Study of the Antioxidant Activity and Total Phenolic Content of Crude Extract from *Michelia alba* Flower

Charinthorn Fakkham[1], Anongnooch thumpad[2, *], Phanthipha Phuttamek[3], Salinthip Kunsilarak[4] and Saengsit Kritsadee[5]

[1-5]Department of Applied Thai Traditional Medicine, College of Allied Health Sciences, Suan Sunandha Rajabhat University;
charinthorn.fa@ssru.ac.th (C.F.); anongnooch.tu@ssru.ac.th (A.T.) phanthipha.ph@ssru.ac.th (P.P.); salinthip.ku@ssru.ac.th (S.K.); saengsit.kr@ssru.ac.th (S.K.)
*Corresponding author

Abstract

Antioxidants decrease the amount of free radicals and reactive oxygen species in vivo. Herbal medicines have been reported for their significant antioxidant activities. *Michelia alba* (*M. alba*) is a flowering tree best known for its essential oil, which has been used for a long time as a fragrance ingredient in perfumes, cosmetics, and traditional medicine. The objectives of this study were to determine the antioxidant activity and the total phenolic content of the flower *M. alba*, which was extracted by maceration with 95% ethanol. The antioxidant test was used 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method and total phenolic compounds by the Folin-Ciocalteu method. The antioxidant activity results of *M. alba* by the DPPH method showed the antioxidant activity IC_{50} at 0.035 mg/ml. The total phenolic content was 98.85 ± 0.04 mg GAE/mg of dry extract. In conclusion, the results of this study demonstrated the antioxidant activity and contained phenolic compounds and could potentially offer health benefits.

Keywords: *Michelia alba*, Antioxidant, Free radical, Total Phenolic Content

1. Introduction

Michelia alba, a flowering plant in the Magnoliaceae family, is native to southern China and Southeast Asia. Its flowers are highly prized for their fragrance and cultural significance. In Thai traditional medicine, various parts of the plant—including the flowers, leaves, and bark—are used for their therapeutic properties. The fragrance of *Michelia alba* flowers is also valued in cosmetics and medicinal products (Promsorn, T., 2019). Natural products are particularly sought after because they are considered safer and less likely to cause allergic reactions than synthetic chemicals. Therefore, plants are important in the cosmetics industry, not only for their established medicinal properties but also for their potential to be further developed into useful skincare ingredients (Kakatum, N., 2024).

Antioxidants are crucial for protecting cells from oxidative stress, which is linked to chronic diseases such as cancer, cardiovascular diseases, and aging. Plant extracts often exhibit antioxidant potential due to their phenolic compounds, which can neutralize free radicals and prevent oxidative damage. Phenolic compounds, secondary metabolites in plants, significantly contribute to their antioxidant capacity. The total phenolic content of plant extracts, measured using spectrophotometric methods, serves as an indicator of their potential health benefits.

This study aims to evaluate the antioxidant activity and total phenolic content of crude extracts obtained from *Michelia alba* flowers. By assessing these parameters, we seek to determine the plant extract's potential health-promoting properties.

1.1 Objective of the Study

The objective of study is to evaluate the antioxidant activity and total phenolic content of the crude extract obtained from *Michelia alba* flowers.

2. Literature Review

Scientific Name: *Michelia alba*
Common Names: White Champaka, White Sandalwood, White Jade Orchid Tree
Family: Magnoliaceae
Characteristics:

Michelia alba is a tree with grey bark. The branchlets are green and initially covered with dense, appressed grey pubescence, though they soon become almost glabrous. The leaves are evergreen, thin, and leathery, with petioles 1.5–5 cm long, and may be pubescent or glabrous. The stipules have a similar pubescence as the branchlets. Flowers emerge from axillary shoots that are pubescent and have two to three evenly distributed scars. The flowers are numerous, white, and fragrant, with 10–12 lanceolate tepals. The stamens are yellowish-brown, and the gynoecium is stipitate with approximately 10 pubescent carpels. The fruits are 10–13 cm long; while most carpels abort, the ripe ones are red, hairy, and beaked.

Traditional Uses:

The flowers are noted for their pleasant fragrance and bitter taste. In traditional medicine, they are used to strengthen the heart, support bile and blood production (Medthai, 2020).

Traditional Uses and Potential Application of *Michelia alba*

Michelia species have long been utilized in traditional medicine across various cultures. In India, M. champaca has been employed for treating abdominal tumors, while in China, M. hypoleuca and M. officinalis have been used to address carcinomatous sores and leukemia (Chen, C., 2008). *Michelia alba* is renowned for its extensive medicinal and traditional applications, involving various parts of the plant including flowers, bark, roots, and leaves. It has a long history of safe use in traditional medicine, where it has been used to treat fever, syphilis, gonorrhea, malaria, and to prevent bronchitis, prostatitis, and leucorrhea. Additionally, the flower was traditionally used as an abortive agent in some Asian countries (Huang, X., 2009).

The plant is also believed to be effective in treating abnormal vaginal discharge and irregular menstrual cycles. The essential oil of *M. alba* is used for its anti-inflammatory properties and potential cancer treatment (Songsamoe, S., 2009). Furthermore, *M. alba* has been noted for its ability to suppress cough, aid in expectoration, and treat bronchitis.

The flower of *M. alba* is prized for its strong, sweet floral scent, making it popular in traditional ceremonies and religious offerings in Asia. It is also used in aromatherapy for treating mental disorders, and the dried flowers are employed in Thailand for heart and nerve care, as well as for alleviating motion sickness. Beyond medicinal uses, extracts from *M. alba* leaves have been reported as repellent agents.

In Thailand, the flowers and leaves are said to reduce mucus production, prevent bad breath, and help in breaking down kidney stones. Additionally, the bark has been used

to treat malaria, syphilis, and gonorrhea, and to alleviate fever symptoms. In the Philippines, *M. alba* is used for treating inflammatory disorders, including gout, rheumatism, and asthma (Cheng, K., 2022).

DPPH Assay for Antioxidant Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating the antioxidant activity of plant extracts and other substances. DPPH is a stable free radical with a deep violet color, and its reduction can be measured spectrophotometrically. The principle behind the DPPH assay is based on the ability of antioxidants to donate electrons or hydrogen atoms to the DPPH radical, thereby neutralizing it and causing a change in color from violet to yellow. The DPPH assay is valued for its simplicity, reproducibility, and ability to provide a quick assessment of antioxidant activity. Its results contribute to the broader understanding of the antioxidant capacity of plant extracts and their potential applications in health and disease prevention.

Total Phenolic Content (TPC)

Total Phenolic Content (TPC) is a crucial parameter in evaluating the antioxidant potential of plant extracts. Phenolic compounds are secondary metabolites found in plants that have been recognized for their antioxidant properties. They include a diverse group of compounds such as phenolic acids, flavonoids, tannins, and lignans. The high antioxidant activity of phenolic compounds is largely due to their ability to donate hydrogen atoms or electrons to neutralize free radicals, thereby reducing oxidative stress. The most common method for quantifying TPC is the Folin-Ciocalteu assay. This colorimetric assay is based on the reduction of a phosphomolybdenum-phosphotungstate complex by phenolic compounds. In this method, plant extracts are reacted with the Folin-Ciocalteu reagent, which forms a blue complex with phenolics. The intensity of the color, measured spectrophotometrically at around 765 nm, is proportional to the total phenolic content.

Antioxidant Activity: Phenolic compounds play a significant role in the antioxidant activity of plant extracts. High TPC is often associated with potent antioxidant properties, which are beneficial for protecting cells from oxidative damage and may contribute to preventing various chronic diseases.

Health Benefits: Plants with high TPC are often studied for their potential health benefits, including anti-inflammatory, anti-cancer, and cardiovascular protective effects.

Traditional Medicine: In traditional medicine, plants with high phenolic content are frequently used for their therapeutic properties. The TPC of such plants can provide scientific validation for their traditional uses.

3. Methodology

Sample Preparation and Extraction

Sample Preparation:

- Select mature *Michelia alba* flowers.
- Dry the flowers in an oven at 50°C for 48 hours.
- Once dried, grind the flowers into a coarse powder using a herbal grinder.

Preparation of Crude Extract:

- Weigh 50 grams of the coarse powder of *Michelia alba* flowers.
- Perform extraction using maceration with 95% ethanol. Allow the mixture to sit at room temperature for 5 days.
- After the extraction period, filter the mixture to separate the liquid extract from the solid residues using Whatman No. 1 filter paper.
- Evaporate the ethanol from the filtered extract using a rotary evaporator.
- Dry the resulting crude extract by placing it in an oven set at 50°C until it is completely dry.
- Transfer the dried crude extract to an airtight container and store it in a freezer at -20°C until further use.

Antioxidant Activity Testing Using the DPPH Radical Scavenging Assay

The antioxidant activity is evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH is a stable synthetic free radical with a deep purple color. When DPPH reacts with antioxidants, which are dissolved in ethanol, the purple color of DPPH fades to yellow. This color change indicates the scavenging activity of the antioxidant. (Thaipong, K., et al. 2006; Noreen, H., et al. 2017; Thamwiriyasati, N., et al. 2022)

Preparation of 2.0 mM DPPH Reagent Solution

- Weigh DPPH: Accurately weigh 7.9 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) using an analytical balance.
- Transfer to Volumetric Flask: Place the weighed DPPH into a 100 mL volumetric flask.
- Dissolve in Ethanol: Add ethanol to the flask, Swirl or shake the flask gently to ensure that the DPPH is fully dissolved.
- Adjust Volume: Continue adding ethanol until the total volume reaches the 100 mL mark on the flask.
- Mix Thoroughly: Mix the solution well to ensure homogeneity.
- Measurement of Antioxidant Activity for Standard and Herbal Extracts
- Prepare a 96-well plate for the assay.
- Add 100 µL of the standard solution and 100 µL of the herbal extract solution into the appropriate wells of the 96-well plate.
- Add 100 µL of the DPPH radical solution to each well containing the standard solution and herbal extract.
- Incubate the plate in the dark for 30 minutes to allow the reaction to proceed.
- Measure the absorbance of the solution at 520 nm using a UV-Vis spectrophotometer.
- Perform the assay in triplicate to ensure accuracy and reproducibility.

- Calculate the percentage inhibition of DPPH radical scavenging activity using the following formula:

$$\% \text{ Inhibition } (\%I) = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Control OD (DPPH + Solvent)
Sample OD (DPPH + Herbal Extract)

Next, calculate the average % inhibition (%I) for each concentration and perform linear regression to determine the concentration required to 50% inhibitory concentration: IC_{50}

Analysis of Total Phenolic Content

To determine the total phenolic content, use the Folin-Ciocalteu method and measure the absorbance with a microplate reader at 765 nm. The procedure is as follows:

- Prepare gallic acid standard solutions in ethanol at the following concentrations: 10, 20, 40, 80, and 100 µg/mL.
- Prepare the herbal extract solution at a concentration of 1 mg/mL in ethanol.

Preparation of 10% Folin-Ciocalteu Reagent (FCR)

- Measure 10 mL of Folin-Ciocalteu Reagent (FCR) and transfer it into a 100 mL volumetric flask. Add distilled water and shake well to mix, and adjust the volume to the 100 mL mark for the preparation of 75% sodium carbonate (Na_2CO_3).
- Weigh 7.5 g of sodium carbonate (Na_2CO_3) and place it into a 100 mL volumetric flask. Add water, shake well to dissolve, and adjust the volume to the 100 mL mark.

Experimental Procedure

- **Add Sample Extract**: Pipette 20 µL of the sample extract into each well of a 96-well plate.
- **Add Folin-Ciocalteu Reagent**: Add 100 µL of 10% Folin-Ciocalteu Reagent to each well of the 96-well plate. Shake well, and let it stand in the dark for 5 minutes.
- **Add Sodium Carbonate**: Add 80 µL of 75% sodium carbonate (Na_2CO_3) to each well of the 96-well plate. Shake well, and let it stand in the dark for 30 minutes.
- **Measure Absorbance**: Measure the absorbance at 765 nm using a microplate reader.
- **Replicates**: Perform the experiment in triplicate and calculate the average.
- **Calculate Total Phenolic Content**: Compute the total phenolic content in the extract, expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).
- **Construct Calibration Curve**: Plot the absorbance values obtained against a standard calibration curve of gallic acid and perform linear regression.
- **Determine Phenolic Content**: From the gallic acid standard curve, determine the concentration of phenolic compounds in the sample. Express the total phenolic content as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g).

Calculation Formula

TPC (% w/w GAE) =PS x V x D x 10^{-6} x 100/W
PS = phenolic content of the solution (µg/ml)
V = Total volume of sample (mL)
D = dilution factor
W = sample weight (g)

4. Data Analysis

In this study, each sample was tested in triplicate (n=3). The average value ($\bar{x}$) and standard deviation (S.D.) were calculated. Differences between groups were compared using ANOVA with a 95% confidence level.

5. Results

A 50-gram sample of *Michelia alba* flower was extracted using maceration with 95% ethanol. The extract was then dried using a rotary evaporator, yielding 17.84 grams of crude extract. The percentage yield of the crude extract relative to the weight of the plant material (% yield) is shown in Table 1.

Table 1: Percentage Yield of Crude Extract

Thai name	scientific name	part of used	Sample Weight (g)	Crude Extract Weight (g)	% yield (w/w) 95% Ethanol
Champee	*Michelia alba*.	Flower	50	8.92	17.84

Antioxidant Activity Testing Using the DPPH Radical Scavenging Assay

The antioxidant activity of *Michelia alba* flower extract, obtained using ethanol as the solvent, and the standard BHT (Butylated Hydroxytoluene) is shown in Table 2. The results indicate that the *Michelia alba* flower extract has an IC_{50} value of 0.035 mg/mL, while the standard BHT has an IC_{50} value of 0.008 mg/ml.

Figure 1: Graph of 50% Inhibitory Concentration (IC_{50}) for Michelia alba Flowers

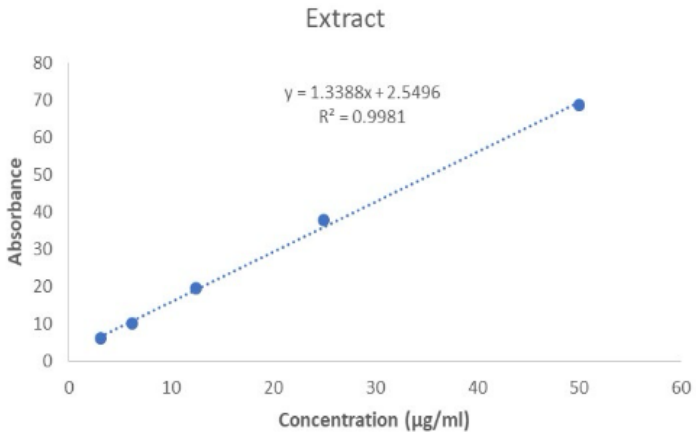

Table 2 shows the 50% inhibition values for oxidation (50% inhibitory concentration: IC_{50})

Sample	IC_{50} values of DPPH radical scavenging activity (mg/ml)
Michelia alba Extract	0.035
BHT (Standard)	0.008

In this context, IC_{50} (the concentration required to inhibit 50% of the DPPH radicals) is used to quantify the antioxidant activity, with lower IC_{50} values indicating stronger antioxidant activity.

Determination of Total Phenolic Content

Results of Total Phenolic Content Analysis**,** The total phenolic content of *Michelia alba* flower extract was determined and compared with the standard gallic acid (R^2 = 0.9998), as shown in Figure 1. The analysis revealed that the *Michelia alba* flower extract has a total phenolic content of 98.85±0.04 mg GAE/mg of dry extract. The results are summarized in Table 3.

Figure 2: Gallic Acid Standard Curve

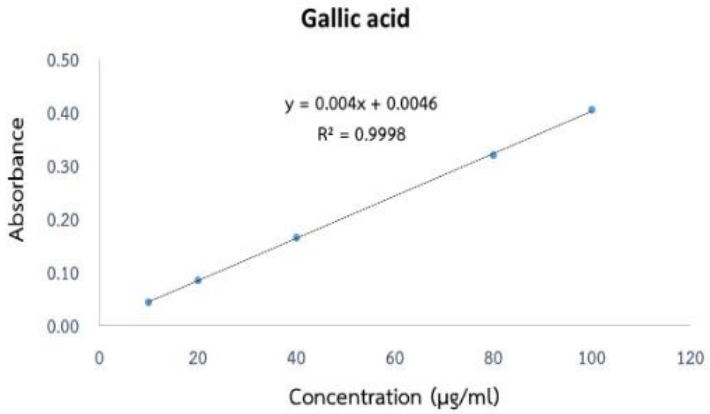

Table 3: Total Phenolic Content of Michelia alba Flower Extract

Extract	Absorbance Value	Total Phenolic Content (mg GAE/mg of dry extract)
Michelia alba	51.35	98.85 ± 0.04

The total phenolic content is expressed in milligrams of gallic acid equivalents per milligram of dry extract (mg GAE/mg), indicating the concentration of phenolic compounds in the sample. The high R^2 value (0.9998) reflects the accuracy and reliability of the calibration curve used for quantification.

5. Discussion

The *Michelia alba* flower extract exhibits antioxidant activity. Overall, both the antioxidant activity and the total phenolic content suggest a correlation, as phenolic compounds are primarily responsible for antioxidant effects. However, differences in antioxidant activity measured by the DPPH radical scavenging assay and the total phenolic content may arise due to factors such as the plant's variety, cultivation source, or harvest season. These variations could result in differences in the antioxidant levels of the extract.

6. Conclusion

The results of this study indicate that *Michelia alba* flowers possess antioxidant activity and a significant amount of total phenolic compounds. To further develop this herbal medicine for potential therapeutic use, it is essential to conduct additional research on other pharmacological effects. This should include studies on the safety of the herbal medicine both in vitro and in animal models.

7. Acknowledgements

The author sincerely thanks Suan Sunandha Rajabhat University, Bangkok, Thailand, for their support and extends gratitude to the Science and Technology Services Center and the College of Allied Health Sciences for providing essential facilities throughout this work.

References

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne D H. Comparison

of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal. 2006;19:669-675.

Noreen H, Semmar N, Farman M, McCullagh S.O. J. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus. Asian Pac J Trop Med. 2017;10(8):792–801.

Thamwiriyasati N, Chancharoen P, Klarod K. Enzyme and non-enzyme antioxidant activities of cherry tomato, Sida pink tomato, and big red tomato fruit extracts. Thai Science and Technology Journal. 2022;30(1):163-173.

Thanya Promsorn. (2019). Medicinal properties of fragrance flowers in Suan Sunandha Royal Garden. ICBTS & ICTBH 2019 Conference in London, 48-51.

Narin Kakatum. (2024) Antioxidant activity and phenolic compounds and determination of quercetin as a biomarker using high-performance liquid chromatography of Butea monosperma (Linn.). ICBTS 2024 Conference in Paris, 61-69.

Medthai (2020). “Champee”, URL: https://medthai.com/

Cheng, K. K., Nadri, M. H., Othman, N. Z., Rashid, S. N. A. A., Lim, Y. C., & Leong, H. Y. (2022). Phytochemistry, Bioactivities and Traditional Uses of Michelia × alba. Molecules (Basel, Switzerland), 27(11), 3450.

Chen, C.; Huang, L.; Chen, L.; Lo, W.; Kuo, S.; Wang, Y.; Kuo, S.; Hsieh, T. Chemical constituents from the leaves of *Michelia alba*. Chem. Nat. Compd. 2008, 44, 137–139.

Huang, X.; Yin, Y.; Liu, X.; Duan, L.; Liu, T.; Yang, Y.; Yu, M. Studies on chemical constituents of volatile oils from the flowers and leaves of *Michelia alba* D.C. in Yunnan. *Chem. Ind. For. Prod.* 2009, *29*, 119–123.

Songsamoe, S.; Matan, N.; Matan, N. Antifungal activity of *Michelia alba* oil in the vapor phase and the synergistic effect of major essential oil components against *Aspergillus flavus* on brown rice. *Food Control* 2017, *77*, 150–157.