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Molecular Insight and Mode of Inhibition of α-Glucosidase and α-Amylase by Pahangensin A from *Alpinia pahangensis* Ridley

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The inhibition of carbohydrate-hydrolysing enzymes in human digestive organs is crucial in controlling blood sugar levels, which is important in treating type 2 diabetes. In the current study, Pahangensin A (**1**), a bis-labdanic diterpene previously characterized in the rhizomes of *Alpinia pahangensis* Ridley, was identified as an active dual inhibitor for α-amylase (IC_{50} = 114.80 μM) and α-glucosidase (IC_{50} = 153.87 μM). This is the first report on the dual α-amylase and α-glucosidase inhibitory activities of a bis-labdanic diterpene. The Lineweaver-Burk plots of compound **1** indicate that it is a mixed-type inhibitor with regard to both enzymes. Based on molecular docking studies, compound **1** docked in a non-active site of both enzymes. The dual inhibitory activity of compound **1** makes it a suitable natural alternative in the treatment of type 2 diabetes.

Keywords: *Alpinia pahangensis* Ridley; Pahangensin A; bis-labdanic diterpene; dual-inhibitor; α-glucosidase; α-amylase

Introduction

Diabetes mellitus is a metabolic disorder characterized by high plasma glucose levels, classified as either type 1 or 2. Type 1, or insulin-dependent diabetes, is due to failure of the pancreas to secrete insulin, while type 2, or non-insulin-dependent diabetes, is the result of insufficient insulin production. Type 2 diabetes receives more attention than type 1 diabetes because it is considered to be preventable. The former is caused by an imbalance between blood sugar absorption and insulin secretion. Post-prandial hyperglycaemia plays an important role in the development of type 2 diabetes. Controlling plasma glucose levels is essential for delaying or preventing type 2 diabetes.[1]

α-Amylase and α-glucosidase inhibitors belong to a class of anti-diabetic drugs that control the sudden rise in blood sugar levels after meals. α-Amylase is in a class of enzymes that hydrolyse polysaccharides to oligosaccharides, while α-glucosidase catalyses the final step in carbohydrate hydrolysis to release the absorbable monosaccharides.[2] Both of these carbohydrate-hydrolysing enzymes are secreted in the small intestine, while α-amylase is also found in the saliva.[1] Thus, the inhibition of these enzymes plays an important role in the management of diabetic complications, particularly type 2 diabetes.[2-3] Antidiabetic drugs such as acarbose, miglitol and voglibose act by inhibiting the activities of α-amylase and α-glucosidase. Although these drugs are effective, continuous use may lead to undesirable side effects, which include liver toxicity and adverse gastrointestinal symptoms.[4] Hence, discovering new potential inhibitors from plants may offer reduced side effects during long-term use.

A large number of traditional medicinal plants and plant-derived constituents, such as terpenes, alkaloids, curcuminoids, anthocyanins, flavonoids, quinones, phenols, phenylpropanoids, acylphenols and dimeric acylphenols, are known to exhibit α-amylase and α-glucosidase inhibitory activities.[2] [5-8] However, to date, bis-labdanic diterpenes have never been investigated for their potential to inhibit these enzymes. Therefore, herein, we report the dual inhibiting potentials of Pahangensin A (**1**) (*Figure 1*), a bis-labdanic diterpene previously

isolated and characterized from the rhizomes of *Alpinia pahangensis* Ridley, against α-amylase and α-glucosidase.[9] Kinetic studies were subsequently carried out on compound **1** to determine its mode of inhibition against each enzyme. Additionally, molecular docking was performed to provide insights into the binding interactions between compound **1** and the enzymes.

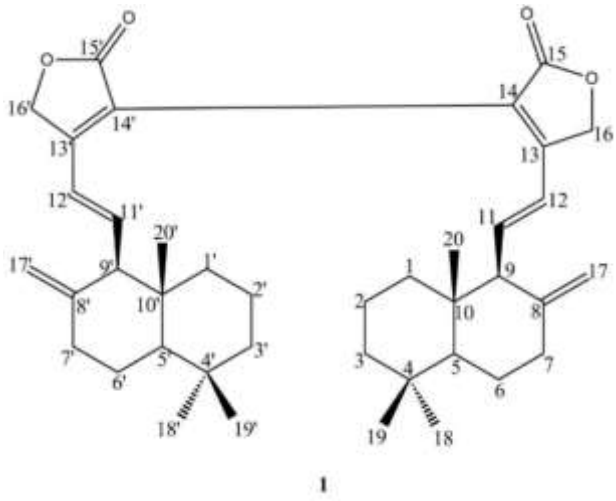

Figure 1: Structure of compound **1**

Results and Discussion

The IC_{50} values of compound **1** and the positive control, acarbose are given in Table 1. The inhibition potential of compound **1** against the carbohydrate-hydrolysing enzymes was higher compared to acarbose, thus making compound **1** a more effective inhibitor than the positive control.

Table 1: Carbohydrate hydrolyzing enzymes inhibition of compound **1** and acarbose.

Compounds	IC_{50} (μM)[a]	
	α-Amylase	α-Glucosidase
1	114.80 ± 8.49	153.87 ± 9.28
Acarbose	280.47 ± 10.19	1449. 67 ± 46.52

[a] Data presented as Mean ± SD (n = 3).

As illustrated by Lineweaver-Burk plot analyses (*Figure 2*), compound **1** displayed mixed-mode inhibition against α-amylase and α-glucosidase, as indicated by the intersect in the second quadrant of each respective plot. This finding suggested that compound **1** will be able to bind to the free enzymes and enzyme-substrate complexes.[5] [10-11]

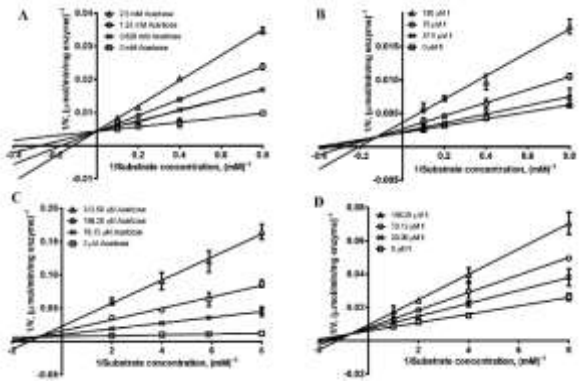

Figure 2: Lineweaver-Burk plots for **(A and B)** α-glucosidase and **(C and D)** α-amylase in the presence of acarbose and compound **1**, respectively. Mode of inhibition was determined by analysing the Lineweaver–Burk plots, which was obtained from the Michaelis–Menten kinetics (*Supp 1*).

The free enzyme inhibition of compound **1**, which was smaller than its enzyme-substrate complex inhibition constants (*Table 2*), enabled us to postulate that compound **1** has a higher affinity towards the free enzymes rather than the enzyme-substrate complexes, which is typical for mixed-mode inhibitors.[11] In the current study, acarbose was identified as a competitive inhibitor of α-glucosidase and a mixed-mode inhibitor of α-amylase, which is consistent with previous reports.[5] [12]

Table 2: Mode of inhibition and inhibitory constants of compound **1** and acarbose against α-amylase and α-glucosidase.

Compound	α-Amylase			α-Glucosidase		
	Mode of Inhibition	Ki_1 (μM)	Ki_2 (μM)	Mode of Inhibition	*Ki_1 (μM)	*Ki_2 (μM)
1	Mixed-mode	47.66 ± 8.24	68.78 ± 11.75	Mixed-mode	124.77 ± 12.16	140.93 ± 20.49
Acarbose	Mixed-mode	26.04 ± 2.67	61.18 ± 10.45	Competitive	568.47 ± 107.04	-

*Ki_1 and Ki_2 indicate the affinity of the inhibitors to the free enzyme and the enzyme-substrate complex, respectively.

The crystal structures of α-amylase (PDB ID: 1OSE)[13] and α-glucosidase (PDB ID: 3A4A)[14] have identified active sites and based on the molecular docking studies (*Figures 3 and 4*), acarbose and compound **1** showed favourable interactions with residues of the enzyme's binding pockets. Acarbose docked in the active site of α-glucosidase, but only interacted with a non-active site in α-amylase. This finding in turn provided supportive data of our experimental results that acarbose inhibited α-glucosidase and α-amylase in competitive and mixed-mode manners, respectively. As shown in Figure 3, the docked conformation of acarbose revealed the formation of two hydrogen bonds with the important active site residues of α-glucosidase, GLU277 and ASP352. On the other hand, compound **1** docked into the non-active site of both α-glucosidase and α-amylase (*Figure 4*), which is in agreement with our experimental data suggested that compound **1** was a mixed-mode inhibitor. In contrast to acarbose, compound **1** has only one hydrogen bond with α-amylase (GLY306). The majority of the interactions involved alkyl-alkyl and pi-alkyl interactions with ILE235, LYS 200, HIS201, ALA307, ILE148 and VAL163. Similar types of interactions were also observed with α-glucosidase, with only one hydrogen bond to ARG176 and the majority being alkyl-alkyl and pi-alkyl interactions (PRO149, PHE166, TRP238, PHE173, PRO151 and LYS148). The results also revealed better binding energies for compound **1** (-9.8 and -11.0 kcal/mol) compared to acarbose (-8.8 and -8.4 kcal/mol) for α-amylase and α-glucosidase, respectively.

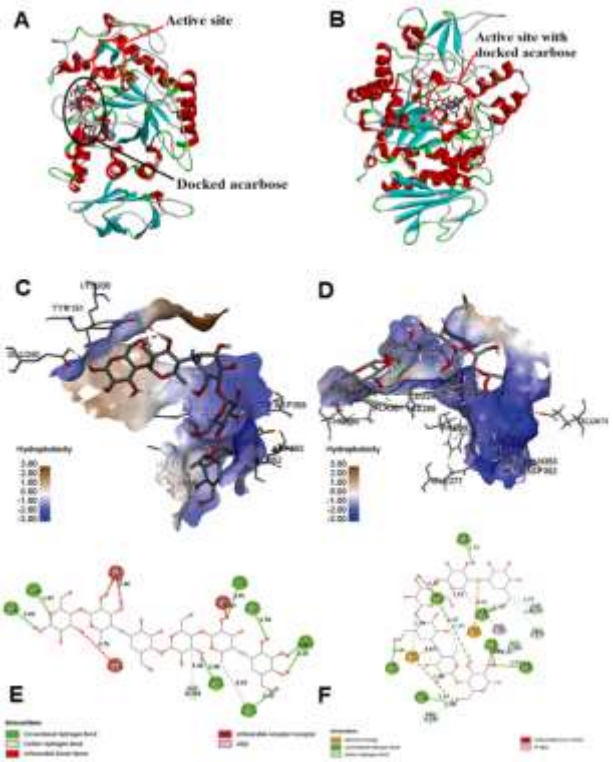

Figure 3: The docked acarbose in (A, C and E) α-amylase (PDB: 1OSE)[13] and (B, D and F) α-glucosidase (PDB: 3A4A)[14] is shown in 3-D and 2-D. The results show acarbose is docked into the α-glucosidase's active

site with 2 important active site residues, Glu277 and Asp352 via hydrogen bonding. However, acarbose only docked into the non-active site of the α-amylase enzyme.

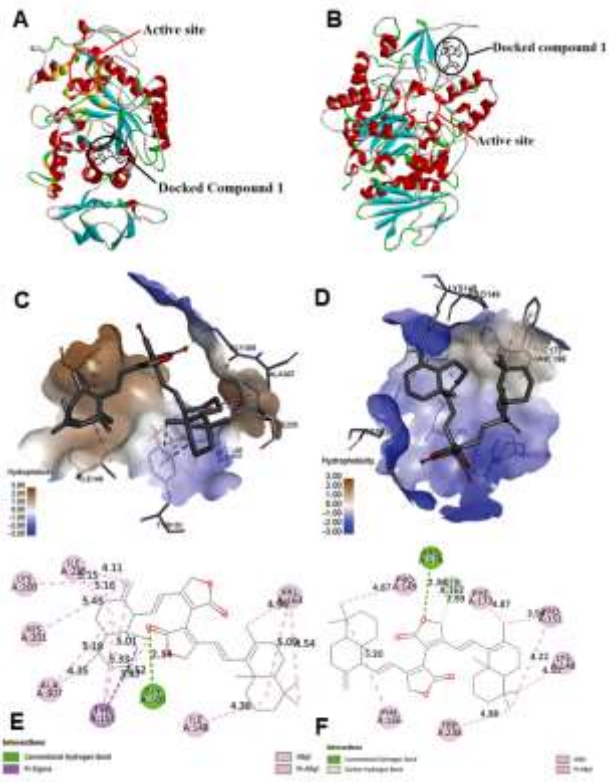

Figure 4: The docked compound **1** in (A, C and E) α-amylase (PDB: 1OSE)[13] and (B, D and F) α-glucosidase (PDB: 3A4A)[14] is shown in 3-D and 2-D. The results show compound **1** is docked into the non-active site of the α-amylase and α-glucosidase enzymes.

Conclusions

Diabetes mellitus is a metabolic disorder that leads to comorbidities such as neuropathy, nephropathy, retinopathy and cardiovascular diseases.[15] Control of postprandial glucose levels is one way to reduce postprandial spikes.[16] Hence, controlling the activities of α-amylase and α-glucosidase seems to be useful in preventing a postprandial spike.[16] In the present study, compound **1** exhibited stronger inhibitory effects towards α-amylase and α-glucosidase in comparison to acarbose. Molecular docking revealed that the interactions of compound **1** with both enzymes involved mainly hydrophobic interactions (alkyl-alkyl and pi-alkyl). The weak inhibition of α-glucosidase by acarbose is known to cause adverse effects, such as flatulence from the breakdown of undigested carbohydrates by intestinal bacteria.[15] Therefore, a compound with dual inhibition may elicit its pharmacological effects while minimizing its side effects.[18] The results presented here indicate that compound **1** is a promising α-amylase and α-glucosidase inhibitor for further development as a potential alternative to acarbose. Since, the enzymes used are non-human types, compound **1** can be a lead compound for medicinal chemists to further develop analogues, which may potentially inhibit human α-amylase and α-glucosidase.

Experimental Section

Chemicals and reagents

Analytical and preparative TLC were carried out on Merck 60 F_{254} silica gel plates (absorbent thickness: 0.25 and 0.50 mm). Column chromatography was performed using silica gel (Merck 230-400 mesh, ASTM). The IR spectrum was recorded using a Perkin-Elmer Spectrum 400 FT-IR Spectrometer. NMR spectra were recorded in $CDCl_3$ (Merck, Germany) with tetramethylsilane as an internal standard using a JEOL ECA 400 MHz NMR spectrometer. The LCMS-IT-TOF spectrum was recorded on a UFLC Shimadzu Liquid Chromatograph with an SPD-M20A diode array detector coupled to an IT-TOF mass spectrometer. The UV spectrum was recorded using

a Shimadzu 1650 PC UV-Vis Spectrophotometer. All solvents were of analytical grade and were distilled prior to use.[9]

The α-glucosidase enzyme (EC 3.2.1.20) was obtained from the yeast *Saccharomyces cerevisiae,* while the α-amylase enzyme (EC 3.2.1.1) was obtained from the porcine pancreas. All reagents, chemicals and enzymes were purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA).

Plant material

A. pahangensis was collected from Pahang in 2011. The plant was identified by one of our authors, Professor Halijah Ibrahim, and a voucher specimen (KU001) was deposited with the University of Malaya herbarium.[9]

*Extraction, isolation and characterization of compound **1***

Dried powdered rhizomes (1.0 kg) of *A. pahangensis* were extracted with CH_2Cl_2 (5.0 L, 2×) followed by MeOH (5.0 L, 2×) at room temperature, giving 15.55 g and 19.04 g of extracts, respectively. The CH_2Cl_2 extract was fractionated over a silica gel column eluting with mixtures of hexane:CH_2Cl_2 in proportions of 50:50 (v/v, 450 mL), 25:75 (v/v, 550 mL), and 0:100 (v/v, 1950 mL), followed by mixtures of CH_2Cl_2:MeOH in proportions of 95:5 (v/v, 400 mL) and 85:15 (v/v, 400 mL) to yield 10 main fractions (AP1-AP10). Repeated preparative TLC of fraction AP 4 (1.00 g; eluted with hexane:CH_2Cl_2 [25:75 v/v]) with hexane:EtOAc (95:5 v/v) led to the isolation of **1** (20.0 mg).[9] The purity of Pahangensin A is 98.2%, determined from the peak area of the LCMS-DAD-IT-TOF analysis (*Supp 2*).

Pahangensin A (= **bis-labda-8(17),11,13-trien-16,15-olide**; **1**): Yellow oil, UV λ_{max} (MeOH) nm (log ε): 205 (7.58), and 281 (11.80); IR λ_{max} (NaCl) cm-1: 3069, 2928, 2863, 2852, 1738, 1646, 1459, 1367, 889; LCMS-IT-TOF m/z : 599.4071 [M + H]+ (calcd.for $C_{40}H_{55}O_4$ 599.4095); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (3H, *s*, H-20/H-20'), δ 0.85 (3H, *s*, H-19/H-19'), δ 0.90 (3H, *s*, H-18/H-18'), δ 1.05 (1H, *m*, H-1*α*/H-1'*α*), δ 1.13 (1H, *dd*, *J* = 12.4, 2.3 Hz, H-5*α*/H-5'*α*), δ 1.20 (1H, *m*, H-3*α*/H-3'*α*), δ 1.37-1.45 (4H, *m*, H-1*β*/H-1'*β*, H-2*α*/H-2'*α*, H-3*β*/H-3'*β*, H-6*α*/H-6'*α*), δ 1.52 (1H, *m*, H-2*β*/H-2'*β*), δ 1.73 (1H, *m*, H-6*β*/H-6'*β*), δ 2.10 (1H, *m*, H-7*α*/H-7'*α*), δ 2.44 (1H, *m*, H-7*β*/H-7'*β*), δ 2.48 (1H, *brd*, *J* = 11.0 Hz, H-9*α*/H-9'*α*), δ 4.42 (1H, *d*, *J* = 1.2 Hz, H-17a/H-17'a), δ 4.78 (1H, *d*, *J* = 1.2 Hz, H-17b/H-17'b), δ 4.88 (2H, *brs*, H-16/H-16'), δ 6.00 (1H, *dd*, *J* = 16.5, 10.0 Hz, H-11/H-11'), δ 6.36 (1H, *d*, *J* = 16.5 Hz, H-12/H-12'); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 15.3 (C-20/C-20'), δ 19.3 (C-2/C-2'), δ 22.2 (C-19/C-19'), δ 23.5 (C-6/C-6'), δ 33.8 (C-4/C-4', C-18/C-18'), δ 36.9 (C-7/C-7'), δ 39.6 (C-10/C-10'), δ 41.2 (C-1/C-1'), δ 42.4 (C-3/C-3'), δ 54.8 (C-5/C-5'), δ 62.2 (C-9/C-9'), δ 68.2 (C-16/C-16'), δ 108.5 (C-17/C-17'), δ 120.9 (C-12/C-12'), δ 127.5 (C-13/C-13'), δ 135.5 (C-11/C-11'), δ 135.7 (C-14/C-14'), δ 149.6 (C-8/C-8'), δ 171.3 (C-15/C-15'). [9]

α-Glucosidase inhibitory assay

Compound 1 was initially dissolved in dimethyl sulfoxide (DMSO) and further diluted with the respective assay buffers to yield a final concentration of 15% DMSO in buffer. Acarbose was directly dissolved in the respective assay buffers. Negative controls with 15% DMSO in the respective assay buffers were included to account for the effect of the solvent. A total volume of 40 µL of compound **1** at different concentrations (0.02 – 2.5 mM) was pre-incubated with 80 µL of potassium phosphate buffer (pH 6.8) containing 67 mM potassium phosphate and 2.0 units/mL α-glucosidase in a 96-well plate for 10 minutes at 25 °C. Subsequently, 40 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution (p-NPG) in potassium phosphate buffer was introduced and incubated for another 10 minutes. After incubation, 60 µL of 100 mM sodium carbonate was added to terminate the reaction, and the absorbance was measured at a wavelength of 415 nm using a microplate reader (Infinite 200, Tecan). Acarbose was utilized as a positive control. The concentration of compound **1** resulting in 50 % inhibition of the α-glucosidase activity (IC_{50}) was determined using the GraphPad Prism 5 statistical package (GraphPad Software, USA). All data are expressed as the mean ± standard deviation of triplicate determinations.[5] [19]

α-Amylase inhibitory assay

Porcine α-amylase (1.0 unit/mL) in a volume of 100 µL was pre-incubated with 10 µL of compound **1** at different concentrations (0.02 – 2.5 mM) for 10 minutes at 25 °C. Next, 100 µL of 0.5 % (w/v) starch solution containing 0.5 % (w/v) potato starch in 20 mM sodium phosphate buffer (pH 6.9) with 6.7 mM sodium chloride was added to the solution and incubated for 8 minutes at 25 °C. Then, 100 µL of DNS colour agent solution containing 96 mM 3,5-dinitrosalicylic acid solution and 5.31 M sodium potassium tartrate in 2 M sodium hydroxide was added into the solution and incubated again for 15 minutes at 85 °C. After incubation, the absorbance was measured at a wavelength of 540 nm using a microplate reader (Infinite 200, Tecan). The experiment was also carried out using the positive control, acarbose. Similarly, the concentration of compound **1** resulting in 50 % inhibition of the α-amylase activity (IC_{50}) was determined using the GraphPad Prism 5 statistical package (GraphPad Software, USA). All data are expressed as the mean ± standard deviation of triplicate determinations.[20]

Mode of α-glucosidase and α-amylase inhibition

The inhibition modes of the compound **1** and acarbose samples against α-glucosidase and α-amylase were measured at different concentrations of their respective substrates (p-NPG or potato starch) in the presence or absence of samples at various concentrations. The mode of inhibition was obtained by Lineweaver-Burk plot analysis and calculated using Michaelis-Menten kinetics. A Dixon plot and the Y-intercept of the Lineweaver-Burk plot versus [inhibitor] were used to determine the inhibition constants (K_i). All data are expressed as the mean ± standard deviation of triplicate determinations.[5] [19] [21] The pathlength of the microplate reader (Infinite 200, Tecan) is 0.511 cm and extinction coefficient of compound **1** is 2.74 L mol^{-1} cm^{-1}.

Molecular docking

Molecular docking was performed to investigate the binding mode between the samples (compound **1** and acarbose) and the enzymes (α-amylase and α-glucosidase) using Autodock Vina 1.1.2. The 3-D structures of compound **1** and acarbose were drawn and energy minimized using ChemBio3D Ultra 12.0. The crystal structures of α-amylase (PDB ID: 1OSE)[13] and α-glucosidase (PDB ID: 3A4A)[14] were prepared using AutoDockTools 1.5.6 to remove water molecules and ligands (acarbose, α-D-glucose and β-D-glucose) and to add missing hydrogens. Although the active sites are identified in the crystal structures, the enzymes were enclosed in a grid box with 1.00 Å spacing, and the search exhaustiveness value was set to 100 to perform blind docking encompassing the whole enzyme structure. The top best-scoring pose from the AutoDock Vina results was analysed using Discovery Studio visualizer 4.5.[22]

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Author Contribution Statement

K.Y. Loo conducted the inhibitory assays, kinetic studies and molecular docking studies and analysed the data. Y. Sivasothy isolated and elucidated the structure of compound **1**. K.H. Leong, K.Y. Loo and Y. Sivasothy wrote the manuscript. K.H. Leong and K. Awang designed the experiments. H. Ibrahim collected and identified the plant material.

References

1. K.T. Kim, L.E. Rioux, S.L. Turgeon, 'Alpha-amylase and alpha-glucosidase inhibition is differently modulated by fucoidan obtained from *Fucus vesiculosus* and *Ascophyllum nodosum*', *Phytochem.* **2014**, *98*, 27–33.
2. D. Kumar, N. Gupta, R. Ghosh, R.H. Gaonkar, B.C. Pal, 'α-Glucosidase and α-amylase inhibitory constituents of *Carex baccans*: Bio-assay guided isolation and quantification by validated RP-HPLC-DAD', *J. Funct. Foods* **2013**, *5*, 211–218.
3. A. Tabussum, N. Riaz, M. Saleem, M. Ashraf, M. Ahmad, U. Alam, B. Jabeen, A. Malik, A. Jabbar, 'α-Glucosidase inhibitory constituents from *Chrozophor plicata*,' *Phytochem. Lett.* **2013**, *6*, 614–619.
4. S. Lordan, T.J. Smyth, A. Soler-Vila, C. Stanton, R.P. Ross, 'α-Amylase and α-glucosidase inhibitory effects of Irish seaweed extracts,' *Food Chem.* **2013**, *141*, 2170–2176.
5. Y. Sivasothy, K.Y. Loo, K.H. Leong, M. Litaudon, K. Awang, 'A potent alpha-glucosidase inhibitor from *Myristica cinnamomea* King', *Phytochem.* **2016**, *122*, 265–269.
6. J. Yan, G. Zhang, J. Pan, Y. Wang, 'α-Glucosidase inhibition by luteolin: Kinetics, interaction and molecular docking,' *Int. J. Biol. Macromol.* **2014**, *64*, 213–223.
7. Z. Yin, W. Zhang, F. Feng, Y. Zhang, W. Kang, 'α-Glucosidase inhibitors isolated from medicinal plants*', Food Sci. and Hum. Well.* **2014**, *3*, 136–174.
8. P.P Reddy, A.K. Tiwari, R.R. Rao, K. Madhusudhana, V.R.S. Rao, A.Z. Ali, K.S. Babu, J.M. Rao, 'New labdane diterpenes as intestinal α-glucosidase inhibitor from antihyperglycemic extract of *Hedychium spicatum* (Ham. Ex Smith) rhizomes', *Bioorg. Med. Chem. Lett.* **2009**, *19,* 2562–2565.
9. Y. Sivasothy, H. Ibrahim, A.S. Paliany, S.A. Alias, K. Awang, 'Pahangensin A and B, two new antibacterial diterpenes from the rhizomes of *Alpinia pahangensis* Ridley', *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6280–6285.
10. S.M. Abdul Wahab, Y. Sivasothy, S.Y. Liew, M. Litaudon, J. Mohamad, K. Awang, 'Natural cholinesterase inhibitors from *Myristica cinnamomea* King', *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3785–3792.

11. Y.H. Chu, S.H. Wu, J.F. Hsieh, 'Isolation and characterization of α-glucosidase inhibitory constituents from *Rhodiola crenulata*', *Food Res. Int.* **2014**, *57*, 8–14.
12. H. Kashtoh, S. Hussain, A. Khan, S.M. Saad, J.A.J. Khan, K.M. Khan, S. Perveen , M.I. Choudhary, 'Oxadiazoles and thiadiazoles: novel α-glucosidase inhibitors', *Bioorg. Med. Chem. Lett.* **2014**, *22*, 5454–5465.
13. C. Gilles, J.P. Astier, G. Marchis-Mouren, C. Cambillau, F. Payan, Crystal structure of pig pancreatic alpha-amylase isoenzyme II, in complex with the carbohydrate inhibitor acarbose. *Eur. J. Biochem.* **1996**. 238, 561–569.
14. K. Yamamoto, H. Miyake, M. Kusunoki, S. Osaki. Crystal structures of isomaltase from *Saccharomyces cerevisiae* and in complex with its competitive inhibitor maltose. FEBS. **2010**. 277, 4205–4214.
15. A. Ramachandran, 'Know the signs and symptoms of diabetes', *Indian J. Med. Res*. **2014**, *140*, 579–581.
16. H. Gao, Y.N. Huang, B. Gao, J. Kawabata, 'Chebulagic acid is a potent alpha-glucosidase inhibitor', *Biosci. Biotech. Bioch.* **2008**, *72*, 601–603.
17. T. Fujisawa, H. Ikegami, K. Inoue, Y. Kawabata, T. Ogihara, 'Effect of two α-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms', *Metabolism* **2005**, *54*, 387–390.
18. M. Figueiredo-González, C. Grosso, P. Valentão, P.B. Andrade, 'α-Glucosidase and α-amylase inhibitors from Myrcia spp.: a stronger alternative to acarbose?', *J. Pharmaceut. Biomed.* **2016**, *118*, 322–327.
19. M.I. Kazeem, J.V. Ogunbiyi, A.O. Ashafa, '*In vitro* studies of the inhibition of α-amylase and α-glucosidase by leaf extracts of *Picralimanitida* (Stapf).', *Trop. J. Pharm. Res.* **2013**, *12*, 719–725.
20. H. Ali, P.J. Houghton, A. Soumyanath, 'α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*', *J. Ethnopharmacol.* **2006**, *107*, 449–455.
21. M. A. T. Phan, J. Wang, J. Tang, Y.Z. Lee, K. Ng, 'Evaluation of α-glucosidase inhibition potential of some flavonoids from *Epimedium brevicornum*', *Food Sci. Technol.* **2013**, ***53*,** 492–498.
22. O. Trott, A.J. Olson, 'AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading', *J. Comput.Chem.* **2010**, *31*, 456–461.