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Research article

Morinda scabrida Craib: An unexplored species with antibacterial potential and inhibition of acetylcholine esterase and α-glucosidase

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Abstract

Importance of the work: The bioactivity levels of pure compounds isolated from the plant *Morinda scabrida* Craib have not been investigated.

Objectives: To explore the bioactivity of active compounds isolated from *Morinda scabrida* Craib, an unexplored species of the *Morinda* plant.

Materials & Methods: The roots of *Morinda scabrida* Craib were processed and subjected to maceration, chromatographic separation and structural elucidation using spectroscopic techniques. Five pure compounds were obtained and tested for their antibacterial activity and inhibitory effects against acetylcholine esterase and α-glucosidase. Molecular docking simulations further illustrated the binding modes to their biomolecular targets.

Results: Gallic acid and four ester derivatives were obtained from the root extract of *Morinda scabrida* Craib. Among these five compounds, M05 (octyl gallate) had antibacterial activity against Gram-positive and Gram-negative bacteria. In addition, M05 (100 µM) showed 71% inhibition against α-glucosidase at 100 µM. Furthermore, all isolated compounds (M01–M05) exhibited inhibitory effects (57–65% inhibition) against acetylcholine esterase at 100 µM. Finally, the molecular docking simulations elucidated the binding energies of all isolated compounds toward acetylcholine esterase and α-glucosidase and the best-docked conformation of the most active compound (M05) in the active sites of these two enzyme targets was illustrated, showing the key intermolecular interactions responsible for ligand binding.

Main finding: This was the first report on the antibacterial, anti-acetylcholine esterase and anti-α-glucosidase activities of five chemical constituents isolated from *Morinda scabrida* Craib. The results demonstrated the medicinal potential of *M. scabrida* Craib, which could be developed further for use as a traditional medicine.

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Introduction

Morinda citrifolia (noni), a plant belonging to the *Morinda* genus, has a long history of traditional medicinal use for over two thousand years (Wang et al., 2002). The *Morinda* genus is composed of approximately 40 distinct species, with 9 of them being native to Thailand (Kesonbuaa, 2013). Other research has isolated numerous groups of compounds from various *Morinda* species, encompassing benzoids, flavones, fatty acids, flavonols, iridoid monoterpenes, quinoids, steroids and triterpenes (Assi et al., 2017).

Beyond their chemical diversity, *Morinda* plants have been associated with various biological activities, making them particularly noteworthy in medicinal contexts. These activities include antibacterial (Natheer et al., 2012; Wang et al., 2016; Torres et al., 2017; De La Cruz-Sanchez et al., 2019; Zhai et al., 2019) and antifungal (Wang et al., 2002; Jainkittivong et al., 2009; Assi et al., 2017; Torres et al., 2017) properties, contributing to their potential to combat microbial infections. Additionally, *Morinda* plants have exhibited anti-inflammatory effects (Torres et al., 2017), hinting at their potential use in reducing inflammation-related conditions. In addition, their antiprotozoal activity signifies possible applications against protozoan infections (Kwofie et al., 2016). Most importantly, their anticancer properties have sparked interest in exploring their role in cancer therapy (Furusawa et al., 2003; Brown, 2012; Sharma et al., 2016; Li et al., 2019; Latifah et al., 2021; Chee et al., 2022; Li et al., 2022). As a result of the vast array of medicinally relevant compounds and biological activities exhibited by *Morinda* plants, they are regarded as rich sources of natural products with substantial therapeutic potential. Researchers continue to dedicate efforts to further explore the beneficial properties of *Morinda* plants in therapeutics.

Morinda scabrida Craib was initially discovered in Thailand in 1932 (Craib, 1932). Subsequently, in 2013, the key to the species was officially published (Kesonbuaa, 2013). Despite its long history, the chemical constituents and bioactivity of *M. scabrida* Craib have not been elucidated, prompting the current study to investigate its bioactive compounds. The primary objectives involved identifying bioactive chemicals within this plant and exploring their potential effects on three biological activities: antibacterial activity, anti-acetylcholine esterase activity and anti-α-glucosidase activity. The last two are particularly relevant to Alzheimer's disease and diabetes mellitus (Amenta et al., 2001; Chatterjee and Davies, 2015; Dirir et al., 2022).

Materials and Methods

Processing, extraction and purification of plant material

The roots of *M. scabrida* Craib were collected from a site (14°40′5.2″N 98°35′43.9″E) in Tongpaphum district, Kanchanaburi province. Species identification was examined by consulting the previous report (Kesonbuaa, 2013) and voucher specimens collected in the Forest Herbarium-BKF (BKF.No.196975). Then, the newly collected plant materials were ground into small pieces and air-dried at 45°C for 3 d in a hot-air oven. Subsequently, 1 kg of the dried *M. scabrida* Craib roots were subjected to successive macerations with 3 L of methanol (MeOH) for 2 d to obtain 39.2 g of viscous methanolic extracts after the removal of the solvent under vacuum. Subsequently, the crude extracts were suspended in 200 mL of water and sequentially partitioned using 200 mL of ethyl acetate (EtOAc) thrice. The combined organic layers were evaporated to dryness to yield 3 g of the crude extracts, which were subjected to a series of chromatographic separations and recrystallization with dichloromethane (DCM) to provide five pure compounds (M01–M05). The spectroscopic analysis identified them to be gallic acid (M01), methyl gallate (M02), ethyl gallate (M03), propyl gallate (M04) and octyl gallate (M05).

Gallic acid (M01): white solid (100 mg), ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.92 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.9, 146.0, 138.6, 121.1, 109.5. HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_7H_6NaO_5$: 193.0119; found: 193.0107

Methyl gallate (M02): white solid (90 mg), ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.94 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.4, 145.6, 138.5, 119.4, 108.6, 51.7. HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_8H_8NaO_5$: 207.0284; found: 207.0264

Ethyl gallate (M03): white solid (50 mg), ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.94 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.9, 145.6, 138.4, 119.6, 108.5, 60.1, 14.3. HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_9H_{10}NaO_5$: 221.0441; found: 221.0420.

Propyl gallate (M04): white solid (60 mg), ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95 (s, 2H), 4.11 (t, J = 6.6 Hz, 2H), 1.66 (q, J = 7.1 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C NMR

(100 MHz, DMSO-d_6): δ 166.0, 145.6, 138.4, 119.6, 108.5, 65.5, 21.8, 10.5. HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{12}NaO_5$: 235.0604; found: 235.0577.

Octyl gallate (M05): white solid (59 mg), ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.94 (s, 2H), 4.14 (t, J = 6.5 Hz, 2H), 1.64 (p, J = 6.5 Hz, 2H), 1.29 (m, 12H), 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.9, 145.6, 138.4, 119.6, 108.5, 64.0, 31.3, 28.69, 28.68, 28.3, 25.6, 22.1, 14.0. HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calcd for $C_{15}H_{22}NaO_5$: 305.1371; found: 305.1359.

Antibacterial evaluation

The antibacterial activity levels of M01–M05 were assessed against four human pathogens: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (DMST 4212) and *Pseudomonas aeruginosa* (DMST 4739) using a disc diffusion method. The inoculation level was 1×10^6–1×10^7 colony forming units (CFU). Stock solutions of M01–M05 were prepared at 10 mg/mL. Penicillin (6 µg/mL) and ciprofloxacin (10 µg/mL) were used as positive controls for Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria, respectively. Dimethyl sulfoxide (DMSO) was used as a negative control and to prepare test compound and positive control stocks. Samples (each 20 µL) of the stock solution were loaded on a disc in each experiment. The inhibition zone diameter was measured after 24 hr incubation at 37°C.

Acetylcholine esterase inhibition

A colorimetric assay was utilized to determine the AChE activity with slight modifications (Ellman et al., 1961). Briefly, the experiment was carried out using 96-well microtiter plates. Each reaction was conducted in 100 mM phosphate buffer saline (PBS) pH 8.0 containing 40 mU/mL acetylcholine esterase (EC 3.1.1.7, type VI-S from *Electrophorus electricus*), 120 µM 5,5′-dithiobis(2-nitrobenzoic acid) and in the presence or absence of the test compound (100 µM final concentration). The reactions were incubated for 10 min at 37°C. Then, acetylthiocholine was added to the final concentration of 120 µM (200 µL total reaction volume) and the reaction was further incubated for 20 min at 37°C. The absorbance was measured at a wavelength of 412 nm using a microplate reader (MPR; Spark™ 10M). Tacrine (0.5 µM) was used as a positive control. The experiments were conducted in triplicate and the percentage inhibition was calculated using Equation 1:

$$\text{Inhibition (\%)} = \left(\frac{Ab - As}{Ab}\right) \times 100 \quad (1)$$

where *As* is the absorbance of the reaction with the test compound and *Ab* is the absorbance of the reaction without the test compound.

α-Glucosidase inhibition

Inhibition of α-glucosidase was monitored according to the reported protocol (Pluempanupat et al., 2007). In brief, the assays were conducted in a 100 µL reaction mixture containing 50 mM PBS pH 6.8, 40 mU/mL α-glucosidase (EC 3.2.1.20, type I from *Saccharomyces cerevisiae*), 200 mM 4-nitrophenyl-α-D-glucopyranoside (PNPG) and in the presence or absence of the test compound (100 µM final concentration). After 15 min, the reaction was quenched by adding 50 µL of 400 mM Na_2CO_3. Subsequently, the absorbance was measured at 400 nm using a microplate reader (MPR; Spark™ 10M). Acarbose (1.6 mM) was used as a positive control. The experiments were done thrice and the percentage inhibition was calculated using Equation 1.

Molecular docking simulations

The 3D structures of M01–M05 were obtained from the DFT calculations using the M062X method with the 6-31g(d,p) basis set implemented in the Gaussian09W package (Frisch et al., 2009). The 3D structures of tacrine and acarbose were obtained from the crystal structures 4BDS (Nachon et al., 2013) and 5NN8 (Roig-Zamboni et al., 2017), respectively. The protein crystal structures of acetylcholine esterase (PDB: 4EY7; from Cheung et al., 2012) and α-glucosidase (3A4A; from Yamamoto et al., 2010) were acquired from the Protein Data Bank (https://www.rcsb.org/). The ligands and the proteins were prepared using the AutoDockTools 1.5.6 package (Sanner, 1999). Water molecules and small molecules were removed from the protein structures and the Gasteiger charges were assigned to generate the PDBQT files. The grid box dimensions were 20 Å × 20 Å × 20 Å and centered at the active site of the enzyme. Then, the AutoDock Vina program (Eberhardt et al., 2021; Trott and Olson, 2010) was executed and the results were analyzed using the AutoDockTool 1.5.6 software. Then, the best-docked conformations were visualized using the Discovery Studio Visualizer V21.1.0.20298 package (BIOVIA, 2020).

Results and Discussion

Identification of Morinda scabrida Craib

Morinda scabrida Craib was first reported in Thailand in 1932 (Craib, 1932). However, the key to the species was officially reported in 2013 (Kesonbuaa, 2013) as follows: *M. scabrida* Craib is a bushy plant growing 1–2 m tall. Its stem is straight and has peeling bark with rough or fissured ridges covered in brown scales. The crown is rounded, and the leaves are alternate, arranged in opposite pairs, with parallel or oblique margins. They are 4–9 cm wide and 15–32 cm long, tapering to a sharp point or having a pointed tail. The base of the leaf is clasping and the leaf margin is wavy. The leaf blade is thin, with brownish hairs approximately 0.2 mm long covering the upper surface densely along the midrib. The lower surface has scattered, brownish hairs about 0.5 mm long. The leaf has 6–10 secondary veins on each side, and the tips of the veins are joined together. The leaf stalk is 0.5–1 cm long and covered in dense hairs. The leaf auricle is triangular, 0.7–1 cm wide and 0.8–1 cm long, with a tapering and sharp tip. The auricle has scattered hairs. The inflorescence is densely packed in compact spikes. The small flowers are borne on secondary inflorescence axes at the tip of the main inflorescence. The flower stalk is 0.5–0.7 cm long and covered in hairs. The flower is perfect and has a tubular calyx, approximately 1.5–2 mm long, with cut or fringed tips. The flower stalk has short, stiff hair and is concave inside. The outer petals of the flower are purplish-pink, while the fully bloomed flower is white. The corolla tube is tubular, approximately 2–3 cm long, with five spreading lobes 5 mm wide and 7–9 mm long. The outside of the corolla lobes is covered in hairs, while the inside is smooth. The male flowers have five stamens attached to the corolla tube near the base of the lobes. The stamen filaments are 1 mm long and attached to the back of the corolla tube. They have oblong appendages 3–4 mm long, breaking away easily. The female flowers have a single pistil attached to the corolla tube. The pistil stalk is 15–18 mm long, and the stigma is divided into two branches that are 3 mm long. The ovary is swollen and immersed in the hollow corolla tube. The fruit is a rounded, 3–8 cm long, greenish-dark structure. The fruit contains 11–20 fused fruits, covered in short hairs, and turns black when ripe. The brown-colored seeds are ribbed, approximately 3–7 mm wide and 4–6 mm long. Fig. 1A shows the flowers and stem of the plant and Fig. 1B shows *M. scabrida* Craib for the specimen comparison.

Compound extraction, purification and characterization

After processing into small pieces and drying, the roots of *M. scabrida* Craib were subjected to maceration, followed by partition extraction using an aqueous-EtOAc mixture. Then, the organic-soluble crude mixture (3 g) was subjected to column chromatography using a gradient of MeOH/DCM as eluents to provide six fractions (F1–F6), as illustrated in Scheme 1.

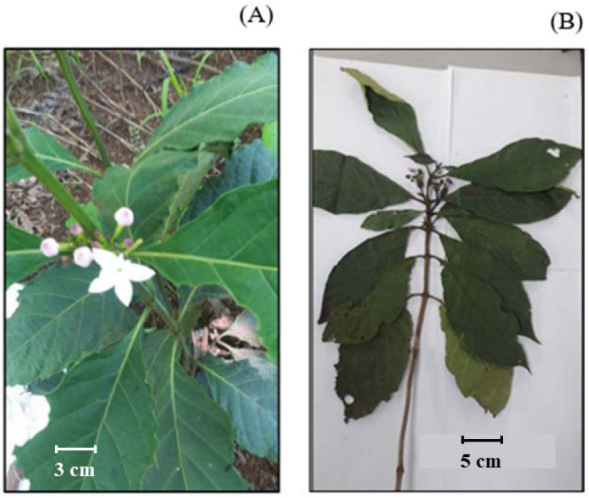
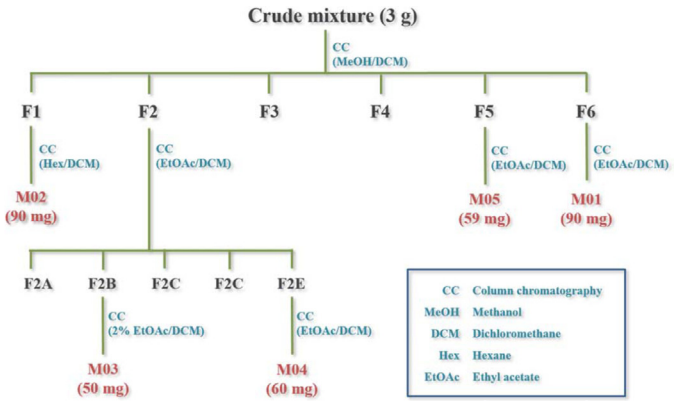


Fig. 1 Components of *Morinda scabrida* Craib: (A) flowers and stem; (B) plant specimen for comparison



Scheme 1 Purification of EtOAc crude extract from the roots of *Morinda scabrida* Craib

Fraction F1 was further purified using column chromatography with a hexane-to-DCM ration of 1:1 as an eluent to produce M02 (90 mg) as a white solid. Fraction F2 was further purified using column chromatography with a gradient elution of EtOAc in DCM. All fractions were collected and combined based on their thin layer chromatography characteristics to give five sub-fractions (F2A–F2E). Subsequently, F2B was chromatographed with 2% EtOAc/DCM to yield 50 mg of M03 as a white solid. Fraction F2E was chromatographed with a gradient system of EtOAc/DCM to produce 60 mg of M04 as a white solid. Fraction F5 was further purified using column chromatography with a gradient of EtOAc/DCM to produce 59 mg of M05 as a white solid. Finally, Fraction F6 was chromatographed with a gradient of EtOAc/DCM to yield 90 mg of M01 as a white solid.

The isolated pure compounds were subjected to ^{1}H and ^{13}C NMR and high-resolution mass spectroscopic analysis. In addition, the results were compared to reported spectra (Mehla et al., 2011; Chaudhuri et al., 2015; Lopez-Martinez et al., 2015; Savi et al., 2005) and the compounds were identified as gallic acid (M01), methyl gallate (M02), ethyl gallate (M03), propyl gallate (M04) and octyl gallate (M05). Their chemical structures are illustrated in Fig. 2.

Antibacterial activity

Gallic acid and its esters (M01–M05) were subjected to antibacterial screening using the disc diffusion method. Both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria were tested and the results are illustrated in Table 1.

Gallic acid is present in many plants and fruits and has been demonstrated to have several health benefits and bioactivities, such as antioxidation, anti-inflammatory, anticancer, and antibacterial activities (Borges et al., 2013; Badhani et al., 2015; Lu et al., 2016; Bai et al., 2021). Its medicinal importance has led to several applications, such as the development of gallic acid-anchored, phthalocyanine-doped, silica nanoparticles for use as antibiofilm materials (Magadla et al., 2023). In addition, alkyl esters of gallic acid exhibit antimicrobial activities against human pathogens (Wang et al., 2021). In particular, octyl gallate (M05) has been used as an antioxidant with antibacterial potency in the food industry (Santativongchai et al., 2022). In the current study, gallic acid (M01) did not exhibit a clear zone under the experimental conditions. However, alkyl gallate with higher alkyl carbon, such as octyl gallate (M05), exhibited clear zones with both Gram-positive and Gram-negative bacteria. Presumably, having a bigger hydrophobic moiety attached to the gallic acid core structure enhanced cell permeability, therefore enhancing the antibacterial effects. The current results affirmed the importance of *M. scabrida* Craib as a medicinally valuable plant, whose extracts could be developed to be used as antibacterial agents.

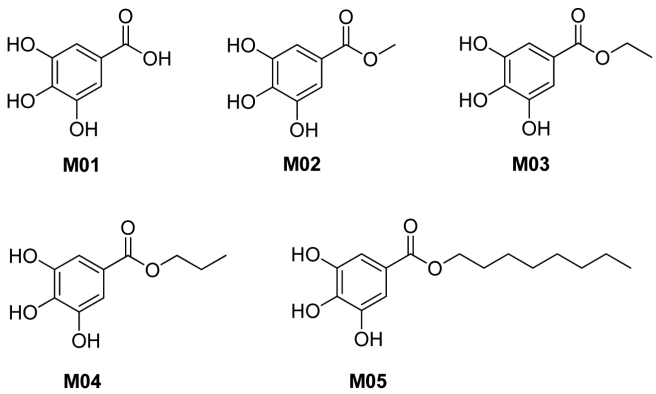


Fig. 2 Chemical structures of M01–M05

Table 1 Antibacterial activity of M01–M05

Compound	Zone diameter (mm)			
	B. subtilis ATCC 6633	*S. aureus* ATCC 25923	*E. coli* DMST 4212	*P. aeruginosa* DMST 4739
M01	0.00	0.00	0.00	0.00
M02	0.00	0.00	17.72	0.00
M03	0.00	0.00	21.60	0.00
M04	0.00	0.00	24.82	0.00
M05	19.04	22.22	21.58	9.92
Penicillin* (Gram-positive)	9.60	23.56	-	-
Ciprofloxacin** (Gram-negative)	-	-	33.84	26.32
DMSO***	0.00	0.00	0.00	0.00

* = positive control (Gram-positive); ** = positive control (Gram-negative); *** = negative control

Acetylcholine esterase inhibition

Acetylcholine esterase plays a crucial role in several human diseases, especially Alzheimer's disease, which involves impairment of cholinergic neurotransmission (Herholz, 2008). Although the mechanism of Alzheimer's disease is complex, the cholinergic hypothesis appears to be well-accepted (Bartus et al., 1982) and supported by the demonstration of decreased acetylcholine biosynthetic enzyme in the cerebral cortex of Alzheimer's patients (Amenta et al., 2001). Therefore, inhibition of acetylcholine esterase could benefit the treatment of Alzheimer's disease.

M01–M05 effectively inhibited acetylcholine esterase, as illustrated in Fig. 3. The degree of inhibition was similar to the positive control (tacrine), although at a much higher concentration (100 μM) compared to tacrine (0.5 μM). Although there are no published reports on anti-acetylcholine esterase activity from alkyl gallate derivatives, epigallocatechin gallate, a glycosyl ester of gallic acid found abundantly in green tea extract, improved the cognitive function of aged rat brain (Srividhya et al., 2012). In addition, epigallocatechin gallate is regarded as a promising candidate for Parkinson's disease treatment (Wang et al., 2022). Therefore, varying the alcohol moiety of the gallate ester could ultimately yield more potent inhibitors against acetylcholine esterase, with such efforts ongoing in the laboratory of the current authors.

α-Glucosidase inhibition

Diabetes mellitus, a metabolic disorder, is characterized by unusually high levels of blood glucose (Alrefai et al., 2002). The manifestations of diabetes mellitus have resulted in health problems worldwide. A peptide hormone, insulin, is responsible for normal glucose homeostasis; however, inadequate insulin production or insulin resistance lead to hyperglycemia, a condition described as abnormally high levels of blood glucose (Wilcox, 2005). One of the methods to combat such conditions is to reduce the blood glucose level by reducing glucose absorption through the inhibition of the enzyme α-glucosidase that is responsible for the liberation of glucose molecules from more complex carbohydrates (Chatterjee and Davies, 2015; Dirir et al., 2022). Therefore, inhibition of α-glucosidase is regarded as one of the strategies to fight diabetes mellitus.

Medicinal plants are rich sources of biologically active compounds with inhibitory activity against α-glucosidase (Wangkiri et al., 2021). In particular, gallic acid (Oboh et al., 2016; Choudhary et al., 2020; Xue et al., 2020; Kokila et al., 2022), theaflavin-3-gallate (Li et al., 2021) and epigallocatechin gallate (Li et al., 2007; Xu et al., 2019; Guan et al., 2023) exhibited anti α-glucosidase activity. Consequently, it is anticipated that M01–M05 would exhibit inhibitory activity against α-glucosidase. Satisfyingly, M01–M04 demonstrated modest inhibitory activity against α-glucosidase, while octyl gallate (M05) had a promising inhibitory effect, as illustrated in Fig. 4. In this case, the alkyl chain length of the natural

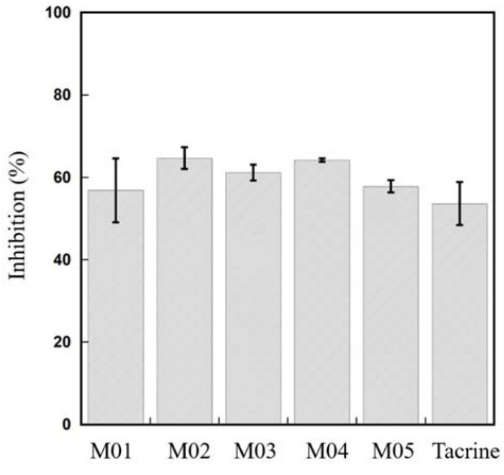


Fig. 3 Percentage inhibition of M01–M05 against acetylcholine esterase, where concentrations of M01–M05 were all 100 μM, tacrine (0.5 μM) was used as positive control, all experiments were performed in triplicate and error bars indicate SD (n = 3)

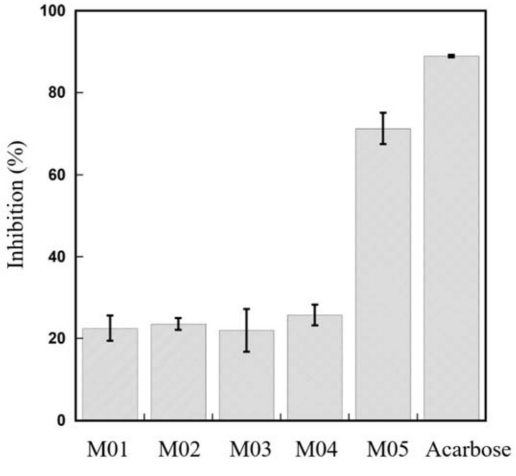


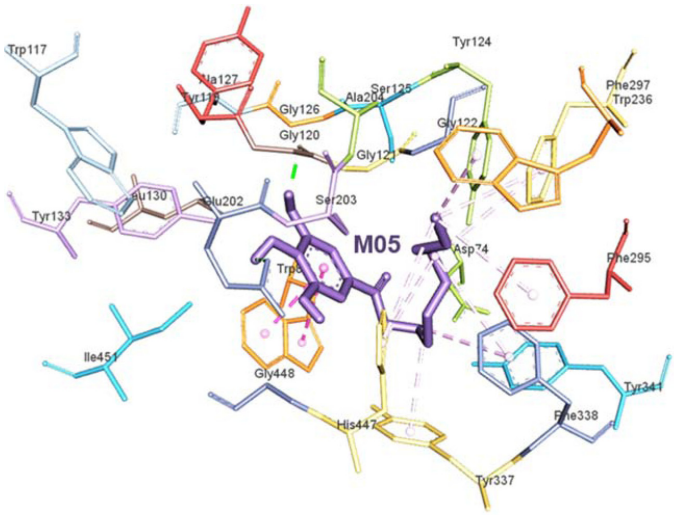
Fig. 4 Percentage inhibition of M01–M05 against α-glucosidase, where concentrations of M01–M05 were all 100 μM, acarbose (1.6 mM) was used as positive control, all experiments were performed in triplicate and error bars indicate SD (n = 3)

compounds affects the inhibitory activity of the compound. Specifically, alkyl groups with three carbons or less (M02–M04) provide a similar inhibitory effect to gallic acid (M01). However, the inhibition substantially increases with the eight-carbon alkyl chain (M05). Undoubtedly, the alcohol moiety of the gallate esters plays a crucial role in α-glucosidase inhibition. Current efforts are underway by the current authors to chemically diversify such a moiety to achieve a higher inhibitory effect, with the results to be reported in due course.

Molecular docking simulations

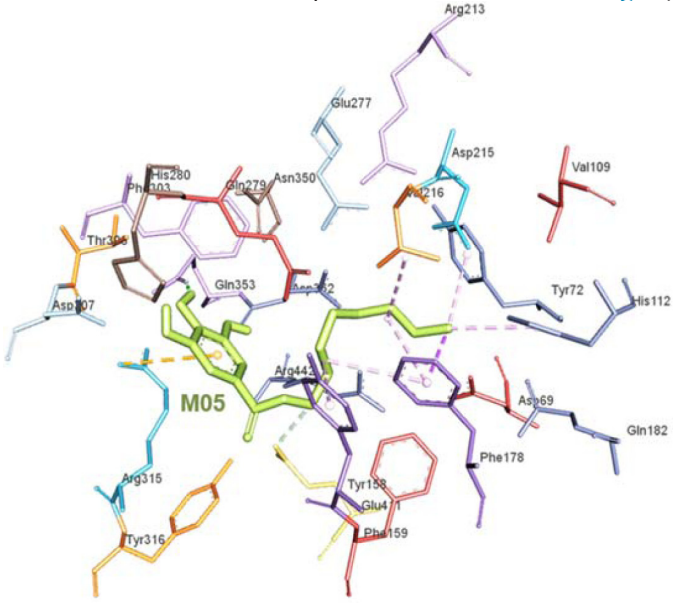
Molecular docking simulations were used to elucidate the binding mode of gallic acid (M01) and its esters (M02–M05) to the active sites of the acetylcholine esterase and α-glucosidase enzymes. First, the structures of M01–M05 were optimized and docked into the active sites of the two enzymes (See Materials and Methods above for details). Notably, only the interactions between M05, the most effective ligand in this study, are illustrated for clarity. M05 interacts with acetylcholine esterase via conventional hydrogen bonding (Gly120 and Glu202), π-π stacking (Trp86) and a set of weaker contacts, van der Walls and π-alkyl interactions, as shown in Fig. 5 (See Supplementary Data for details). Furthermore, M05 had the lowest (best) binding energy (-8.1 kcal/mol) toward the acetylcholine esterase enzyme. However, the experimental data demonstrated similar inhibitory potency among the M01–M05 compounds. Presumably, the discrepancies between the *in vitro* inhibition study and the *in silico* results arose because the enzyme sources were different. Acetylcholine esterase from *Electrophorus electricus* was used in the *in vitro* experiments. In contrast, the crystal structure of the human enzyme was used in the *in silico* study, due to the unavailability of a high-resolution enzyme structure from *Electrophorus electricus*. Therefore, some discrepancies could be anticipated.

The interactions between M01–M05 and the α-glucosidase enzyme were further examined to better understand the binding modes of these compounds. Fortunately, the crystal structure of α-glucosidase comes from the same organism as that utilized in our *in vitro* assays. Therefore, reasonable correlations between the experimental and computational simulation data could be expected. As illustrated in Fig. 6,



Compound	Affinity (kcal/mol)
M01	-6.7
M02	-6.7
M03	-7.1
M04	-7.6
M05	-8.1
Tacrine	-8.8

Fig. 5 Best-docked conformation of M05 on active site of acetylcholine esterase (PDB: 4EY7)



Compound	Affinity (kcal/mol)
M01	-5.8
M02	-5.9
M03	-6.2
M04	-6.7
M05	-7.0
Acarbose	-6.9

Fig. 6 Best-docked conformation of M05 on active site of α-glucosidase (3A4A)

through several intermolecular forces, including conventional hydrogen bonding (Asp352 and Gln353), the π-cation (Arg315) and weaker interactions, such as van der Waals, π-sigma and π-alkyl interactions (See Supplementary Data for details). In addition, M05 had the lowest binding energy (-7.0 kcal/mol) compared to the other compounds (M01–M04). These results indicated the importance of the alkyl moiety of the gallate esters on active site binding. Although the inhibition results demonstrated a similar inhibitory effect among M01–M03 and an abrupt increase for M04, the molecular docking simulations suggested a gradual increase in binding affinity as the alkyl chain grows larger. It is yet to be seen whether a longer alkyl chain will demonstrate stronger binding affinity *in silico* and higher inhibitory efficiency *in vitro*.

In summary, the root extracts from *Morinda scabrida* Craib, an unexplored species first reported in Thailand, were purified to homogeneity. Five compounds (gallic acid and its four esters) were identified spectroscopically and tested for antibacterial, anti-acetylcholine esterase and anti-α-glucosidase activities. All isolated compounds had levels of anti-acetylcholine esterase activity, while octyl gallate had notable anti-α-glucosidase activity. Molecular docking simulations were conducted to further suggest binding interactions between the isolated compounds and the two molecular targets. The results were in good agreement with the experimental data in the case of α-glucosidase but showed slight discrepancies for acetylcholine esterase, presumably due to difference in the enzyme sources. The current results emphasized the medicinal significance of *M. scabrida* Craib, suggesting its potential as a traditional medicine due to its diverse bioactivities.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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