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Research article

Potential alpha-glucosidase inhibitory activity of root extracts from *Morinda citrifolia* L.

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Abstract

Importance of the work: *Morinda citrifolia* L., called 'yo-ban' in Thai, is a medicinal plant that is widely used in traditional medicine. This is the first report on the phytochemical analysis and *in-vitro* α-glucosidase inhibitory activity of the roots of *M. citrifolia*.

Objectives: To explore the main chemical components of the root extracts and investigate their *in-vitro* α-glucosidase inhibitory activity.

Materials & Methods: The dried root powders were macerated with an increasing polarity of solvents (hexane, dichloromethane, ethyl acetate and ethanol). The levels were evaluated of the inhibition of α-glucosidase by all root extracts. The main chemical components of the bioactive extracts were investigated using gas chromatography-mass spectrometry (GC-MS); molecular docking of four promising GC-MS-detected compounds was conducted.

Results: All the phytochemical assays of the root extracts confirmed the presence of bioactive secondary metabolites such as terpenes, steroids, phenolics and flavonoids. Among the four crude extracts, the dichloromethane root extract had the highest α-glucosidase inhibitory activity against rat intestinal sucrase with a half-maximal inhibitory concentration (IC_{50}) value of 0.943 mg/mL. Furthermore, this extract inhibited yeast α-glucosidase with an IC_{50} value of 0.646 mg/mL, having greater activity than the standard drug, Acarbose® (IC_{50} = 1.122 mg/mL). Additionally, analysis of the main chemical components of the dichloromethane extract based on GC-MS revealed the presence of Neophytadiene as a promising bioactive component. Molecular docking of the four promising GC-MS-detected compounds suggested the feasibility of these compounds as viable α-glucosidase inhibitors.

Main finding: This study is the first to demonstrate the presence of an antidiabetic diterpene in the plant using GC-MS analysis. Therefore, this plant has high potential for further research into antidiabetic medical applications.

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Introduction

Diabetes mellitus (DM) is presently one of the most frequent chronic and noncommunicable diseases, with symptoms gradually increasing and escalating until it becomes permanently chronic over time, resulting in significant long-term implications on the lifestyle of patients and family caregivers (Ogurtsova et al., 2017). In 2015, there were approximately 415 million people who suffered from diabetes worldwide, and the number of diabetic patients is increasing and is estimated will be approximately 642 million in 2040. Diabetes is a syndrome caused by a metabolic disease disorder in which the body is unable to produce enough insulin hormone, which is released by the pancreas, resulting in excessively high blood sugar (glucose) levels in the body (Barrett, 2019)

Diabetes is divided into two types: insulin-dependent (Type 1) and insulin-resistant (Type 2) diabetes (Chaudhuri and Dandona, 2011). Approximately 90% of all patients are obese with type 2 DM and there are several approaches for treatments to reduce blood sugar levels (Tuomi, 2005). In the first stage of this disease, diabetic patients improve their health by controlling their blood glucose levels in various ways such as a healthy diet, exercise and weight loss (Costanian et al., 2014). Importantly, effective treatment for Type 2 DM is to use antidiabetic medications that slow the digestion of polysaccharides to create glucose, resulting in slower glucose absorption and lower blood sugar levels by inhibiting alpha-glucosidase. Currently, there are various antidiabetic drugs with alpha-glucosidase inhibitory potential, such as acarbose (Precose®), miglitol (Glyset®) and *N*-butyl-1-deoxynojirimycin (Zavesca®) (Koning et al., 2016) and voglibose (Basen®) (American Diabetes Association, 2009), but these drugs have side effects on patients, such as flatulence, diarrhea and kidney damage. Consequently, many countries globally are interested in using natural plant products as alternative traditional medicine therapies in addition to manufactured medications for the treatment of diabetes. For example, *Ceriops tagal* (Bhandari et al., 2008), *Vitex glabrata* (de Melo et al., 2006), and *Zizyphus rugosa* (Xiaolong et al., 2006) were among the Thai medicinal herbs that inhibited alpha-glucosidase activity. The current work investigated the chemical and biological characteristics the of bioactive constituents found in the Thai plant, 'yo-ban' (*Morinda citrifolia*).

M. citrifolia L. (Rubiaceae**)** is a shrub and a valuable traditional plant distributed in many regions in Asia (Chan-Blanco et al., 2006). The root, barks, stems, leaves and fruits of *M. citrifolia* have long been involved in foods and folklore medicine to treat a variety of ailments. For example, the leaves are used to cure diarrhea and fever, the fruit juice is used to treat nausea and the roots are decocted and used in diabetic treatment. The juices of other fruits (such as noni fruit) been used as health supplements for antidiabetic medications (Nerurkar et al., 2015). Many chemical substances of members of the genus *Morinda* have been identified from fruits and roots, including iridoids, iridoid glucosides, flavonoids, flavonol glycosides, anthraquinones, anthraquinones glycosides, lignan and coumarins (Inoue et al., 1981; Kamiya et al., 2010; Kanchanapoom et al., 2002; Pawlus et al., 2005). Fruits of *M. citrifolia* have been shown to contain biological activities such as anti-inflammatory, quinone reductase induction, antibacterial and antioxidant properties (Sang et al., 2003; Deng et al., 2007). Furthermore, Sam-ang et al. (2020) showed that the ethanolic root extract of *M. citrifolia* had relatively high antioxidant and acetylcholinesterase inhibitory activities compared to their stem and leaf extracts. However, there have been few reported studies on the root of this species.

Consequently, this is the first report on the α-glucosidase inhibitory activity and phytochemical screening of *M. citrifolia* L. root extract in hexane, dichloromethane, ethyl acetate and ethanol. In addition, GC-MS analysis was used to investigate the main bioactive components and molecular docking of promising GC-MS-detected compounds.

Materials and Methods

Plant material

The *M. citrifolia* plant was collected from Nong Mae Taeng subdistrict, Sai Ngam district, Kamphaeng Phet province in Thailand in January 2017. The plant sample was authenticated to issue a voucher specimen (Queen Sirikit Botanic Garden [QBG] No. 105894) and was deposited at QBG), Chiang Mai, Thailand.

Preparation of crude extracts

The *M. citrifolia* roots were air-dried and ground into fine powders before extraction. Finely ground and dried powders (887 g) were sequentially extracted using hexane, chloromethane, ethyl acetate and ethanol, respectively based on maceration. The residue was reextracted using a new solvent and the extraction and filtration steps were repeated until the extract solution was colorless. Each extract solution was filtered twice, once using muslin cotton wool and once using Whatman® No.1 filter paper.

Each solution was concentrated to dryness in a vacuum at 40 °C using a rotary evaporator to obtain a crude hexane extract (2.66 g, 0.30%), a crude dichloromethane extract (3.66 g, 0.41%), a crude ethyl acetate extract (10.86 g, 1.23%) and a crude ethanol extract (18.06 g, 2.04%). All crude extracts were placed in amber glass vials and subsequently stored at 4 °C for further analysis.

Phytochemical tests

All crude extracts were subjected to phytochemical screening assays to determine the types of secondary metabolites (Yadav et al., 2014; Gul et al., 2017). For terpenes (Salkowski's test and copper (II) acetate test) and steroids (Salkowski's test and Liebermann Burchard's test), every crude extract (0.4 g) was diluted in chloroform (2.0 mL). Each crude extract (0.4 g) was also diluted in methanol (2.0 mL) to test for phenolic compounds (ferric (II) chloride test), flavonoids (alkaline test and Shinoda's test), and tannins (ferric (II) chloride test). The crude extract (0.2 g) was combined with water (1.0 mL) for the saponin test (foam test), then shaken and settled for 1–2 min. For the alkaloid tests (Dragendorff's test, Wager's test and Hager's test), each crude extract in chloroform (3.0 mL) was mixed with 1.0 mL of 28% NH_3 solution and allowed to settle for 1–2 min. The chloroform layer was collected and mixed with 1.0 mL of 1% HCl solution before being shaken and settled for 1–2 min. The acid layer was recovered and used for alkaloid testing. The colorimetric measurement was performed by monitoring the color of the original root extract.

In vitro α glucosidase inhibitory assay

Baker's yeast α-glucosidase inhibition assay was performed according to the slightly modified method of Worawalai et al. (2012). The α-glucosidase inhibitory activity was measured in 96-well plates and then read with a microplate reader. All crude extracts were made in dimethyl sulfoxide (DMSO) at concentrations of 0.04, 0.2 and 1.0 mg/mL. α-Glucosidase (0.1 U/mL) and *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) substrate (1 mM) were dissolved in 0.1 M phosphate buffer, pH 6.9. Each crude extract solution (10 µL) was preincubated with 40 µL of α-glucosidase at 37 °C for 10 min. Then, the substrate solution (50 µL) was added to the reaction mixture and further incubated at 37 °C for 20 min. The reaction mixture was terminated by adding 100 µL of 1 M Na_2CO_3 solution. Enzymatic activity was quantified by measuring the absorbance at a wavelength of 405 nm. The percentage of yeast α-glucosidase inhibition was estimated using Equation 1:

$$\% \text{ Inhibition} = [(Abs_{blank} - Abs_{sample})]/Abs_{blank}] \times 100 \quad (1)$$

where: Abs_{blank} is the absorbance of the DMSO solution and Abs_{sample} is the absorbance of each crude extract solution.
The half-maximal inhibitory concentration (IC_{50}) value was determined by plotting sample concentrations (x-axis) against the percentage of yeast α glucosidase inhibition (y-axis). Acarbose® as a medication and DMSO were applied as positive and negative controls, respectively. The experiment was performed in triplicate.

Rat intestinal α-glucosidase inhibitory activity was modified by the reported method of Ramadhan and Phuwapraisirisan (2015). The rat intestinal acetone powder was purchased from Sigma-Aldrich (St. Louis, MO, USA) containing maltase and sucrase with specificities of 0.09 units/mL protein and 0.45 units/mL protein, respectively. To make the crude enzyme solution, 1.0 g of rat intestinal powder was dissolved in 30 mL of 0.9% NaCl solution. The produced enzyme solution was centrifuged for 30 min at 12,000 revolutions per minute and 4 °C. Unless it was subjected to assay, the supernatant was collected and stored at -20 °C. Briefly, the test samples (concentration of 1 mg/mL in DMSO, 10 µL) were mixed with 30 µL of 0.1 M phosphate buffer (pH 6.9), 20 µL of the substrate solution (maltose: 2 mM; sucrose: 20 mM) in 0.1 M phosphate buffer, 80 µL of glucose assay kit and 20 µL of the crude enzyme solution. Then, the reaction mixture was incubated at 37 °C for 10 min (for maltose) and 40 min (for glucose and sucrose). The absorbance of the quinoneimine produced (500 nm) was measured using a Bio-Rad microplate reader model 3550 UV to determine enzymatic activity. As a positive control, Acarbose® was used. Each set of studies was carried out in triplicate. The percentage inhibition from the enzymatic activity in the rat small intestine was calculated using Equation 1.

Gas chromatography-mass spectrometry analysis of chemical compositions from M. citrifolia root extract

GC-MS analysis of bioactive compounds found in the *M. citrifolia* root dichloromethane extract was performed using Clarus 690 gas chromatography and a PerkinElmer, Elite-5ms Capillary Column (30 m × 250 µm with 0.25 µm film thickness). The following conditions were programmed: helium (He) gas as a carrier at a flow rate of 1.0 mL/min, initial temperature at 45 °C increased at a rate of 10 °C/min until reaching the maximum temperature of 250 °C. At 230 °C, 1 µL of crude extract was supplied in a 20:1 ratio, split mode. The spectrometer was operated using an electron impact system

with an electron energy of 70 eV and a scan mass range of 33–550 amu. The ion source temperature was 230 °C, and the quadrupole temperature was set at 150 °C. Each putative compound detected in the chromatogram was identified based on its retention time (RT) and by matching the fragmentation pattern of its mass spectra against the National Institute of Standards and Technology database library (NIST, 2017).

Molecular docking of possible α-glucosidase inhibitors detected in dichloromethane extract

To correlate the in vitro α-glucosidase inhibitory activity of the dichloromethane extract to a list of putative compounds detected using GC-MS analysis, preliminary molecular studies were undertaken to predict the binding affinity of these compounds at the active site of the enzyme. Three-dimensional structures of the targeted enzymes were obtained from the RCSB Protein Data Base (PDB) as α-glucosidase I purified from *Saccharomyces cerevisiae* S288C (PDB code 4j5t, resolution = 2.04; Barker and Rose, 2013). Only four putative compounds with the highest matching scores to the library search (> 80%) were selected from those compounds detected using GC-MS. These compounds were p-menthane, 2,3-dibromo-8-phenyl-; 6betabicyclo[4.3.0]nonane, 5beta-iodomethyl-1beta-isopropenyl-4alpha,5alpha-dimethyl; 2,4-dimethyl decane and Neophytadiene (PubChem CID: 576882, 573073, 520357, 10446, respectively). Acarbose® (PubChem CID: 444254) was included in the studies as a control group. A simple structure of each selected compound was obtained from a conformational search based on the Universal Force Field (UFF) parameterization provided in Open Babel (O'Boyle et al., 2011) where the structure with a minimized energy was selected for subsequent docking studies.

Statistical analysis

To evaluate any significant differences for the measured attributes, the data were analyzed using analysis of variance with the SPSS software package (version 16.0; SPSS Inc.; Chicago, IL, USA). Duncan's multiple range test was used to test for significant differences among means at $p < 0.05$.

Results and Discussion

Phytochemical test

The phytochemical screening test results (Table 1) showed that all crude extracts contained terpenes, flavonoids and phenolic compounds. On the other hand, alkaloids were exclusively found in the ethyl acetate and ethanol extracts.

Table 1 Phytochemical composition of all bioactive *Morinda citrifolia* L. root extracts

Phytochemical test	*M. citrifolia* L. root extract			
	Hexane	Dichloromethane	Ethyl Acetate	Ethanol
Terpenes				
Salkowski's test	+	+	+	+
Copper(II) acetate test	+	+	+	+
Steroids				
Salkowski's test	+	+	+	+
Liebermann–Burchard's test	-	-	-	-
Saponin				
Foam test	-	-	-	-
Phenolic compounds				
Ferric(II) chloride test	+	+	+	+
Flavonoids				
Alkaline test	+	+	+	+
Shinoda's test	-	+	-	-
Tannins				
Ferric(II) chloride test	+	+	+	+
Alkaloids				
Dragendorff's test	-	-	+	+
Hager's test	-	-	-	-
Wager's test	-	-	-	-

+ = present; - = absent

In-vitro α-glucosidase inhibitory activities

Four *M. citrifolia* root extracts (hexane, dichloromethane, ethyl acetate and ethanol) inhibited α-glucosidase enzymes (rat intestinal sucrase and maltase, and yeast α-glucosidase), as listed in Table 2. The results for sucrase inhibitory activity showed that the dichloromethane extract had stronger inhibitory action than the other extracts, with an IC_{50} value of 0.943 mg/mL.

Table 2 α-Glucosidase inhibitory activities of *Morinda citrifolia* L. root extracts

Extract	α-Glucosidase inhibitory activity (IC_{50}, mg/mL) (mean ± SD, n = 3)		
	Sucrase	Maltase	Baker's yeast
Hexane	NI	NI	1.118±0.035[b]
Dichloromethane	0.943±0.051[b]	NI	0.646±0.038[a]
Ethyl acetate	NI	NI	NI
Ethanol	NI	NI	NI
Acarbose®*	0.201±0.030[a]	0.016±0.002	1.122±0.200[b]

IC_{50} = half-maximal inhibitory concentration; * = positive control; NI = % inhibition less than 30% at 2 mg/mL

Values (mean ± SD) in the same column superscripted with different lowercase letters are significantly different ($p < 0.05$).

However, no crude extract was active against maltase. Interestingly, for the yeast α-glucosidase inhibitory activity, the dichloromethane extract (IC_{50} = 0.646 mg/mL) was more active than the standard drug, Acarbose® (IC_{50} = 1.122 mg/mL). On the other hand, the hexane extract inhibited yeast α-glucosidase (IC_{50} = 1.118 mg/mL). The findings from the α-glucosidase inhibitory experiments revealed that the dichloromethane extract had the strongest inhibitory activity against rat intestinal sucrase and yeast α-glucosidase.

Gas chromatography-mass spectrometry analysis of dichloromethane extract

The dichloromethane extract was subjected to further GC-MS analysis; a representative total ion chromatogram is illustrated in Fig. 1. All peaks appearing at each RT were identified by comparing the fragmentation patterns of their mass spectra to those registered in a mass library published by NIST. In addition, the amount of each putative compound was estimated in terms of the peak area percentage (PA%), as shown in Table 3. The GC-MS analysis of the

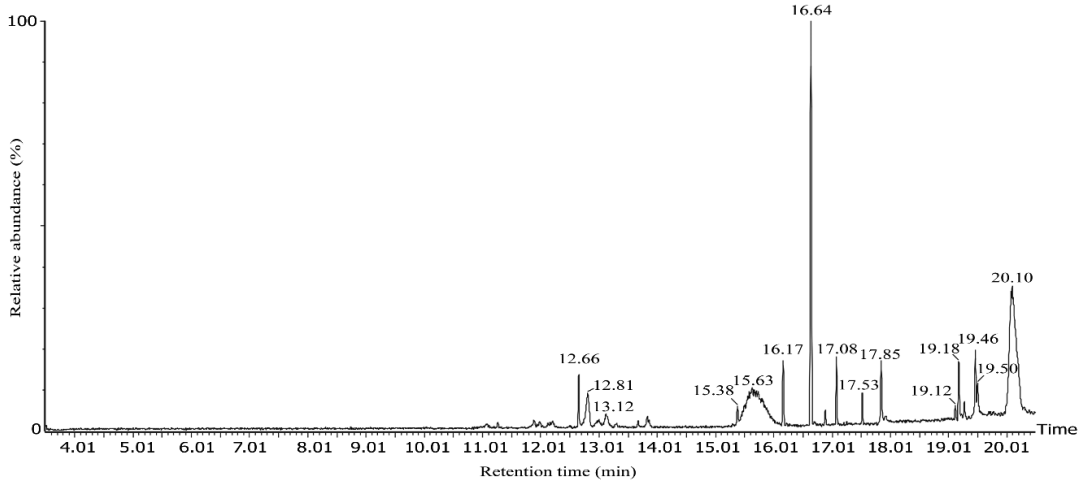

Fig. 1 Gas chromatography-mass spectrometry chromatogram of *Morinda citrifolia* L. root dichloromethane extract

Table 3 Phytochemical constituents detected with high confidence* in *Morinda citrifolia* L. root dichloromethane extract using gas chromatography-mass spectrometry analysis

Peak	RT (min)	Putative compound (% match)	MF	PA%
2	12.81	2,4-Dimethyldecane (84.2%)	$C_{12}H_{26}$	3.45
5	16.17	p-Menthane, 2,3-dibromo-8-phenyl- (80.1%)	$C_{16}H_{22}Br_2$	3.13
6	16.64	Neophytadiene (90.3%)	$C_{20}H_{38}$	22.51
14	20.10	6Betabicyclo[4.3.0]nonane,5beta-iodomethyl-1beta-isopropenyl-4alpha,5alpha-dimethyl- (82.7%)	$C_{15}H_{25}I$	31.56

RT = retention time

* level of confidence justified as "high" if matching score between mass spectra and registered mass library > 80%.

dichloromethane extract revealed Neophytadiene as one of the major components (PA% = 22.51%) with the highest matching score (90.3%). Additionally, Neophytadiene belongs to the diterpene secondary metabolite class and is biosynthetically produced from the precursor, farnesyl diphosphate (FPP), which is derived from biochemical reactions between two isopentyl diphosphate molecules and dimethylallyl diphosphate through the mevalonate pathway (Majumder et al., 2020). There have been a few reports of the biological activities of Neophytadiene and its antibacterial activity (Gawade and Farooqui, 2018) and its anti-inflammatory, antioxidant and cardioprotective properties (Bhardwaj et al., 2020). However, the biological activities of Neophytadiene produced from the genus *Morinda* have not yet been identified, although it may be the major component responsible for decreasing blood glucose levels via inhibition of α-glucosidase. In addition, this study detected another three unique compounds with high matching scores (> 80%). Although the isolation of these compounds from *M. citrifolia* has not been reported, the biosynthesis of their core structures is well known. P-menthane, 2,3-dibromo-8-phenyl- is a monoterpene that contains p-menthane as its core structure. Basically, this core compound is usually found in plant species such as *Eucalyptus* spp. (Barasa et al., 2002). P-menthane is biologically synthesized from geranyl diphosphate as the parent compound via the mevalonate pathway (Lange, 2015). A complex 6betabicyclo[4.3.0] nonane, 5beta-iodomethyl-1beta-isopropenyl-4alpha,5alpha-dimethyl- is classified as a brasilane-type sesquiterpene, where its core structure is biosynthesized in planta from FPP as part of the mevalonate pathway (Sato et al., 2020). Finally, 2,4-dimethyldecane is reportedly produced from a fatty acid biosynthetic pathway that involves the elongation of methyl-branched fatty acid and a subsequent loss of a carbonyl carbon, consequently leading to the formation of 2,4-dimethyldecane (Jurenka, 2004). Thus, the isolation of bioactive compounds determined using GC-MS is promising and worth addressing in further works.

Molecular docking

In silico computation of the predicted binding energies of the compound-enzyme docked complexes clearly showed that there could be interactions between the four putative compounds and α-glucosidase, based on the binding affinity of the model where the least values (most negative values) indicate the highest predicted binding affinity. Among the four selected compounds found in the dichloromethane extract, the strongest binding affinity was recorded for p-menthane, 2,3-dibromo-8-phenyl- followed by 6betabicyclo[4.3.0] nonane, 5beta-iodo methyl-1beta-isopropenyl-4alpha,5alpha-dimethyl, 2,4-dimethyl decane and Neophytadiene, respectively (Fig. 2). Only p-menthane, 2,3-dibromo-8-phenyl- strongly bound (-7.8 kcal/mol) to α-glucosidase at a similar binding affinity (-8.6 kcal/mol) to the standard drug, Acarbose®. Based on the current literature, the current report is the first to evaluate the inhibitory activities of p-menthane, 2,3-dibromo-8-phenyl- against α-glucosidase; this finding probably contributed to the observed anti-diabetes effects of dichloromethane root extract. In contrast, a predicted binding energy of a promising α-glucosidase inhibitor, Neophytadiene, was only 57% compared to Acarbose® (-4.9 kcal/mol). In addition, another major component of the dichloromethane extract, 6betabicyclo[4.3.0] nonane,5beta-iodomethyl-1 beta-isopropenyl-4alpha,5alpha-dimethyl, was predicted to bind the enzyme slightly more than Neophytadiene with a predicted binding energy of -5.5 kcal/mol (Table 4).

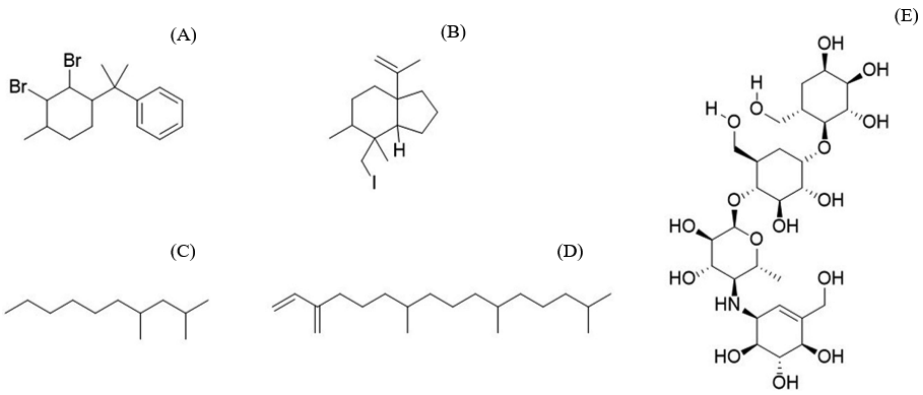

Fig. 2 Chemical structures of selected molecules used in molecular docking: (A) p-menthane, 2,3-dibromo-8-phenyl-; (B) 6betabicyclo[4.3.0] nonane,5beta-iodomethyl-1beta-isopropenyl-4alpha,5alpha-dimethyl; (C) 2,4-dimethyldecane; (D) Neophytadiene; (E) Acarbose®

Table 4 Evaluation of predicted binding energies for selected molecules from *Morinda citrifolia* L. root dichloromethane extract detected using gas chromatography-mass spectrometry analysis

Molecule	PubChem CID	MW (g/mol)	Predicted binding affinity (kcal/mol)
p-Menthane, 2,3-dibromo-8-phenyl-	576882	374.20	-7.8
6Betabicyclo[4.3.0]nonane,5beta-iodomethyl-1beta-isopropenyl-4alpha,5alpha-dimethyl	573073	332.26	-5.5
2,4-Dimethyldecane	520357	170.33	-5.4
Neophytadiene	10446	278.50	-4.9
Acarbose®	444254	645.60	-8.6

Visualization of the compound-enzyme docked complexes facilitated a better understanding of the nature of the binding interactions between the putative compounds and α-glucosidase (Fig. 3). Of all the selected compounds, the best binding model for each putative compound (indicated by the lowest binding energy) was surprisingly found to dock α-glucosidase at different locations. This discrepancy in preference for their favorite docking sites was likely due to the distinct variation in their molecular sizes and types of functional groups (Sliwoski et al., 2014). For example, 2,4-dimethyldecane was merely a short hydrocarbon chain with a low molecular weight (170.33 g/mol) and was 2.2-fold lower than that of a cyclic compound, p-menthane, 2,3-dibromo-8-phenyl- (374.20 g/mol), as shown in Table 4.

However, the computational simulation showed that all putative compounds were able to dock onto the Acarbose® binding site, but with slightly lower predicted binding energies compared to their best values (Table 4). For example, the best orientation of Neophytadiene to dock onto α-glucosidase at the suitable docking pocket resulted in a predicted binding affinity of -4.9 kcal/mol. The sharing of the Acarbose® binding site in the current study suggested a possibility that one or all of the four selected compounds were responsible for the inhibitory effects of dichloromethane extract on α-glucosidase in vitro. The lower predicted binding energy of all the putative compounds compared to the standard drug, Acarbose®, led to a detailed analysis of the bonding formation between the compounds and their favorite active sites. Clearly, only Acarbose® strongly docked to α-glucosidase using both hydrogen bonds (H-bonds) and hydrophobic bonds (π-π interactions) whereas all four putative compounds extracted from the roots of *M. citrifolia* only formed hydrophobic bonds to their corresponding sites (Fig. 4). This result provided a logical explanation for the relatively low binding affinities of the four selected compounds in the current study.

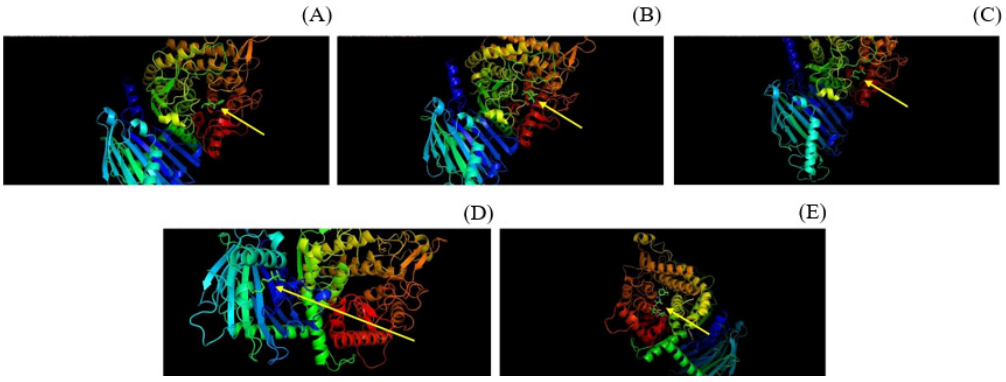

Fig. 3 Compound-enzyme docked complexes between α-glucosidase: (A) p-menthane, 2,3-di bromo-8-phenyl-; (B) 6betabicyclo[4.3.0]nonane, 5beta-iodomethyl-1beta-isopropenyl-4alpha ,5alpha-dimethyl; (C) 2,4-dimethyldecane; (D) Neophytadiene; (E) Acarbose®, where yellow arrows indicate location of compound

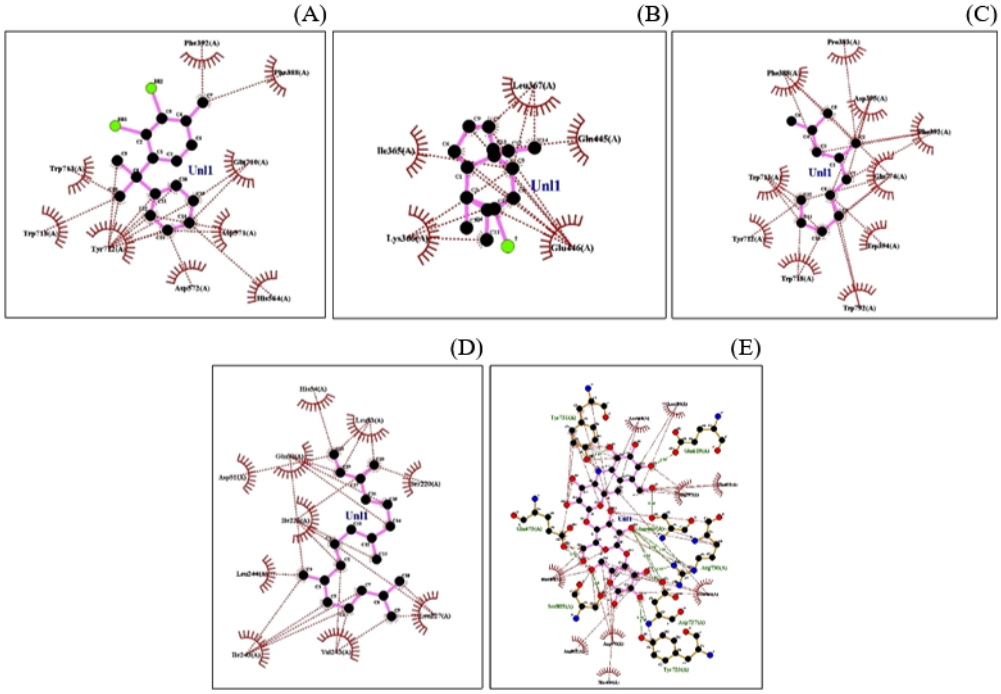

Fig. 4 Predicted binding interactions between possible active sites of α-glucosidase and molecules: (A) p-menthane, 2,3-dibromo-8-phenyl-; (B) 6betabicyclo[4.3.0]nonane, 5beta-iodomethyl-1beta-isopropenyl-4alpha,5alpha-dimethyl; (C)2,4-dimethyldecane; (D) Neophyta diene; (E) Acarbose®, where chemical bonding between atoms of putative compounds are represented in pink and hydrogen bonds and hydrophobic bonds between putative compounds and amino acid residues in active sites are represented by green and black broken lines, respectively.

Furthermore, a possible synergistic or combined effect of these compounds to exert strong inhibitory effects on α-glucosidase could be not overlooked. For example, the synergistic effects were demonstrated of Neophytadiene, phytol and caryophyllene from a methanolic extract of *Ageratum conyzoides* at the binding site of α-amylase (Oso and Olaoye, 2020). As can be seen in Fig. 1, several compounds were present in the dichloromethane extract; however, further investigation is required to determine how these compounds inhibit α-glucosidase. Therefore, a thorough analysis of the dichloromethane extract from the roots of *M. citrifolia*, using a better separation and identification technique such as liquid chromatography high resolution tandem mass spectrometry is recommended so that the broadest range of compounds can be detected. *M. citrifolia* still has promise of additional therapeutic effects on diabetes and is worth more extensive investment to elucidate its inhibitory mechanism against α-glucosidase, which could lead to the development of antidiabetic treatments.

In conclusion, although *Morinda citrifolia* L. roots have been used in folk medicine for diabetic treatment for ages, the current study was the first to investigate the α-glucosidase inhibitory activities of *M. citrifolia* root extracts using hexane, dichloromethane, ethyl acetate, and ethanol as solvents. The crude dichloromethane extract was the most active against *in-vitro* rat intestinal sucrase and yeast α-glucosidase. GC-MS analysis of this extract revealed the presence of Neophytadiene as a promising bioactive component. Thus, the roots of *M. citrifolia* have the potential to be a viable candidate for further development of plant-derived antidiabetic medications. Furthermore, this plant could be effective as a herbal supplement for diabetes management.

Conflict of Interest

The authors declare that there are no conflicts of interests.

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References

American Diabetes Association. 2009. Diagnosis and classification of diabetes mellitus. Diabetes Care 33: S62–S69. doi: 10.2337/dc09-S062

Barker, M.K., Rose, D.R. 2013.Specificity of processing α-glucosidase I is guided by the substrate conformation: Crystallographic and *in silico* studies. J. Biol. Chem. 288: 13563–13574. doi.org/10.1074/jbc.M113.460436

Barrett, K.E. 2019. Ganong's Review of Medical Physiology, 26th ed. McGraw-Hill Education. New York, NY, USA.

Barasa, S.S., Ndiege, I.O., Lwande, W., Hassanali, A. 2002. Repellent activities of stereoisomers of p-menthane-3,8-diols against *Anopheles gambiae* (Diptera: Culicidae). J. Med. Entomol. 39: 736–741. doi.org/10.1603/0022-2585-39.5.736

Bhandari, M.R., Jong-Anurakkun, N., Hong, G., Kawabata, J. 2008. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). Food Chem. 106: 247–252. doi.org/10.1016/j.foodchem.2007.05.077

Bhardwaj, M., Sali, V.K., Mani, S., Vasanthi, H.R. 2020. Neophytadiene from *Turbinaria ornata* suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and Sprague Dawley rats. Inflammation 43: 937–950. doi.org/10.1007/s10753-020-01179-z

Chan-Blanco, Y., Vaillant, F., Pérez, A., Reynes, R., Brillouet, J.-M., Brat, P. 2006. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. J. Food Compos. Anal. 19: 645–654. doi.org/10.1016/j.jfca.2005.10.001

Chaudhuri, A., Dandona, P. 2011. Effects of insulin and other antihyperglycaemic agents on lipid profiles of patients with diabetes. Diabetes Obes. Metab. 13: 869–879. doi:10.1111/j.1463-1326.2011.01423.x

Costanian, C., Bennett, K., Hwalla, N., Assaad, S., Sibai, A.M. 2014. Prevalence, correlates and management of type 2 diabetes mellitus in Lebanon: Findings from a national population-based study. Diabetes Res. Clin. Pract. 105: 408–415. doi: 10.1016/j.diabres.2014.06.005

de Melo, E.B., Gomes, A.d.S., Carvalho, I. 2006. α- and β-Glucosidase inhibitors: Chemical structure and biological activity. Tetrahedron 62: 10277–10302. doi.org/10.1016/j.tet.2006.08.055

Deng, Y., Chin, Y.W., Chai, H., Keller, W.J., Kinghorn, A.D. 2007. Anthraquinones with quinone reductase-inducing activity and benzophenones from *Morinda citrifolia* (noni) roots. J. Nat. Prod. 70: 2049–2052. doi.org/10.1021/np070501z

Gawade, B., Farooqui, M. 2018. Screening of phytochemicals and *in vitro* antidiabetic activity of *Bauhinia racemosa* Lam. leaves. Asian J. Pharm. Clin. Res. 11: 190–193. doi: 10.22159/ajpcr.2018.v11i6.24038

Gul, R., Jan, S.U., Faridullah, S., Sherani, S., Jahan, N. 2017. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. Sci. World J. 2017: 5873648. doi.org/10.1155/2017/5873648

Inoue, K., Nayeshiro, H., Inouyet, H., Zenk, M.1981.Anthraquinones in cell suspension cultures of *Morinda citrifolia*. Phytochemistry 20: 1693–1700. doi.org/10.1016/S0031-9422(00)98557-1

Jurenka, R. 2004. Insect pheromone biosynthesistopics. In: Schulz, S. (Ed.). The Chemistry of Pheromones and Other Semiochemicals I. Topics in Current Chemistry, Vol. 239. Springer. Berlin, Germany, pp. 97–132. doi.org/10.1007/b95450

Kamiya, K., Hamabe, W., Tokuyama, S., Hirano, K., Satake, T., Kumamoto-Yonezawa, Y., Yoshida, H., Mizushina, Y. 2010. Inhibitory effect of anthraquinones isolated from the Noni (*Morinda citrifolia*) root on animal A-, B- and Y-families of DNA polymerases and human cancer cell proliferation. Food Chem. 118: 725–730. doi.org/10.1016/j.foodchem.2009.05.053

Kanchanapoom, T., Kasai, R., Yamasaki, K. 2002. Iridoid and phenolic glycosides from *Morinda coreia*. Phytochemistry 59: 551–556. doi.org/10.1016/S0031-9422(01)00426-5

Koning, S.H., Hoogenberg, K., Lutgers, H.L., van den Berg, P.P., Wolffenbuttel, B.H. 2016. Gestational diabetes mellitus: Current knowledge and unmet needs. J. Diabetes 8: 770–781. doi.org/10.1111/1753-0407.12422

Lange, B.M. 2015. Biosynthesis and biotechnology of high-value *p*-menthane monoterpenes, including menthol, carvone, and limonene. In: Schrader, J., Bohlmann, J. (Eds.). Biotechnology of Isoprenoids. Advances in Biochemical Engineering/Biotechnology, Vol. 148. Springer. Cham, Switzerland, pp. 319–353. doi.org/10.1007/10_2014_289

Majumder, S., Ghosh, A., Bhattacharya, M. 2020. Natural anti-inflammatory terpenoids in *Camellia japonica* leaf and probable biosynthesis pathways of the metabolome. Bull. Natl. Res. Cent. 44: 141. doi.org/10.1186/s42269-020-00397-7

Nerurkar, P.V., Hwang, P.W., Saksa, E. 2015. Anti-diabetic potential of Noni: The yin and the yang. Molecules 20: 17684–17719. doi.org/10.3390/molecules201017684

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R. 2011. Open babel: An open chemical toolbox. J. Cheminform. 3: 33. doi.org/10.1186/1758-2946-3-33

Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., et al. 2017. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res. Clin. Pr. 128: 40–50. doi.org/10.1016/j.diabres.2017.03.024

Oso, B., Olaoye, I. 2020. Comparative *in vitro* studies of antiglycemic potentials and molecular docking of *Ageratum conyzoides* L. and *Phyllanthus amarus* L. methanolic extracts. SN Appl. Sci. 2: 629. doi.org/10.1007/s42452-020-2275-5

Pawlus, A.D., Su, B.N., Keller, W.J., Kinghorn, A.D. 2005. An anthraquinone with potent quinone reductase-inducing activity and other constituents of the fruits of *Morinda citrifolia* (Noni). J. Nat. Prod. 68: 1720–1722. doi.org/10.1021/np050383k

Sam-ang, P., Surangkul, D. Savaspun, K., Phanumartwiwath, A. 2020. Antioxidant and acetylcholinesterase inhibitory activities of *Morinda citrifolia* L. extracts. Agr. Nat. Resour. 54: 173–179. doi.org/10.34044/j.anres.2020.54.2.08

Sato, H., Hashishin, T., Kanazawa, J., Miyamoto, K., Uchiyama, M. 2020. DFT study of a missing piece in Brasilane-type structure biosynthesis: An unusual skeletal rearrangement. J. Am. Chem. Soc. 142: 19830–19834. doi.org/10.1021/jacs.0c09616

Ramadhan, R., Phuwapraisirisan, P. 2015. New arylalkanones from *Horsfieldia macrobotrys*, effective antidiabetic agents concomitantly inhibiting α-glucosidase and free radicals. Bioorg. Med. Chem. Lett. 25: 4529–4533. doi.org/10.1016/j.bmcl.2015.08.069

Sang, S., Liu, G., He, K., Zhu, N., Dong, Z., Zheng, Q., Rosen, R.T., Ho, C.-T. 2003. New unusual iridoids from the leaves of noni (*Morinda citrifolia* L.) show inhibitory effect on ultraviolet B-induced transcriptional activator protein-1 (AP-1) activity. Bioorg. Med. Chem. 11: 2499–2502. doi.org/10.1016/S0968-0896(03)00180-9

Sliwoski, G., Kothiwale, S., Meiler, J., Lowe, E.W.Jr. 2014. Computational methods in drug discovery. Pharmacol. Rev. 66: 334–395. doi.org/10.1124/pr.112.007336

Tuomi, T. 2005. Type 1 and type 2 diabetes: What do they have in common? Diabetes 54: S40–S45. doi.org/10.2337/diabetes.54.suppl_2. S40

Worawalai, W., Rattanangkool, E., Vanitcha, A., Phuwapraisirisan, P., Wacharasindhu, S. 2012. Concise synthesis of (+)-conduritol F and inositol analogues from naturally available (+)-*proto*-quercitol and their glucosidase inhibitory activity. Bioorg. Med. Chem. Lett. 22: 1538–1540. doi.org/10.1016/j.bmcl.2012.01.007

Xiaolong, C., Yuguo, Z., Yinchu, S. 2006. Voglibose (Basen®, AO-128), one of the most important α-glucosidase inhibitors. Curr. Med. Chem. 13: 109–116. doi.org/10.2174/ 092986 7 06789803035

Yadav, M., Chatterji, S., Gupta, S., Watal, G. 2014. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int. J. Pharm. Sci. 6: 539–542.