# Pancreatic Lipase Inhibitory Effects of Murraya koenigii Root Extracts

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Obesity is a growing health problem in many countries including Malaysia. Murraya koenigii, also known as curry tree, is a traditional herbal remedy from the family of Rutaceace valued for its wide range of traditional uses and pharmacological properties. While previous studies have shown its effectiveness as an anti-obesity agent in live animals through in vivo testing, the phytochemical components of this plant responsible for this effect remain relatively unexplored. The hexane and dichloromethane extracts of the roots of M. koenigii and their fractions were evaluated for their pancreatic lipase inhibitory activity via in vitro experiment. The dichloromethane (DCM) extract exhibited slightly higher inhibitory activity of 48.03%, followed by the hexane extract (37.08%), with FTIR analysis revealing key functional groups indicative of potential bioactive phytochemicals in the DCM extract. In addition, the DCM extract was further fractionated into ten fractions, namely Fraction 1 (DCM) to Fraction 10 (DCM), by column chromatography. Among all 10 fractions, Fractions 4 to 10 showed positive PL inhibition within the range of 61.83% to 95.78% at the concentration of 10 mg/mL. The results highlighted Fraction 7 (DCM), which exhibited the highest activity of 95.78%, with an IC<sub>50</sub> value of 492.77 ± 54.72 μg/mL. The crude extracts derived from the roots of M. koenigii (L.) Spreng (Rutaceae) and their fractions exhibit a significant potential as inhibitors of pancreatic lipase.

**Keywords**: *Murraya koenigii*; antiobesity; pancreatic lipase inhibitory assay; maceration; *p*-nitrophenyl butyrate

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Overweight and obesity are complex and widespread public health problems with significant and often unpredictable consequences for individuals' health. These conditions are closely linked to the development of non-communicable diseases, particularly cardiovascular diseases such as myocardial infarction and stroke, as well as type 2 diabetes mellitus, which are among the leading causes of death worldwide. Obesity develops when energy intake consistently exceeds energy expenditure through basal metabolism and physical activity, resulting in the excessive accumulation of adipose tissue. According to a report by World Health Organization (WHO), projected for 2025, approximately 35 million children under the age of five are projected to be overweight, defined by a body mass index (BMI) equal to or greater than the age-appropriate threshold [1]. This alarming trend reflects the influence of obesogenic environments, which promote weight gain due to a combination of structural determinants, such as urbanization, socioeconomic inequality, inadequate access to healthcare, and the widespread availability of energydense, nutrient-poor foods.

Excess caloric intake, especially dietary fat, is the primary cause of most overweight and obesity. Pancreatic lipase is a key digestive enzyme that facilitates the digestion of lipids in the small intestine, hydrolyzing triglycerides into monoglycerides and free fatty acids [2]. These breakdown products are subsequently re-esterified in enterocytes and packaged into chylomicrons, which are then transported to the adipose tissue via the lymphatic system and stored in white adipocytes, leading to cell hypertrophy and proliferation [3]. Excess visceral fat, particularly that is rich in triglycerides, is associated with increased lipolysis. In certain pathological conditions, such as acute pancreatitis, pancreatic lipase abnormally penetrates adipose tissue and catalyzes the hydrolysis of triglycerides, leading to adipocyte necrosis and an inflammatory cascade [4]. Importantly, the use of pancreatic lipase inhibitors has been shown to reduce dietary fat absorption [5], thereby mitigating some of the metabolic consequences of obesity.

Murraya koenigii, commonly known as curry tree, has garnered significant interest as a natural antiobesity agent, largely due to its abundant carbazole alkaloids that exhibit diverse pharmacological activities. In one pivotal preclinical study, ethyl acetate, and dichloromethane extracts, along with alkaloidenriched fractions, consistently demonstrated substantial anti-obesity and lipid-lowering effects in both highfat diet-induced obese rodents and 3T3-L1 adipocyte models. When administered at 300 mg/kg/day for two weeks, these extracts significantly curtailed body weight gain and reduced plasma total cholesterol and triglyceride levels in HFD-fed rats [6]. A recent study demonstrated significant anti-obesity effects in high-fat diet-induced Wistar rats treated with 200 mg/kg and 400 mg/kg doses of ethanolic leaf extract of M. koenigii [7]. A notable reduction was observed in the triglyceride (Tg) and low-density lipoprotein cholesterol levels along with a marked increase in the high-density lipoprotein cholesterol level. Collectively, this spectrum of activities highlights the multifaceted pharmacodynamics of M. koenigii; its carbazole alkaloids not only inhibit lipid absorption [10] but also modulate adipogenesis [8] and reduce inflammation [9], presenting a promising multi-targeted strategy for managing obesity. In addition, numerous studies provide valuable insights into the anti-obesity potential of the plant, particularly focusing on the leaves of M. koenigii, such as Birari et al. [10] and Pinto et al. [11]. Nevertheless, the roots of M. koenigii remain largely underexplored within this domain, despite the leaves demonstrating significant antiobesity potentials across numerous studies. This study continues our extensive research on M. koenigii [12–15] and successfully explores the potential of M. koenigii roots as a source of anti-obesity drugs.

# MATERIALS AND METHODS

# **Plant Material**

Roots of Murraya koenigii (L.) Spreng (TM1008) were collected from Sibu, Sarawak, Malaysia. This plant material was authenticated by the Phytochemistry Group, Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Malaysia. The respective plant materials were air-dried and ground into fine powder for further use.

# **Chemicals and Materials**

All chemicals utilized were of analytical grade. The chemicals and materials utilized were hexane (HmbG Chemical, HXE-0723-00-4E-131023), dichloromethane (DCM) (Fisher Scientic, 241022.1), p-nitrophenyl butyrate (Sigma-Aldrich, 1003589987), orlistat (Sigma-Aldrich, LRAC7141), porcine pancreatic lipase (Sigma-Aldrich, 282239), potassium dihydrogen phosphate (Supelco, AM 1797473223) and di-potassium hydrogen phosphate (Supelco, AM1779504207), silica gel (0.040-0.064 mm) (Merck, TA5016985845), TLC silica gel 60 F<sub>254</sub> (Merck, HX98205654), and 96-well tissue culture plates (Biofil, 230718-078-F).

# **Preparation of Crude Extracts and Isolation**

The dried root powder of M. koenigii (780 g) was subjected to sequential maceration with hexane and dichloromethane at room temperature, repeated three times to ensure exhaustive extraction. The combined extracts were concentrated under reduced pressure to afford a dark brown viscous residue, yielding 11.64 g of hexane extract and 27.38 g of dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) extract. Following solvent evaporation under ambient conditions, both crude extracts were chromatographed on silica gel using a gradient elution system of hexane, dichloromethane, and methanol. This process resulted in the isolation of ten dichloromethanederived sub-fractions, designated as Fraction 1 (DCM) through Fraction 10 (DCM), via column chromatography.

#### **Pancreatic Lipase Inhibitory Activity**

Pancreatic lipase activity was evaluated using the modified method based on Jeong et al. [16] in a 96well plate. It was assayed using porcine pancreatic lipase, with p-nitrophenyl butyrate (pNPB) as the substrate and orlistat as the standard. Potassium phosphate buffer was prepared at the concentration of 0.1 M and adjusted to pH 8. Porcine PL enzyme was dissolved in potassium phosphate buffer to obtain the concentration of 10 mg/mL. In addition, p-nitrophenyl butyrate was prepared in dimethyl sulfoxide to give a 10 mM stock solution for later use. The enzyme and substrate solutions were stored at -20°C and below 4°C, respectively.

In the 96-well plate, 50 μL of DMSO was first added to each well. Then, the extracts and fractions were dissolved in DMSO at a concentration of 10 mg/mL and serially diluted to obtain the final concentrations of 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.156 mg/mL, with 50 µL of each dilution added per well. After performing the serial dilution in the 96-well plate, each sample was mixed 25 µL of the porcine pancreatic lipase (PL) enzyme solution. Then, the reaction mixture was incubated at 37°C for 30 minutes. Subsequently, 25 µL of the substrate solution (pNPB) or DMSO (for negative control without substrate) was added to the reaction mixture. Finally, 100 µL of buffer was added to each well to reach a final volume of 200 µL, followed by a second incubation at 37°C for 10 minutes. This reaction involves the hydrolysis of p-nitrophenyl butyrate to p-nitrophenol and butyric acid. The absorbance of p-nitrophenol was measured at 405 nm using a microplate reader. Hence, the calculation was made by following the formula below. All the experiments were performed in triplicate.

Lipase inhibitory activity (%) = 
$$100 - \left(\frac{Abs (A) - Abs (a)}{Abs (B) - Abs (b)} \times 100\right)$$

Where, B=activity without inhibitor, b=negative control without inhibitor, A=activity with inhibitor,

and a=negative control with inhibitor. DMSO was used as the negative control.

#### Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR analysis was performed using a Shimadzu IR Tracer 100 spectrometer. A small quantity of the extracts was placed on the sample holder, and the spectra were recorded over a wavenumber range of 4000-600 cm<sup>-1</sup>, using 32 scans per sample.

#### **Statistical Analysis**

All data were recorded as means  $\pm$  SD of triplicate measurements. Statistical analyses of the data were performed using GraphPad Prism software. IC<sub>50</sub> values and statistical significance (p-values) were determined using appropriate non-linear regression and statistical tests.

#### RESULTS AND DISCUSSION

#### Percentage Yield and Fractionation of Crude Extracts

The dried M. koenigii roots (780 g) were powdered and subjected to maceration in hexane and dichloromethane (DCM), yielding 11.64 g of hexane crude extract (1.49%) and 27.38 g of DCM crude extract (3.51%). Among the solvents evaluated, dichloromethane (DCM) showed a higher extraction yield, suggesting that moderately polar solvents are more effective for extracting bioactive compounds from M. koenigii roots.

### **Pancreatic Lipase Inhibitory Activity**

Both crude extracts were subjected to pancreatic lipase (PL) inhibition assay at various concentrations: 0.039, 0.078125, 0.15625, 0.3125, 0.625, 1.25, and 2.5 mg/mL. According to the results shown in **Table 1**, the DCM crude extract exhibited a slightly higher PL inhibition activity of 48.03%, compared to 37.6% for the hexane extract. Orlistat was used as the standard reference drug, and its pancreatic lipase inhibitory activity was used to compare with that of the fractions. The calculated IC50 value of orlistat was 44.065 µM (equivalent to 21.85 μg/mL).

The inhibitory activity of the fractions was weaker than that of the standard drug orlistat and this was expected. The extracts comprise of a diverse array of phytochemical constituents, hence the DCM crude extract was chosen to be fractionated via column chromatography with an increasing gradient of hexane: DCM followed by DCM: methanol. A total of 10 fractions were collected based on the tested TLC patterns. The weight and pancreatic lipase inhibitory activity, as percent inhibition, of the ten fractions were recorded and are presented in Table 1.

Among the fractions, six exhibited strong inhibition, with percent inhibition values exceeding 70%. Fractions 7, 8, 9, and 10 showed inhibition percentages greater than 85%, indicating their high potential as natural pancreatic lipase inhibitors, warranting further isolation to identify the active constituents. While the fractions demonstrated weaker activity than the standard drug orlistat, this was anticipated due to the presence of a complex mixture of phytochemicals. Thus, additional fractionation is needed to isolate the more potent active compounds. M. koenigii is particularly noted for its abundance of carbazole alkaloids, which are likely to be key bioactive constituents responsible for its pharmacological effects.

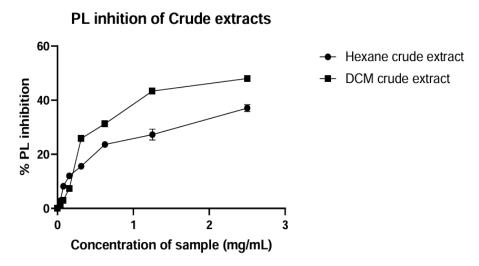


Figure 1. Pancreatic lipase inhibition activity of *Murraya koenigii* root extracts.

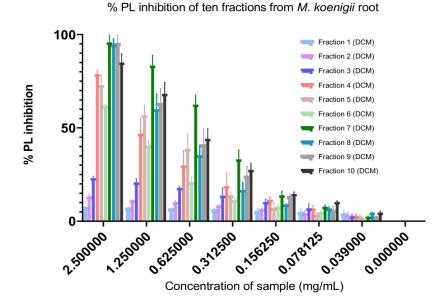


Figure 2. Pancreatic lipase inhibition activity of ten fractions of M. koenigii roots.

**Table 1.** Comparison of the yields and pancreatic lipase inhibitory effects of hexane and dichloromethane (DCM) extracts and their subfractions from Murraya koenigii roots.

Test Sample	Weight of fraction (mg)	% of PL inhibition (Conc: 2.5 mg/mL)	IC <sub>50</sub> value (μg/mL)
Hexane	11640	37.06	-
Dichloromethane (DCM)	27380	48.03	-
Fraction 1 (DCM)	193.7	7.55	-
Fraction 2 (DCM)	11456.3	13.22	-
Fraction 3 (DCM)	2172	23.06	-
Fraction 4 (DCM)	260.8	78.73	1345.58±275.92
Fraction 5 (DCM)	564	72.81	987.62±182.46
Fraction 6 (DCM)	240.8	61.83	1799.8077±90.26
Fraction 7 (DCM)	370.7	95.78	492.77±54.72
Fraction 8 (DCM)	2732.6	94.53	1017.40±155.42
Fraction 9 (DCM)	281.6	92.52	$838.95 \pm 107.62$
Fraction 10 (DCM)	1475	85.01	772.93±104.64

Note: Percentage inhibition was measured at 2.5 mg/mL and IC<sub>50</sub> values were determined for the most active fraction.

As shown in Figure 1, the DCM extract of the M. koenigii roots exhibited 48.03% PL inhibitory activity at a concentration of 2.5 mg/mL, which was slightly higher than that of the hexane extract (37.6%). As illustrated in Figure 2, a dose-dependent pancreatic lipase inhibitory activity was observed across the ten

DCM fractions derived from the M. koenigii roots. A clear trend was observed, where Fraction 7 (represented in dark green) exhibited the highest pancreatic lipase inhibitory activity across all tested concentrations, showing 95.78% inhibition, approaching 100% at the highest tested concentration (2.5 mg/mL). In addition,

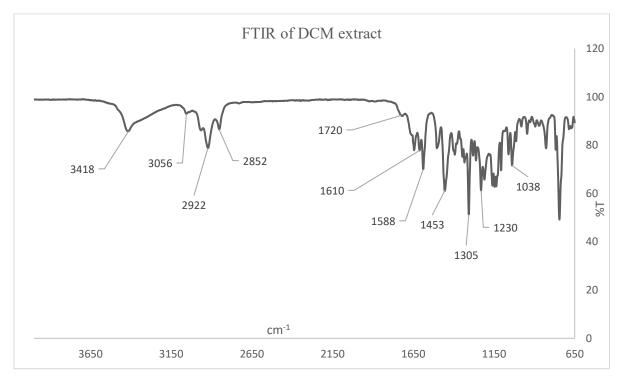
Fraction 10 (black) showed the second-highest PL inhibition at 2.5 mg/mL and maintained relatively strong activity at lower concentrations. In contrast, Fractions 1 to 3 exhibited low inhibition levels, all below 50%. The data referenced in **Figure 2** are further summarized in **Table 1**.

This study represents the first investigation into the roots of M. koenigii as a source of pancreatic lipase inhibitors. As shown in Table 2, the pancreatic lipase inhibitory activity of the DCM fractions varied significantly among the ten fractions. Fractions 7 to 10 exhibited the highest inhibitory activity, with Fraction 7 showing the strongest inhibition at 95.78% and an IC<sub>50</sub> value of 492.77  $\pm$  54.72 µg/mL. This was followed closely by Fraction 8 with 94.53% inhibition and an IC<sub>50</sub> value of 1017.40  $\pm$  155.42  $\mu$ g/mL, and Fraction 10 with 85.01% inhibition and an IC<sub>50</sub> value of  $3.0917 \pm 1.42$  mg/mL. Moderate inhibition was observed in Fractions 4 to 6, which exhibited 78.73%, 72.81%, and 61.83% inhibition, respectively, suggesting the presence of lipase-inhibitory compounds at lower concentrations or in less active forms. In contrast, Fractions 1 to 3 showed minimal activity, with inhibition values of only 7.55%, 13.22%, and 23.06%, respectively, indicating a lower abundance or absence of effective inhibitory constituents in these early fractions. Although Phatak et al. [18] have linked the antihyperlipidemic properties of M. koenigii to phytoconstituents such as alkaloids, saponins, and flavonoids, further investigations are required to determine which specific compounds are most significant to pancreatic lipase inhibition. The

pancreatic lipase (PL) inhibitory activity of Fraction 7 (IC<sub>50</sub>=492.77  $\pm$  54.72 µg/mL) was compared with that of reported carbazole alkaloids from *M. koenigii*. Mahanimbine (IC<sub>50</sub> = 17.9 µM) and koenimbine (IC<sub>50</sub> = 168.6 µM) have been shown to inhibit PL [19], and both compounds can be isolated from a dichloromethane (DCM) extract of *M. koenigii* [14, 20, 21]. The IC<sub>50</sub> value of fraction 7 was comparable to that of carbazole alkaloids from *M. koenigii*, suggesting that its biological activity may be at least partially attributable to these carbazole components. Ongoing work in our laboratory is focused on isolating these bioactive components, particularly the alkaloids, from their most active fractions.

# Fourier-Transform Infrared (FTIR) Spectroscopy Analysis

The FTIR spectrum (**Table 2**, **Figure 3**) revealed several key functional groups indicative of bioactive phytochemicals. In this study, the FTIR spectrum of the DCM extract of *M. koenigii* exhibited absorption peaks within the range of 4000-600cm<sup>-1</sup>, which correspond to various molecular vibrations. The observed peak values are consistent with prior studies (Majid and Harun [22], Aniqa and Kaur [23], Khurana et al. [24], Umeanadu and Ezumezu [25]). A strong peak at 3418 cm<sup>-1</sup> suggests N–H stretching, commonly associated with alkaloids and amides that may act at enzyme binding sites [25]. Peaks at 3056.7 and 2922.36 cm<sup>-1</sup> indicate C–H and sp<sup>3</sup> C–H/O–H stretching, respectively, characteristic of alkenes, flavonoids, and saponins, which can form hydrogen bonds with pancreatic lipase.



**Figure 3.** FTIR spectrum of the DCM extract of *M. koenigii* roots.

Wave number (cm <sup>-1)</sup> )	Intensity estimation	Group or function class	Type of vibration	Functional group
3418.92	Strong	O-H/N-H	Stretching	Alcohol/ Amine
3056.7	Medium	С-Н	Stretching	Alkene
2922.36	Strong	Sp <sup>3</sup> C-H /OH stratching	Stretching	Alkene
2852.95	Broad	N-H	Stretching	Alkane
1720.7	Weak	C=O/C-N/N-H	Stretching	Carbonyl/Nitrile/amines
1610.80	Strong	C=C/C=O	Stretching	Aliphatic/aromatic
1588.50	Strong	С-Н	Bending	Aromatic
1455.66	Strong	C=O/C=C/N-H	Stretching	Carbonyl/alkenes/amides/ amines
1305.81	Strong and sharp	C-O	Stretching	Aromatic
1230.29	Strong and sharp	C-N	Stretching	Amine
1038.41	Strong and sharp	C-N	Stretching	Aliphatic ammine

**Table 2.** FTIR spectrum of the DCM extract of *M. koenigii* roots.

A broad absorption band at 2852.95 cm<sup>-1</sup> further supports the presence of nitrogen-containing compounds such as alkaloids. A weak peak at 1720.7 cm<sup>-1</sup> is attributed to C=O, C-N, or N-H stretching, suggesting the presence of carbonyl and amide groups, possibly derived from tannins or peptides [26]. Strong peaks at 1610.80 and 1588.50 cm<sup>-1</sup> correspond to aromatic C=C stretching and C-H bending, indicative of flavonoids and other aromatic compounds capable of  $\pi$ - $\pi$  stacking interactions with lipase. Collectively, these spectral features confirm the presence of multiple phytochemical classes, such as alkaloids, flavonoids, saponins, and tannins, all known to inhibit pancreatic lipase through hydrogen bonding, steric hindrance, and hydrophobic interactions. These findings support the observed inhibitory activity in the DCM root extract fractions of M. koenigii.

# CONCLUSION

In this study, both hexane and dichloromethane (DCM) extracts of Murraya koenigii roots were evaluated for their pancreatic lipase inhibitory activity. Both extracts demonstrated moderate inhibition, with the DCM extract showing a slightly higher inhibitory effect (48.03%) compared to the hexane extract (37.08%). Following fractionation of the DCM extract, Fraction 7 exhibited the most potent activity, with a 95.78% inhibition rate, making it a promising candidate for further development as a natural anti-obesity agent targeting pancreatic lipase. Ongoing fractionation and compound isolation are being conducted to identify the specific bioactive phytochemicals responsible for this activity. These efforts aim to elucidate the mechanism of inhibition and support the potential clinical application of M. koenigii root extracts in obesity management.

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