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Antioxidant, Antidiabetic and Protective Properties of *Lawsonia Inermis* Linn. Extracts on Sodium Nitroprusside-Induced Oxidative Damage in Isolated Pancreas: *in vitro, ex-vivo* and *in silico* studies

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ABSTRACT

Lawsonia inermis is a prominent medicinal herb used for treatment of diabetes mellitus. The study aimed at evaluating the antioxidant, antidiabetic and protective properties of different solvent extracts of *Lawsonia inermis* on sodium nitroprusside (SNP) - induced oxidative damage on isolated pancreas. The results showed that the all the extracts possess antioxidant, antidiabetic and protective property against SNP –induced oxidative damage in pancreas. Acetone extract has the highest activities, followed by methanol extract and aqueous extract. The HPLC (DAD) analysis revealed several bioactive compounds from the different solvent extracts of *Lawsonia inermis.* Docking simulation of the phytochemicals detected in these extracts with human pancreatic α-amylase (HPA) and human α-glucosidase (HG) showed good binding affinity and amino acid interactions with the active sites of enzymes. Given the roles of key residues that define the active site of HPA (ASP197, GLU233 and ASP300), the interactions of Lawsonia compounds with these amino acids may hamper the action of ASP197 as a nucleophile, GLU233 which participate in the acid–base catalysis and that of ASP300 which helps in the proper orientation of the polymeric substrate. Assessment of the stability of the ligand-protein complexes through a 50-ns full atomistic MD simulation alongside the unbound enzyme revealed the stability of the complexes as indicated by the RMSD, RMSF, RoG, SASA and number of hydrogen bonds. This study revealed the antioxidant and antidiabetic activity of solvent extracts of *Lawsonia inermis* and provided insight into the mechanism of action *insilico*.

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Introduction

Oxidative stress has been implicated in the pathogenesis of acute pancreatitis, a severe and incapacitating inflammation of the pancreas that resulted to loss of function of pancreas and significant mortality (Lourdes *et al*., 2021). Oxidative stress is triggered by a combination of amplified formation of reactive oxygen species (ROS) and reduced antioxidant functions. (Grzybowski 2016). Excessive oxidative stress in pancreas resulted into its loss of function and subsequently development of diabetes mellitus (Karigidi and Olaiya 2019). Pancreatic β cells are particularly prone to oxidative stress due to their high endogenous production of reactive oxygen species (ROS) and their low antioxidant capacity (Gurgul-Convey *et al*., 2016).

Recently, people have focused on use of medicinal plants because they are readily available, have safety concern and capable of biological activity in mammals (Karigidi et al., 2022; Pizzino *et al*., 2017). The pharmacological activities of medicinal plants are due to presence of many phytochemicals such as polyphenols, alkaloids, flavonoids, tannins, terpenoids (Habbal *et al*., 2011; Misganaw 2022). An example of well used medicinal plants is *Lawsonia inermis Linn*. (Fapetu et al 2023).

Lawsonia inermis Linn. This plant has long been used as an herbal treatment (Salih *et al*., 2017). In addition, analysis conducted among the diabetes patients in Medan, North Sumatera, Indonesia, showed that its leaves is used as an alternative to regulate the blood glucose level (Widyawati *et al*., 2015).

In silico modelling techniques are veritable tools for providing mechanistic insights into the biological activity of food herbs, spices and medicinal plants. They are also valuable for drug discovery and development as they are employed for screening interactions of potential drugs with specific targets. They are particularly useful for providing insightful information on the binding characteristics of such bioactive compounds in atomistic details, which are often difficult to obtain in experimental procedures. Such tools have revealed that, plant-derived compounds, which are characterized by large-scale structures, possess high efficiency and ability to bind to carbohydrate digesting enzymes (Dandekar et al., 2021, Ogunyemi et al., 2022a). Thus, this study is aimed at investigating the antioxidant, antidiabetic, and protective effects of acetone, aqueous and methanol extracts of Lawsonia *inermis* on sodium nitroprusside induced oxidative damage on isolated pancreas of Wistar rats.

Materials and Methods

Plant Materials

Lawsonia inermis leaves used in this study were plucked from a farm in Okitipupa (6° 33'N 4°43'E) local government area of Ondo state, Nigeria. They were identified and authenticated in the Biological sciences Department, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State. The Voucher name (OAUSTECH/H/0679) was deposited there.

Preparation of extract

The leaves were washed, sorted and dried at room temperature 4 weeks; thereafter they were milled to powdery form. Fifty (50 g) grams of the powdered samples were soaked separately in 500 mL of acetone, methanol and distilled water for 48 hours, filtered and concentrated with rotary evaporator.

In vitro antioxidant capacity and enzyme inhibition analyses

Total phenolics of the sample were determined according to the method of Kim et al., (2003) as modified by Karigidi et al (2018). Total flavonoids of the sample were evaluated with the method of Park et al., (2008). Total antioxidant capacity of the sample was determined with the Prieto et al., (1999) method. DPPH radical scavenging activity of the sample was determined with the Gyamfi et al., (1999). Ferric reducing antioxidant potential of the sample was determined with the procedure of Benzie and strain (1996). Reducing power of the samples was assayed using Oyaizu (1986) method. Amylase inhibition of the samples was evaluated as described by Worthington (1993) while glucosidase inhibition was evaluated as described by Apostolidis et al., (2007).

Experimental animals

Four healthy male Wistar rats (120-140) g were purchased from the Physiology Department, University of Ibadan. The animals were acclimatized for 14 days given pellet diet and water *ad libitum*. The procedure for the experiment was done as highlighted in the guidelines of National Institute of Health on the handling and use of laboratory animals (NIH Publication No. 80-23) revised in 2011. This protocol was approved by the Research Ethics Committee of Olusegun Agagu University of Science and Technology (OAUSTECH/ETHC-BCH/2022/005).

Preparation of homogenate

The pancreas homogenate was prepared using the method by Karigidi and Olaiya (2019). The experimental rats were anesthetized and decapitated using sodium pentobarbitone. The pancreas tissue was removed and weighed on ice. The tissue was homogenized with cold normal saline (1:4 w/v) on ice. The homogenate was centrifuged at 3,000 rpm for 10 min and supernatant was used for assays.

Induction of oxidative stress (ex-vivo)

Oxidative stress was induced as described by Prigol et al (2009). 100 µL of the supernatant was added to 30 µL of 0.1 M Tris-HCl buffer, 100 µL extract and 30 µL of freshly prepared Sodium nitroprusside (10 mM). The reaction mixture was incubated for 120 mins at 37 °C.

Determination of oxidative stress markers

Lipid peroxidation was evaluated by the method of Ohkawa et al (1973). Nitric oxide was determined by the procedure of Green et al (1982) and glutathione content was evaluated with procedure of Jollow et al (1974).

Phytochemical profiling of the extracts using HPLC-DAD

The phytochemical profiling was done using the method as described by Karigidi et al (2022).

Molecular docking analysis of *in silico* analysis inhibitory potentials of phytochemicals

The crystal structures of human pancreatic amylase (PDBID: 4GQR) and human glucosidase (PDBID: 5NN8) were downloaded from the Protein Data Bank website: http://www.rcsb.org. The native ligands and molecules of water associated with the protein structures were removed, while missing hydrogen atoms were included using MGL-AutoDockTools (ADT, v1.5.6) The Kollman charges were included as the partial atomic charges(Morris et al., 2009). This procedure was applied to the two target enzymes and then saved as the dockable protein databank extension pdbqt for docking simulations.

The 3D structures of the Lawsonia compounds,

the reference inhibitors acarbose and the co-crystallized compound myricetin were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov) in structure file format (SDF). The chemical structures were then converted to mol2 format through Open babel (O'Boyle et al., 2011). Then polar hydrogen charges of the Gasteiger-type were assigned to atoms, while the non-polar hydrogen molecules were merged with the carbon atoms. The ligands were then converted to the PDBQT format using AutoDock Tools for molecular docking simulation and molecular dynamics simulations.

The bioactive compounds alongside reference inhibitor and co-crystalized compound were docked into the active region the enzymes using AutoDock Vina docking tool in PyRx 0.8 (Trott and Olson, 2010). The structures of the bioactive compounds were imported and energy minimization was carried out using OpenBabel (Trott and Olson, 2010) in PyRx 0.8 applying the Universal Force Field (UFF) as the energy minimization parameter, and the conjugate gradient descent as the optimization algorithm. The parameters of the regions enclosing the active sites of HPA were defined by a grid box size of 18.90 × 10.24 × 54.04 Å centered at (x, y, z) of (20.59, 19.94, 21.58) Å, . Those of HG were defined by a grid box size of 18.90 × 10.24 × 54.04 Å centered at (x, y, z) of (20.59, 19.94, 21.58) Å. The 3D structures of the enzymes were imported into the docking tool and the docking simulation was performed with all other parameters kept as default. The docked complexes were obtained and then visualized using the Discovery Studio Visualizer version 16 in order to detect and document the molecular interactions.

Molecular Dynamics Simulation of top complexes

The unliganded HPA (PDBID: 4GQR), and the top HPA-ligand complexes were subjected to a full atomistic 50 ns Molecular Dynamic (MD) simulation using GROMACS 2019.2 and GROMOS96 43a1 forcefield on the WebGRO (Oostenbrink et al., 2004, Abraham et al., 2015) as performed earlier (Olawale et al., 2023). The required topology files of the ligand molecules were generated using PRODRG webserver (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg) (Schüttelkopf and van Aalten, 2004). The solvation modeling of the apoHPA and HPA-ligand complexes within a cubic box of the transferable intermolecular potential was carried out with a four-point (TIP4P) water model, using the periodic boundary conditions at a physiological concentration of 0.154 M set by neutralized NaCl ions. The biomolecular systems were minimized for 10000 steps through steepest descent algorithm in constant number of atoms, volume, and temperature ensemble (NVT) ensemble for 0.3 nanosecond followed by 0.3 nanosecond of equilibration in constant atom number, constant pressure and constant temperature (NPT). The constant temperature was maintained using 310 K using velocity rescale, and the constant pressure set to 1 atm using Parrinello-Rahmanbarostat. The Leap-frog integrator was employed with a time step of 2 femtosecond. For the 50 ns production run performed for each system, 0.1 ns a snapshot was saved with a total of 1000 frame from each system. The thermodynamic parameters such as RMSD, RMSF, SASA, RoG, and number of H-bond were computed from the trajectory files using VMD TK console scripts (Humphrey et al., 1996).

Statistical analysis

Results were presented as the mean ± SD of triplicate reading, analyzed by ANOVA, and the means were separated by least significant ($p < 0.05$) difference.

Result

Antioxidant and antidiabetic activities

The antioxidant activity of different solvent extracts of *Lawsonia inermis* are presented in Table 1 and Figure 1.

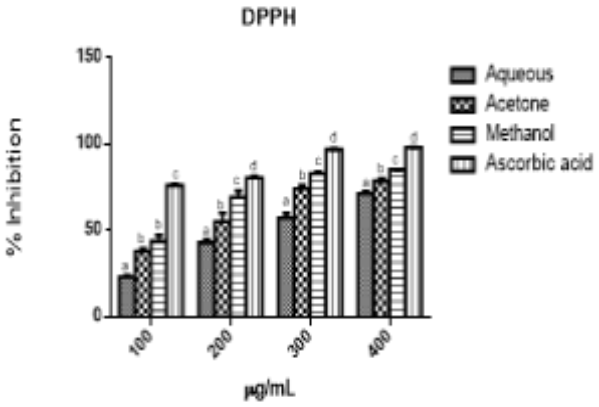

Figure 1: Effect of different solvent extracts of *Lawsonia inermis* on DPPH scavenging property.

Table 1: Antioxidant activity of different solvent extracts of *Lawsonia inermis*

	Aqueous	Acetone	Methanol
Phenolics (mg GAE/100g	16.22±0.38[a]	20.85±1.33[b]	18.14±1.08[a]
Flavonoids (mg QUE/100g)	27.16±0.09[a]	41.42±2.11[b]	28.36±0.70[a]
TAC (mg AAE/100g)	5.75±0.12[a]	13.31±0.64[c]	7.17±0.39[b]
FRAP (mg Fe^{2+}/100g)	15.25±0.29[a]	19.43±0.18[b]	15.01±0.12[a]
RP (mg AAE/100g)	25.71±2.06[a]	31.67±2.46[b]	26.47±0.64[a]

Mean ± Standard deviation. Values with same alphabet across the row are not significantly different ($p < 0.05$).

In the antioxidant assays, acetone extract exhibited significant ($p < 0.05$) higher activity when compared with aqueous and methanol extracts. The ability of the extracts to inhibit amylase and glucosidase was shown in Figure 2 and their IC_{50} is presented in Table 2.

Table 2: The IC_{50} values of the extracts of *Lawsonia inermis* against DPPH, **α**-amylase and **α**-glucosidase enzymes

	DPPH	**α**-amylase	**α**-glucosidase
Aqueous	165.84 ±1.23[a]	148.64±3.65a	180.05±2.08a
Acetone	151.6± 2.10[b]	140.30±2.15b	153.6±3.25b
Methanol	218.56± 1.90[c]	190.50±2.01c	145.80±3.10c
Ascorbic acid (µg/mL)	22.40±0.23*	-	-
Acarbose (µg/mL)	-	64.93±0.88*	45.33±0.65*

Mean ± Standard deviation. Values with same alphabet across the column are not significantly different ($p < 0.05$).

The total phenolics and flavonoids were presented in Table 1 while the phytochemical constituent was presented in Table 3.

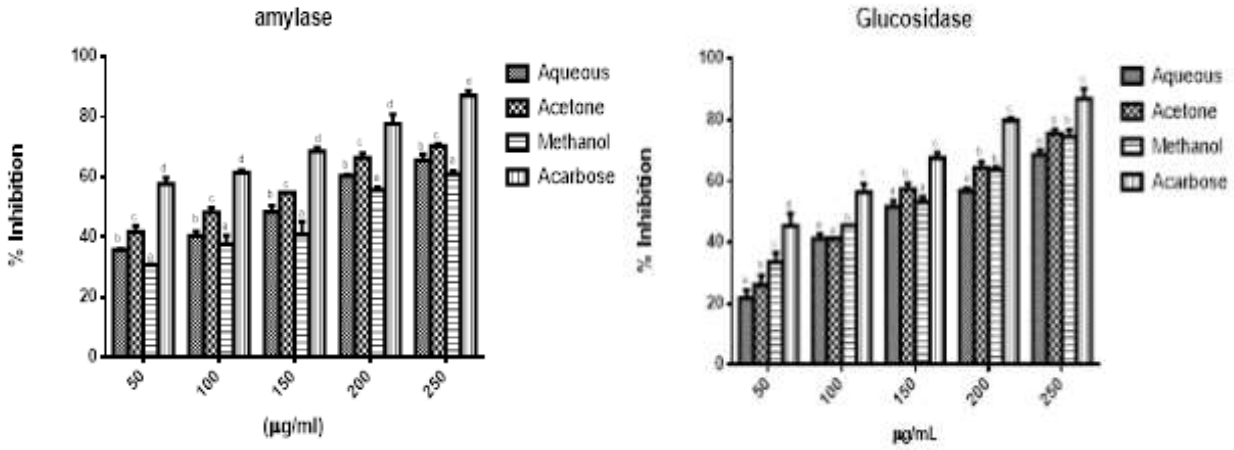

Figure 2: Effect of different solvent extracts of *Lawsonia inermis* on amylase and glucosidase inhibition

Table 3: Phytochemicals identified in different solvent extracts of *Lawsonia inermis*

	Aqueous (µg/mL)	Acetone (µg/mL)	Methanol (µg/mL)
p-Coumaric acid	74.81	88.80	84.04
Betulin	164.98	202.30	191.49
Betulinic acid	40.71	62.29	62.62
Gallic acid	12.53	28.27	30.63
Tannic acid	6.03	19.60	22.99
Alpha-lonone	5.82	8.08	5.82
β-sitosterol	5.06	6.34	5.06
Stigmasterol	5.63	7.15	7.15
Lawsone	656.41	716.12	687.92
Lawsoniaside	247.65	274.93	272.89
Luteolin	154.26	248.13	237.26
Apigenin	5.49	9.53	7.56
Lupeol	15.45	48.19	38.22
Esculetin	5.70	7.21	5.10
Lallioside	5.30	7.61	5.36
Acacetin	5.20	8.72	5.39
Apiin	-	6.73	8.67

Mitigation of induced oxidative stress

The effect of the extracts of *Lawsonia inermis* on SNP-induced oxidative stress in pancreas is presented in Figure 3. The levels of malondialdehyde (MDA) and nitric oxide (NO) were significant reduced upon incubation with the extracts. However, acetone extract exhibited the highest reduction at 400µg/mL in level of MDA while aqueous extract at 400µg/mL exhibited the highest reduction in NO. The level of glutathione was also presented, incubated pancreas treated with acetone extract possess relatively higher concentration of glutathione

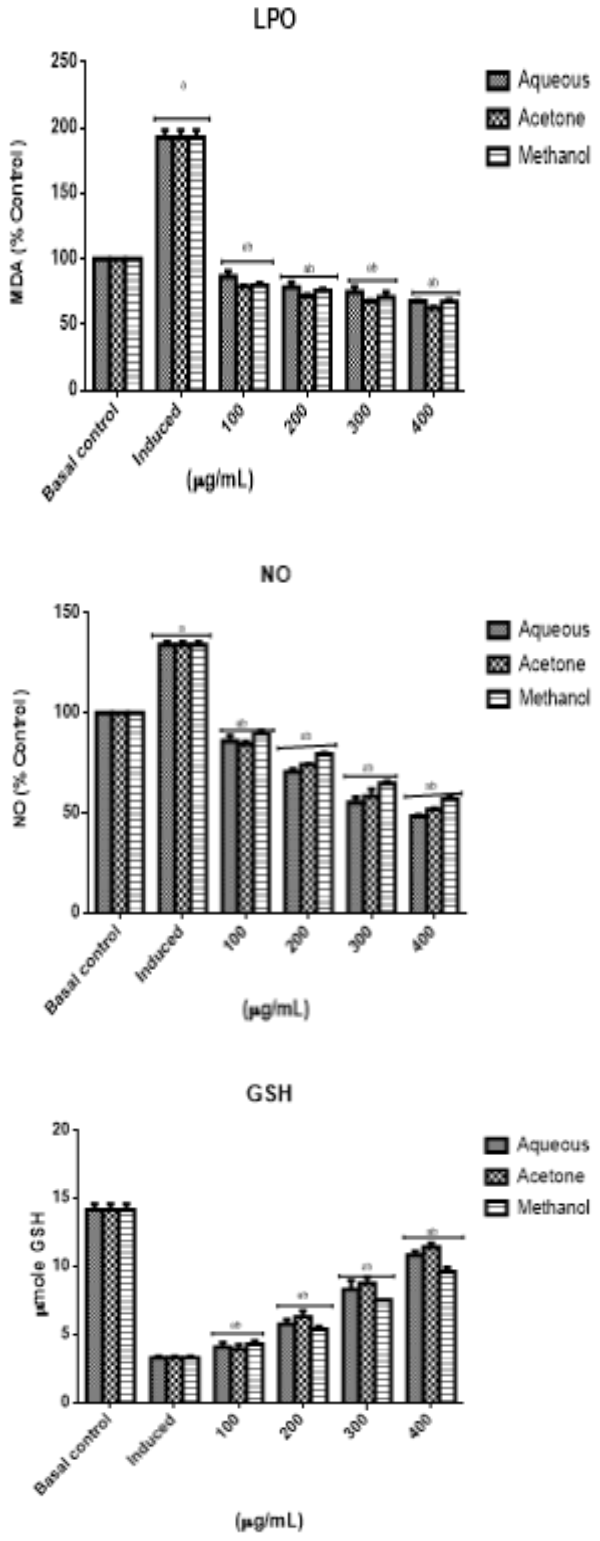

Figure 3: Effect of different solvent extracts of *Lawsonia inermis* on LPO, NO and GSH of SNP-treated pancreas

Molecular docking simulations of bioactive compounds against human pancreatic **α**-amylase and human **α**-glucosidase

Docking of the bioactive compounds with the target enzymes employing the scoring function in AutoDock Vina provided estimate of the binding energies ΔG (Gibbs energy) and interactions of the compounds with the enzymes as shown in the Table 4.

Table 4: Binding affinity of bioactive compounds with human pancreatic **α**-amylase and human **α**-glucosidase

Compounds	Docking score (Kcal/mol)	
	Amylase	Glucosidase
Acarbose	-6.9	-5.9
Myricetin	-7.9	-7.2
Lupeol	-9.1	-7.7
Betulinic Acid	-8.9	-6.7
Stigmasterol	-8.8	-8.1
B-Sitosterol	-8.8	-7.8
Betulin	-8.8	-6.8
Luteolin	-8.4	-7.0
Apigenin	-8.2	-6.8
Apiin	-7.8	-7.3
Lawsoniaside	-7.7	-6.7
Acacetin	-7.5	-6.8
Lalioside	-6.7	-6.1
Esculetin	-6.6	-6.0
P-Coumaric Acid	-6.1	-5.7
Alpha-Ionone	-6.1	-5.7
Lawsone	-6.0	-5.6
Gallic Acid	-5.4	-5.5

The result revealed that, acarbose, the reference inhibitors, had binding affinity -6.9 Kcal/mol for the active region of α-amylase. The co-crystalised myricetin had binding affinity -7.9 Kcal/mol for the active region of α-amylase. Using the reference compounds and ranking

based on negative and low value of ΔG, Lupeol is the top most docking compound with -9.1 Kcal/mol, which was followed by betulinic acid (-8.9 Kcal/mol). acarbose, had binding affinity -5.9 Kcal/mol for the active region of HG. Stigmasterol and beta-sitosterol had the highest binding affinity with HG.

The docked complexes were screened for favourable interactions with the active sites of HPA defined by the catalytic residues ASP197, GLU233, and ASP300 and that of HG defined by the catalytic residues ASP 518 and ASP 616. Also, Figure S1 shows that Luteolin, luteolin, apigenin, apiin, lawsoniaside has the best interactions with HPA. Figure S2 shows that, apiin, luteolin, apigenin, acacetin, lawsoniaside had best interactions with HG. The amino acid interactions of the target enzymes are documented in Table 5.

Luteolin, was found to utilize multiple interactions with the binding and catalytic residues of the enzyme. It interacted via hydrogen bonds with GLU233, Arg195 and Gln63, Electrostatic interactions with Asp300 and hydrophobic interactions with Trp59 and Tyr62. Apigenin, and apiin also conducted strong hydrogen bond with Asp197. The ligand- HPA complexes involve multiple interactions such as hydrogen bonds, and hydrophobic interactions, depending on the amino acid composition of binding sites and chemical properties of the compounds. In the case of apiin as shown in Table 5 and Figure 4, the compound conducted hydrogen bonds with Ala284, Phe525, Asp518, Leu283, Ser676 and Asp616. In addition, apigenin and lawsoniaside made hydrogen bonds with Asp518.

Molecular Dynamics Simulations of top enzyme-inhibitor complexes

The 50 ns atomistic MD simulation analysis revealed the stability of the enzyme-inhibitor complexes as compared stability and structural conformation of the unbound HPA (apo enzyme) in a dynamic environment as indicated through the thermodynamic parameters computed and plotted from the MDS trajectory files. The root mean square deviation (RMSD) of the backbone C-α atoms of all the HPA systems showed a stable trend after equilibration as depicted in Figure 5.

The plot shows that, all the biomolecular systems attained stability around 20 ns which was maintained throughout the simulation period. The apigenin-enzyme complex showed the greatest stability as compared with the apoenzyme, acarbose-enzyme and apiin-enzyme complexes. The RMSD of the backbone C-α atoms of the apo-HPA showed an increasing trend in the first 19 ns before stabilizing at around an average value of 2.71 Å. The HPA-apigenin system showed the rising value of RMSD until 11.5 ns at which stability around 2.4 Å was attained. The HPA-apiin complex showed stability beyond 20.8 ns, averaging at 2.7 Å.

The RMSF in Angstrom (Å) was plotted for the apo-HPA and in complex with the isolated steroidal pregnanes

The plot shows that, amino acid residues around key regions of the active site of the enzyme showed flexibility and interactions potential towards binding the bioactive compounds.

The surface accessible surface area (SASA) plot indicates the level of the solvent accessibility surface of the protein is shown in Figure 7. Stability of the apoprotein and the ligand-protein complexes.

Table 5: amino acid interactions of compounds with human pancreatic **α**-amylase and human **α**-glucosidase

Compounds	Enzyme	Hydrogen bonds interactions		Electrostatic interactions		Hydrophobic interactions	
		Number	Residues	Number	Residues	Number	Residues
Acarbose	HPA	7	Arg195, Asp300 (2), Glu233, Gln63, His 305, Asp197	0	Nil	0	Nil
Myricetin		2	Arg195, Gln63	1	Asp300	4	Trp59 (2), Trp58, Tyr62
Luteolin		3	Glu233, Arg 195, Gln63	1	Asp300	4	Trp59 (3), Tyr62
Apigenin		2	Asp197, Gln63	1	Asp300	4	Trp59 (3), Tyr62
Apiin		6	Asp197, Glu233, His305 (2), Asp300, Trp59	1	Asp300	3	Trp59 (3), Leu165
Lawsonia-side		5	Tyr 151, His 201 (2), Gln63, Trp59	2	Asp197, Asp300	2	Tyr62, Leu162
Acarbose	HG	7	Arg600(2), Asp282 (2), Asp 518, Asp616	0	Nil	2	Phe525, Trp481
Apiin		6	Ala284, Phe525, Asp518, Leu283, Ser676, Asp616	2	Asp282(2)	1	Ala555
Luteolin		1	Ser523	1	Asp616	3	Trp481 (2), Phe525
Apigenin		4	Arg600, His674, Asp404, Asp518	2	Asp616(2)	4	Trp481(3), Phe649
Acacetin		2	Ala484, Asp404	3	Asp282 (2), Asp616	5	Trp481, Trp376, Trp516, Phe649, His674
Lawsonia-side		7	Asp404(2), Asp282, Asp523 (2), Asp518(2)	0	Nil	3	Trp481(3)

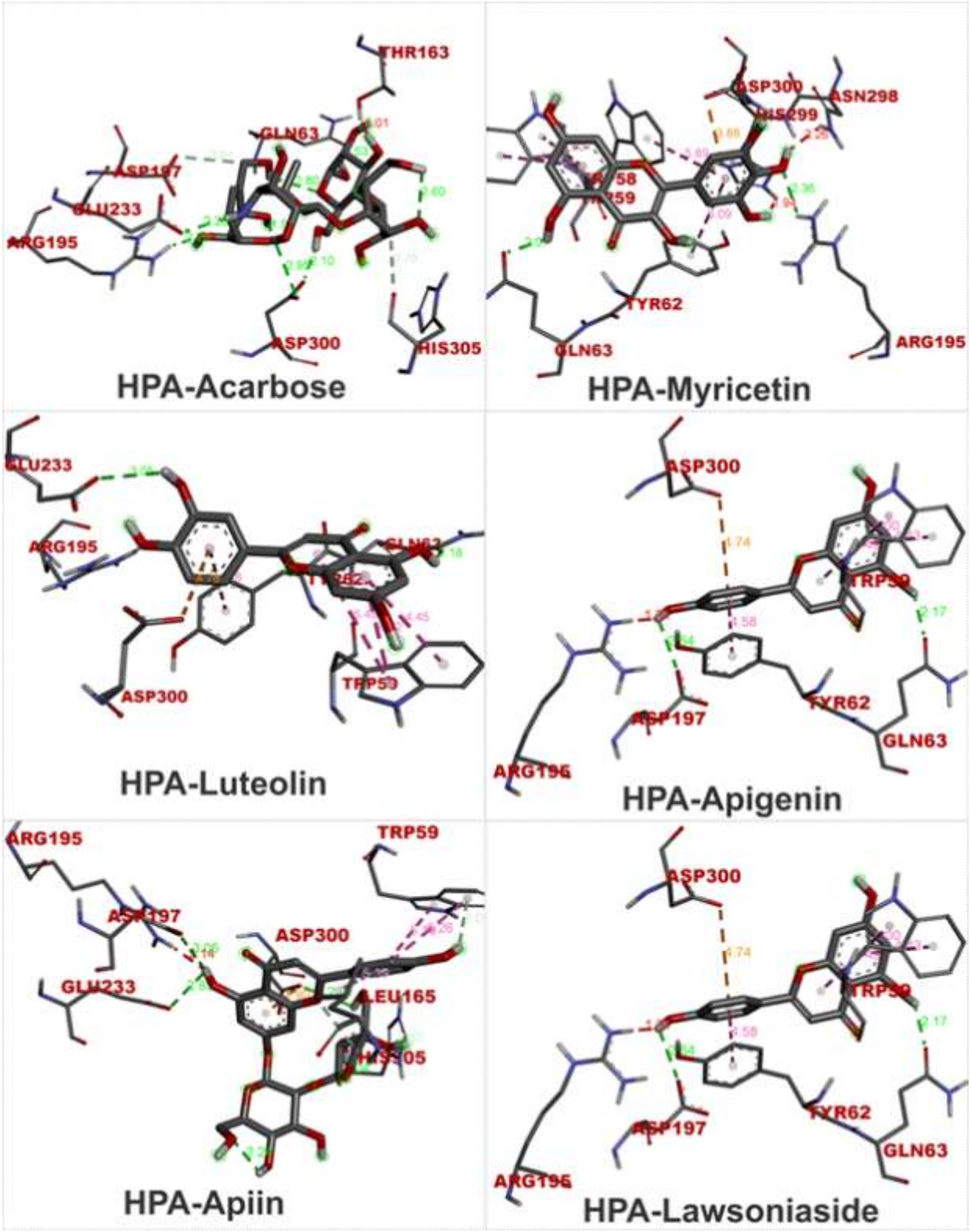

Figure 4: Interactions of bioactive compounds with human pancreatic **α**-amylase

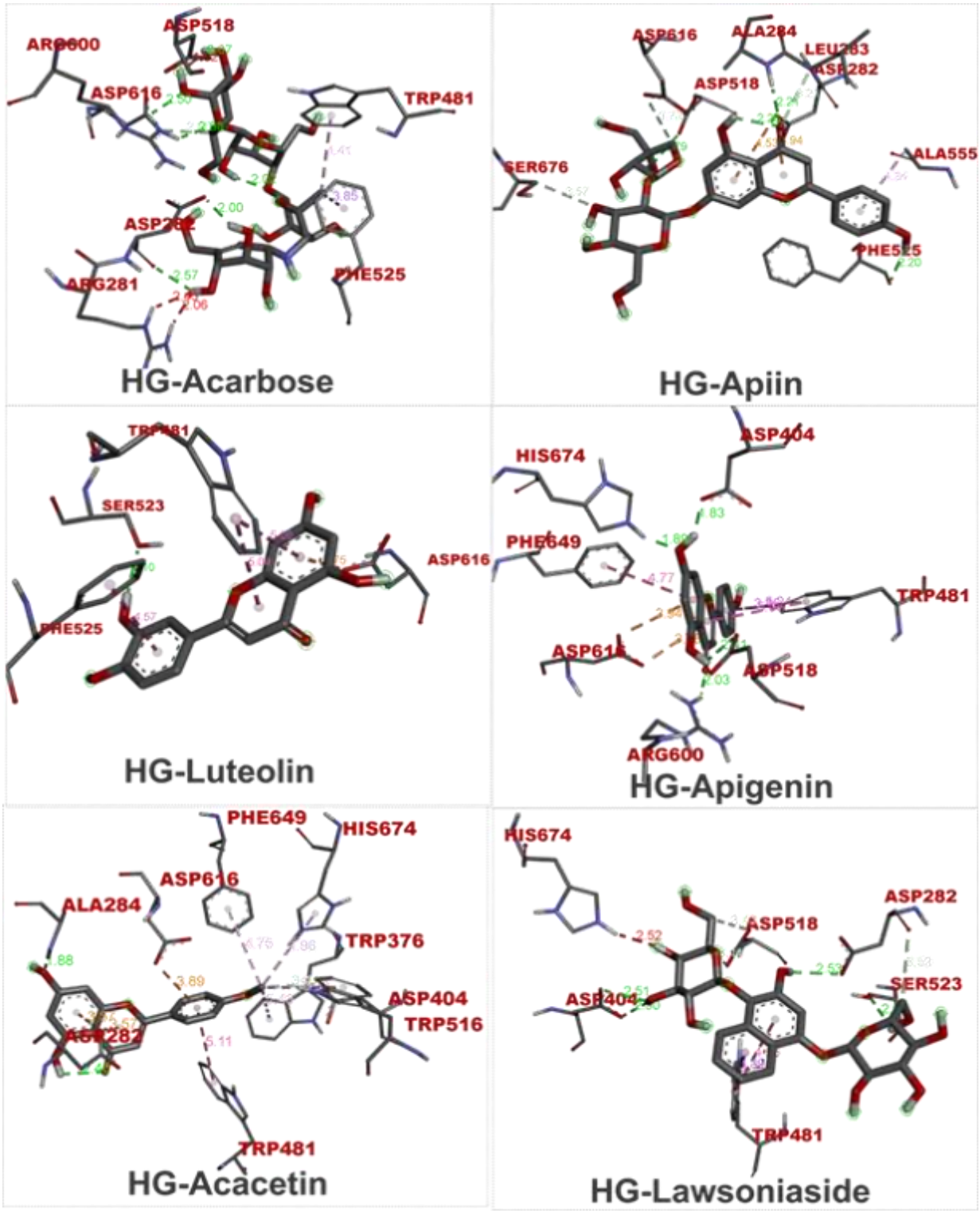

Figure 5: Interactions of bioactive compounds with human **α**-glucosidase

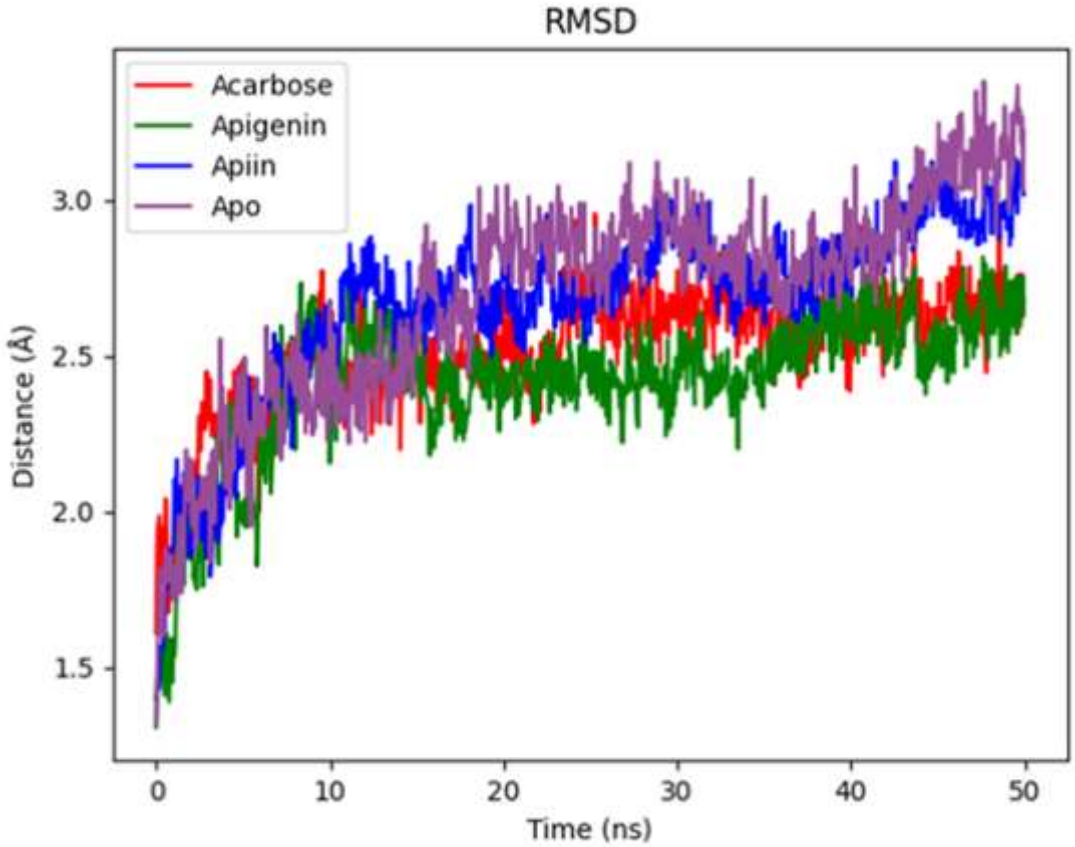

Figure 6: The Backbone-Root Mean Square Deviation (RMSD) plot of the apo enzyme and ligand-protein complexes

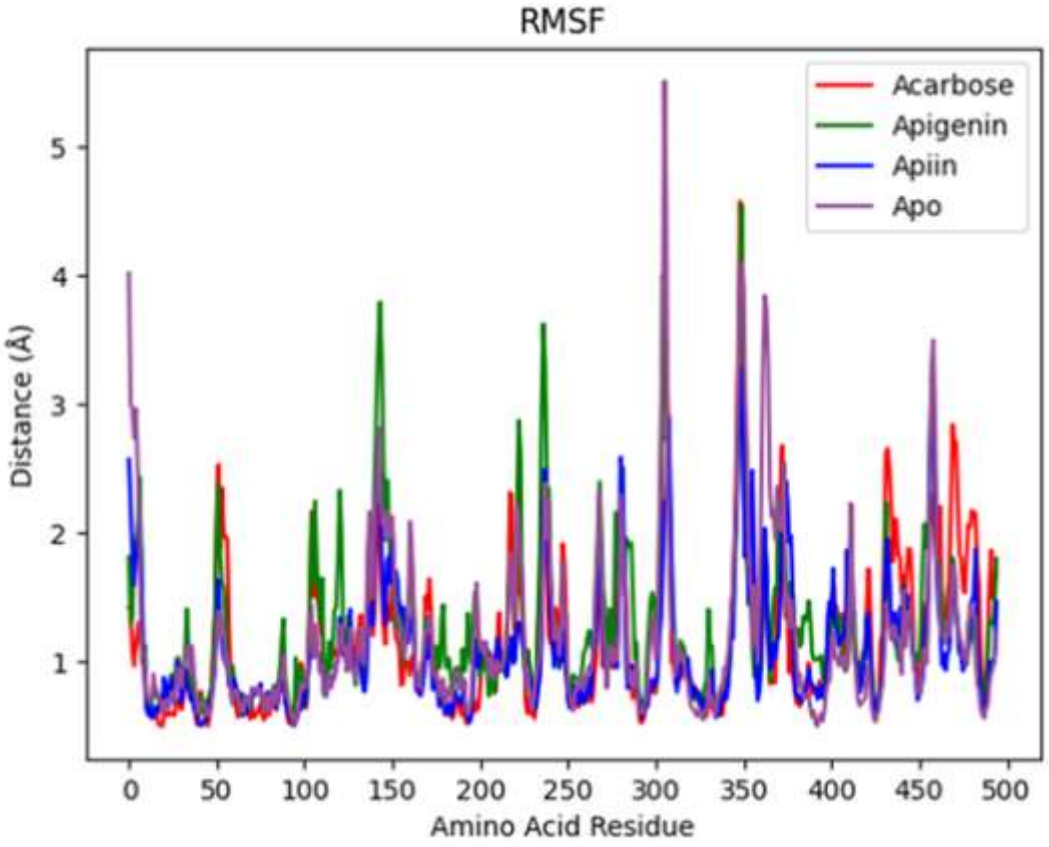

Figure 7: the per Residue Root Mean Square Fluctuations (RMSF) plots of molecular dynamics (MD) simulation of top complexes

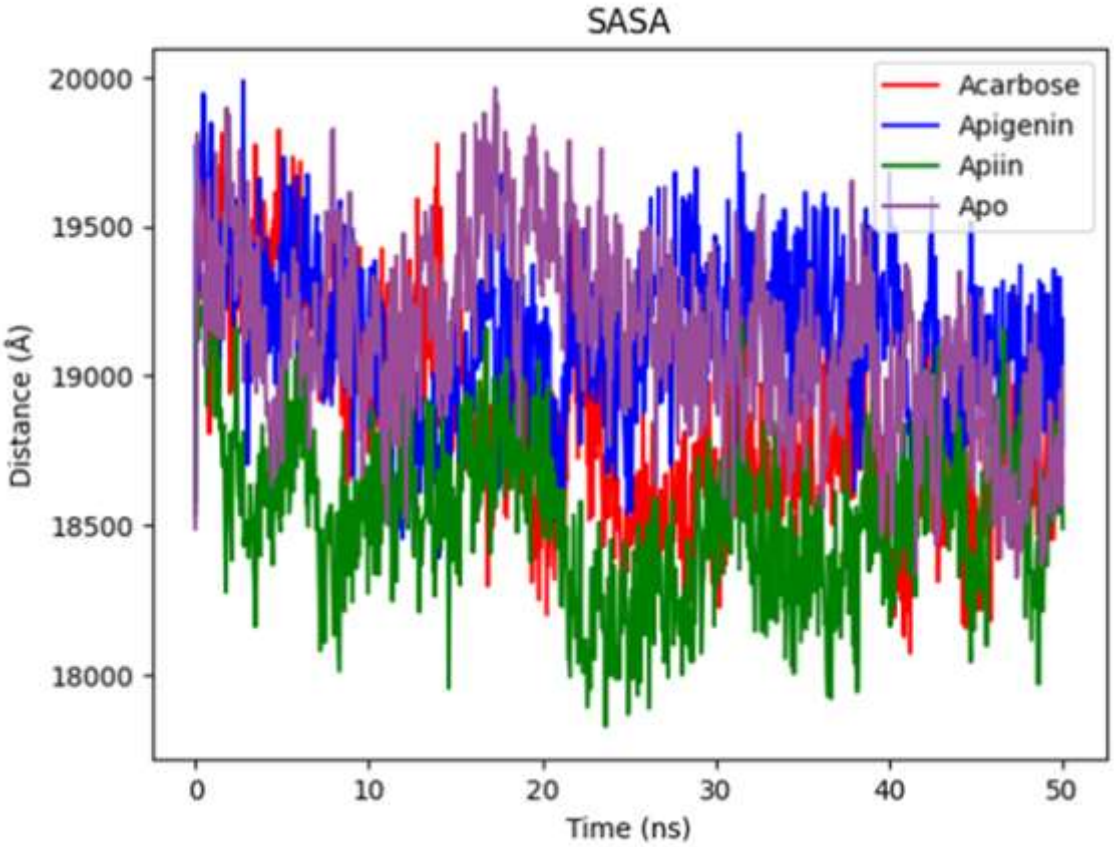

Figure 8: surface accessible surface area (SASA)

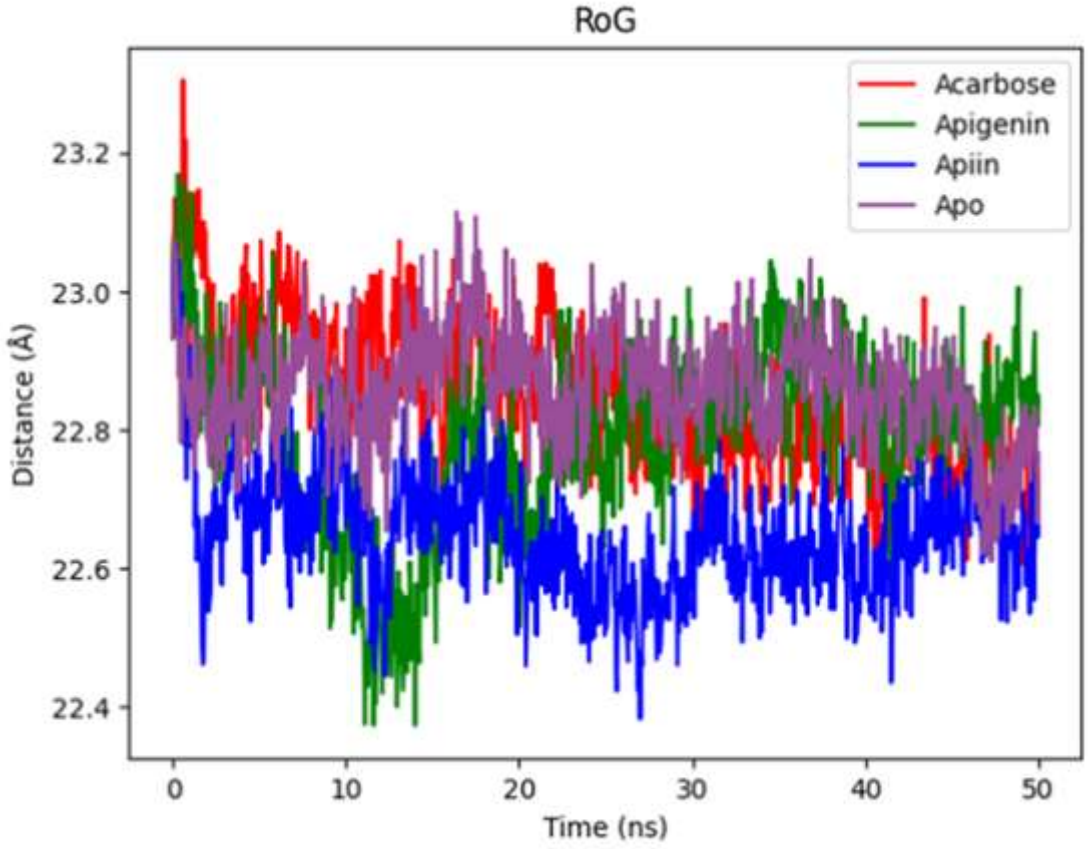

Figure 9: Radius of gyration plot (RoG)

DISCUSSION

The phytochemical composition of plants differs with species, geographical conditions, and parts of the plants, the extraction method and solvent used for extraction (Hayat et al 2020). In this study it was observed that the acetone solvent extract of *Lawsonia inermis* contained higher phenolics content compared to the aqueous and methanol extract. Furthermore, different researchers have reported that the phenolic contents differs with respect to type of solvent used, method of extraction, type of plant, part used and analysis method (Shan *et al*., 2005). Flavonoids are one of the major secondary metabolites in the plant kingdom. They have shown anticancer activities such as, inhibition of cancer cell growth, antioxidant activity, apoptosis induction and cancer cell cytotoxicity (Greenwell and Rahman, 2015). This indicate that the extraction solvent have significant effect on the flavonoids contents of the plants.

The concept behind the total antioxidant assay (phosphomolybdenum method) and reducing power assay (RPA) is reduction. While phosphomolybdenum method involves the reduction of molybdenum to a green coloured molybdenum complex (Prieto et al., 1999). RPA includes the ability to reduce Fe^{3+} to Fe^{2+} (Juntachote and Berghofer, 2005). The total antioxidant capacity expressed in ascorbic acid equivalents and ferric reducing ability of the extracts. Acetone extracts of *Lawsonia inermis* shows an excellent reducing power than the aqueous and methanol extraction solvents respectively as presented in Table 1. The effective reduction by the acetone extract shows its efficiency in preventing oxidation. Naturally occurring reductants are involved in oxidative defence mechanisms and the reducing ability may act as a significant indicator of its potential antioxidants (Meir et al., 1999). Thus the antioxidant capacity of the extracts based on its ability to reduce can be given in the following descending order: Acetone> Methanol > Aqueous. On an average all the extracts were able to scavenge DPPH radicals. The IC_{50} of methanol was very close to that of the ascorbic acid. The scavenging of DPPH radical by acetone and aqueous may be due to the presence of trace amounts of phenolics as presented in Figure 1.0. Based on the IC_{50} values the ability of the extracts to scavenge the DPPH free radical can be given in the following order Aqueous < Acetone< Methanol. The inhibitory activities of different solvents (Acetone, Aqueous and methanol) extract of *Lawsonia inermis*, against alpha-glucosidase and alpha amylase were evaluated and the results obtained are shown in Figure 2. Furthermore, it was noted that the three solvents used were able to have higher inhibition against alpha-glucosidase and alpha-amylase leading to reduced side effects. This assay was evaluated based on the development of yellow colour of p-nitrophenol by employing p-NPG as the substrate (Rege and Chowdhary, 2014). This suggested that the leaves extracts have higher potential for anti-diabetic activity. From this study, it can be inferred that L. inermis is a promising plant candidate for further investigations to be developed into anti-diabetic agents. This observation corroborated with the report by Kim et al. (2009) stating that, generally, natural inhibitors from plant extracts have displayed higher inhibition against alpha-glucosidase enzyme and alpha-amylase enzyme with minimal side effects. Lipid peroxidation is a basic membrane damage process which involves free radicals leading to oxidative degradation of polyunsaturated fatty acids and resulting in adverse effects. Reactive oxygen species are mainly generated in the mitochondria

as byproduct of cellular metabolic processes and can affect biomolecules by causing damage (Riess, 2004). Reactive intermediates resulting from oxidative stress can target membrane bilayers by causing lipid peroxidation. Polyunsaturated fatty acids in the membranes undergo lipid peroxidation resulting in lipoperoxyl radical (LOO.) generation, which attack lipids to form lipid hydroperoxides (LOOH) and lipid radicals. In this present study, it was observed that the different solvents (Acetone, Aqueous and methanol) extraction of *Lawsonia inermis* leaves were able to inhibit lipid peroxidation, which might be due to the presence of various polyphenolic content present in it.

Nitric oxide (NO) is derived from the oxidation of L-arginine by NO synthase (NOS) and is a mediator in the inflammatory response involved in host defense (Geller, 1998). This present investigation showed that the extraction solvent method (Acetone, Aqueous and methanol) for *lawsonia inermis* were not able to inhibit nitric production as the concentration increases as presented in Figure 4.0. In the glutathione cycle, GSH is regenerated *via* glutathione reductase as an 'auxiliary mechanism' that facilitates ther egulation of GSH homeostasis, in conjunction with other enzymes like gamma-glutamyl cysteine synthetase and glucose 6-phosphate. Henna leaf extract was effective in elevating GR activity only at lower dose level. Reduced glutathione (GSH) is the most abundant antioxidant in the cell. Its protective action is based on oxidation of the thiol group of its cysteine with the formation of oxidized glutathione (GSSG) (Tzeng *et al*., 1994). GSH is the most important non-protein thiol present in animal cells as well as in most plants and bacteria. Most of the intracellular GSH exists in the thiol form. Although mixed disulfides (mainly GSS-protein), thioethers and to a lesser extent, GSH disulfide (GSSH) contribute to the total cellular pool of GSH (Jernstrom et al., 1993). GSH plays an important role in detoxification mechanisms and in the protection of cellular constituents against ROS. Lawsonia inermis leaf extract (Acetone, Aqueous and methanol) effectively elevated the GSH level at both the dose levels in pancreas.

The enzyme alpha-amylase (1,4-α-Dglucan glucanohydrolase, EC 3.2.1.1) and glucosidase are key therapeutic targets that have been exploited for developing several synthetic drugs such as acarbose, voglibose, and miglitol (Alqahtani et al., 2019). Employing molecular docking in this study, the phytochemicals detected in the different solvent extracts of *Lawsonia inermis* showed good binding affinity and amino acid interactions with the active sites of HPA and HG. Given the roles of key residues that define the active site of HPA (ASP197, GLU233 and ASP300), the interactions of lawsonia compounds with these amino acids may hamper the action of ASP197 as a nucleophile, GLU233 which participate in the acid–base catalysis and that of ASP300 which helps in the proper orientation of the polymeric substrate. The study therefore suggests that, the compounds may act as competitive inhibitors of this enzyme. A similar report by Williams et al. (2012) revealed that, myricetin as a HPA inhibitor can binds at the active site of α-amylase via hydrogen bonds and hydrophobic interactions with critical amino acid residues. In the case of glucosidase, the interactions of acarbose and the lawsonia compounds with HG are dominated by hydrogen bonds and hydrophobic interactions particular involving Asp518 and Asp616.

Binding of inhibitors with specific enzymes is a dynamic process which can be assessed

through MD simulations. In this study, the stability of the ligand-protein complexes were assessed through a 50-ns full atomistic MD simulation alongside the unbound enzyme (apoenzyme). After the equilibration, the RMSD values of the apo-HPA and the ligand–HPA complexes were maintained within 2 Å. This result indicates that, there exist the formation of a stable complex. The RMSF plots indicate the role of the critical amino acid residues in the protein in attaining stable conformations with the lawsonia compounds. A loop structure around Asp300 showed high flexibility beyond indicating a high interaction potential with the ligands. Earlier studies have revealed such interaction potential of several loops around the critical amino acids with inhibitors (Borah et al., 2019, Ogunyemi et al., 2022b). Furthermore, maintenance of RoG of the apoprotein and the ligand–HPA complexes within 2 Å throughout the simulation period indicate that, the complexes exist in a well folded form. The SASA plots also indicate high tendency of solvent accessibility surface of the ligand-protein complexes. The high number of hydrogen bonds detected in the compound-enzyme complexes also revealed the stability of the complexes.

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