chatan., a new species that is endemic to Thailand. It found in Phulangkha National Park, Nakhon Phanom Province.
- GC-MS analysis revealed the presence of 19 phytochemical constituents. The major chemical constituents were innositol (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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