Received: February 20, 2024 | Revised: September 25, 2024 | Accepted: September 26, 2024

Phytochemical screening and toxicity assessment of compounds isolated from the leaves of *Mangifera indica* L. for the control of *Spodoptera litura* (Lepidoptera; Noctuidae)

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ABSTRACT

This study aimed to analyze the phytochemical composition and antioxidative capabilities of mango leaves (*Mangifera indica* L., Nam Dok Mai), indigenous to Sa Kaeo Province, Thailand. Various solvents with differing polarities, including *n*-hexane, DCM, ethyl acetate, and MeOH, were utilized for leaf extraction. The findings revealed the existence of eight groups of phytochemical compounds: alkaloids, flavonoids, coumarins, saponins, tannins, terpenoids, steroids, and cardiac glycosides. The MeOH crude extract exhibited the highest concentration of total phenolic compounds at 409.88 ±0.02 mg GAE/g. Furthermore, the MeOH crude extract demonstrated the strongest antioxidant activity, with an IC_{50} value of 0.52 ±0.02 µg/ml, as determined by the DPPH method. High-performance liquid chromatography (HPLC) was employed to identify gallic acid and mangiferin in the MeOH crude extract. Laboratory tests were conducted using the topical application method to evaluate the toxicity of the *M. indica* leaf crude extract on 2nd instar *Spodoptera litura* larvae. The MeOH crude extract exhibited high efficacy, with an LD_{50} value of 10.58 µg per larvae within 24 hours. Gallic acid and mangiferin were identified as the primary active ingredients, with LCD_{50} values of 1.19 µg per larvae and 1.90 µg per larvae, respectively, within 24 hours. Additionally, the impact on detoxification enzymes (24 hours post-treatment) was assessed in surviving 2nd instar *S. litura* larvae using the topical application method. The MeOH extract treatment resulted in 1.31-fold inhibition of carboxylesterase (CE), 1.31-fold inhibition of glutathione-*S*-transferase (GST), and 1.32-fold inhibition of acetylcholinesterase (AChE).

Keywords: Spodoptera litura, *Mangifera indica*, Phytochemicals, Biocides, Detoxification enzymes

INTRODUCTION

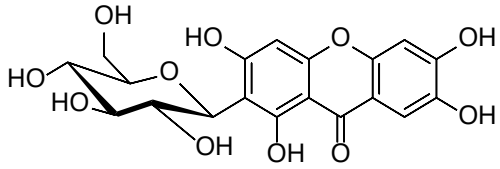
The tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae), is a destructive insect that causes damage to more than 180 agricultural crops, including cotton, maize, sunflower, rice, cereal, and vegetables [1, 2]. Currently, conventional insecticides such as organophosphates, carbamates, deltamethrin, cypermethrin, chlorpyrifos, and prophenofos are being utilized to manage herbivorous insects like *S. litura*. However, the prolonged use of these insecticides can lead to the development of resistance in insects. Additionally, using these chemicals poses risks to the ecosystem and non-target organisms. Therefore, developing natural insecticides promptly and reducing chemical contamination in the environment is crucial to exploring new strategic approaches for monitoring this insect.

Plant-derived phytochemicals, including extracts from *Piper ribesioides*, and *Acorus calamus*, as well as allelochemicals such as pinocembrin, pinostrobin,

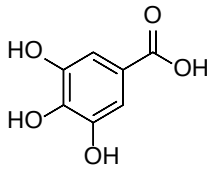
DOI: 10.60101/jarst.2024.255634

methyl cinnamate, chrysin, pachypophyllin, and galangin, have been used to prevent lepidopteran insects such as *Spodoptera exigua* and *S.litura* [3-5]. Furthermore, plants possess a variety of secondary metabolites, such as alkaloids, phenolics, terpenoids, and polyacetates, which serve as biological defenses against herbivorous insects. These compounds exhibit a range of activities, including repelling insects, acting as antitumor agents, attracting food sources, and displaying antimicrobial properties [6].

Mango, scientifically known as *Mangifera indica* L., is a popular fruit that is widely cultivated in subtropical and tropical regions, including Thailand. In fact, Thailand is one of the world's top five exporters of the fresh mango variety Nam Dok Mai [7]. The leaves of *M. indica* have also been found to contain various secondary substances, such as phenolic acids, flavonoids, terpenoids, and carotenoids [8]. These extracts from *M. indica* have shown potential biological activities, including anti-allergic, antidiabetic, and antioxidant properties [9 -10]. This study is the first to investigate the properties of *S. litura* larvae in Thailand, a major agricultural center known for its mango production and export. However, the production of mangoes also generates agro-industrial waste in the form of mango leaves and unripe fruits. Hence, the objective of this study is two-fold: to explore the potential utilization of mango waste as a biopesticide and to assess the larval eradication capabilities of *M. indica* extract and its allelochemicals against *S. litura* larvae through acute toxicity tests. Additionally, the study aims to examine the biochemical reactions triggered by the *M. indica* extract, particularly the activity of detoxification enzymes and neuronal enzymes such as carboxylesterase, glutathione-*S*-transferase, and acetylcholinesterase.



(a)



(b)

Figure 1 Chemical Structures of (a) Mangiferin and (b) Gallic Acid.

Mangiferin (figure 1a) is a major component found in many species of mangoes. It is a phytochemical that has been found to have various biological activities, including anti-inflammatory, anti-oxidative, immune-modulatory, anti-obesity, anti-bacterial, anti-viral, anti-depression, and anti-diabetic properties [11-14]. It also modulates the expression of key enzymes involved in inhibiting lipolysis and promoting-fatty acid oxidation in the liver [15, 16]. Additionally, mangiferin has shown larvicidal activity against *Culex pipiens* L [17].

Gallic acid (figure 1b) is a well-known polyphenol with antioxidant potential, making it an effective bioactive element in treating gastrointestinal diseases and oral health problems [18]. Furthermore, gallic acid exhibits the same larvicidal activity against *Culex pipiens* L. as mangiferin [19].

MATERIALS AND METHODS

Plant extraction

The leaves of *M. indica* were washed and dried in the shade. These samples were collected from Sa Kaeo province, Thailand in June 2022. A hot-air oven (Memmert UM300 oven) was used to dry the samples. Subsequently, a commercial blender (Zhejiang HC-2000Y) was used to powder the dried samples. A total of 257 g of *M. indica* leaf powder was obtained. For extraction, maceration was performed using different organic solvents (*n*-hexane, dichloromethane (DCM), ethyl acetate, and methanol (MeOH)) in a sequential 7-day process. Each extract was filtered through a suction filter connected to a vacuum pump. The resulting solutions were concentrated using a rotary evaporator (BÜCHI R-215). The percentage yield of each extract was calculated. The crude plant extracts were then stored at 4°C until further use in phytochemical detection and biological assays.

Phytochemical screening

This study aims to identify the phytochemicals in Nam Dok Mai mangoes from Sa Kaeo and compare them with those cultivated in other regions.

Alkaloids: 0.20 g of crude extract was dissolved in 1 mL of a 10% ammonia solution and then extracted with 3 mL of DCM. The DCM was evaporated until it became dry, and the remaining residue was dissolved in 3 mL of diluted H_2SO_4. Mayer's reagent was added to the test tube, and the presence of alkaloids was indicated by observing an opalescent or yellow precipitate [18].

Flavonoids: A crude extract weighing 0.20 g was dissolved in 1 mL of 50% MeOH by heating. Then, magnesium metal and 5-6 drops of concentrated HCl were added to the solution. In this experiment, red represents flavanols, while orange represents flavone [18].

Anthraquinone: A total of 0.20 g of crude extract was dissolved in 4 mL of DCM and then heated in a steam bath for 5 minutes. The hot extract was filtered and allowed to cool. Afterward, an equivalent volume of 10% ammonia solution was added to the filtrate. When shaken, the upper aqueous layer turned a bright pink color, indicating the presence of anthraquinone [18].

DOI: 10.60101/jarst.2024.255634

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Coumarins: A test tube with 0.2 g of crude extract was covered with filter paper. Then, a drop of 10% NaOH was added to the paper, and the mixture was heated in a water bath for 5 minutes. Next, the filter paper was examined under a 365 nm UV light, revealing a greenish-blue spot. This observation confirms the presence of coumarins [18].

Saponins: The crude extract (0.2 g) was vigorously shaken with 10 mL of deionized water for 5 minutes until it foamed. If the foam remains in the test tube after resting for 10 minutes, it indicates the presence of saponins [18].

Tannins: A few drops of a 5% $FeCl_3$ solution were added to the 0.2 g of crude extract. The presence of a green color indicated the presence of gallotannins [18].

Terpenoids: The crude extract weighing 0.2 g was mixed with 2 mL of DCM. Then, 3 mL of concentrated H_2SO_4 was added to create a layered effect. The appearance of a red-brown color at the interface confirmed the presence of terpenoids [18].

Steroids: A total of 0.2 g of crude extract was dissolved in 5 mL of DCM. Then, 1 mL of acetic anhydride was added to the mixture. Next, 1 mL of concentrated H_2SO_4 was carefully added to the side of the test tube, forming a distinct layer. The presence of steroids was indicated by the green color observed at the bottom of the test tube [18].

Cardiac Glycosides: A brown ring appeared between layers when a solution of glacial acetic acid (4.0 mL) containing 1 drop of 2.0% $FeCl_3$ was mixed with 0.2 g of crude extract and 1 mL of concentrated H_2SO_4. This brown ring represents the presence of cardiac steroidal glycosides [18].

The contents of total phenolics, total flavonoids, and total tannin determination

The Folin-Ciocalteu method was used to determine the total content. A 0.02 mL portion of a 2.0 mg/mL extract solution was combined with 0.2 mL Folin-Ciocalteu reagent and 2.0 mL of distilled water. After 3 minutes, 1.0 mL of sodium carbonate was added. The mixture was then incubated at room temperature for 20 minutes. The absorbance at 765 nm was measured using a microplate reader. The total phenolic content was calculated using a gallic acid standard curve. A stock standard solution of gallic acid at a concentration of 1.0 mg/mL was prepared by dissolving gallic acid in distilled water. The results were reported as milligrams of gallic acid equivalent (mg GAE) per gram of extract [20].

The total flavonoid content of the extracts was determined using a modified version of the method described by Arvouet-Grand, Vennat, Pourrat, and Legret. Quercetin was prepared in MeOH to create the standard solutions. To measure the flavonoid content, 1.0 mL of sample (1.0 mg/mL)was mixed with 1 mL of 2% $AlCl_3$ in MeOH. The mixture was then incubated at room temperature for 10 minutes. The absorbance was measured at 415 nm using a microplate reader, with a blank sample consisting of 1 mL sample solution mixed with 1 mL of MeOH without $AlCl_3$. A calibration line was created by plotting the absorbance against the quercetin concentration. The flavonoid content is expressed as quercetin equivalents (mg QE) per gram of extract [20].

Tannins were determined using the Folin-Denis method. An extract of approximately 0.1 mL was added to a volumetric flask with a total volume of 10.0 mL. This flask contained 7.5 mL of distilled water, 0.5 mL of Folin-Denis reagent, and 1.0 mL of 35% Na_2CO_3. The mixture was then diluted to a final volume of 10.0 mL with distilled water. After shaking the mixture, it was left at room temperature for 30 minutes. A standard solution of tannic acid was prepared in the same manner. The absorbance of both the assay and standard solution was measured at 700 nm against a blank using a microplate reader. The tannin content is as milligrams of tannic acid equivalent (mg TAE) per gram of the extract [20].

DPPH radical-scavenging activity

To evaluate the antioxidant activity of 2,2-diphenyl-1-picryl-hydrazyl (DPPH), we employed it as a free radical. Different concentrations of DPPH were prepared to ascertain IC_{50}. The total volume in each well of the 96-well plate was 100 µL, comprising 90.0 µL of the DPPH solution and 10.0 µL of the crude extract solution. Using a microplate reader, the mixture was thoroughly blended and incubated at 37°C for 30 minutes to measure the absorbance at 517 nm. A negative control using a DPPH solution and a reference standard using Trolox were implemented. The DPPH scavenging activity percentage was calculated using equation (1).

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}} \quad (1)$$

where the absorbance of the control represents the overall radical activity without an inhibitor, while the absorbance of the sample reflects the activity found in the sample extract [21].

HPLC analysis of gallic acid and mangiferin

The HPLC analysis of *M. indica* leaf extracts was characterized and identified using reverse phase HPLC (HPLC 1100 Agilent). The separation was conducted on a Varian column (250 x 4.6 mm) with a C_{18} guard column. Elution was performed on an isocratic system using a mixture of acetonitrile in 0.2% orthophosphoric acid (10:90, v/v). The flow rate was set at 1.2 mL/min, and the temperature was maintained at 40°C. The diode array detector was set to a wavelength of 242 nm, and the injection volume was 10 µL.

DOI: 10.60101/jarst.2024.255634

Insect maintains

S.litura larval egg colonies were obtained from the National Center for Genetic Engineering and Biotechnology, Bangkok. The colonies were maintained in controlled laboratory conditions at 26 ±2°C, with a relative humidity of 60% to 75% and a photoperiod of 14 hours of light to 10 hours of darkness until the larvae hatched. Subsequently, the larvae were transferred to a new plastic box (25 x 15 cm) and were fed a modified artificial diet based on Pengsook [3]. This diet included commercial green bean powder (100 g; Raithip®), agar (10 g), ascorbic acid (1 g), sorbic acid (1 g) methylparaben (1.5 g), yeast (8.0 g), mixed vitamins (15 mL), formaldehyde (1 mL), and distilled water (600 mL). Pupae were then transferred to a net cage (45 × 40 cm, 45 cm high) for adult emergence, with wax paper sheets provided for moth oviposition. The adults were fed honey solutions consisting of a 30% sugar solution via cotton swabs. Daily, the egg colonies were collected and briefly immersed in a 10% formaldehyde solution (for 10 seconds) to prevent bacterial infection.

Larval bioassay

The isolated compounds, mangiferin, and gallic acid, were utilized in this study. Leaf extract and active ingredients from *M. indica* were prepared using acetone. The solutions were applied topically to the 2nd instar *S. litura* larvae at a rate of 2 µL per larva, with larvae prepared in three replicates (n = 90). The larvae were transferred to a petri dish containing an artificial diet after exposure. The treated insects were maintained under laboratory conditions as described above. The acute toxicity of the compounds was assessed after 24 and 48 hours. Mortality was determined based on LD_{50} values using the STATPLUS program (Probit Analysis, version 2019).

Detoxification enzyme analysis

Enzyme source: The enzyme source was prepared from *S. litura* larvae that survived for 24 hours. Nobsathian outlined the procedure for extracting the enzymes [22]. The larvae were homogenized in a microtube with a phosphate buffer (100 mM, pH 7.2) and triton x-100 (0.5%). After homogenization, the mixture was centrifuged at 12,000 rpm (4°C) for 15 minutes. The resulting supernatants were collected and stored at -20°C for biochemical assays.

General Esterase: *Carboxylesterase* (CE) activity was determined using a modified procedure based on the method described by Nobsathian [22] and Kumrungsee [23]. To begin, 50 µL samples of the enzyme were collected from the enzyme source and pre-incubated at 30°C for 30 minutes. A substrate homogenate was then prepared: para nitrophenyl acetate (10 mM, 50 µL) and phosphate buffer (50 mM, 3.0 mL). Next, the enzyme-substrate mixture was transferred to the 96-well microreader plates. CE activity was measured at 400 nm for 3 minutes at 25°C. The enzyme activity was assessed in triplicates.

Glutathione-S-transferase (GST): To prepare GST, an enzyme solution (20 µL) was collected. The substrate solution consisted of 0.1 M phosphate buffer (pH 7.2, 1150 µL) with the addition of 1-chloro-2,4-dinitrobenzene (150 mM, 10 µL). The homogenized enzyme and substrate were mixed well. The enzyme activity was measured using a microplate reader in kinetic mode at an absorbance of 340 nm, at a temperature of 25°C for a duration of 3 minutes. Three biological replicates were analyzed.

Acetylcholinesterase (AChE): AChE activity was determined using the modified Ellman procedure [24]. The homogeneous substrate consisted of 100 mM phosphate buffer (pH 7.2; 50 µL), 5,5'-dithiobis (2-nitrobenzoic acid) with 0.1 M ethylenediamine tetraacetic acid and 100 mM acetylthiocholine-iodide. The enzyme solution was collected from the enzyme source and preincubated at 30°C for 30 minutes. The enzyme and substrate mixture solutions were homogenized, and the AChE reaction was measured at a wavelength of 412 nm. The enzyme activity was calculated using an extinction coefficient of 1.36×10^4 M^{-1} cm^{-1}. Three biological replicates were performed.

RESULTS AND DISCUSSION

Plant extracts

M. indica leaves were extracted using organic solvents in the following sequence: *n*-hexane, DCM, ethyl acetate, and MeOH. The extract with the highest amount was obtained using MeOH. The percentages of yield and their corresponding characteristics are listed in Table 1.

Phytochemical screening

The preliminary phytochemical examination of *M. indica* leaf extracts was conducted using various solvents, as shown in Table 2. The results revealed the presence of flavonoids, coumarins, tannins, terpenoids, steroids, and cardiac glycosides in the whole-leaf extract. However, alkaloids and saponins were only found in the MeOH extract. Notably, anthraquinones were not found in any leaf extracts.

The variation in the phytochemical constituents of different extracts may be due to the use of different extraction solvents. This can affect the extraction of various bioactive compounds. An examination of the phytochemicals in *M. indica* extract reveals the presence of pharmacological constituents such as tannins, saponins, cardiac glycosides, flavonoids, steroids, and alkaloids. This research supports previous findings by Pintu [25], who also found tannins, alkaloids, steroids, glycosides, and flavonoids in *M. indica* leaf extract. However, this research differs from the finding of Olasehinde [26], who reported that steroids were not present in the leaf extracts obtained using water and ethanol. The content

DOI: 10.60101/jarst.2024.255634

of plant compounds in mango cultivars can vary depending on factors such as acid synthesis, degradation, utilization, partitioning, and external factors like temperature, light, fertilization, and water supply [27].

Table 1 Characteristic of *M. indica* leaf extracts and yield obtained from organic solvents through maceration.

Solvents used for extraction	Characteristics	Yield[a] of crude extract (%w/w)
n-hexane	Dark green and sticky	2.0241
DCM	Dark green and sticky	3.0600
Ethyl acetate	Dark green and sticky	2.9662
MeOH	Dark green and sticky	4.0084

[a]Yield of extracts was calculated as $W/W_0 \times 100$ (W = Total weight of dried extract; W_0 = Weight of the specimen of *M. indica* L. leaf after extractions).

Table 2 Preliminary Phytochemical Screening of *M. indica* leaf extracts.

Phytochemical constituents	Solvent extract[a]			
	n-hexane	DCM	Ethyl acetate	MeOH
Alkaloids	-	-	-	+
Flavonoids	+	+	+	+
Anthraquinones	-	-	-	-
Coumarins	+	+	+	+
Saponins	-	-	-	+
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Cardiac Glycosides	-	-	+	+

[a] (+): presence, (-): absence of phytochemical

Table 3 Contents of total phenolics, total flavonoids and total tannins in leaf extracts of *M. indica*.

Extracts	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Total tannins (mg TAE/g)
n-hexane	9.63±0.00[a]	250.93±0.08[a]	7.82±0.00[a]
DCM	22.59±0.00[b]	284.24±0.03[b]	18.46±0.00[a]
Ethyl acetate	150.95±0.02[d]	172.21±0.02[c]	123.76±0.02[b]
MeOH	409.88±0.02[c]	158.77±0.03[c]	336.34±0.02[c]

Values (averages of three replicates) labeled with different letters indicate significant differences at $p < 0.05$.

Determination of total phenolics, total flavonoids and total tannins content

The total phenolic content was determined using the Folin-Ciocalteu method, which involves quantification of a blue chromophore formed through UV-Vis spectrophotometry at 765 nm. The results in Table 3 indicate the phenolic content in various extraction solvents. The content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g). The total phenolic content ranged from 9.63 to 409.88 mg GAE/g in leaves, with the highest value observed in MeOH leaf extract (409.88±0.02 mg GAE/g), while the lowest content was found in the leaf extract using *n*-hexane (9.63±0.00 mg GAE/g).

The total flavonoid content was measured using aluminum chloride, based on forming acid-stabilized complexes between flavonoids and aluminum chloride, with an absorption peak at 415 nm. The total flavonoid content was expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g). As shown in Table 3, the total flavonoid content ranged from 158.77 to 284.24 mg QE/g. The DCM leaf extract found the highest content of 284.24((0.03 mg QE/g), while the lowest content of 158.77±0.03 mg QE/g was found in the MeOH leaf extract.

Total tannin content was determined using Folin-Denis with sodium carbonate. The Folin-Denis reagent reacts with tannins, forming a mixture of blue oxides that can be measured using UV-VIS spectrophotometry at an absorption peak of 700 nm. The total tannins were expressed in milligrams of tannic acid equivalent per gram of extract (mg TAE/g). As shown in Table 3, the total tannin content ranged from 7.82 to 336.34 mg GAE/g. The MeOH leaf extract had the highest tannin content extract (336.34±0.02mg TAE/g),

DOI: 10.60101/jarst.2024.255634

while the *n*-hexane leaf extract had the lowest (7.82 ±0.00 mg TAE/g).

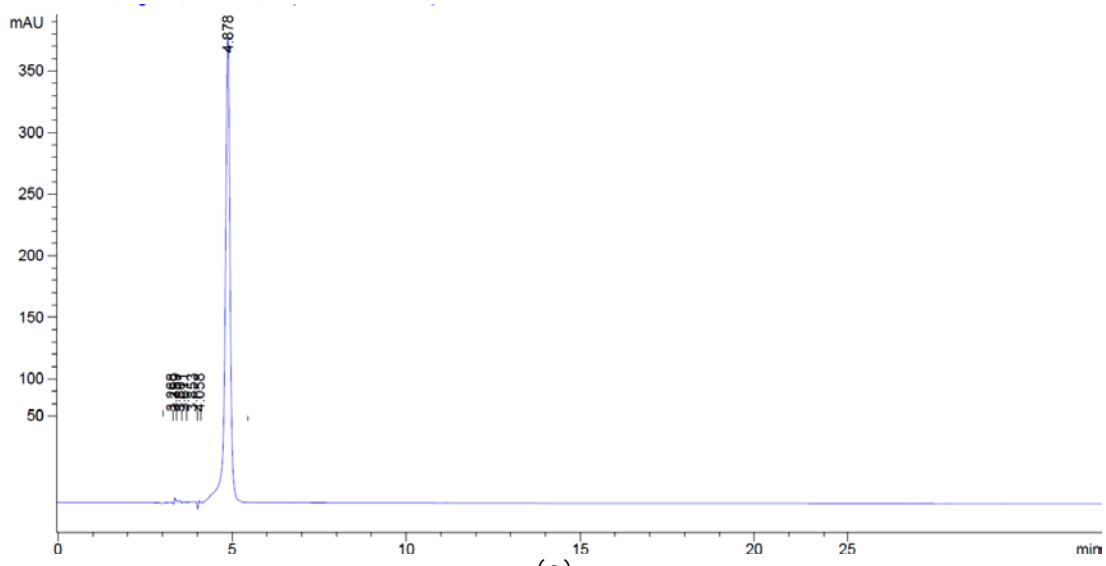
DPPH radical-scavenging activity

The antioxidant activities of *M. indica* extract were assessed using the DPPH assay. This DPPH method is based on the reduction of purple DPPH radicals through electron acceptance from antioxidants, resulting in the formation of reduced DPPH (DPPH-H). The color of the solution changes from purple to yellow upon reduction, which can be measured at 520 nm using UV-VIS Spectrophotometry. The concentration of each extract required to inhibit 50% of the DPPH radicals (IC_{50}) was determined and compared to the antioxidant standard (Trolox). The results presented in Table 4 demonstrate that the MeOH extraction exhibited the highest antioxidant activity (0.52±0.02 µg/mL), followed by ethyl acetate (13.84±0.10 µg/mL), DCM (184.52 ±0.03 µg/mL) and *n*-hexane (1141.41 ± 0.01 µg/mL), respectively.

Table 4 Scavenging activity expresses as median inhibitory concentration (IC_{50}), in the DPPH test with leaf extracts of *M. indica.*

Extract	DPPH radical scavenging assay; IC_{50} (μg/mL)
n-hexane	1141.41±0.01^{d}
DCM	184.52± 0.03^{c}
Ethyl acetate	13.84±0.10^{b}
MeOH	0.52±0.02^{a}

Values (averages of three replicates) labeled with different letters indicate significant differences at $p < 0.05$.



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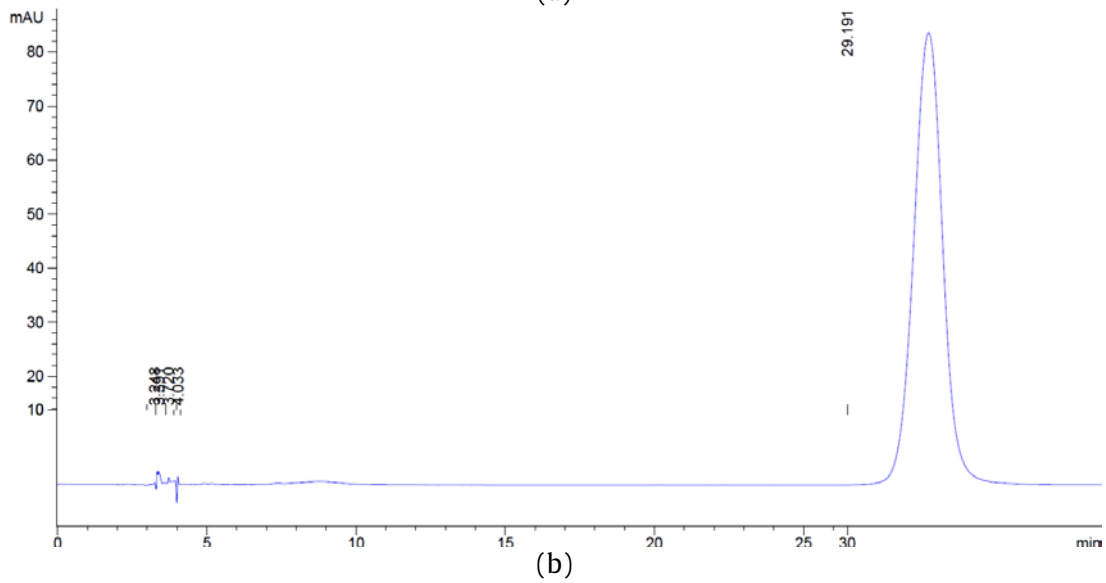

(b)

Figure 2 (a) HPLC chromatogram of standard of gallic acid and (b) mangiferin.

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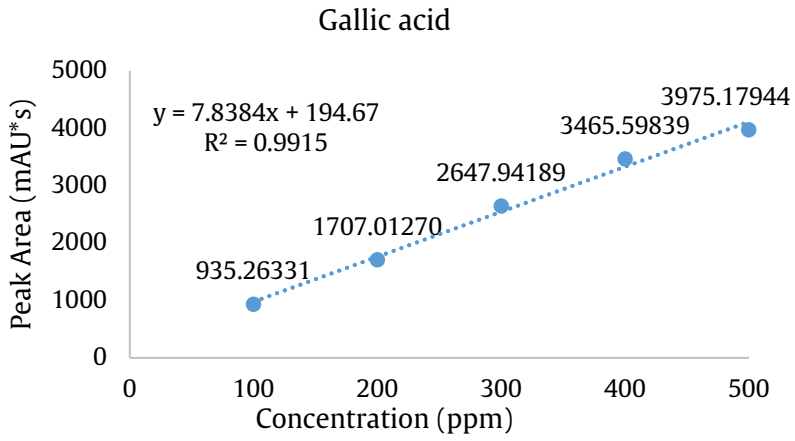

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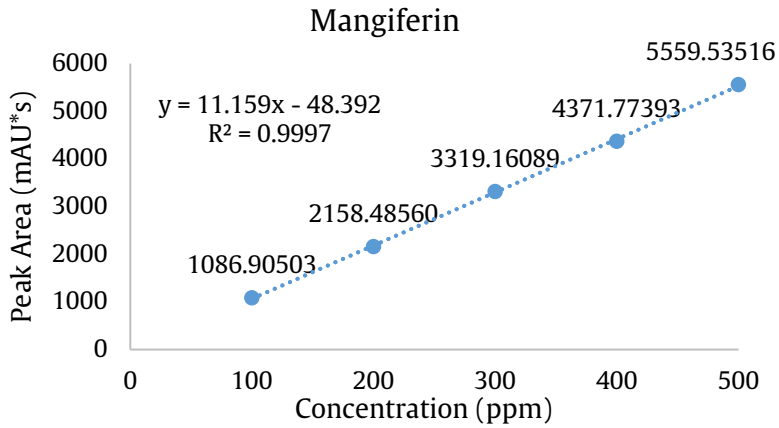

(b)

Figure 3 Calibration curve of (a) gallic acid and (b) mangiferin.

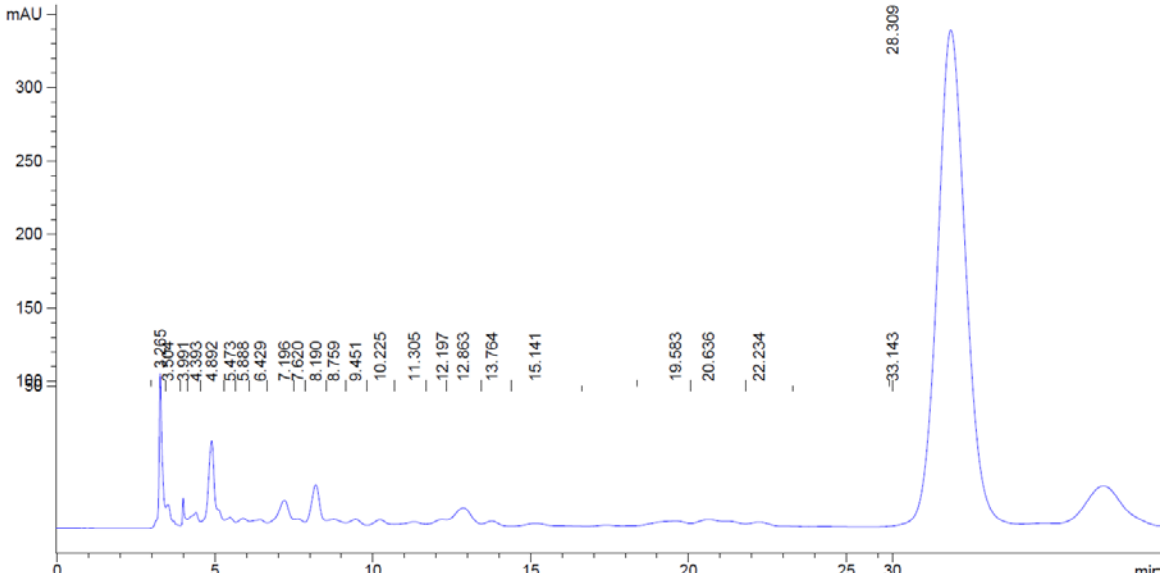

Figure 4 HPLC chromatogram of crude *M. indica* leaf MeOH extract.

According to Fitmawati [28], the wild mango demonstrated the highest antioxidant activity in terms of total antioxidant activity of mangoes by DPPH assay. In this study, the MeOH leaf extract of the wild mango exhibited the highest antioxidant activity (0.52±0.02 µg/mL). Additionally, Fitmawati found that the wild mango displayed significant antioxidants in both its leaf (0.88 µg/mL) and bark (33.24 µg/mL).

HPLC analysis of gallic acid and mangiferin content

The chromatogram in Figure 2 demonstrates the elution of gallic acid at 4.88 minutes and mangiferin

DOI: 10.60101/jarst.2024.255634

at 29.19 minutes. Both gallic acid and mangiferin showed a linear response within the 100-500 mg/L concentration range. The linearity was confirmed with a correlation coefficient (R^2) of 0.9915 and 0.9997 for gallic acid and mangiferin, respectively. The calibration curve can be seen in Figure 3. Figure 4 presents the chromatograms of gallic acid and mangiferin contents in the MeOH extract. The analysis of the crude extracts revealed several peaks with different retention times (R_t) and peak areas. Two peaks were observed at approximately 4.9 and 28 minutes, which correspond closely to the standard gallic acid and mangiferin peaks, respectively. Consequently, the HPLC analysis results indicated that the MeOH extract contained 8.9978 mg/g of gallic acid and 188.0275 of mangiferin, as shown in Table 5.

Table 5 HPLC analysis of gallic acid and mangiferin content in the crude leaf extract MeOH fraction from *M. indica.*

Compound	Retention time (R_t)		Area (mAU*s)	Content (mg/g)
	Standard	MeOH fraction		
Gallic acid	4.878	4.892	899.95129	8.9978
Mangiferin	29.191	29.309	20933.60	188.0275

Table 6 The insecticidal properties of *M.indica* leaf extract and isolated compounds after treatment with 2nd *S.litura* at 24 and 48 hours h via topical.

Compound	Time (h)	LD_{50} (µg/cm²)[a]	LCL-UCL (µg/cm²)[b]	Chi square	*P*-value
n-hexane extract	24	21.53	12.49-62.65	1.05	0.79
	48	0.86	0.80-1.40	2.87	0.41
DCM extract	24	11.10	4.65-55.09	15.88	0.00
	48	0.69	0.41-0.98	7.43	0.06
Ethyl acetate extract	24	11.79	9.56-15.29	2.62	0.45
	48	6.63	5.34-8.42	0.74	0.86
MeOH extract	24	10.58	7.26-18.71	0.96	0.81
	48	0.88	0.49-1.28	3.19	0.36
Mangiferin	24	1.90	0.96-7.41	0.76	0.94
	48	0.77	0.30-2.18	0.54	0.97
Gallic acid	24	1.19	0.36-3.90	0.79	0.94
	48	0.55	0.22-1.21	0.39	0.98

[a]LD_{50}: Lethal Dosage means the compound kills 50% of the exposed larvae, expressed in µg/cm²
[b]Lower Confidence Limit-Upper Confidence Limit (µg/cm²)

Acute toxicity

The larvicidal killing potential of the *M. indica* leaf extracts was determined by applying them topically and measuring the LD_{50} after 24 hours. The MeOH extract had the highest LD_{50} of 10.58 µg per larvae, followed by DCM, ethyl acetate, and *n*-hexane. The specific LD_{50} values can be found in Table 6.

This study found that gallic acid and mangiferin, when isolated, showed LD_{50} values of less than 2 µg per larvae at 24 and 48 hours after topical application. These results suggest these substances may have highly toxic and larvicidal properties when applied topically. A previous study also reported that *Alpinia galanga*, a plant extract, effectively controlled *S. litura* larvae with LD_{50} values of 1.68 and 1.25 µg per larvae after 24 and 48 hours [3]. Moreover, a previous report highlighted the insecticidal efficacy of gallic acid in inhibiting the growth of the oriental fruit fly, *Bactrocera dorsalis* [29].

Impact on detoxification enzyme activity general esterase

The extract from *M. indica* leaves showed inhibition of MeOH (1.31-fold), followed by the *n*-hexane extract (0.98-fold), DCM, and ethyl acetate extracts (both 0.91-fold) against *S. litura*. Additionally, mangiferin and gallic acid exhibited 2.56-fold and 1.27-fold inhibition of CE activity, respectively (Table 7).

Glutathione-S-Transferase (GST)

After exposure to insects, the enzyme reaction was inhibited 1.23-fold by the MeOH extract. Mangiferin exhibited the highest inhibitory activity, with a 1.28-fold increase. However, the *n*-hexane extract caused a 0.94-fold increase in GST levels, which varied (Table 7).

Acetylcholinesterase (AChE)

The leaf extract of *M. Indica* in MeOH showed a 1.31-fold inhibition of AChE, while gallic acid exhibited the highest inhibitory activity with 3.98-fold increase.

DOI: 10.60101/jarst.2024.255634

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However, the *n*-hexane extract caused a 0.99-fold increase in the AChE reaction (Table 7).

Conventional pesticides leave residues in the environment, soil, air, and water, and they negatively impact insect development through detoxification enzymes. However, botanical insecticides offer a safer alternative [30]. Plant extracts, including secondary metabolites, play a crucial role in controlling growth, exterminating larvae, and preventing insect feeding [5]. The metabolism of detoxification enzymes relies on xenobiotic compounds' hydrophilicity and catalytic activity, leading to their elimination through the excretory system. Insects possess cytochrome P450 mono-oxygenase (P450s), a versatile agent for detoxifying foreign compounds such as allelochemicals, common pesticides, and pollutants. Other foreign enzymes like glutathione-*S*-transferase (GST), carboxylase (CE), and acetylcholinesterase Ares (AChE) also play a significant role as key metabolites, inducing or neutralizing plant toxins [31-33]. Although the MeOH extract, which contains mangiferin and gallic acid, was isolated in the present phytochemical study, these compounds inhibited CE levels in *S. litura.*

Table 7 Detoxification enzyme reactions after *M. indica* leaf extracts and allelochemical application on 2nd instar *S. litura* larvae after 24 hours.

Compound	Carboxylesterase		Glutathione-*S*-transferase		Acetylcholinesterase	
	reaction[a]	CF[b]	reaction[a]	CF[b]	reaction[a]	CF[b]
Control	0.0563[c]	-	0.2529 [bc]	-	0.1916[e]	-
n-hexane extract	0.0572[c]	0.98	0.2680 [c]	0.94	0.1940[e]	0.99
DCM extract	0.0620 [c]	0.91	0.2275 [abc]	1.11	0.1740[c]	1.10
Ethyl acetate extract	0.0621 [c]	0.91	0.2276 [ab]	1.10	0.1540[bc]	1.24
MeOH extract	0.0430[a]	1.31	0.2063 [a]	1.23	0.1457[d]	1.32
Mangiferin	0.0220 [b]	2.56	0.1974 [ab]	1.28	0.1741[a]	1.10
Gallic acid	0.0445[b]	1.27	0.2085 [a]	1.21	0.0482 [b]	3.98

[a]Mean values within a column followed by the same letter were not significantly different ($P < 0.05$) by Tukey's test. Carboxylesterase (CE) measured as *p*-nitrophenol/min/mg esterase in nM. Glutathione-*S*-transferase (GST) measured as glutathione conjugated product/min/mg in nM. Acetylcholinesterase (AChE) measured as µM/min/mg of protein.

[b]Correction factor (CF) of detoxification enzyme activities calculated as enzyme activity in controls / enzyme activity in treatments.

In contrast, the extracts of *n*-hexane, DCM, and ethyl acetate induce the activity of CE in *S. litura* by 0.91 to 0.98-fold after exposure. CE is a hydrolase enzyme that is widely distributed in organisms, including bacteria and animals. It hydrolyzes water-soluble small molecules of ester and acylglycerol bonds in insects, making it an important detoxification enzyme involved in the development of insecticides [34, 35]. Similarly, the MeOH extract and its isolated compounds inhibit the levels of GST in *S. litura*. GST is an enzyme found in the metabolism of both eukaryotes and prokaryotes, and it is involved in the processing of endogenous and foreign substances. Although this study demonstrates that other parts of *M. indica* induce GST activity in *S. litura* after exposure, it remains unclear whether *M. indica* itself induces GST activity. GST also catalyzes glutathione peroxidase and plays a role in regulating cellular mechanisms [36]. Almost all fractions of *M. indica*, except the *n*-hexane extract, exhibit larvicidal properties by inhibiting the AChE reaction in *S. litura*. AChE is an essential enzyme in both vertebrates and invertebrates and is used in biochemical sensors to induce the AChE pathway or recurrent excitation. ChE inhibitors are useful for treating neurological symptoms caused by acetylcholine (Ach) deficiency. However, it is necessary to have an inducer of AChE inhibition in order to block inappropriate Ach neurotransmitters [36]. Therefore, pest strategies need to be economically and ecologically addressed in order to reduce the harm caused by conventional chemical compounds. Plant extracts contain allelochemicals and active metabolites, which are toxic compounds that protect plants against herbivorous organisms. This indicates that phytochemicals can be applied to insects and have a phytochemical effect that is effective against specific or multiple target sites through a biochemical pathway.

CONCLUSIONS

This study aimed to analyze the phytochemical composition and antioxidative capabilities of mango leaves (*M. indica* L., Nam Dok Mai), indigenous to Sa Kaeo Province, Thailand. Leaf extraction was performed using different solvents with varying polarities: *n*-hexane, DCM, ethyl acetate, and MeOH. The results revealed the presence of eight groups of phytochemical compounds: alkaloids, flavonoids, coumarins, saponins, tannins, terpenoids, steroids, and cardiac glycosides. The MeOH crude extract exhibited the highest

DOI: 10.60101/jarst.2024.255634

concentration of total phenolic compounds at 409.88 ±0.02 mg GAE/g. Moreover, the MeOH crude extract demonstrated the strongest antioxidant activity with an IC_{50} value of 0.52 ±0.02 μg/ml, as determined by the DPPH method. High-performance liquid chromatography (HPLC) was employed to identify gallic acid and mangiferin in the MeOH crude extract. To assess the toxicity of the *M. indica* leaf crude extract on 2nd instar *S. litura* larvae, laboratory tests were conducted using the topical application method. The MeOH crude extract exhibited high efficacy, with an LD_{50} value of 10.58 μg per larvae within 24 hours. Gallic acid and mangiferin were identified as the primary active ingredients, with LCD_{50} values of 1.19 μg per larvae and 1.90 μg per larvae, respectively, within 24 hours. Additionally, using the topical application method, the impact on detoxification enzymes (24 hours post-treatment) was evaluated in surviving 2nd instar *S. litura* larvae. The MeOH extract treatment resulted in a 1.31-fold inhibition of carboxylesterase (CE), a 1.31-fold inhibition of glutathione-*S*-transferase (GST), and a 1.32-fold inhibition of acetyl-cholinesterase (AChE).

Mango leaves contain beneficial substances that can effectively 2nd instar *S. litura* larvae. This makes them a potential source for developing insecticide products. Furthermore, it is recommended that other active botanical compounds found in *M. indica* for their insecticidal properties be investigated further in the future.

ACKNOWLEDGEMENT

This research was supported by Fundamental Fund (FF) from Thailand Science Research and Innovation. Thanks to Department of Zoology and Department of Chemistry, Faculty of Science, Kasetsart University including Science Center, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage for supporting and using analytical equipment in this research.

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DOI: 10.60101/jarst.2024.255634