

In-vitro anti-diabetic activity and *in-silico* studies of binding energies of palmatine with alpha-amylase, alpha-glucosidase and DPP-IV enzymes

Patrick Okechukwu¹, Mridula Sharma¹, Wen Hui Tan¹, Hor Kuan Chan¹, Kavita Chirara¹, Anand Gaurav¹, Mayasah Al-Nema¹

¹ UCSI Universiti, Kuala Lumpur, Malaysia

Corresponding author: Patrick Okechukwu (patrickn@ucsiuniversity.edu.my)

Received 5 September 2020 ♦ **Accepted** 18 October 2020 ♦ **Published** 27 November 2020

Citation: Okechukwu P, Sharma M, Tan WH, Chan HK, Chirara K, Gaurav A, Al-Nema M (2020) *In-vitro* anti-diabetic activity and *in-silico* studies of binding energies of palmatine with alpha-amylase, alpha-glucosidase and DPP-IV enzymes. Pharmacia 67(4): 363–371. <https://doi.org/10.3897/pharmacia.67.e58392>

Abstract

Palmatine a protoberberine alkaloid has been previously reported to possess *in vivo* antidiabetic and antioxidant property. The aim of the experiment is to evaluate the *in vitro* antidiabetic activity and *in-silico* studies of the binding energies of Palmatine, acarbose, and Sitagliptin with the three enzymes of alpha-amylase, alpha-glucosidase, and dipeptidyl peptidase-IV (DPP-IV). The *in vitro* antidiabetic study was done by evaluating the inhibitory effect of palmatine on the activities of alpha-amylase, alpha-glucosidase, and DPP-IV. Acarbose, and sitagliptin was used as standard drug. The molecular docking study was performed to study the binding interactions of palmatine with alpha-glucosidase, a-amylase, and DPP-IV. The binding interactions were compared with the standard compounds Sitagliptin and acarbose. Palmatine with IC_{50} ($1.31 \pm 0.27 \mu\text{M}$) showed significant difference of (< 0.0001) higher inhibiting effect on alpha-amylase and weak inhibiting effect on alpha-glucosidase enzyme with IC_{50} ($9.39 \pm 0.27 \mu\text{M}$) and DPP-IV with IC_{50} ($8.7 \pm 1.82 \mu\text{M}$). Palmatine possess inhibition effect on the three enzymes.

Keywords

Alpha-glucosidase, alpha-amylase, dipeptidyl peptidase-IV, diabetes mellitus, palmatine

Introduction

Diabetes mellitus (DM) is a metabolic multiple etiology disease associated with high blood sugar levels (Kharroubi and Darwish 2015). DM is associated with minor and major complications such as renal function, liver function, dyslipidemia, and cardiovascular disease (Rangel et al. 2019). According to the International Diabetes Federation Atlas 2017, the globally estimated prevalence of diabetes

was 8.4% in 2017 and predicted to grow to 9.9% in 2045 (Cho et al. 2017). There is an increase interest towards the discovery of new leads from plants with strong antidiabetic property to be developed as potential antidiabetic therapy. There are many mechanisms of antidiabetic drugs such as to suppress hepatic glucose production (Biguanides), stimulate insulin secretion (sulfonylureas), improve the sensitivity of insulin receptor and peripheral glucose uptake (thiazolidinedione's) and delay digestion and ab-

sorption of intestinal carbohydrates to maintain the postprandial glucose levels (Meneses et al. 2015). One of the therapeutic approaches for DM management is to reduce the postprandial hyperglycemia by delaying the digestion and absorption of carbohydrates such as alpha-glucosidase and alpha-amylase enzyme in the digestive tract. Inhibitors of alpha-glucosidase and alpha-amylase enzyme delays the digestion of carbohydrate and prolong the carbohydrate digestion rate, which causes a reduction in the absorption rate of glucose and consequently decrease the postprandial hyperglycemia (Ahmed et al. 2016; Akuba et al. 2018; Tar et al. 2020). Researchers are equally interested in antidiabetic drugs that target incretin pathway. Incretins are hormones predominantly glucose-dependent insulin tropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) produced by the intestinal mucosa that enhance glucose-induced insulin secretion and lower blood glucose concentration levels (Thakare et al. 2017). About 60% of the insulin release in the body is through incretin pathway via the activity of GLP-1 and GIP (Salehi et al. 2012). Therefore, there are two main approaches in the GLP-1 therapies have been used for the treatment of diabetes. Both treatments aim to extend the time of GLP-1 circulation in the liver. Dipeptidyl peptidase-IV (DPP-IV) is serine protease enzyme that is responsible for the rapid degradation of incretins GLP-1 and GIP-1 (Lin et al. 2019).

Palmatine, is a bioactive protoberberine alkaloid, which was isolated from *Coscinium fenestratum* (CF) stem extract. CF belongs to the plant family name Menispermaceae and commonly known as 'tree turmeric'. Previous report has shown that the stem extract of CF and Palmatine possess *in vivo* antidiabetic and antioxidant properties. This study was aimed to evaluate the *in-vitro* antidiabetic activity and *in-silico* studies of the binding energies of palmatine and the standard compounds with the three active centers of alpha-amylase, alpha-glucosidase, and DPP-IV enzymes.

Materials

Abbreviation's

ADT = AutoDockTools, DM = Diabetes mellitus; DNS = 3,5-Dinitrosalicylic acid DPP-IV = Dipeptidyl peptidase-IV; GIP = Glucose-dependent insulin tropic polypeptide; GLP-1 = glucagon-like peptide 1; NaOH = sodium hydroxide.

Chemicals

Palmatine (Sigma Aldrich, USA), alpha-glucosidase from *Saccharomyces cerevisiae* (E.C. 3.2.1.20) (Sigma Aldrich, USA), alpha-amylase from porcine amylase ((E.C. 3.2.1.1) (Sigma-Aldrich, USA) DPP-IV enzyme (Sinar Scientific, Malaysia), starch (Friendmann Schmdit, Austria), sucrose (EMD, Millipore Chemicals, France), 6N HCL (Sinar Scientific, Malaysia), Sodium phosphate buffer (Sigma

Aldrich, USA), 3,5-dinitrosalicylic acid (System, Malaysia), sodium potassium tartrate tetrahydrate (Hopkins and Williams, UK), Sodium hydroxide (System, Malaysia), Rebaudioside (Sigma Aldrich, USA), Tris buffer (Bio-Rad, United States), 25% glacial acetic acid (Merck, Germany), Acarbose (Sigma-Aldrich, USA), Sitagliptin (Januvia, MSD Pharmaceutical Pvt. Ltd., India), Glimepiride (Amaryl, Sanofi India Pvt. Ltd., India), Metformin (Glucophage XR, Merck, Malaysia).

Equipment

Vortex mixer (LMS CO., LTD, Japan); Centrifuge machine (Zentrifugen, Germany); pH meter (Mettler Toledo, Switzerland); UV-Vis spectrophotometer (SECOMAM, France); Water bath (Copens Scientific (M) Sdn Bhd, Malaysia); Electronic balance (Mettler Toledo, Switzerland); FLUOstar Omega Plate Reader (Thermofisher, USA).

Methodology

In-vitro Anti-diabetic studies

Alpha-Amylase inhibiting activity

The inhibiting activity of alpha-amylase was performed according to the method by González-Montoya et al. 2018. The reaction mixture contained 1mL of Palmatine solution at concentrations of 29.2, 58.4, 87.61, 116.81 and 146.02 μ M and 1mL of alpha – amylase solution (2U/mL). The mixture was pre-incubated for 30 minutes and then 1 mL of the starch solution was added to the reaction and incubated at 37 °C for 10 minutes. The reaction mixture was stopped by adding 1 mL of DNS solution 12.0 g of sodium potassium tartrate tetrahydrate in 8 ml of 2M NaOH and 96 mM of 3,5-dinitrosalicylic acid solution and the mixture was boiled for 5 minutes. The negative control was prepared without sample and acarbose was used as standard drug (positive control), the following equation was used to determine the percentage of inhibition of each compound using equation 3.1. Inhibitory concentration 50% (IC_{50}) of alpha – amylase enzyme inhibiting activity of each compounds were calculated using Graph Pad Prism version 7.0.

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

Alpha-Glucosidase inhibiting activity

The alpha-glucosidase inhibiting activity was performed according to a method described by Pandithurai et al. 2015. 1mL of 2% w/v of sucrose solution was added as the substrate in 0.2 M of Tris buffer (pH 8.0). 1mL of palmatine at concentrations of 29.2, 58.4, 87.61, 116.81 and 146.02 μ M was added to the reaction mixture and incubated for 5 minute at room temperature. 1 mL of the alpha – glucosidase enzyme (10U/mL) was added into the reaction mixture for initiation of the reaction followed by incubation for 40 minutes at 35 °C. The reaction was terminated by the addition

of 2 mL of 6N HCl. The intensity of the color was measured at 540 nm. Acarbose was used as standard drug control. The absorbance was measured at 540 nm. The % inhibition was calculated as given equation 3.1. IC_{50} of alpha – glucosidase enzyme inhibiting activity of each compounds were calculated using Graph Pad Prism version 7.0.

Dipeptidyl peptidase IV inhibiting activity (DPP-IV)

The inhibiting activity of dipeptidyl peptidase IV (DPP-IV) was performed according to the method by González-Montoya et al. 2018 with slight modifications. DPP-IV inhibiting activity was performed with a pre-incubation volume 200 μ L containing 50 μ L of DPP-IV enzyme (500 μ g/mL) and 25 μ L of 29.2, 58.4, 87.61, 116.81 and 146.02 μ M concentration of Palmatine drugs on 96-microwell plates. This mixture was incubated at 37 °C for 5 minutes, followed by the addition of 100 μ L rebaudioside (substrate). The reaction mixture was incubated for 15 minutes at 37 °C and the reaction was terminated with 25 μ L of 25% glacial acetic acid. Absorbance was measured at 405 nm. The negative control was without palmatine and sitagliptin was used as standard drug. The percentage of inhibition was calculated as given in equation 3.1. IC_{50} of DPP-IV enzyme inhibiting activity of each compounds were calculated using Graph Pad Prism version 7.0.

In-silico studies

The molecular docking study was performed using AutoDock Vina 1.1.2. The X-ray crystallographic structures of the target receptors, alpha – glucosidase (PDB ID: 3W37), alpha – amylase (PDB ID: 4W93) and DPP-IV (PDB ID: 4A5S), bound with their co-crystallized ligands were retrieved from the protein data bank (PDB) at resolutions of 1.70 Å, 1.35 Å and 1.62 Å, respectively. The protein structures were prepared for docking using Biovia Discovery Studio (DS) and AutoDock Tools (ADT) 1.5.6. All the water molecules, co-crystallized ligands and other heteroatoms were removed and polar hydrogen atoms, as well as Gasteiger charges, were added. Following the protein preparation, the structures of palmatine and the standard compounds, acarbose and Sitagliptin, were downloaded from PubChem and converted into their respective 3D structures using Biovia DS. The ligands were imported into AutoDock where ADT was used to add charges, set the rotatable bonds, and allow all the torsions to rotate for the ligands. Then, the amino acid residues of the prepared protein structures that involved in the interactions with the co-crystallized ligands were determined in which the binding site of each protein structure was identified based on the determined residues. ADT was used to determine the Grid Box that covers the entire binding site of the protein. The coordinates and size of the Grid Box were saved in the input parameter file. Finally, the prepared ligands were docked into the active sites of the prepared proteins' structures. All the docking parameters have remained as default settings. The binding energies of the ligands were

recorded and compared (the more negative value indicates the higher affinity). The binding poses of the ligands and the mode of interactions of the protein-ligand complexes were studied thoroughly using Biovia DS.

Statistical analysis

All data were presented as mean (\pm) standard deviation (SD) in triplicates ($n = 6$) using the XL. STAT 7.0. The data were statistically analyzed by One-Way ANOVA followed by Dunnet's Test. Values were considered statistically significant and denoted as; $p < 0.05 = a$; $p < 0.01 = b$; $p < 0.001 = c$; $p < 0.0001 = d$ and data which were not found significantly different from each other, denoted by same superscript letter.

Results

In-vitro anti-diabetic activities

Alpha-amylase enzyme inhibiting activity

The effect of palmatine on the alpha-amylase activity was investigated using starch substrate. Acarbose was used as positive control and glimepiride and metformin was used as standard drug. On the basis of IC_{50} , Palmatine with IC_{50} ($1.31 \pm 0.27 \mu$ M) showed significant (< 0.0001) higher inhibiting effect on alpha-amylase followed by metformin ($2.5 \pm 0.47 \mu$ M), acarbose ($2.94 \pm 0.36 \mu$ M) and glimepiride ($4.98 \pm 0.47 \mu$ M) as shown Figure 1. However, metformin and acarbose had similar inhibitory effect on alpha-amylase enzyme.

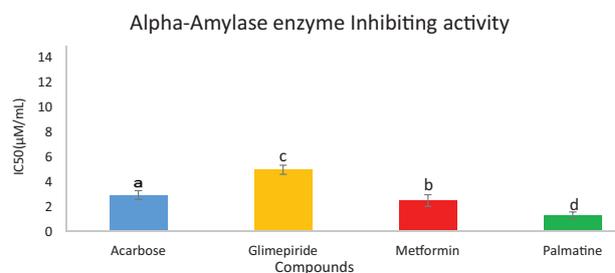


Figure 1. Alpha-amylase enzyme inhibiting activity (IC_{50} (μ M)) of palmatine, glimepiride, metformin and standard drug (acarbose). The result are shown as Mean \pm standard deviation in triplicates ($n = 3$). The data were statistically analyzed by One-way ANOVA followed by Dunnet's post hoc test. Graph showed that acarbose(a) vs. glimepiride = $p < 0.001 = c$; acarbose vs. metformin = $p < 0.01 = b$; acarbose vs. palmatine = $p < 0.0001 = d$. Glimepiride vs. Metformin = $p < 0.001 = c$, glimepiride vs. palmatine = $p < 0.0001 = d$, metformin vs. palmatine = $p < 0.0001 = d$.

Alpha-glucosidase enzyme inhibiting activity

The effect of palmatine on the alpha-glucosidase enzyme activity was investigated using sucrose as substrate. Acarbose was used as positive control drug, glimepiride and metformin was used as standard drugs. On the basis of IC_{50} , palma-

tine had a weak inhibiting effect on alpha-glucosidase enzyme with IC_{50} ($9.39 \pm 0.27 \mu M$). Metformin and glimepiride had similar inhibitory effect with IC_{50} ($9.5 \pm 1.11 \mu M$ and $9.9 \pm 0.22 \mu M$). However, acarbose showed a very strong inhibitory effect with IC_{50} ($1.31 \pm 0.2 \mu M$) as shown in Figure 2.

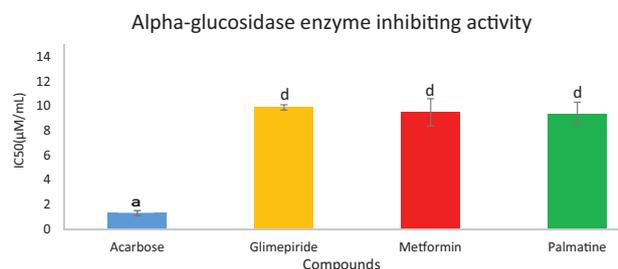


Figure 2. Alpha-glucosidase enzyme inhibiting activity (IC_{50} (μM)) of palmatine, glimepiride, metformin and standard drug (acarbose). The result are shown as Mean ± standard deviation in triplicates (n = 3). The data were statistically analyzed by One-way ANOVA followed by Dunnet's post hoc test. Graph showed that acarbose (a) vs. glimepiride = $p < 0.0001 = d$; acarbose vs. metformin = $p < 0.0001 = d$; acarbose vs. palmatine = $p < 0.0001 = d$. Glimepiride vs. metformin = No significant = d, glimepiride vs. palmatine = No significant = d and metformin vs. palmatine = No significant = d.

Dipeptidyl peptidase enzyme (DPP IV) Inhibiting activity

The effect of palmatine on the dipeptidyl peptidase enzyme activity was investigated using rebaudioside as a substrate. Sitagliptin was used as positive drug, glimepiride and metformin was used as standard drug. On the basis

of IC_{50} , palmatine had a weak inhibiting effect on DPP-IV with IC_{50} ($8.7 \pm 1.82 \mu M$) and sitagliptin showed a very strong inhibitory effect with IC_{50} ($1.07 \pm 0.23 \mu M$) as shown in Figure 3. Metformin and glimepiride did not show any inhibition against DPP-IV enzyme.

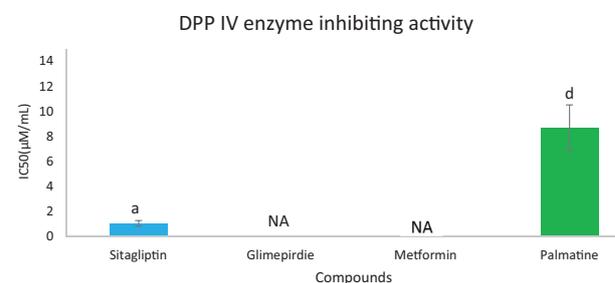
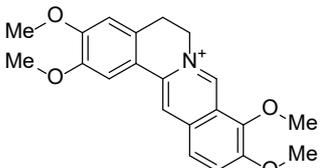
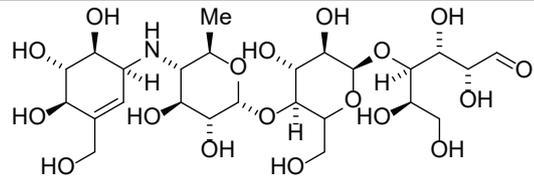
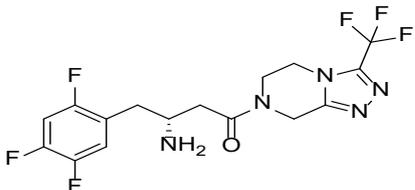


Figure 3. DPP-IV enzyme inhibiting activity (IC_{50} (μM)) of glimepiride, metformin, palmatine and standard drug (sitagliptin). The result are shown as Mean ± standard deviation in triplicates (n = 3). The data were statistically analyzed by One-way ANOVA followed by Dunnet's post hoc test. Graph showed that sitagliptin (a) vs. palmatine = $p < 0.0001 = d$. Glimepiride and metformin = NA = No activity.

In-silico studies

The docking study of palmatine and the standard compounds, acarbose and sitagliptin, allowed to display the affinity and the best binding pose of the respective compounds within the active sites of alpha-glucosidase, alpha-amylase and DPP-IV in addition to the elucidation of the interactions and the amino acids involved in the binding. The results of docking studies are presented in Table 1, the lower

Table 1. The binding energies of palmatine and the standard compounds with the three active centers of alpha-amylase, alpha-glucosidase and DPP-IV enzymes (Table shows the result of the binding energies of palmatine and the standard drug acarbose and sitagliptin).

Compound	Structure	Binding Energy (Kcal/mol)		
		alpha-glucosidase	alpha-amylase	DPP-IV
Palmatine		-6.4	-7.8	-8.0
Acarbose		-8.3	-8.8	-9.4
Sitagliptin		-8.7	-8.7	-9.2

value of the binding energy indicates a higher binding affinity within the active site of the respective protein target.

Discussion

Hyperglycemia is categorized as a common metabolic abnormality in diabetes mellitus. Hyperglycemia is a condition in which an excessive quantity of glucose circulates in the blood plasma due to insufficient secretion or impaired action of insulin (Kortykowski et al. 2012), thus it is essential to maintain the glucose levels. The alpha-amylase enzyme is an endonuclease enzyme which is responsible for the digestion of polysaccharides and alpha-glucosidase is located in the brush border of small intestinal area and responsible for the digestion of oligosaccharides (Telagari and Hullatti 2015) DPP-IV is serine protease enzyme and located in the liver, responsible for the deactivation of incretins (Lin et al. 2019). These enzymes are the key enzymes for the regulating the blood glucose level by delaying digestion and absorption of carbohydrate and incretins. In the present study we have investigated the ability of palmatine to inhibit the enzymatic activity of the alpha-amylase and alpha-glucosidase and observed that palmatine have a strong alpha-amylase inhibiting activity and weak alpha-glucosidase and DPP-IV enzyme inhibiting activity. Several studies have shown that polyphenols, flavonoids, alkaloids are known for the inhibition of carbohydrates enzyme activity (Kidane et al. 2018; Unuofin et al. 2018). Palmatine is an alkaloid, which may interact with the specific position of the enzymes thereby reducing the activity the alpha-amylase and alpha-glucosidase. However, palmatine is a potent inhibitor of alpha-amylase enzyme and weak inhibitor of alpha-glucosidase enzyme. There is a contradictory in the activity of the alpha-glucosidase enzyme of flavonoids

and alkaloids; many studies have shown that flavonoids and alkaloids are the weak inhibitors of alpha-glucosidase enzyme (McDougall et al. 2005) and some studies have shown that flavonoids and alkaloids are potent alpha-glucosidase enzyme (Yin et al. 2014). Several studies have shown that Flavonoids and alkaloids are known as a potent alpha-amylase inhibitor (Kidane et al. 2018, Telagari and Hullatti et al. 2015; Somtimuang et al. 2018) which was in accordance with alpha-amylase inhibitory activity of palmatine found in our study. *In-vitro* DPP-IV inhibiting activity, palmatine showed relatively lower inhibiting activity of DPP-IV compared to sitagliptin. Several recent progresses of DPP-IV inhibitors have shown that chemical components of natural sources were found with potent DPP-IV inhibitory activity (Kim et al. 2018). A study has shown that Berberine which is an alkaloid have potency to inhibit the DPP-IV enzyme, but activity was lower compared to sitagliptin (Beidokhti et al. 2018). which was in accordance with DPP-IV inhibiting activity of palmatine observed in our study. *In-vitro* anti-diabetic studies have demonstrated that palmatine may interfere or delay the digestion or absorption of dietary carbohydrate and incretins leading to the suppression of postprandial hyperglycemia. Hence, it may be useful in the management of diabetes mellitus.

According to the docking results, acarbose has shown the highest binding affinity (binding energy -8.8 kcal/mol) to the alpha-amylase. The high affinity is attributed to the hydrogen bond interactions between the ligand and the active site residues of the receptor. As can be seen in Figure 4, the ligand interacts with alpha-amylase via five hydrogen bonds. Three hydrogen bonds form between two hydroxyl groups of the ligand and the residues TRP59, GLN63 and ASP300, respectively. The oxygen atom in tetrahydropyran forms one hydrogen bond with the residue GLN63 while the amino group of the ligand form one

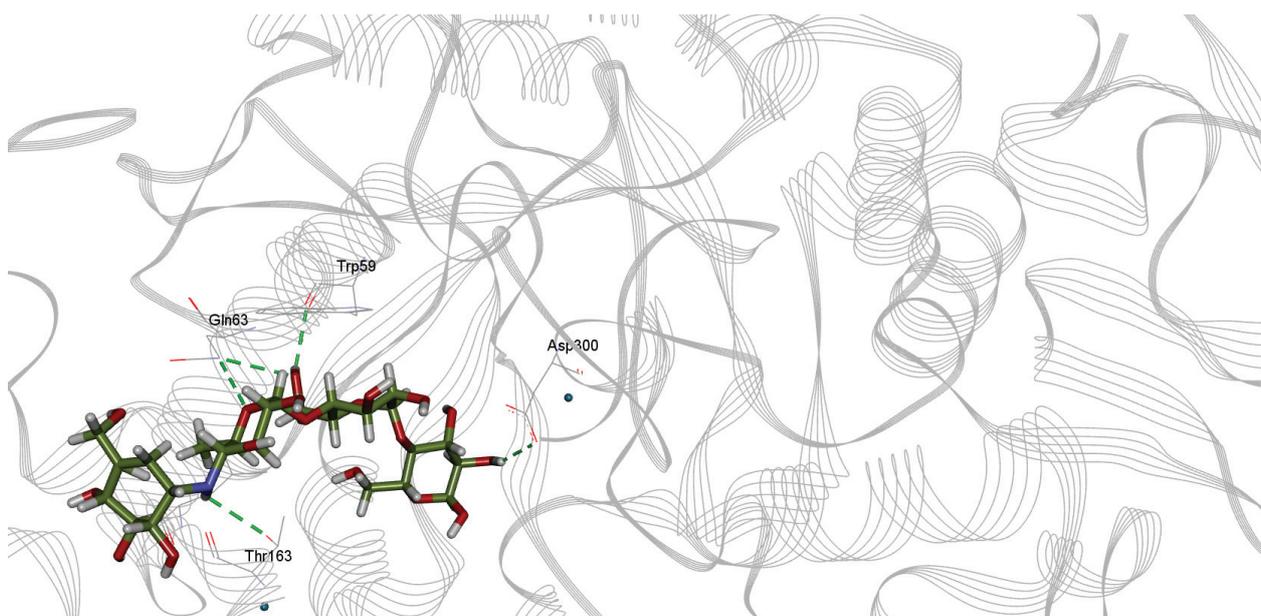


Figure 4. Docking interaction and binding mode of acarbose and alpha-amylase enzyme. The hydrogen bonds are presented in green dotted lines.

hydrogen bond with the residue THR163 of the receptor. All the amino acid residues act as hydrogen bond donors.

In contrast to acarbose, no hydrogen bond interactions were observed between palmatine and alpha-amylase. The isoquinoline group of palmatine displays π - π stacked interactions with the aromatic ring of TRP59. Additionally, the tetrahydronaphthalene group displays another π - π stacked interaction with the aromatic residue TYR62 (Figure 5). The five hydrogen bonds that observed between the acarbose and alpha-amylase play an important role in stabilizing the ligand in the active site of alpha-amylase. Whereas, the lack of hydrogen bond interactions between palmatine and alpha-amylase result in a lower binding affinity.

The binding mode of acarbose within the active site of alpha-glucosidase receptor showed that the ligand forms two hydrogen bond interactions with the receptor. These hydrogen bonds form between the hydroxyl groups of the ligand and the residue MET470 and SER497, respectively, in which the amino acids residues act as hydrogen bond donors. Moreover, the methyl group of the ligand interacts with the aromatic rings of PHE236 and PHE601 (Figure 6). Similarly, palmatine shows two hydrogen bond interactions with alpha-glucosidase. Both hydrogen bonds form between the two methoxy groups of the dimethoxy-tetrahydronaphthalene of the ligand and the residue

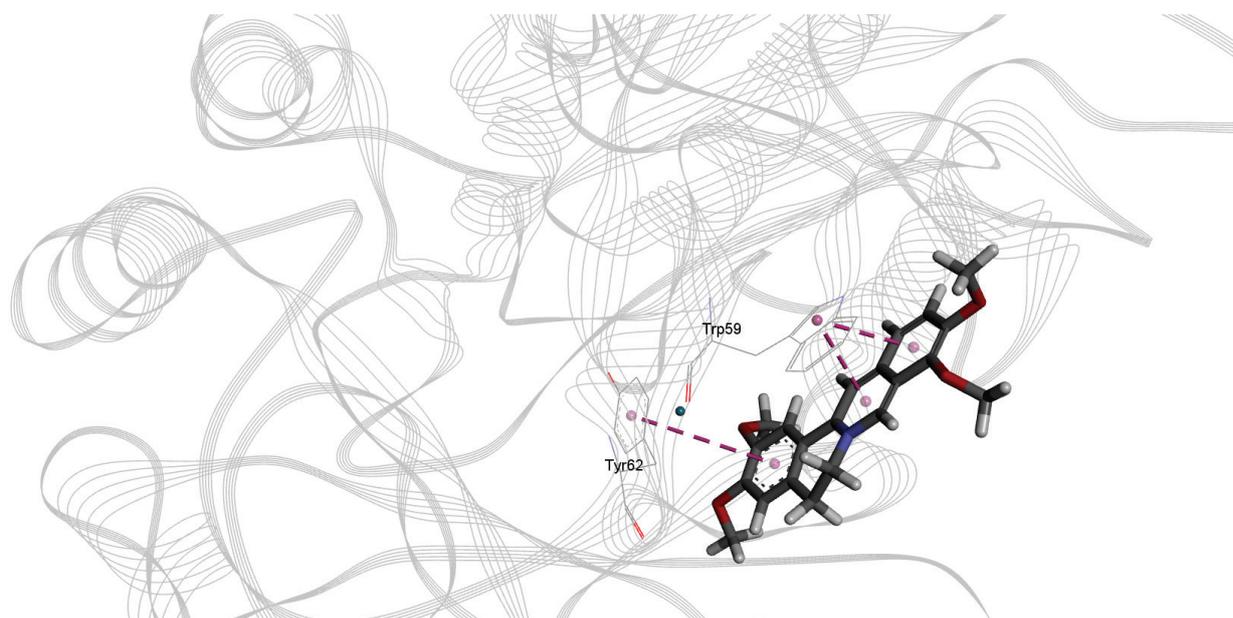


Figure 5. Docking interaction and binding mode of palmatine and alpha-amylase enzyme. The π - π stacked interactions are presented in magenta dotted lines.

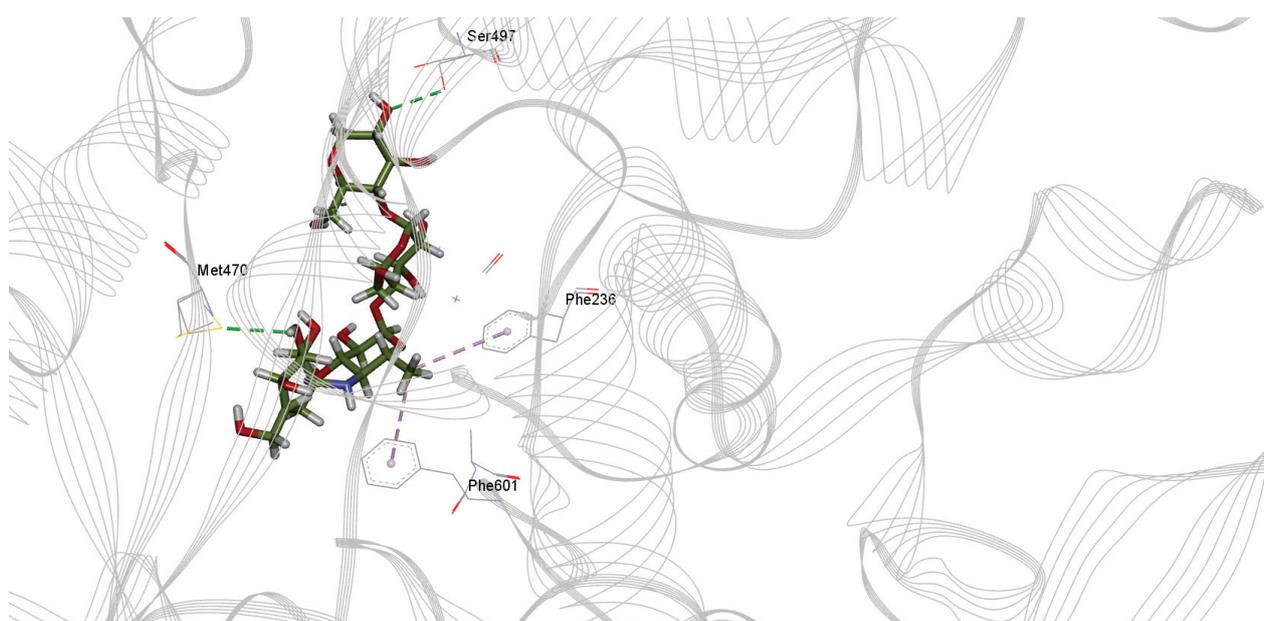


Figure 6. Docking interaction and binding mode of acarbose and alpha-glucosidase enzyme. The hydrogen bonds are presented in green dotted lines and the π -alkyl interactions are presented in pink dotted lines.

SER497. Palmatine also displays aromatic interactions with the receptor (Figure 7). These interactions were observed between the isoquinoline group and the residue ALA234 from one side and the tetrahydronaphthalene group and the residues ILE233 and LYS506 from the other side (Figure 7).

Both acarbose and palmatine displayed similar interactions with alpha-glucosidase. However, acarbose exhibited a higher affinity to the receptor (binding energy -8.3 Kcal/mol) than palmatine (binding energy -6.4 Kcal/mol). The higher affinity can be attributed, to the hydrogen bond interactions with two different resi-

dues MET470 and SER497 which were observed with acarbose in contrast, to the hydrogen bond interactions with only one residue SER497 which, were observed with palmatine.

Sitagliptin was used as a standard compound where it exhibited high affinity towards DPP-IV receptor (binding energy -9.2 Kcal/mol). The ligand involved in several interactions with DPP-IV in which the two fluorine groups of the phenyl ring and the carbonyl group form four hydrogen bonds with the residues ARG358 and ARG125, respectively. The ligand acts as hydrogen bond acceptor where it accepted the hydrogen atoms from ARG. Addi-

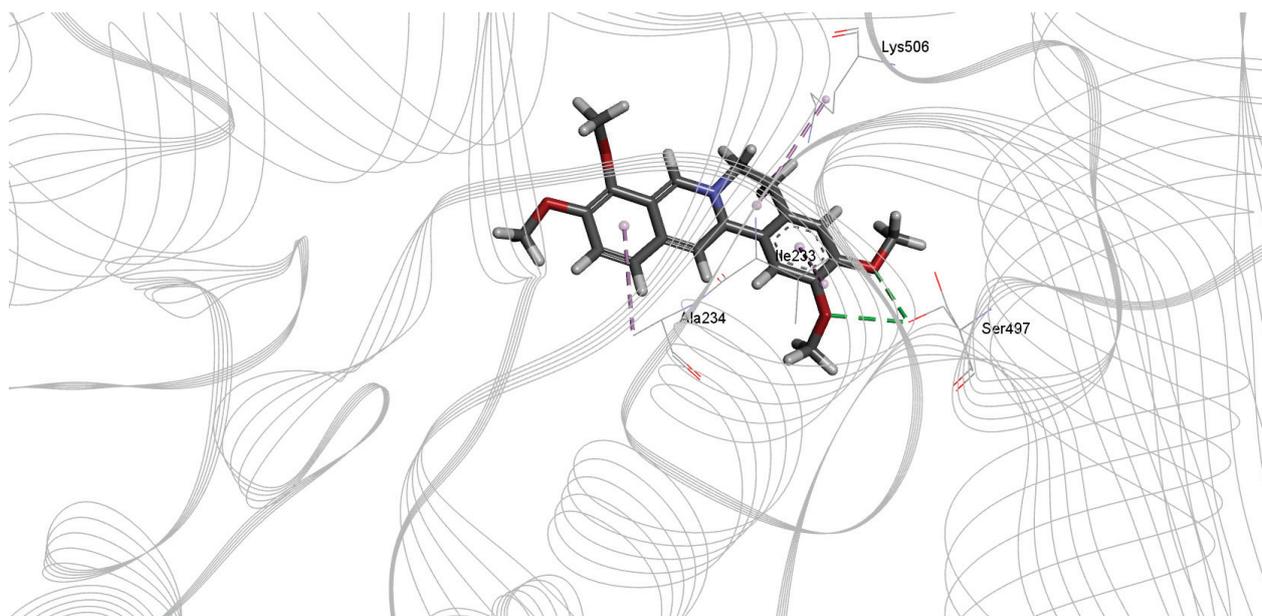


Figure 7. Docking interaction and binding mode of palmatine and alpha-glucosidase enzyme. The hydrogen bonds are presented in green dotted lines and the π -alkyl interactions are presented in pink dotted lines.

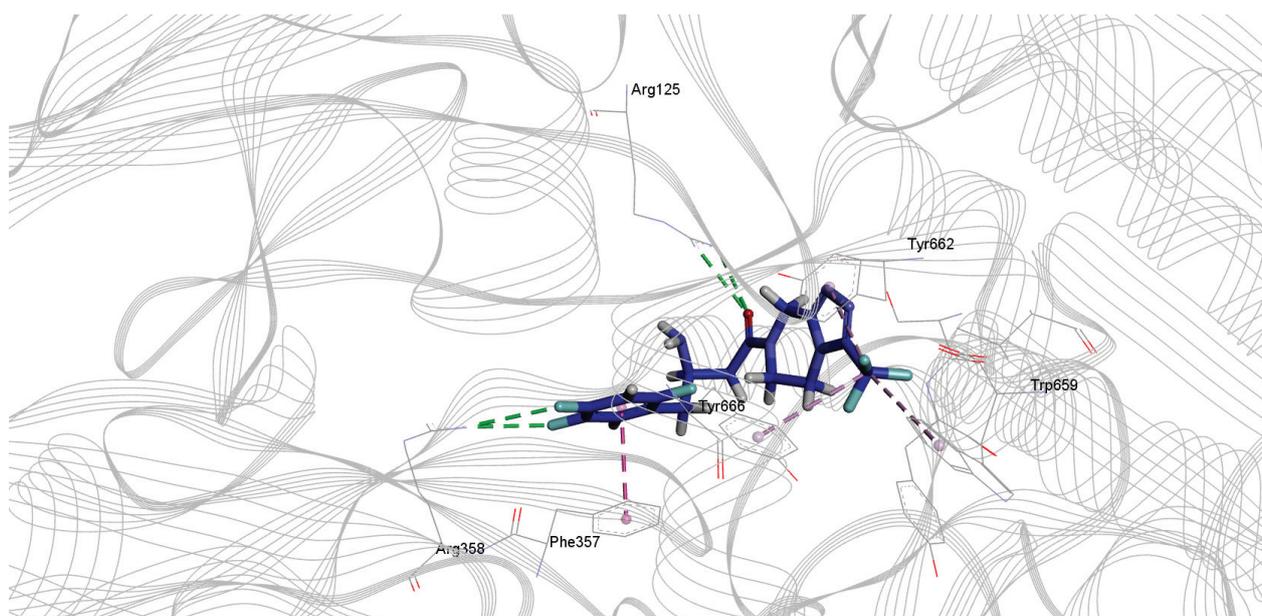


Figure 8. Docking interaction and binding mode of sitagliptin and DPP-IV enzyme. The hydrogen bonds are presented in green dotted lines, the π - π stacked interactions are presented in magenta dotted lines and the π -alkyl interactions are presented in pink dotted lines.

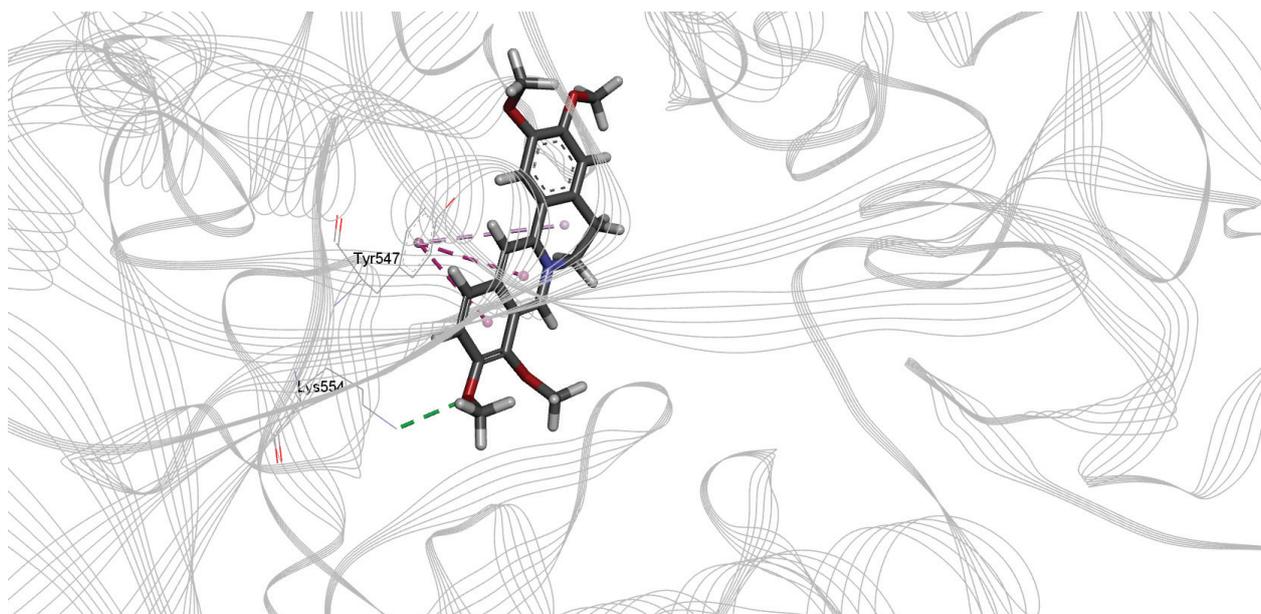


Figure 9. Docking interaction and binding mode of palmatine and DPP-IV enzyme. The hydrogen bonds are presented in green dotted lines, the π - π stacked interactions are presented in magenta dotted lines and the π -alkyl interactions are presented in pink dotted lines.

tionally, the phenyl ring of the ligand displays π - π stacked interactions with the aromatic residue PHE357 and the methyl group interacts with the aromatic residues TRP659, TYR662 and TYR666 (Figure 8). On the other hand, palmatine interacts with DPP-IV through one hydrogen bond that formed between the methoxy group of the ligand and the residue LYS554 where the ligand acted as hydrogen bond acceptor. Besides, three aromatic interactions were observed between the isoquinoline group and tetrahydronaphthalene group of the ligand and the residue TYR547 (Figure 9).

The binding interactions of sitagliptin (four hydrogen bonds and the aromatic interactions) contribute to the strength of binding and the high affinity of the ligand towards the DPP-IV receptor in comparison to palmatine which involved in only one hydrogen bond and three aromatic interactions with the same residue of the receptor.

References

- Ahmed F, Chandra J, Timmaiah N (2009) An In vitro study on the inhibitory activities of *Eugenia jambolana* seeds against carbohydrate hydrolyzing enzymes. *Journal of Young Pharmacists* 1(4): 1–317. <https://doi.org/10.4103/0975-1483.59320>
- Akuba B, Ozougwu V (2018) In vitro Inhibition of Carbohydrate Metabolizing Enzymes and in vivo Anti-hyperglycaemic Potential of Methanol Extract of *Desmodium velutinum* Leaves. *Research Journal of Medicinal Plants* 12(1): 48–56. <https://doi.org/10.3923/rjmp.2018.48.56>
- Beidokhti M, Lobbens E, Rasoavaivo P, Staerk D, Jäger A (2018) Investigation of medicinal plants from Madagascar against DPP-IV linked to type 2 diabetes. *South African Journal of Botany* 115: 113–119. <https://doi.org/10.1016/j.sajb.2018.01.018>
- Cho N, Shaw J, Karuranga S, Huang Y, da Rocha Fernandes J, Ohlrogge A, Malanda B (2018) IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice* 138: 271–281. <https://doi.org/10.1016/j.diabres.2018.02.023>
- González-Montoya M, Hernández-Ledesma B, Mora-Escobedo R, Martínez-Villaluenga C (2018) Bioactive Peptides from Germinated Soybean with Anti-Diabetic Potential by Inhibition of Dipeptidyl Peptidase-IV, α -Amylase, and α -Glucosidase Enzymes. *International journal of molecular sciences* 19(10): e2883. <https://doi.org/10.3390/ijms19102883>
- Kharroubi AT, Darwish HM (2015) Diabetes mellitus: The epidemic of the century. *World journal of diabetes* 6(6): 850–867. <https://doi.org/10.4239/wjd.v6.i6.850>

Conclusion

The results of the present study prove that palmatine is effective alpha-amylase, alpha-glucosidase and DPP-IV inhibitors, which may be helpful to reduce the postprandial glucose level. The binding interactions of palmatine with the alpha-amylase, alpha-glucosidase and DPP-IV identified through docking studies helped to shed light on the mechanism of its binding with these three antidiabetic targets. These findings can be extremely useful in designing new semisynthetic derivatives of palmatine as antidiabetic compounds.

Acknowledgement

The authors acknowledge UCSI University for providing the fund and facility for this research work.

- Kidane Y, Bokrezion T, Mebrahtu J, Mehari M, Gebreab Y, Fessehaye N, Achila O (2018) In Vitro Inhibition of α -Amylase and α -Glucosidase by Extracts from *Psiadia punctulata* and *Meriandra bengalensis*. Evidence-Based Complementary and Alternative Medicine 1–9. <https://doi.org/10.1155/2018/2164345>
- Kim B, Kim H, Choi I, Kim J, Jin C, Han A (2018) DPP-IV Inhibitory Potentials of Flavonol Glycosides Isolated from the Seeds of *Lens culinaris*: In Vitro and Molecular Docking Analyses. Molecules 23(8): e1998. <https://doi.org/10.3390/molecules23081998>
- Kortykowski M, McDonnell ME, Umpierrez GE, Zonszein J (2012) Patient Guide to Managing Hyperglycemia (High Blood Sugar) in the Hospital. The Journal of Clinical Endocrinology Metabolism 97(1): 27A–28A. <https://doi.org/10.1210/jcem.97.1.zeg27a>
- Lin SR, Chang CH, Tsai MJ, Cheng H, Chen JC, Leong MK, Weng CF (2019) The perceptions of natural compounds against dipeptidyl peptidase 4 in diabetes: from *in silico* to *in vivo*. Therapeutic advances in chronic disease 10: e2040622319875305. <https://doi.org/10.1177/2040622319875305>
- McDougall G, Shpiro F, Dobson P, Smith P, Blake A, Stewart D (2005) Different Polyphenolic Components of Soft Fruits Inhibit α -Amylase and α -Glucosidase. Journal of Agricultural and Food Chemistry 53(7): 2760–2766. <https://doi.org/10.1021/jf0489926>
- Meneses M, Silva B, Sousa M, Sá R, Oliveira P, Alves M (2015) Antidiabetic Drugs: Mechanisms of Action and Potential Outcomes on Cellular Metabolism. Current Pharmaceutical Design 21(25): 3606–3620. <https://doi.org/10.2174/1381612821666150710145753>
- Pandithurai M, Murugesan S, Bhuvanewari S, Thennarasan S (2015) In-vitro alpha-amylase and alpha glucosidase inhibition activity of methanolic extract of marine brown alga *Spatoglossum asperum*. Advances in Pharmaceutics 4(5).
- Rangel É, Rodrigues C, de Sá J (2019) Micro- and Macrovascular Complications in Diabetes Mellitus: Preclinical and Clinical Studies. Journal of Diabetes Research 2019(2161085): 1–5. <https://doi.org/10.1155/2019/2161085>
- Salehi M, Aulinger B, D'Alessio D (2012) Effect of Glycemia on Plasma Incretins and the Incretin Effect During Oral Glucose Tolerance Test. Diabetes 61(11): 2728–2733. <https://doi.org/10.2337/db11-1825>
- Somtimuang C, Olatunji O, Ovatlarnporn C (2018) Evaluation of in Vitro α -Amylase and α -Glucosidase Inhibitory Potentials of 14 Medicinal Plants Constituted in Thai Folk Antidiabetic Formularies. Chemistry & Biodiversity 15(4): e1800025. <https://doi.org/10.1002/cbdv.201800025>
- Tay Y, Bakar M, Azmi M, Saad N, Awang K, Litaudon M, Kassim M (2020) Inhibition of Carbohydrate Hydrolysing Enzymes, Antioxidant Activity and Polyphenolic Content of *Beilschmiedia* Species Extracts. IOP Conference Series: Materials Science and Engineering 716: 012007. <https://doi.org/10.1088/1757-899X/716/1/012007>
- Telagari M, Hullatti K (2015) In-vitro α -amylase and α -glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. Indian journal of pharmacology 47(4): 425–429. <https://doi.org/10.4103/0253-7613.161270>
- Thakare V, Shende S, Shirure P, Swami O (2017) Role of conventional oral antidiabetic drugs in management of type 2 diabetes mellitus. International Journal of Research in Medical Sciences 5(3): e749. <https://doi.org/10.18203/2320-6012.ijrms20170619>
- Unuofin J, Otunola G, Afolayan A (2018) In vitro α -amylase, α -glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of *Kedrostis africana* (L.) Cogn. Heliyon 4(9): e00810. <https://doi.org/10.1016/j.heliyon.2018.e00810>
- Yin Z, Zhang W, Feng F, Zhang Y, Kang W (2014) α -Glucosidase inhibitors isolated from medicinal plants. Food Science and Human Wellness 3(3–4): 136–174. <https://doi.org/10.1016/j.fshw.2014.11.003>