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Original Research Article

Phytochemical Screening, Total Flavonoid and Phenolic Contents, and Antioxidant Activities of Thai Mango (*Mangifera indica* L.) Extracts

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ABSTRACT

Mangifera indica L. (mango) is widely recognized for its rich and diverse bioactive compounds, with potential nutraceutical and pharmaceutical applications in health, food, and cosmetics. Research interest in this plant has increased significantly due to its diverse chemical constituents and bioactivities. This study aims to investigate the phytochemical profiles, total flavonoid and phenolic contents, and antioxidant activities of Thai *M. indica* L. extracts from shoots, leaves, and flowers using ethanol, methanol, and water as solvents. The results showed that ethanol consistently produced the highest extraction yield among all crude extracts, with yields of 12.33%, 6.89%, and 9.00% for ES, EL, and EF, respectively. Phytochemical screening revealed the presence of flavonoids, saponins, phenolics, and alkaloids across all extracts. The total flavonoid content was highest in the ethanol shoot (ES) crude extract (48.96 ± 3.08 mg QE/g), whereas the total phenolic content peaked in the ethanol flower (EF) crude extract (62.28 ± 1.77 mg GAE/g). The methanol flower (MF) crude extract exhibited the strongest DPPH antioxidant activity (SC_{50} 1.4 ± 0.3 μg/mL). The methanol leaf (ML) crude extract displayed the highest ABTS antioxidant activity (5.64 ± 0.13 mM TE/g). The MF crude extract showed the greatest FRAP antioxidant activity (0.190 ± 0.007 mg AE/g). These findings highlight the potential of *M. indica* L. as a valuable source of natural antioxidants and phytochemicals for further exploration in health-related applications.

Keywords: *Mangifera indica* L., Phytochemical Screening, Total Flavonoid and Phenolic Contents, Antioxidant Activities

Introduction

The exploration of phytochemicals from natural sources has gained significant attention due to their potential applications in health, medicine, and industry. Phytochemicals, which include flavonoids, phenolics, saponins, and alkaloids, are bioactive compounds found in plants that can exhibit various biological activities such as anti-inflammatory, antimicrobial, and antioxidant effects.[1] Antioxidants, in particular, play a crucial role in neutralizing free radicals, thereby protecting cells from oxidative stress and reducing the risk of chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative disorders.[2] Mango (*Mangifera indica* L.), commonly known in Thailand as "Nam Dok Mai Si Thong", is one of the most popular tropical fruits, valued not only for its taste but also for its rich phytochemical content.[3-6] Various parts of the mango plant—such as leaves, stems, and fruits—contain valuable bioactive compounds.

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The primary bioactive compounds present in mangoes include polyphenols such as mangiferin, catechins, quercetin, kaempferol, gallic acid, and benzoic acid, which have medicinal properties. These compounds are linked to the prevention of degenerative conditions like cancer, cardiovascular diseases, and diabetes.[7-10] Recent studies have focused on assessing the antioxidant potential of different mango cultivars, revealing that these plants contain high levels of phenolic and flavonoid compounds, which contribute to their significant antioxidant activities.[11-13] However, the effectiveness of extracting these compounds depends on several factors, including the solvent used. The choice of solvent plays a critical role in the extraction efficiency and phytochemical yield of plant extracts. Solvents like ethanol, methanol, and water are commonly used due to their polarity and ability to dissolve a wide range of phytochemicals. Ethanol and methanol are particularly effective in extracting phenolic and flavonoid compounds due to their intermediate polarity, which allows them to interact with both polar and nonpolar compounds in the plant matrix.[14]

Although *M. indica* L. from Chachoengsao province is widely recognized for its unique flavor, aroma, and economic significance, research on its phytochemical composition, total flavonoid and phenolic contents, and antioxidant activities remains limited, particularly in plant parts such as shoots, leaves, and flowers. Most existing studies have primarily focused on the fruit, leaving a gap in understanding the bioactive potential of these overlooked parts. To bridge this gap, the present study investigates the phytochemical composition and bioactive properties of *M. indica* L. extracts from different plant parts. The

findings could contribute to the development of natural health products and expand the potential applications of *M. indica* L. from Chachoengsao in the medical, food, and cosmetic industries.

Materials and Methods

Plant materials

Leaves, shoots, and flowers of *M. indica* L. were collected from Suan Kaew Wong Nu Kun, Bang Khla District, Chachoengsao Province, Thailand (13°40′56.7″N 101°09′22.6″E), in April 2015 and used for this experiment.

Preparation of crude extracts

The shoots, leaves, and flowers of *M. indica* L. were cleaned, air-dried, and ground using a blender. Each part (10 g) was wrapped in a muslin cloth for Soxhlet extraction with 250 mL of ethanol for 3 hours at the solvent's boiling point. The extraction was then repeated sequentially using methanol and water as solvents. The crude extracts were concentrated using a rotary vacuum evaporator to obtain ethanol shoot (ES), ethanol leaf (EL), ethanol flower (EF), methanol shoot (MS), methanol leaf (ML), methanol flower (MF), water shoot (WS), water leaf (WL), and water flower (WF) extracts. The crude extracts were further dried in a desiccator to ensure complete solvent removal, and the dried extracts were weighed to determine the percentage yield.

Preliminary phytochemical screening

The qualitative phytochemical screening of plant extracts was carried out to confirm the presence of major compound classes (flavonoids, steroids, triterpenoids, saponins, tannins, phenolics, and alkaloids) in the crude extracts, using established protocols.[14-18]

Total flavonoid content assay

Using the aluminum chloride colorimetric method,[19] quercetin was used as a standard. A calibration curve was plotted with absorbance at 520 nm against quercetin concentrations. Sample flavonoid contents were determined based on this standard curve using the formula:

$$C = C_1 \times (V/m) \times DF$$

Where C is the amount of flavonoid compounds in the sample solution (mg of quercetin/g of crude extract), C_1 is the amount of flavonoid compounds in the sample solution derived from the standard graph (mg/mL), V is the volume of the stock solution prepared (mL), m is the weight of the crude extract used to prepare the stock solution (g), and DF is the dilution factor.

Total phenolic content assay

The Folin-Ciocalteu method[19] was used with gallic acid as the standard. A calibration curve was generated at 750 nm, allowing sample absorbances to be expressed as gallic acid equivalents. The phenolic content of each sample was calculated using this standard curve and the previously mentioned formula.

DPPH radical scavenging assay

Using a method adapted from a previous study,[19] crude extracts at concentrations of 0.05, 0.02, and 0.01 mg/mL were tested for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The samples and blanks were incubated with DPPH solution, and absorbance was measured at 520 nm after 30 minutes. The percentage of inhibition was calculated using the following formula:

$$\% \text{ DPPH inhibition} = [(A_{blank} - A_{sample}) \times 100]/A \text{ blank}$$

Where A_{blank} and A_{sample} are the absorbance of the blank and sample, respectively. The concentration required to scavenge 50% of free radicals (SC_{50}) was derived from the plot of radical scavenging percentages against crude extract concentrations.

ABTS antioxidant capacity assay

The ABTS radical scavenging activity assay was performed following a previously described method,[20] with slight modifications for microplate evaluation. First, 7 mM ABTS and 2.5 mM potassium persulfate were prepared separately in distilled water. The solutions were then mixed in a 1:1 ratio and kept in the dark at room temperature for 14-16 hours to produce a radical $ABTS^{\bullet+}$ solution. This $ABTS^{\bullet+}$ solution was diluted approximately 20-fold with distilled water to achieve an absorbance of 0.700-1.000 at 734 nm before use. The extracts were diluted in methanol to a concentration of 1.0 mg/ml, with Trolox (0.2-1.0 mM) used as a standard and methanol as a blank. For the microplate test, 10 μl of each crude extract or standard was mixed with 190 μl of the diluted $ABTS^{\bullet+}$ solution in a microplate and incubated at room temperature in the dark for 30 minutes. The absorbance at 734 nm was measured using a multimode microplate reader. The radical scavenging activity of each crude extract was calculated in comparison to a Trolox calibration curve and expressed as mM Trolox equivalents per gram of dry crude extract weight (mM TE/g extract). Each treatment was performed in triplicate.

FRAP antioxidant assay

The FRAP assay was performed as described by Benzie and Strain,[21] with modifications for a microplate platform. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. The extracts were diluted in methanol to a concentration of 1.0 mg/ml, with various concentrations of ascorbic acid (0.2-1.0 mg/ml) used as a standard. Methanol served as the blank. The reaction mixture consisted of 10 μl of each crude extract or standard and 190 μl of FRAP reagent, incubated at 37°C for 30 minutes. The absorbance was then measured at 593 nm. Antioxidant power was calculated by comparing results to an ascorbic acid calibration curve and expressed as mg of ascorbic acid equivalents per gram of dry crude extract weight (mg AE/g extract). Each treatment was performed in triplicate.

Statistical analysis

All data were expressed as the mean ± standard deviation (SD) from five separate experiments (n = 5). Data were analyzed using one-way ANOVA and Duncan's Multiple Range Test (DMRT) at a 95% confidence level ($p < 0.05$).

Results and Discussion

Extraction yield

In Table 1, ethanol consistently yielded the highest percentage of crude extracts across all parts, with 12.33%, 6.89%, and 9.00% for ES, EL, and EF, respectively. This suggests that ethanol is more efficient at extracting the phytochemicals present in the shoots, leaves, and flowers of *M. indica* L. due to its polarity and ability to solubilize a wide range of compounds.[22] Methanol showed moderate efficiency, while water consistently yielded the lowest percentages. These results align with previous studies on mango extraction, indicating that polar organic solvents tend to be more effective for extracting compounds compared to water.[23]

Table 1: Physical characteristics and percentage yield of the crude extracts

Crude Extract	Characteristic	% Yield (w/w)
ES	Yellow Solid	12.33
MS	Dark Brown Solid	8.53
WS	Brown Solid	6.26
EL	Green Solid	6.89
ML	Dark Green Solid	5.72
WL	Brown Solid	5.82
EF	Brown Viscous Liquid	9.00
MF	Brownish Viscous Liquid	6.31
WF	Dark Brown Solid	4.48

Preliminary phytochemical screening
The phytochemical analysis (Table 2) confirmed the presence of flavonoids, saponins, phenolics, and alkaloids in all *M. indica* L. extracts, while steroids, triterpenoids, and tannins were not detected. Various phytometabolites, including phenols, flavonoids, alkaloids, tannins, terpenoids, carbohydrates, proteins, and mucilage, were identified in the hexane, chloroform, and methanol extracts of *M. indica* L.[3,24] The absence of steroids, triterpenoids, and tannins in this study suggests their low abundance or limited extractability with the polar solvents used.

Table 2: Phytochemical screening results of the crude extracts.

Phytochemical	ES	MS	WS	EL	ML	WL	EF	MF	WF
Flavonoids	+	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-	-	-
Phenolics	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+

Total flavonoid content (TFC)
The shoots of *M. indica* L. showed the highest total flavonoid content, followed by the leaves and flowers (Table 3). The ethanol crude extracts exhibited the highest TFC in the shoots, leaves, and flowers, with values of 48.96 ± 3.08, 35.50 ± 9.26, and 23.04 ± 5.24 mg QE/g, respectively. In contrast, the TFC in water crude extracts was lower than that in methanol extracts. Based on previous studies, ethanol is often considered an effective solvent for extracting flavonoids and phenolics due to its optimal polarity, which facilitates the dissolution of these compounds. Its amphiphilic nature allows it to interact with both hydrophilic and hydrophobic molecules, enhancing the extraction efficiency of a broad spectrum of phytochemicals.[25-27]

Total phenolic content (TPC)
According to Table 3, all flower crude extracts (EF, MF, and WF) exhibited high total TPC values of 62.28 ± 1.77, 48.84 ± 1.21, and 44.72 ± 2.96 mg GAE/g, respectively, followed by the leaves and stems (Table 3). The significant variation in phenolic content across different plant parts, as observed in this study, is consistent with previous research findings.[28,29] These variations are influenced by factors such as plant anatomy and the choice of extraction methods.

DPPH radical scavenging activity
All flower crude extracts (EF, MF, and WF) exhibited the strongest antioxidant activity, with the lowest SC_{50} values in the range of 1.4-3.1 μg/mL, followed by shoots and leaves (Table 4). Among them, MF crude extract exhibited the highest DPPH antioxidant activity with an SC_{50} value of 1.4 ± 0.3 μg/mL. Notably, the observed high DPPH radical scavenging activity in the flower crude extracts aligns with findings from various studies. Previous research on *Cassia fistula* flower extracts demonstrated significant DPPH radical scavenging activity, correlating with their high phenolic content.[30] This supports the notion that flower crude extracts possess potent antioxidant properties, primarily due to their high phenolic content, which contributes to the inhibition of oxidative stress-related damage.

Table 3: Total phenolic and flavonoid contents, as well as ABTS and FRAP antioxidant activities of the crude extracts.

Crude Extract	TFC (mg QE/g)	TPC (mg GAE/g)	ABTS (mM TE/g)	FRAP (mg AE/g)
ES	48.96 ± 3.08	36.39 ± 4.48	5.03 ± 0.10	0.086 ± 0.053
MS	45.78 ± 2.74	39.70 ± 5.51	3.96 ± 0.12	0.101 ± 0.035
WS	42.12 ± 2.71	29.02 ± 0.37	4.84 ± 0.08	0.097 ± 0.069
EL	35.50 ± 9.26	38.80 ± 0.79	3.83 ± 0.07	0.080 ± 0.062
ML	30.98 ± 2.66	29.25 ± 0.80	5.64 ± 0.13	0.097 ± 0.048
WL	25.65 ± 0.58	43.32 ± 0.85	5.28 ± 0.08	0.126 ± 0.021
EF	23.04 ± 5.24	62.28 ± 1.77	4.99 ± 0.59	0.077 ± 0.031
MF	19.59 ± 4.76	48.84 ± 1.21	7.32 ± 0.02	0.190 ± 0.007
WF	17.95 ± 1.02	44.72 ± 2.96	6.58 ± 0.15	0.137 ± 0.113

Table 4: DPPH antioxidant activity of the crude extracts.

Crude Extract	SC_{50} (μg/mL)	Crude Extract	SC_{50} (μg/mL)
ES	4.7 ± 0.2	WL	4.7 ± 0.5
MS	4.3 ± 0.1	EF	2.0 ± 0.3
WS	4.6 ± 0.5	MF	1.4 ± 0.3
EL	4.8 ± 0.2	WF	3.1 ± 0.2
ML	5.8 ± 1.0	Quercetin[a]	0.087 ± 0.1

[a] Positive control

ABTS antioxidant activity
The ABTS assay is widely used to evaluate the antioxidant capacity of plant extracts due to its ability to measure both hydrophilic and

lipophilic antioxidants.[31] This method is highly sensitive and applicable to various plant matrices.[32] In this study, the MF crude extract exhibited the highest activity, with a value of 7.32 ± 0.02 mM TE/g in flowers, while the ES crude extract demonstrated the highest activity in shoots at 5.03 ± 0.10 mM TE/g. For leaves, the ML crude extract showed the best performance, with an antioxidant activity of 5.64 ± 0.13 mM TE/g. These findings suggest that plant parts, particularly flower extracts, may serve as potent natural antioxidant sources.[32] The high antioxidant potential observed is consistent with previous research demonstrating the effectiveness of the ABTS assay in evaluating the radical-scavenging capacity of plant-derived compounds.[33]

FRAP antioxidant activity

The FRAP assay is a widely used method for evaluating the antioxidant capacity of plant extracts based on their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions in an acidic medium.[21] This assay is considered reliable for measuring the total antioxidant potential of plant-based compounds, particularly phenolics and flavonoids.[32] The FRAP assay results (Table 3) showed that, in shoots, the MS crude extract had the highest activity (0.101 ± 0.035 mg AE/g). The WL crude extract was most effective in leaves (0.126 ± 0.021 mg AE/g), and the MF crude extract exhibited the strongest activity in flowers (0.190 ± 0.007 mg AE/g). These results indicate that methanol was an effective solvent for antioxidant extraction, with the MF crude extract showing the highest activity. The effectiveness of methanol in extracting antioxidants aligns with previous studies demonstrating that polar solvents enhance phenolic compound solubility, thereby improving antioxidant activity.[33,34]

Conclusion

In conclusion, the shoots, leaves, and flowers of *M. indica* L. contain significant phytochemicals with antioxidant properties. Ethanol was the most effective solvent for extraction. The presence of flavonoids and phenolics supports the potential health benefits of *M. indica* L. crude extracts, particularly from the flowers, which exhibited the highest antioxidant activity. This study contributes to the growing body of research on mango plant crude extracts and their potential therapeutic applications in combating oxidative stress and related diseases. Future research should explore the specific compounds responsible for these activities and investigate the health benefits of these crude extracts in biological systems

Conflict of Interes

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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