

MOLECULAR DOCKING AND DYNAMICS SIMULATIONS OF FENOLIC CONTENTS ON HENNA PLANT (*Lawsonia inermis* L.) AS ANTIDIBETIC THROUGH INHIBITION OF DIGESTIVE ENZYME α -AMYLASE

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ABSTRACT

Diabetes mellitus is a disease caused by high blood glucose levels, and one way to overcome it is by inhibiting the digestive enzyme, α -amylase. The henna plant (*Lawsonia inermis* L.) has been shown to show potential as an antidiabetic through inhibition of the enzyme digestive, but its molecular mechanism has not been revealed. Therefore, the aim of this study was to reveal the α -amylase enzyme inhibitory activity of phenolic compounds in the henna plant by molecular docking and molecular dynamics. The protein used was a high-resolution α -amylase crystallographic protein with the code PDB: 1XD0, and the ligands used were 20 phenolic compounds that were known to be contained in the henna plant. The phenolic compounds were docking, then a molecular dynamic simulation was carried out, and then compared with acarbose. Molecular docking analysis shows 3 compounds with the best binding energies, e.g. Acacetin-7-o-glucoside, laxanthone-2, and laxanthone-3. Furthermore, tracing the stability of the three compounds using a molecular dynamics simulation based on the parameters of SASA, Rg, PCA, and MM-PBSA binding free energy, obtained good stability and is close to acarbose as reference ligand.

Keywords: Henna Plant (*Lawsonia inermis* L.), Molecular Docking, Molecular Dynamics, Diabetes mellitus, Digestive Enzyme α -amylase

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INTRODUCTION

Diabetes mellitus is a serious long-term (chronic) condition characterized by elevated levels of glucose in the blood.¹ Diabetes mellitus is one of the top 10 causes of death in adults and is estimated to have caused four million deaths globally in 2017.² The prevalence of Diabetes Mellitus in 2019 is around 463.0 million people (9.3% for ages 20-79 years). It is estimated that in 2030 this incidence rate will increase to 578.4 million people (10.2% for ages 20-79 years) and in 2045 it will increase to 700.2 million people (10.9% for ages 20-79 years).^{3,4} Common effects of uncontrolled diabetes cause serious damage to the heart, eyes, kidneys and nerves.^{5,6}

The two most common types of diabetes mellitus are diabetes mellitus types 1 and 2. Type 1 diabetes occurs due to an autoimmune process, the pancreatic β cells are damaged so that they depend on insulin to survive. Type 2 diabetes is characterized by resistance to insulin and the insulin produced tends to be less.^{7,8} One way to control blood glucose levels is through inhibition of α -amylase enzyme.⁹ α -Amylase

hydrolyses α -1,4 glycosidic linkages in starch, which degrade complex carbohydrates into simpler ones, i.e. oligosaccharides and disaccharides which will eventually be converted into even simpler carbohydrates, namely monosaccharides.¹⁰ Therefore, inhibition of these enzymes can limit blood glucose levels by reducing the digestion of complex carbohydrates and their absorption, so they are useful in the management of therapy in type 2 diabetes mellitus.^{11,12}

Acarbose is a drug that has been used to control blood sugar through inhibition of digestive enzymes, especially it has good effectiveness against human pancreatic α -amylase enzyme.¹⁰ Eventually, several studies have shown that the use of antidiabetic drugs such as acarbose has side effects such as gastrointestinal disturbances (diarrhea and flatulence), liver disorders, dizziness, nausea and vomiting.¹³ It is desirable to have safer but more effective drug candidates, for example, drug candidates derived from natural ingredients. Various studies have been conducted on plants or natural compounds to treat diabetes.¹⁴⁻¹⁶ One of the plants that are widely used as traditional medicine is *Lawsonia inermis* L. The leaves, flowers, seeds, bark and roots of plants have the potential to cure headaches, arthritis, diarrhea, fever, diabetes, and various other pharmacological activities.¹⁷ *Lawsonia inermis* L. is a member of the family of Lythraceae which is commonly known as henna.^{18,19} This plant has the main content of active compounds such as flavonoids, phenols, alkaloids, glycosides, saponins, tannins, and essential oils. Phenols and flavonoids are the most active compounds found. The leaves of *Lawsonia inermis* L. reported contain flavonoids (luteolin, apigenin, and glycosides), coumarin (esculetin, fraxetin, scopletin), steroids (β -sitosterol), essential oils, 1,4-naphthoquinone carbohydrates, cardioglycosides, glucose, mannitol, proteins, gallic acid, resin (2-3%), phenolic compounds, tannins (5-10%), terpenoids, alkaloids, quinones (2-hydroxy-1,4-naphtho-quinone), xanthenes, and fatty acids.²⁰

The leaves extract of *Lawsonia inermis* L. plant extracts have been reported to be able to reduce blood glucose levels by *in vivo* assay, which are as effective as antidiabetic drugs on the market.¹⁹ In addition, The histopathological and biochemical results of the extract of *Lawsonia inermis* L., showed no pathological and hematological changes in the tested animals²¹. However, until now there has been no study showing the molecular activity of *Lawsonia inermis* L. on the antidiabetic receptors. Seeing its potential, we are interested in conducting molecular docking and molecular dynamics studies to find metabolites in *Lawsonia inermis* L., which have the potential for activity like acarbose, as inhibitor of a digestive enzyme α -amylase.

EXPERIMENTAL

Preparation of Metabolite Structure

The ligand structure preparation was carried out on 20 compounds contained from *Lawsonia inermis* leaves¹⁷ and a reference compound, acarbose, can be seen in Table-1. Acarbose and the compounds contained from *Lawsonia inermis* leaves were prepared in the form of a two-dimensional (2D) structure which was then made into a three-dimensional (3D) structure using the ChemDraw 8.0 program.

Macromolecule Structure Preparation

One macromolecule used in this study was the human pancreatic α -amylase receptor (PDB code: 1XD0) obtained from the PDB database on the site <https://www.rscb.org/pdb> which is an x-ray crystallographic protein with a high resolution of 2.00 Å. The protein is in complex with acarbose-derived pentasaccharide as inhibitor and reference compound in this study, and also the protein is in complex with various ligands and ions, which then all the ligands were removed. Then into the protein, polar hydrogen, water, and partial charge were added²². The procedures were performed using Discovery Studio 2017 and Autodock tools 1.5.4.²³

Molecular Docking Simulation

The molecular docking simulation was initiated by the validation method. The co-crystal ligand (acarbose) was re-docked into the binding pocket of α -amylase using the AutoDock program.²³ The best pose of the re-docked acarbose was taken and superimposed with the co-crystal acarbose. Then Root-Mean-Square Deviation (RMSD) was calculated. The RMSD value must be less than 2.0Å.²⁴ Furthermore, the docking process of the metabolites in henna plant was carried out based on a validated

method, on the crystal α -amylase structure based on the coordinates of acarbose, with Grid Center: $x = 40$, $y = 40$, $z = 40$ and the size of the Grid Box coordinates: $x = -8,939$, $y = 15,385$, $z = 39,807$.

Molecular Dynamics Simulation

Molecular dynamics (MD) simulation was carried out using the Gromacs 2016.3 software with the AMBER99SB-ILDN force field.²⁵ Topology and ligand parameters were made using ACPYPE.²⁶ The electrostatic force was set using the PME method.²⁷ The system was neutralized by carrying out the Na^+ and Cl^- ions into the complex system. And then the solvation of the system was set by the model of TIP3P water cube.²⁸ The minimization stride in the preparation stage includes the heating to 310 °K, temperature equilibration, pressure equilibration, and followed by a simulation process. Furthermore, 100 ns of MD production was performed with a 2 fs timestep. After the simulation, were calculated by *g_rms*, *g_rmsf*, and *g_rg* functions. Post-MD simulation analysis was done by calculating the RMSD, RMSF, radius of gyration (Rg), and binding free energy using MM-PBSA method, and then the SASA analysis and PCA analysis for the detection of the direction and amplitude of the dominant motions were analyzed.²⁸⁻³¹

RESULTS AND DISCUSSION

Molecular Docking Method Validation

The molecular docking simulation which was initiated by validation of the co-crystal ligand was successfully carried out. The superimposed picture of the co-crystal ligand before and after docking was represented in Fig.-1, the RMSD value is 1.8 Å.

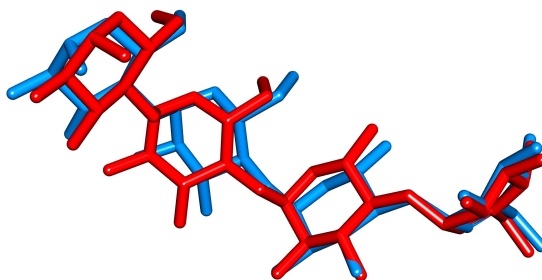


Fig.-1: Superimposed Structure of Co-crystal Ligands Before and After Docking

Molecular Interaction and Binding Energy

The binding energy and the interactions of the compounds in the henna plant at the α -amylase receptor are represented in Table-1. Based on the docking result of compounds contained in the henna plant, the best top-three compounds have the best affinity at the α -Amylase receptor, e.g. Acacetin-7'-O-glucoside of -8.63 kcal/mol, Laxanthone-2 of -7.8 kcal/mol, and Laxanthone-3 of -7.5 kcal/mol. These results indicate that the compounds contained in *Lawsonia inermis* have a binding energy value that is close to the acarbose value of -11.05 kcal/mol.

Acacetin-7'-O-glucoside forms 3 hydrogen bonds with HIS201, ILE235 and ASP197 residues, laxanthone-2 forms 6 hydrogen bonds with HIS305, ASP197, HIS299, ARG195 TYR151 and LYS200 residues, and laxanthone-3 forms 5 hydrogen bonds with ASP197, HIS299, ARG195 TYR151 and LYS200 residues. The three compounds showed hydrogen interaction with the same residue, e.g. ARG197. Meanwhile, the acarbose shows hydrogen bonds with more residue, e.g. THR163, LYS200, TYR151, HIS201, GLU233, ARG195, ASP300, HIS299, HIS305. Here it can be seen that laxanthone-2 and laxanthone-3 exhibit hydrogen bonds that are more similar to acarbose than acacetin-7'-O-glucoside. However, other types of interactions were detected, indicating that acacetin-7'-O-glucoside formed a binding with more residue, e.g. ARG195, TRP59, TYR62, ASP300, HIS299. In addition, the acacetin-7'-O-glucoside also shows a closer hydrogen bond distance than laxanthone-2 and laxanthone-3 in terms of its hydrogen bond. It is possible that this has an impact on the strength of the interactions of these compounds. In further, the stability of the three compounds was analyzed using molecular dynamics simulation.

Table-1: Binding Energy and Molecular Interaction of Metabolites in Henna Plant at α -amylase Receptor compared to Acarbose

No.	Compound	Binding energy (kcal/mol)	Hydrogen bond distance (Å)	Hydrogen bonds	The functional group(s) from compounds that contributed to hydrogen bond	The nearest amino acid residue(s)
1	Acacetin	-7.05	2.14, 2.10 and 1.13	GLU ²³³ , ASP ¹⁹⁷ and HIS ²⁹⁹	-OH and -CO	TYR ⁶² , ARG ¹⁹⁵ , ALA ¹⁹⁸ , TRP ⁵⁹ , ASP ³⁰⁰
2	Acacetin-7'-O-glucoside	-8.63	2.16, 1.79 and 1.87	HIS ²⁰¹ , ILE ²³⁵ and ASP ¹⁹⁷	-CO and -OH	ARG ¹⁹⁵ , TRP ⁵⁹ , TYR ⁶² , ASP ³⁰⁰ , HIS ²⁹⁹
3	Apigenin	-6.65	2.04, 2.15, 1.74 and 2.51	GLU ²³³ , ASP ¹⁹⁷ , ARG ¹⁹ and HIS ²⁹⁹	-OH and -CO	TYR ⁶² , ALA ¹⁹⁸ , TRP ⁵⁹ , ASP ³⁰⁰
4	Apigenin-4'-O-glucoside	-6.65	2.17, 2.84, 2.07, 2.67 and 2.84	THR ¹⁶³ , GLU ⁶³ , HIS ²⁹⁹ , ASP ³⁰⁰ and GLU ²³³	-OH and -CO	TYR ⁶² , ASP ³⁰⁰ , LEU ¹⁶⁵ , ALA ¹⁹⁸
5	Apiin	-5.94	3.06, 1.84, 1.72 and 2.12	GLY ³⁰⁶ , HIS ³⁰⁰ , HIS ²⁹⁹ , ARG ¹⁹⁵	-OH and -CO	TYR ¹⁵¹ , ASP ³⁰⁰ , LEU ¹⁶² , ALA ¹⁹⁸ , ILE ²³⁵ , HIS ²⁰¹
6	Esculetin	-5.94	1.89	GLU ²³³	-OH	TYR ¹⁵¹ , ALA ¹⁹⁸ , ILE ²³⁵ , HIS ²⁰¹ , LYS ²⁰⁰
7	Laloeside	-5.24	1.80, 1.93, 2.09 and 2.67	ASP ³⁰⁰ , ASP ¹⁹⁷ , ILE ²³⁵ , ILE ²³⁵ , GLU ²³³	-OH and -CO	HIS ²⁰¹ , LYS ²⁰⁰
8	Luteolin	-6.6	1.89, 2.11, 2.83, 1.98	GLU ²³³ , THR ¹⁶³ , ARG ¹⁹⁵ , HIS ²⁹⁹	-OH and -CO	ASP ¹⁹⁷ , ASP ³⁰⁰ , ALA ¹⁹⁸ , TYR ⁶² , TRP ⁵⁹ , LEU ¹⁶⁵
9	Luteolin-3-glucoside	-7.21	1.90, 2.15, 1.70, 2.61, 2.24, 2.34	ASP ¹⁹⁷ , HIS ²⁹⁹ , HIS ³⁰⁵ , ALA ¹⁹⁸ , ASP ³⁰⁰ , and GLU ²³³	-OH and -CO	ILE ²³⁵ , HIS ²⁰¹ , LEU ¹⁶²
10	scopoletin	-5.62	1.91	GLU ²³³	-OH	TYR ¹⁵¹ , ALA ¹⁹⁸ , ILE ²³⁵ , HIS ²⁰¹ , LYS ²⁰⁰
11	2-methoxy-3-methyl-1,4-Napthoquinone	-5.89	1.95	ILE ²³⁵	-OH	TYR ¹⁵¹ , ALA ¹⁹⁸ , GLU ²³³ , HIS ²⁰¹ , LYS ²⁰⁰
12	Dihydroxy-Glucosyloxy-Naphtalen	-5.63	2.05, 2.33, 2.01, 2.97, 2.10	ASP ¹⁹⁷ , HIS ²⁹⁹ , GLY ³⁰⁶ , ASP ³⁰⁰ , and GLU ²³³	-OH and -CO	TRP ⁸⁹ , LEU ¹⁶⁵
13	Gallic acid	-4.18	2.20, 2.10, 1.95, 2.05	ARG ¹⁹⁵ , TYR ⁶² , ASP ³⁰⁰ , and GLU ²³³	-OH	ARG ⁸⁵⁴ , TRP ⁷⁹⁶
14	Lacoumarin	-6.11	2.15, 2.09, 2.04, 1.91	ARG ¹⁹⁵ , HIS ³⁰⁵ , ASP ¹⁹⁷ , and GLY ³⁰⁶	-OH	ASP ¹⁹⁷ , LUE ⁷⁹⁷
15	Lawsonide	-5.91	2.23, 2.77, 2.33, 1.83	ARG ¹⁹⁵ , HIS ²⁹⁹ , ASP ¹⁹⁷ , and ASP ³⁰⁰	-OH	TYR ⁶²
16	Lawson	-5.73	1.97, 1.82	HIS ²⁹⁹ and ASP ¹⁹⁷	-CO and -OH	TYR ⁶² , HIS ¹⁰¹ , ARG ¹⁹⁵ , ASP ³⁰⁰
17	Laxanthone-1	-6.68	2.00, 1.98, 3.09, 2.14	HIS ²⁹⁹ , ASP ¹⁹⁷ , HIS ¹⁰¹ , and ASP ³⁰⁰	-CO and -OH	TYR ⁶² , TRP ⁸⁹ , THR ¹⁶³
18	Laxanthone-2	-7.8	2.86, 2.35, 2.30, 2.68, 2.28, 2.00	HIS ³⁰⁵ , ASP ¹⁹⁷ , HIS ²⁹⁹ , ARG ¹⁹⁵ , TYR ¹⁵¹ and LYS ²⁰⁰	-CO and -OH	ASP ³⁰⁰ , ALA ¹⁹⁸ , GLU ²³³ , LEU ¹⁶²
19	Laxanthone-3	-7.5	2.37, 2.21,	ASP ¹⁹⁷ , HIS ²⁹⁹ ,	-CO and -OH	ASP ³⁰⁰ , ALA ¹⁹⁸ , GLU ²³³ ,

			2.65, 2.26, 2.21	ARG ¹⁹⁵ TYR ¹⁵¹ and LYS ²⁰⁰		LEU ¹⁶²
20	P-Coumarin	-4.51	2.10, 2.64, 2.16	ASP ¹⁹⁷ , ILE ²³⁵ and LYS ²⁰⁰	-CO and -OH	ALA ¹⁹⁸ , GLU ²³³ , LEU ¹⁶²
21	Acarbose	-11	2.99, 1.85, 2.39, 3.03, 2.02, 3.07, 2.10, 3.20, 2.92, 2.75, 1.90, 3.20 2.75, 2.43 dan 2.71	THR ¹⁶³ LYS ²⁰⁰ TYR ¹⁵¹ HIS ²⁰¹ GLU ²³³ ARG ¹⁹⁵ ASP ³⁰⁰ HIS ²⁹⁹ HIS ³⁰⁵	-CO	HIS ¹⁰¹ , TRP ⁵⁹ , TYR ⁶²

Molecular Dynamics Simulation

Root-Mean-Square Deviation (RMSD) and Root-Mean-Square Fluctuation (RMSF)

Molecular dynamic simulations were successfully carried out through 100 ns simulation for acarbose and the three best compound, based on molecular docking study, in complex with human pancreatic α -amylase. The RMSD plot was analyzed through 100 ns simulation to identify the changes of the backbone of α -amylase in complex with acarbose as a reference, and the top-3 ranked of docked bioactive compounds, i.e. acacetin-7-O-Glucoside, laxanthone-2 and laxanthone-3 (Fig.-2a). Each complex reaches equilibrium at 5 ns, and is stable until the end of the simulation. From 0-25 ns simulations, the whole complex shows similar fluctuations. However, above 25 ns, the fluctuation of laxanthone-2 compounds is higher than others. Meanwhile, Above 60 ns, acacetin-7-O-Glucoside compounds also showed slightly higher fluctuations than acarbose and laxanthone-3. However, in general, all complexes showed good fluctuation stability and were close to the stability of acarbose at α -amylase. Meanwhile, the RMSF plots were revealed to analyzed the per-residue flexibility from the system trajectory (Fig.-2b). The RMSF analysis represented that the system complex showed an identical fluctuations and the fluctuations have shown in the same residues. The residues with the highest fluctuation were residue numbers 134, 142, 350, 364, and 434, which the residues responsible for the loop region of α -amylase protein. However, the difference in fluctuation is slightly different indicated by laxanthone-3-amylase in residue number 350. The laxanthone-3-amylase complex showed lower fluctuation compared to other ligands.

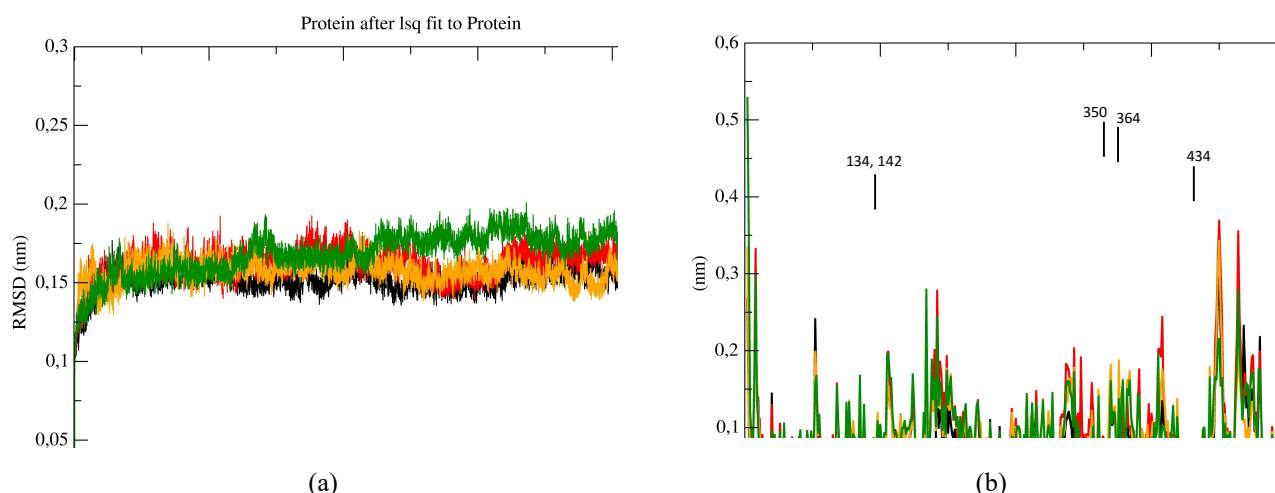


Fig.-2: The plots of RMSD (a) and RMSF (b) during 100 ns of MD simulations. The complexes were represented in different colors, i.e. acarbose-amylase (black), acacetin-7-O-Glucoside-amylase (red), laxanthone-2-amylase (yellow), and laxanthone-3 (green).

Radius of Gyration (Rg)

To measure the complex stability form, folded or unfolded conformation, the radius gyration (Rg) measurement was carried out from the trajectories during the 100 ns simulation. The Rg plots were presented in Fig.-3. The Rg plots of acarbose-amylase complex showed similar characteristics between the complex of acacetin-7-O-Glucoside-amylase, laxanthone-2-amylase, and laxanthone-3-amylase. The

Rg value average of acarbose-amylase, acacetin-7-O-Glucoside-amylase, laxanthone-2-amylase, and laxanthone-3 were 2.309 nm, 2.318 nm, 2.316 nm, and 2.314, respectively. The Rg values of the metabolite showed identical stability of folded structure compared to the acarbose-amylase as reference ligand. This Rg calculation, indicates that the three bioactive compounds increase the cohesiveness of the protein structure, thus increasing the overall stability.

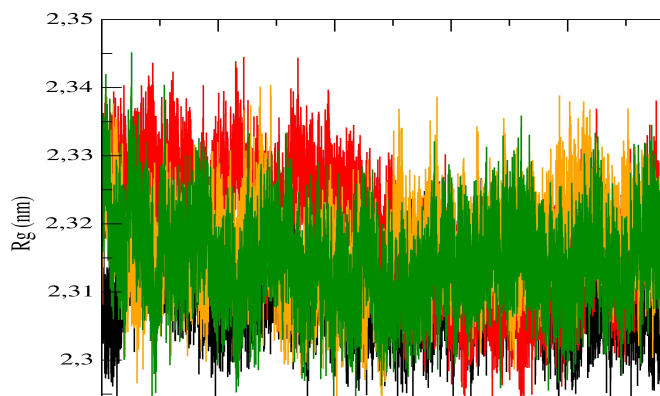


Fig.-3: Evaluation of Radius Gyration of the Acarbose-amylase (black), Acacetin-7-O-Glucoside-amylase (red), laxanthone-2-amylase (yellow), and laxanthone-3 (green) complexes.

Solvent Accessible Surface Area (SASA)

Solvent accessible surface area (SASA) was investigated to do the calculation of the extent of the protein surface, accessible by water molecules. The conformation changes during the simulation of SASA from the complexes were measured during 100 ns of simulation. The SASA plots showed high similarity between the acarbose-amylase and bioactive compounds-amylase complexes (Fig.-4). In addition, the average value of SASA measurement was carried out and compared between the ligands. The average value of SASA was found 191.66 nm² for acarbose-amylase complex, 190.92 nm² for acacetin-7-O-Glucoside-amylase complex, 192.66 nm² for laxanthone-2-amylase complex, and 190.38 nm² laxanthone-3-amylase complexes. The SASA average indicates that the three bioactive compounds have significantly similar compared to the reference (acarbose-amylase complex).

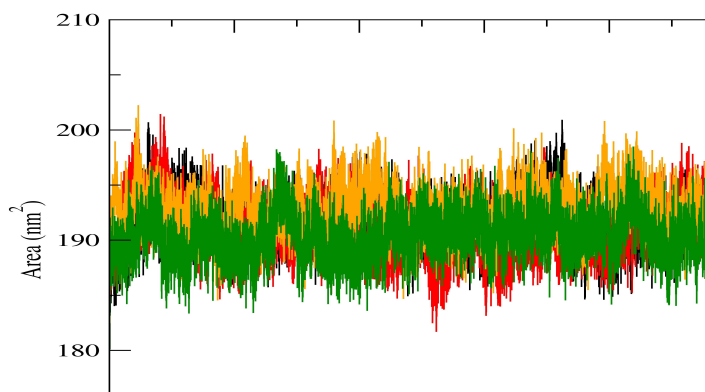


Fig.-4: Evaluation of SASA of the Acarbose-amylase (black), Acacetin-7-O-Glucoside-amylase (red), Laxanthone-2-amylase (yellow), and Laxanthone-3 (green) complexes.

Principal Component Analysis (PCA)

A small number of patterns that reveal the majority of fluctuations were analyzed using PCA, based on the eigenvectors that are responsible and indicate the complex motion during the simulation. PCA is analyzed by creating the porcupine plot which can be seen in Fig.-5a. Eigenvector analysis was performed in the first 10 ns of the simulation. The acarbose-amylase, acacetin-7-O-Glucoside-amylase, laxanthone-2-amylase, and laxanthone-3-amylase complexes eigenvector were 62.45%, 66.46%, 68.08%, and

61.70%, respectively. The acarbose-amylase and laxanthone-3-amylase showed high similarity in eigenvector, Although the acacetin-7-O-Glucoside-amylase and laxanthone-2-amylase showed the percentage was close, compared to the reference ligand. In addition, we also analyzed the dynamics of all the complex by 2D projection of trajectory plot generation that represented in Fig.-5b. Little space in the protein that can be occupied by the system cluster provides a stable complex, and more space occupied represents a less stable of the system complex. The 2D projection pattern showed the laxanthone-3-amylase complex showed a similar pattern compared to acarbose-amylase complex. The laxanthone-2-amylase complexes provided more space occupied than other ligands, and represent a different pattern. The acacetin-7-O-Glucoside-amylase complex showed a different pattern too, but showed less space occupied.

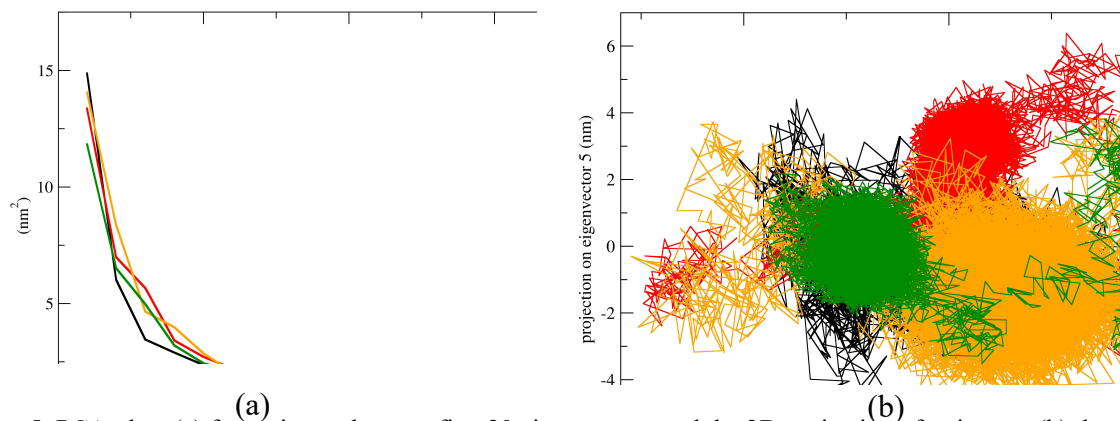


Fig.-5: PCA plots (a) from eigenvalues vs. first 20 eigenvectors and the 2D projection of trajectory (b) that describe the complexes motion in phase space. The complexes were represented in many colors, acarbose-amylase (black), acacetin-7-O-Glucoside-amylase (red), laxanthone-2-amylase (yellow), and laxanthone-3-amylase (green).

Binding Free Energy

The binding free energy calculated by MM-PBSA method was undertaken in post-simulation to calculate each energy contribution to the complex binding stability between ligands and α -amylase. The MM-PBSA binding free energy was calculated from 100 ns of molecular dynamics simulation. The MM-PBSA calculation found the three bioactive compounds, i.e. acacetin-7-O-Glucoside, laxanthone-2, and laxanthone-3 showed good binding free energy. The total energy of the ligand-amylase complexes was -113.083 KJ/mol for acarbose-amylase, -82.207 KJ/mol for acacetin-7-O-Glucoside-amylase, -73.121 KJ/mol for laxanthone-2-amylase, and -81.127 KJ/mol for laxanthone-3-amylase (Table-2). From the three bioactive compounds, acacetin-7-O-Glucoside had a stronger predicted binding affinity, although not as high as acarbose binding free energy. However, the three ligands indicate binding free energy close to acarbose as reference.

Table-2: MM-PBSA Energy Calculation of the Three Bioactive Compounds and Acarbose.

Molecule	Van der Waals energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	S.A.S.A. energy (kJ/mol)	Bond prediction energy (kJ/mol)
Acarbose	-313.077 +/- 20.864	-145.646 +/- 37.847	376.443 +/- 27.584	-30.803 +/- 0.974	-113.083 +/- 20.442
Acacetin-7-O-Glucoside	-172.370 +/- 20.814	-35.306 +/- 30.360	144.805 +/- 46.139	-19.336 +/- 1.680	-82.207 +/- 26.536
Laxanthone-2	-128.237 +/- 28.885	-22.562 +/- 15.355	92.410 +/- 44.630	-14.733 +/- 2.704	-73.121 +/- 23.777
Laxanthone-3	-125.413 +/- 15.421 kJ/mol	-11.634 +/- 9.846 kJ/mol	69.639 +/- 19.085 kJ/mol	-13.719 +/- 1.406 kJ/mol	-81.127 +/- 16.372 kJ/mol

CONCLUSION

By using molecular docking and molecular dynamics simulations, we identified three potential bioactive compounds contained in Henna plant (*Lawsonia inermis* L.) against α -amylase. The three bioactive compounds showed good affinity and stability through the simulation. The lead compounds provide by simulation, can be the basis for further effectiveness analysis, leading to discovering and designing new drugs against diabetes mellitus. Our study provides an impetus for future detailed efficacy analysis research in discovering the activity of oral antidiabetics with a specific mechanism to inhibit α -amylase.

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[RJC-6654/2021]