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Antimetabolic Syndrome Effects of a Flavonoid-Rich Extract from the Leaves of *Newbouldia laevis*: *in Vitro* and *in Silico* Investigations

Hope Ikani[1], Precious A. Idakwoji[1], Aanuoluwa Iyiola[2], Sadiq Bishir[3], Ummulkhairi Tukur[4], Abubakar R. Mannir[3, 8], Hannah Nsude[5], Abdulrahman I. Suleiman[6, 9], Idris K. Zubairu[7]

[1] *Department of Biochemistry, Faculty of Natural Sciences, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria.*
[2] *Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria*
[3] *Department of Biochemistry, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, Katsina, Katsina State, Nigeria.*
[4] *Independent Researcher, Ankara, Turkey. (Formerly at Middle East Technical University, Ankara)*
[5] *Department of Biochemistry, Faculty of Natural and Applied Sciences, Renaissance University, Ugbawka, Enugu State, Nigeria.*
[6] *Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic, Lokoja, Kogi State, Nigeria.*
[7] *Department of Food Science & Technology, Federal University, Dutsin-Ma, Katsina State, Nigeria.*
[8] *Nanomaterials for Biomedical Applications Research Group, Italian Institute of Technology, Genova, Italy.*
[9] *Directorate of Research and Innovation, Kogi State Polytechnic, Lokoja, Kogi State, Nigeria.*

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Abstract

This study evaluates the antimetabolic syndrome potential of a flavonoid-rich extract from the leaves of *Newbouldia laevis* (FRENL) using *in vitro* antioxidant and enzyme inhibition assays, complemented by GC-MS profiling and *in silico* molecular docking. The antioxidant activity of FRENL was evaluated using the ferric reducing antioxidant power (FRAP) and 2,2-di-phenyl-1-picrylhydrazyl (DPPH) assays, while enzyme inhibition assays targeted alpha-amylase, alpha-glucosidase, pancreatic lipase, and angiotensin-converting enzyme (ACE). FRENL demonstrated strong antioxidant activity with IC50 values of 191.60 µg/ml for FRAP and 199.30 µg/ml for DPPH. Enzyme inhibition assays revealed concentration-dependent inhibition of the key metabolic enzymes: alpha-amylase (IC_{50} = 90.46 µg/ml), alpha-glucosidase (IC_{50} = 65.47 µg/ml), pancreatic lipase (IC_{50} = 109.20 µg/ml), and ACE (IC_{50} = 110.00 µg/ml), suggesting potential anti-diabetic, anti-obesity, and anti-hypertensive effects. GC-MS analysis identified major phytochemicals, including kaempferol, apigenin, phytol, and quercetin, which are known for their bioactivity. Molecular docking studies showed strong binding affinities of these compounds to the active sites of the target enzymes. Notably, kaempferol showed the best binding energies against alpha-amylase (-8.4 kcal/mol), alpha-glucosidase (-7.8 kcal/mol), pancreatic lipase (-8.7 kcal/mol), and quercetin against ACE (-7.5 kcal/mol), forming stable hydrogen bonds and hydrophobic interactions with key catalytic residues. These findings support the traditional use of *Newbouldia laevis* in managing metabolic disorders and suggest that its flavonoid-rich extract contains bioactive compounds with multi-target potential. Further *in*

*Email : idakwojipa@gmail.com

vivo and clinical studies are recommended to validate these effects and explore therapeutic applications in metabolic syndrome management.

Keywords: Metabolic Syndrome, Flavonoid-Rich, Extract, *Newbouldia Laevis*, *In Vitro, In Silico*

Introduction

Metabolic syndrome (MetS) is a multifactorial disorder characterized by insulin resistance, obesity, hypertension, and dyslipidaemia [1]. An individual is said to be suffering from metabolic syndrome if he/she is diagnosed with at least three of the following conditions. These include: waist circumference that is greater than 102 cm in men and greater than 88 cm in women, serum triglycerides concentration that is equal to or greater than 150 mg/dL or under treatment for hypertriglyceridemia[1]; serum HDL-C concentration that is equal to or less than 40 mg/ dL in men and less than 50 mg/dL in women or under treatment for hypercholesterolemia, systolic blood pressure greater or equal to 130 mmHg, diastolic blood pressure greater or equal to 85 mmHg or undergoing treatment for hypertension, and fasting glucose greater or equal to 100 mg/ dL or taking oral hypoglycaemic agent [2]. In addition, genetic predisposition, age, unhealthy dietary habits, and lack of physical activities play a role in the development of metabolic syndrome. [1]. It has been reported that lifestyle adjustments can help manage some of these factors, thereby reducing the chances of developing metabolic syndrome in individuals [3].

In addition to lifestyle adjustments, each component of metabolic syndrome is usually managed via the use of conventional drugs that act on specific biochemical pathways, giving rise to that particular condition [4]. A class of anti-hypertensive drugs known as beta-blockers, such as propranolol, HMG-CoA inhibitors like simvastatin, fibrates like gemfibrozil, and sulfonylureas like glibenclamide are among the first-line drugs used for the treatment of metabolic syndrome [4]. However, the use of some of these drugs is usually complicated as a result of numerous adverse reactions and side effects linked to their mechanisms of action. This affects patient compliance and, as a result, does not produce the desired, sustainable therapeutic effect[5]. Also, polypharmacy is usually required in order to be able to address all the pathophysiological aspects of metabolic disorders, and this often leads to drug-drug and drug-disease interactions, especially in elderly patients [6]. Consequently, this has created a gap that needs to be filled by developing other approaches that can effectively manage all the pathophysiological aspects of metabolic syndrome [5]. In recent times, complementary and alternative medicine (CAM) has become the most commonly used alternative to orthodox drugs. The use of CAM is as old as man himself, and it is embraced by both the developed and the developing parts of the world [7, 8]. CAM encompasses a wide range of treatment methods, including the use of medicinal plants [8].
Over the years, scientific investigations have revealed that medicinal plants are a large reservoir of bioactive compounds that can serve as therapeutic agents [9, 10]. The bioactive compounds of medicinal plants have served as leads for the development of drugs with a wide range of pharmacological activities. These include the popular antimalarial drug quinine, anticancer agents vincristine and vinblastine, and a host of others [11]. In line with this approach, many researchers are exploring the option of screening medicinal plants for compounds that can help in the prevention and treatment of MetS [11]. In this regard, Jang *et al.,* [3] studied the effect of a number of medicinal plants and reported their ability to reduce weight gain, hyperglycaemia, dyslipidaemia, and blood pressure, hence giving a clue on their potential ameliorative effects on metabolic syndrome [5]. Sabarathinam *et al.,* [12] also reported that some medicinal plants contain some active principles with diverse mechanisms of action, thus suggesting their capability to address various pathophysiological aspects of

metabolic syndrome [12]. Additionally, Murtala and Akindele, [13] reported that certain medicinal plant extracts can ameliorate the conditions associated with MetS, such as hyperglycemia, insulin resistance, dyslipidaemia, raised systolic and diastolic blood pressure, and reduce weight gain.

Newbouldia laevis is found in the tropical regions of Africa. It is usually referred to as the African hyssop. It is a very popular plant in Africa as a result of its diverse folkloric uses. It is widely utilized in traditional medicine by various cultures in Nigeria, Togo, Cameroun, Senegal, and Ghana [11]. The African hyssop is thus used in the treatment of several ailments such as: ear infections, malaria, dysentery, conjunctivitis, hypertension, diabetes, diarrhoea, constipation, epilepsy, gastrointestinal ulcer and Buruli ulcer; sickle cell anemia dysmenorrhea, fever, cough and pain-related conditions, such as arthritis, chest pain, otalgia and migraine [13]. Despite the wide range of pharmacological activities of the plant that have been reported in the literature, to the best of our knowledge, there has not been any study that evaluated the *in vitro* activity of flavonoid-rich extract against key enzymes associated with the pathophysiology of the components of metabolic syndrome. Additionally, there is no report of an *in silico* study of the compounds present in the extract of *Newbouldia laevis* against molecular targets implicated in metabolic syndrome. This study, therefore, seeks to evaluate the in vitro antimetabolic syndrome potentials of the flavonoid-rich extract of *Newbouldia laevis* leaves and also to carry out molecular docking of some GC-MS-identified compounds against key molecular targets implicated in metabolic syndrome.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and reagents

The chemicals and reagents used were of analytical grade and include: 1% Thiobarturic acid, acetone, aluminium chloride, ammonia, EDTA, heparin, ascorbic acid, Bismuth carbonates butanol, chloroform, potassium dichloromate, glacial acetic acid, Drangendorff's reagent, lead acetate solution, Mayer's reagent, picric acid, potassium hydroxide, sodium dodecyl sulphate, sodium hydroxide, trichloroacetic acid (TCA), trichloroacetic acid, tungstic acid, Turk's solution (20% glacial acetic acid), and Wagner's reagent.

2.1.2 Equipment and instruments

The following equipment and instruments were used: UV-vis spectrophotometer, Whatman filter paper, hot air oven, vortex mixer, round-bottom flasks, centrifuges, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, and PC spectrophotometer.

2.1.3 Enzymes and substrates

Porcine pancreatic α-Amylase (Sigma-Aldrich, UK), yeast α-glucosidase (Sigma-Aldrich, UK), angiotensin converting enzyme, porcine pancreatic lipase (Sigma-Aldrich, UK), soluble potato starch, P- nitrophenyl- α-D-glucopyranoside (Sigma-Aldrich, UK), P-Nitrophenyl butyrate, Hip-puryl-L-histidyl-L-leucine (Sigma-Aldrich, UK).

2.1.4 Drugs

Acarbose® (Bayer), Orlistat, Lisinopril

2.2 Methods

2.2.1 Collection and preparation of plant material

The leaves of *Newbouldia laevis* were harvested from its natural habitat at the bank of Meme River, Otokiti Area of Lokoja, Kogi State, Nigeria. The leaves were identified at the

Herbarium unit of the Department of Biological Sciences, Federal University, Lokoja, by an ethnobotanist, and a voucher specimen was deposited for future reference.

2.2.2 Extraction of Newbouldia laevis leaves

Fresh leaves of *Newbouldia laevis* were properly rinsed with clean tap water to remove sand debris. It was dried in the shade in the laboratory for 7 days and blended using an electric blender. Two kilograms of the blended sample were cold-macerated in 7.5 L of absolute ethanol for 3 days. After this period, the mixture was filtered using a mesh and then a Whatman No. 1 filter paper. The resulting filtrate was concentrated in a rotary evaporator (temperature: 45 °C, pressure: 100 mbar) to obtain the crude ethanol extract of *Newbouldia laevis* leaves.

2.2.3 Preparation of the flavonoid-rich extract of Newbouldia laevis leaves

The flavonoid-rich extract of *Newbouldia laevis* leaves was prepared adopting the method of Chu *et al.,* [14]. Nine grams of the crude ethanol extract was placed into a beaker, 60 ml of 10% tetraoxosulphate-iv acid (H_2 SO_4) was added, the mixture was stirred and heated on a water bath for 30 min at 100 °C for complete hydrolysis to take place. Subsequently, the solution was placed on ice for 15 min to enhance the precipitation of flavonoids alongside aglycones. The precipitate, which now contains a flavonoids and aglycones mixture, was placed in a 100 ml volumetric flask and 50 ml of 95% ethanol was added to allow dilution. The volume was subsequently made up to the mark with 95% ethanol. This was centrifuged, filtered, and the filtrate collected was concentrated using a rotary evaporator to obtain the flavonoid-rich extract of *Newbouldia laevis leaves* (FRENL), which was stored in the freezer until required for use.

2.2.4 Phytochemical analysis of the FRENL

The presence of major phytochemicals, including alkaloids, phenols, flavonoids, saponins, glycosides, tannins, terpenoids, and steroids, was analysed following the methods described by Harborne [15] and Trease and Evans [16].

2.2.5 DPPH radical scavenging assay

The DPPH radical scavenging assay was modified according to a previous method described by Munteanu and Apetrei [17].

2.2.6 Ferric reducing antioxidant power assay

The FRAP assay was performed according to a previous method by Ishola *et al.,* [18].

2.2.7 α-amylase inhibitory assay

The inhibitory assay was performed according to the method of Huang *et al.,* [19].

2.2.8 α-glucosidase inhibitory assay

The α-glucosidase inhibitory assay was performed according to the method of Zumaider *et al.,* [20].

2.2.9 Pancreatic lipase inhibitory assay

Pancreatic lipase activity was determined by measuring the hydrolysis of *p*-nitrophenyl butyrate (*p*-NPB) to *p*-nitrophenol using a method previously reported by Jeong *et al.,* [21].

2.2.10 Angiotensin converting enzyme (ACE) inhibitory assay

The activity of ACE was determined using Hip-puryl-L-histidyl-L-leucine (HHL) as the substrate with some modifications [22].

2.2.11 GC-MS analysis

Chemical composition of the FRENL was determined by GC/MS-QP-2010 plus Ultra (Shimadzu, Kyoto, Japan). Each component was identified based on its column retention time relative to computer-based matching of mass spectra with those of standards (NIST and Wiley libraries for the GC-MS system).

2.2.12 In silico investigation of GC-MS- identified against key metabolic enzymes

Protein target retrieval: 3D Structures of *α*-amylase (PDB ID: 1B2Y), *α*-glucosidase (PDB ID: 2JKP), Lipase (PDB ID: 10IL) , and angiotensin converting enzyme, ACE (PDB ID: 1O86), were obtained from the protein databank (www.rcsb.org). These protein structures were refined using Discovery Studio software. Test ligand: Two of the GC-MS-identified compounds, kaempferol and quercetin were selected for this study. Ligand retrieval: The 3D format of kaempferol and quercetin and the standard drugs (acarbose, orlistat and lisinopril) were downloaded from the Pubchem database in SDF file. These SDF files were converted to PDB using Pymol software prior to molecular docking studies [23].

Molecular docking using AutoDock Vina Software: The enzymes and GC-MS- identified ligands were prepared prior to molecular docking using AutoDock Vina. The active site investigation was performed using Site finder of Molecular Operating Environment [24]. Molecular docking was performed using AutoDock Vina Software [25]. The 2D views of the protein-ligand interactions renderings were done using Discovery Studio software and Pymol software, respectively [25].

2.2.13 Statistical analysis

Data were expressed as Mean ± standard deviation. Statistical differences between means were determined by one-way analysis of variance (ANOVA) followed by Duncan's *post–hoc* test for multiple comparison tests, and values were considered significant at $p \leq 0.05$. The IC_{50} values were determined through a nonlinear regression analysis of the log concentration-inhibition curve. All analyses were carried out using GraphPad version 10.0.

3. Results

3.1 Qualitative phytochemical analysis of the FRENL

FRENL was analysed qualitatively for its phytochemical components. Results showed the presence of phenols, flavonoids, and terpenoids in FRENL (Table 1).

Table 1: Phytochemical Composition of FRENL

Phytochemicals	FRENL
Alkaloids	ND
Phenols	+
Flavonoid	+++
Saponin	ND
Glycosides	ND
Tannins	ND
Terpenoids	+
Steroids	ND

Key: + trace, ++ moderate, +++ abundant, ND- Not detected

3.2 Ferric reducing antioxidant power and 2,2-di-phenyl-1-picrylhydrazyl inhibitory activity of the FRENL

Ferric Reducing Antioxidant Power (FRAP) and 2,2-di-phenyl-1-picrylhydrazyl (DPPH) Inhibitory assays were carried out in this study. FRENL demonstrated strong antioxidant activity with median inhibitory concentration (IC_{50}) values (calculated from % inhibition) of 191.60 µg/ml for FRAP and 199.30 µg/ml for DPPH (Table 2).

Table 2: FRAP and DPPH inhibitory activity of FRENL

Concentration (µg/ml)	FRAP % Inhibition	DPPH % Inhibition
50	17.63±1.46	12.81±1.47
100	32.48±2.35	40.43±2.15
200	50.81±2.13	49.78±2.78
400	69.45±3.25	68.21±3.12
800	77.23±5.08	75.53±4.15
IC_{50} (µg/ml)	191.60	199.30

n=3

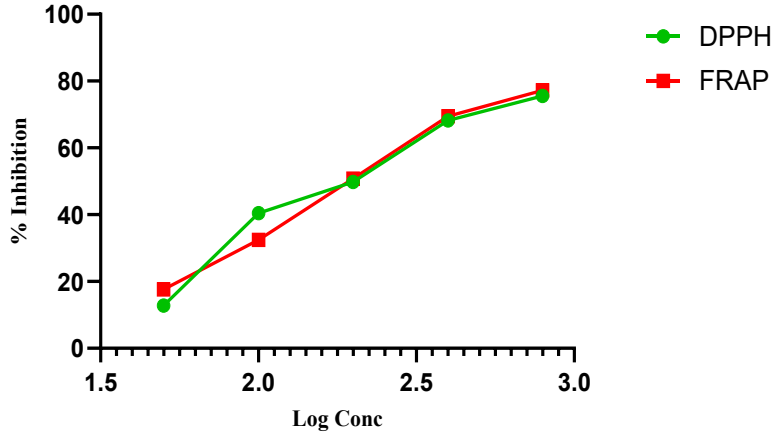

Figure 1: Log concentration of FRENL vs percentage inhibition curve

3.3 Alpha-amylase inhibitory activity of the FRENL

A concentration-dependent inhibitory effect on *α*-amylase was observed with FRENL and acarbose (Table 3). The IC_{50} of FRENL for *α*-amylase was estimated to be 90.46 µg/ mL, while that of acarbose was 73.50 µg/ mL (Figure 2).

Table 3: Alpha-amylase inhibitory activity of the FRENL

Concentration (µg/ml)	FRENL % Inhibition	Acarbose % Inhibition
10	0.53±0.01	3.28±0.38
20	2.56±0.34	10.11±1.29
30	10.01±1.23	16.73±1.45
40	12.36±1.48	25.15±1.37
50	17.24±1.26	33.01±1.84
60	25.81±2.11	39.18±1.99
70	33.26±2.05	42.20±2.01
80	40.14±2.31	50.12±2.36
90	49.11±2.26	61.36±2.51
100	59.25±2.71	70.59±2.81
IC_{50} (µg/ml)	90.46	73.50

n=3

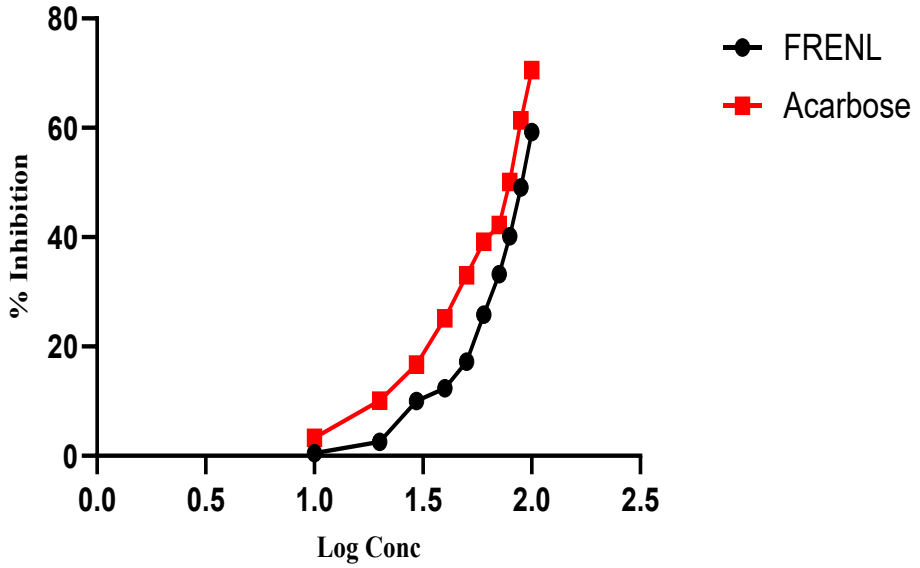

Figure 2: Log concentration of FRENL and acarbose vs percentage inhibition curve

3.4 Alpha-Glucosidase Inhibitory Activity of the Flavonoid-Rich Extract of Newbouldia laevis Leaves (FRENL)

For alpha-glucosidase, a concentration-dependent inhibitory effect was also observed with both FRENL and acarbose (Table 4). The IC_{50} of FRENL for α-glucosidase was estimated to be 65.47 µg/ mL, while that of acarbose was 50.93 µg/ mL (Figure 3)

Table 4: Alpha-glucosidase inhibitory activity of the FRENL

Concentration (µg/ml)	FRENL % Inhibition	Acarbose % Inhibition
10	7.12±0.26	15.43±0.21
20	15.43±0.47	23.91±0.86
30	28.31±1.38	32.02±1.25
40	31.96±1.29	38.21±1.33
50	39.02±1.66	44.33±1.40
60	46.17±1.71	51.07±1.06
70	51.43±1.25	58.48±1.09
80	58.26±1.37	63.67±2.14
90	61.01±1.28	76.11±2.81
100	65.27±2.47	81.08±3.01
IC_{50} (µg/ml)	65.47	50.93

n=3

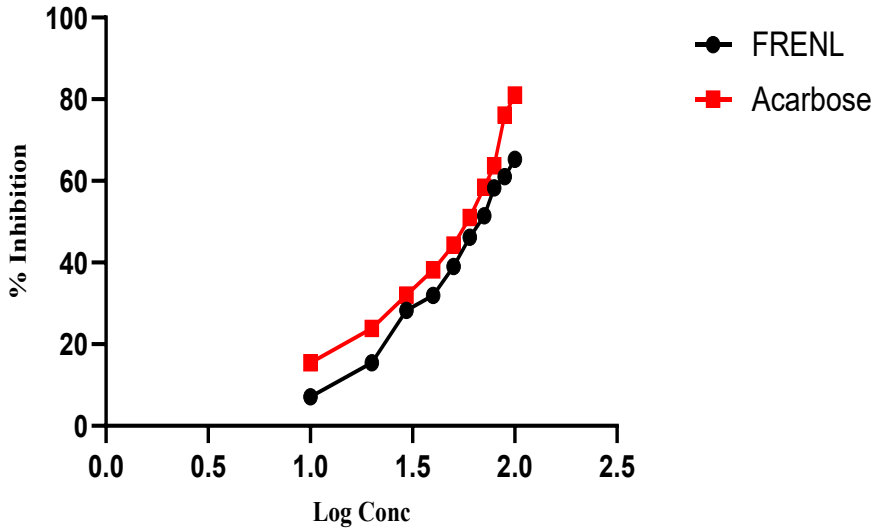

Figure 3: Log concentration of FRENL and acarbose vs percentage inhibition curve

3.5 Pancreatic lipase inhibitory activity of the FRENL

In this study, a concentration-dependent inhibitory effect on pancreatic lipase was observed with FRENL and orlistat (Table 5). The IC_{50} of FRENL for pancreatic lipase was estimated to be 109.20 µg/ mL, while that of orlistat was 119.50 µg/ mL (Figure 4).

Table 5: Pancreas lipase inhibitory activity of the FRENL

Concentration (µg/ml)	FRENL % Inhibition	Orlistat % Inhibition
10	0.62±0.04	0.35±0.06
20	0.89±0.06	1.63±0.11
30	1.50±0.12	1.92±0.21
40	5.28±0.79	3.22±0.55
50	10.36±0.95	5.21±0.85
60	17.11±1.21	12.05±1.31
70	25.09±1.36	21.25±1.04
80	31.72±1.04	25.15±1.22
90	38.58±1.80	32.11±1.34
100	41.33±2.61	35.21±1.72
IC_{50} (µg/ml)	109.20	119.50

n=3

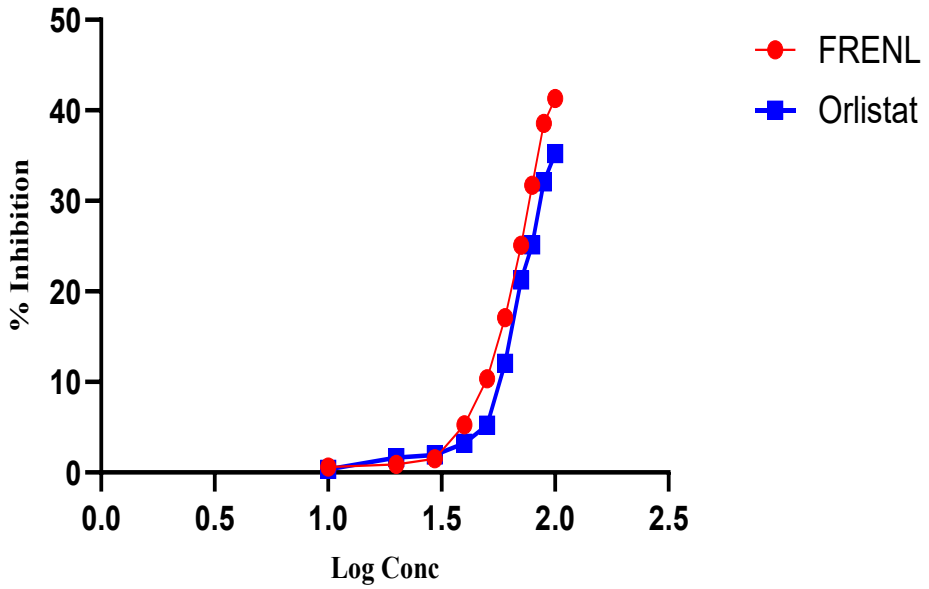

Figure 4: Log concentration of FRENL and orlistat vs percentage inhibition curve

3.6 Angiotensin Converting Enzyme (ACE) Inhibitory Activity of the FRENL

The results showed that a concentration-dependent inhibitory effect on ACE was observed with FRENL and lisinopril (Table 6). The IC_{50} of FRENL for ACE was estimated to be 110.00 µg/ mL, while that of lisinopril was 97.19 µg/ mL (Figure 5).

Table 6: ACE inhibitory activity of the FRENL

Concentration (µg/ml)	FRENL % Inhibition	Lisinopril % Inhibition
10	0.81±0.02	5.15±0.33
20	1.26±0.46	9.09±0.41
30	5.35±0.38	13.26±0.63
40	13.08±0.94	18.44±0.79
50	17.21±1.23	27.03±1.66
60	20.39±1.58	31.05±1.41
70	26.84±1.94	36.98±1.18
80	33.33±1.01	41.66±1.50
90	38.11±1.46	48.32±1.01
100	46.25±2.04	51.45±1.71
IC_{50} (µg/ml)	110.60	97.19

n=3

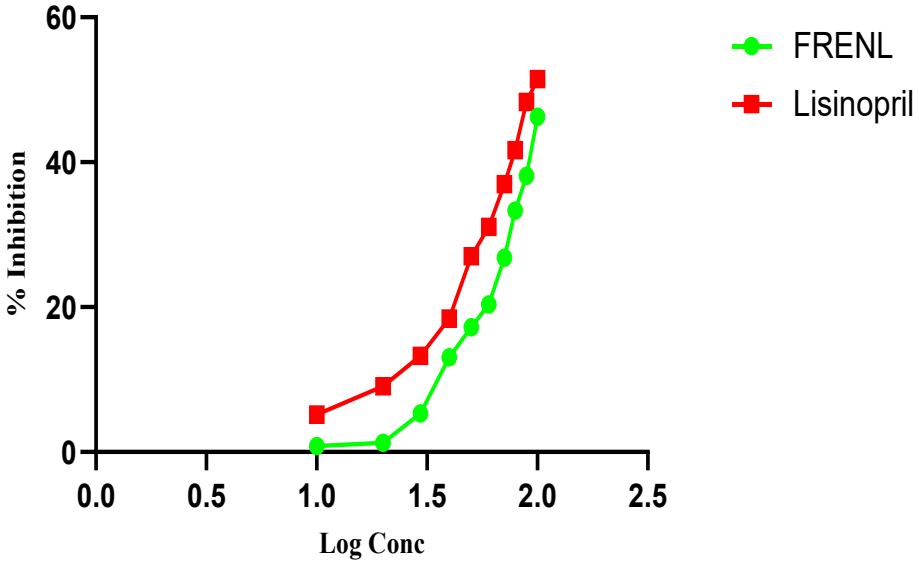

Figure 5: Log concentration of FRENL and lisinopril vs percentage inhibition curve

3.7 GC-MS analysis of the FRENL

GC-MS analysis revealed the presence of 15 compounds, as shown in Table 7.

Table 7: GC-MS- identified compounds

S/N	Compound Name	RT	Peak Area	Molecular formula	Molecular Weight
1	Proline	4.447	2.55	C5H9NO2	115
2	1,3,5-Cycloheptatriene	5.427	2.26	C7H8	92
3	Apigenin	6.823	2.33	C15H10O5	270
4	Heptadecane, 8-methyl-	25.785	1.19	C18H38	254
5	Quercetin	27.354	1.29	C15H10O7	302
6	Ethylallylphthalate (Phthalic acid)	28.458	2.37	C13H14O4	234
7	acrylic acid tetradecanyl ester	30.625	1.09	C17H32O2	268
8	Palmitic acid	35.141	8.10	C16H32O2	256
9	kaempferol	32.345	1.51	C15H10O6	286
10	Tetracosane	31.980	1.32	C24H50	338
11	Docosane	30.980	1.11	C22H46	310
12	Ethyl linoleolate	37.085	5.20	C20H36O2	308
13	Phytol	36.850	2.57	C20H40O	296
14	Phytol isomer	34.643	1.87	C20H40O	296
15	Nonadecane	26.927	1.16	C19H40	268

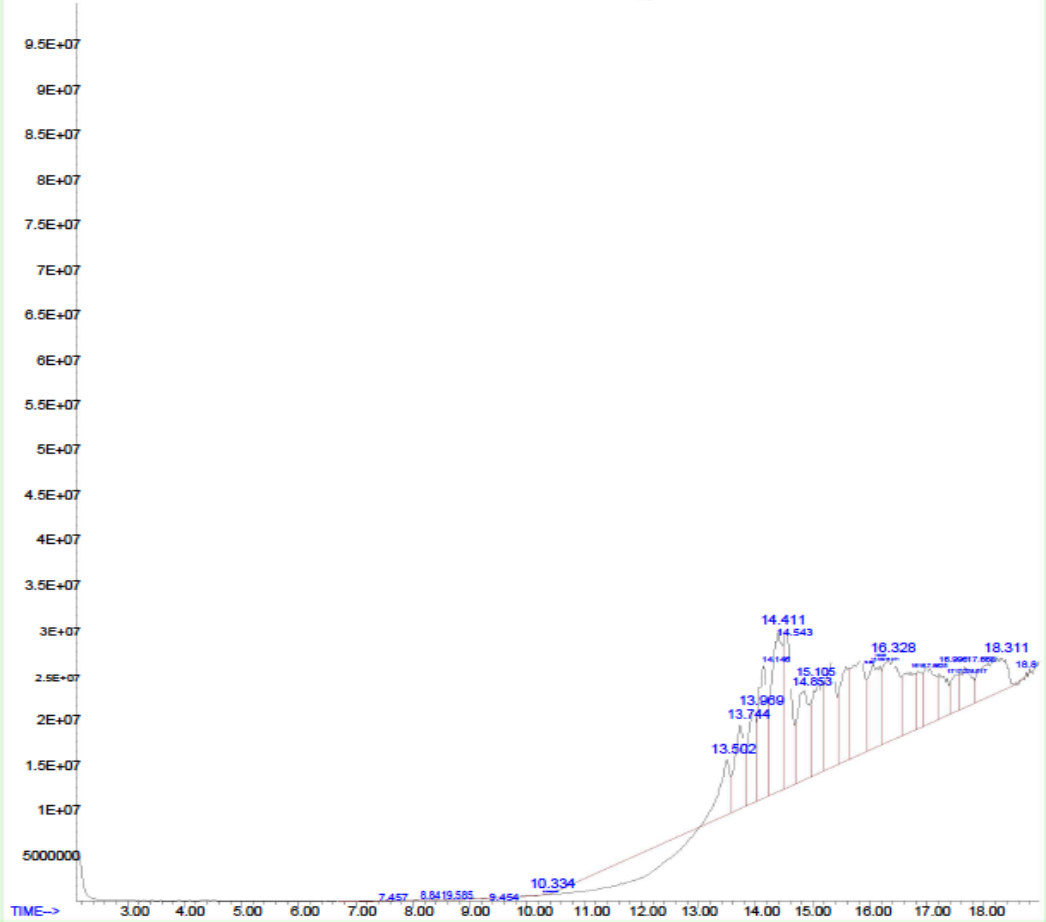

Figure 6: GC-MS chromatogram of FRENL

3. 8 In silico investigation of GC-MS- identified against key metabolic enzymes

Molecular docking studies showed strong binding affinities of these compounds to the active sites of the target enzymes. Notably, kaempferol showed the best binding energies against alpha-amylase (-8.4 kcal/mol), alpha-glucosidase (-7.8 kcal/mol), pancreatic lipase (-8.7 kcal/mol), and quercetin against ACE (-7.5 kcal/mol), forming stable hydrogen bonds and hydrophobic interactions with key catalytic residues.

Table 8: Binding affinity of ligands against target proteins

Target Proteins	Acarbose	Orlistat	Lisinopril	Kaempferol	Quercetin
Alpa-Amylase (PDB ID: 1B2Y)	7.9 Kcal/mol	-	-	-8.4 Kcal/mol	-6.2 Kcal/mol
Alpa-Glucosidase (PDB ID: 2JKP)	7.6 Kcal/mol	-	-	-7.8 Kcal/mol	-6.4 Kcal/mol
Pancreatic Lipase (PDB ID: 10IL)	-	9.5 Kcal/mol	-	-8.7 Kcal/mol	-5.8 Kcal/mol
Angiotensin-Converting Enzyme, ACE (PDB ID: 1O86)	-	-	8.8 Kcal/mol	-6.5 Kcal/mol	-7.5 Kcal/mol

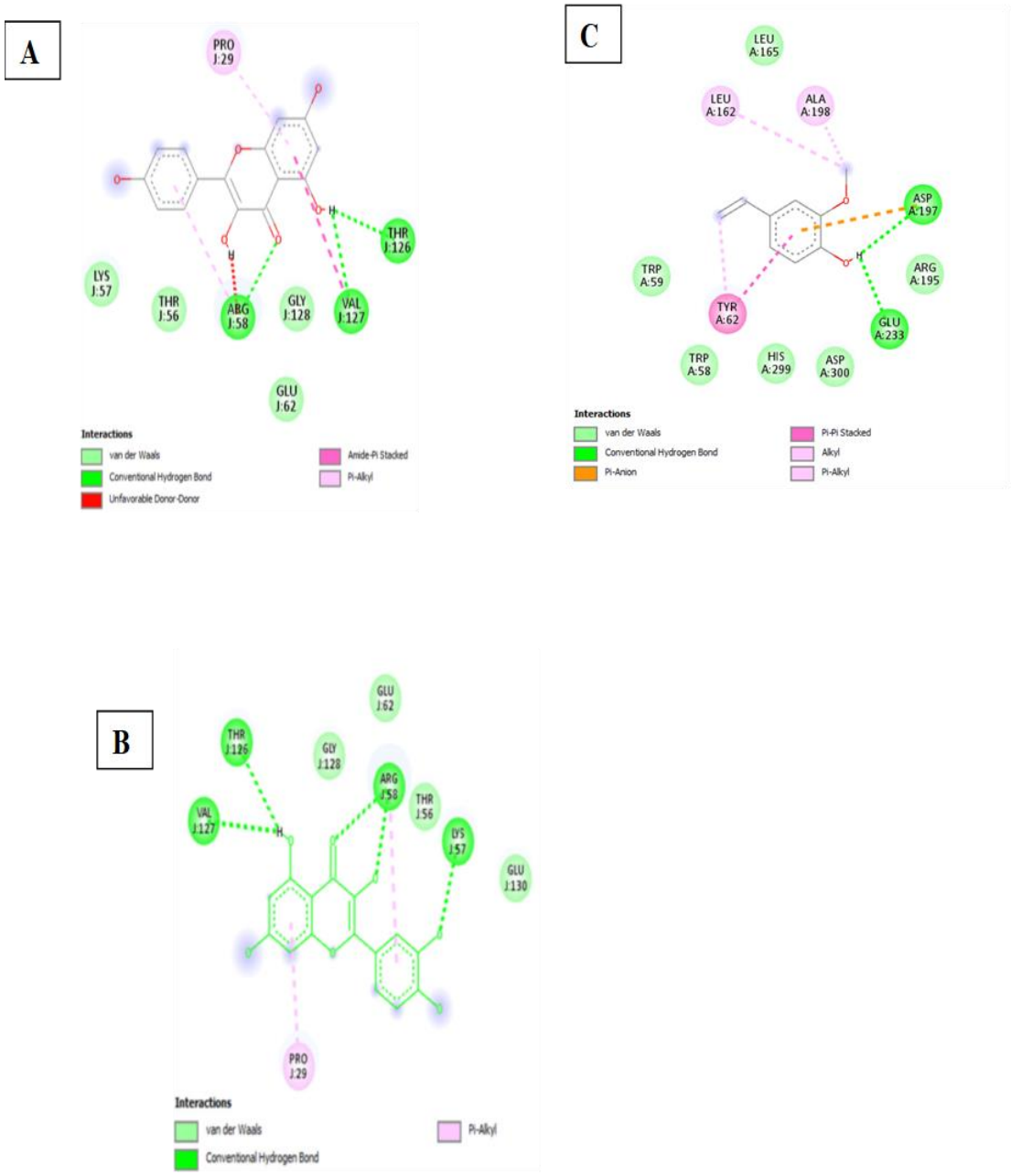

Figure 7: 2-D views of the molecular interactions of amino acid residues of Alpha-amylase with (A) Kaempferol (B) Quercetin (C) Acarbose

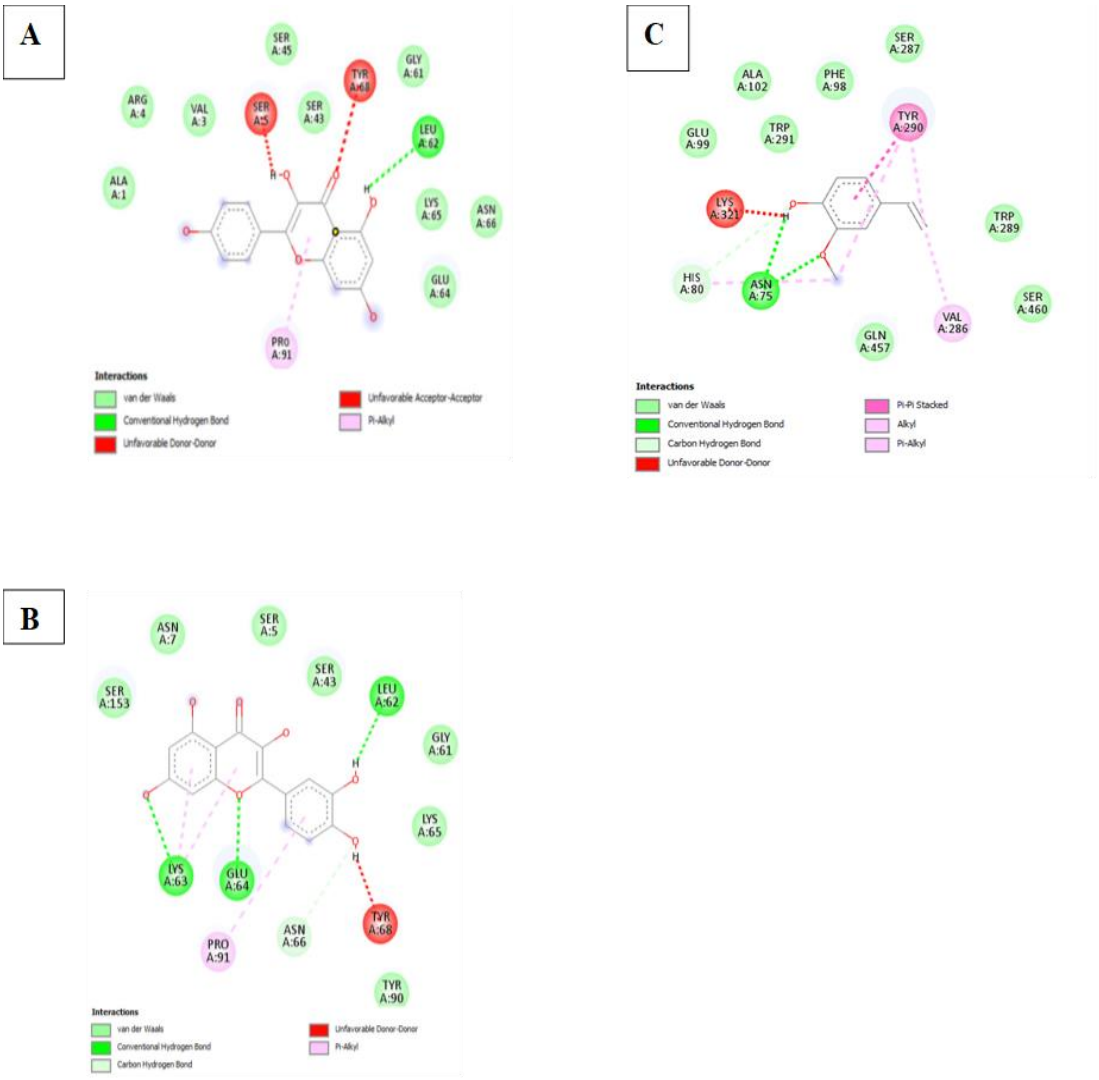

Figure 8: 2 -D views of the molecular interactions of amino acid residues of Alpha-glucosidase with (A) Kaempferol (B) Quercetin (C) Acarbose

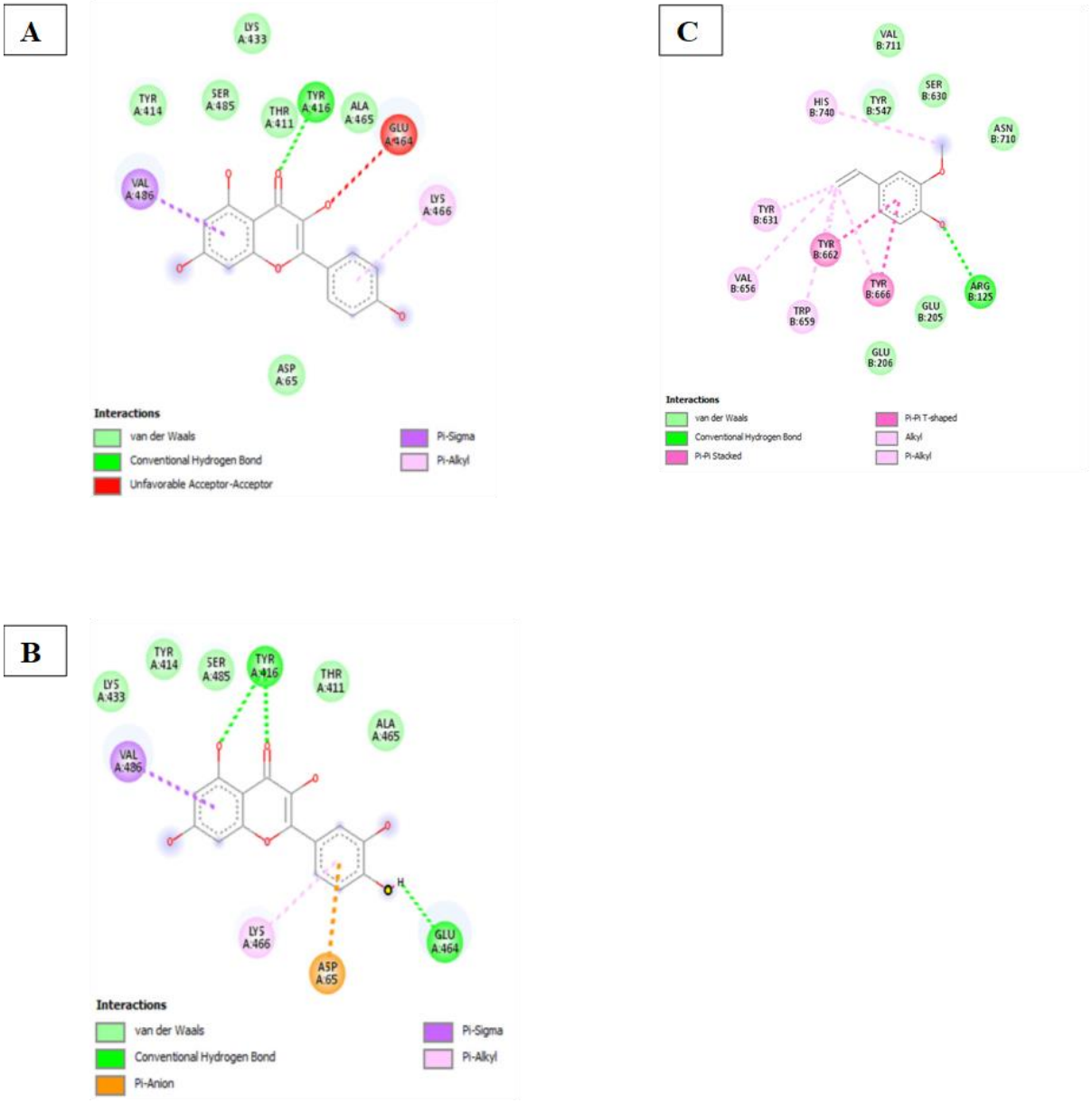

Figure 9: 2-D views of the molecular interactions of amino acid residues of Pancreatic lipase with (A) Kaempferol (B) Quercetin (C) Orlistat

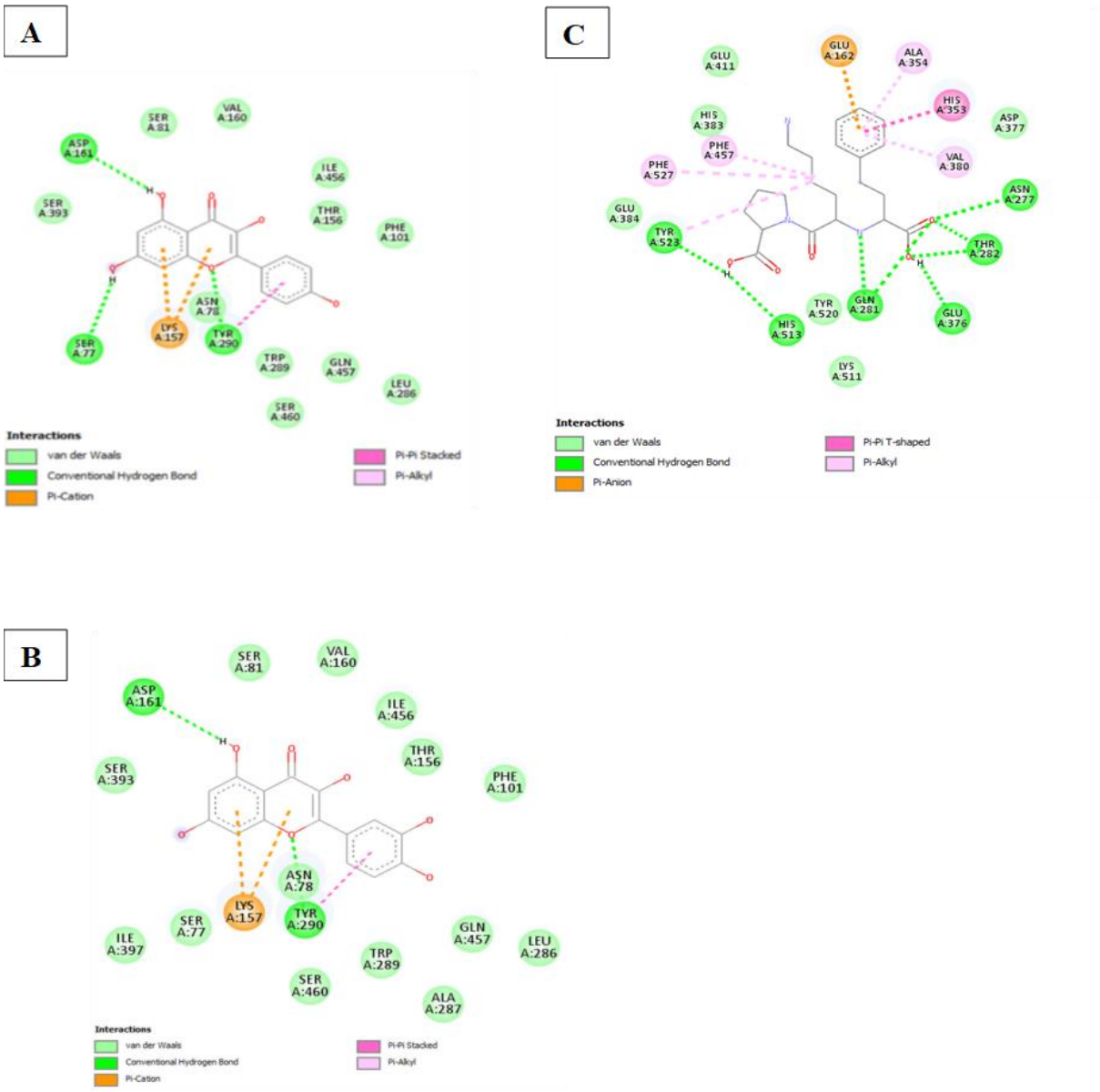

Figure 10: 2 -D views of the molecular interactions of amino acid residues of Angiotensin-converting Enzyme (ACE) with (A) Kaempferol (B) Quercetin (C) Lisinopril

4. Discussion

Metabolic syndrome is a group of metabolic disturbances which include hyperglycaemia, insulin resistance, excessive body weight, high blood pressure, and dyslipidaemia, which collectively increase the risk of cardiovascular disease (CVD), type 2 diabetes, and other related disorders [1]. This syndrome not only impacts the health of individuals but also places a major burden on healthcare systems and societies around the world, leading to higher disability and mortality rates. This highlights the need for a proper knowledge of its risk factors to guide effective prevention and management strategies [26]. This study evaluated the antimetabolic syndrome effects of the FRENL using a combination of *in vitro* and *in silico* methods.

The phytochemical analysis results revealed a predominant presence of flavonoids and phenols as the major compounds in FRENL. Flavonoids and other polyphenolic compounds are well-documented for their diverse biological activities, including anti-inflammatory, antioxidant, and metabolic regulatory effects [26]. Their abundance in the extract strongly indicates that they may contribute significantly to the observed antioxidant and potential antimetabolic effects [26].

Antioxidant assays using DPPH and FRAP methods demonstrated a high antioxidant activity of FRENL. The DPPH assay, which measures the free radical scavenging ability, showed substantial inhibition of free radicals, reflecting the extract's capacity to neutralize oxidative stress [27]. Similarly, the FRAP assay, which assesses the reducing power of the extract, indicated a strong electron-donating ability, further supporting its potent antioxidant profile [27]. These findings align with the high flavonoid content, as flavonoids are known to exhibit antioxidant effects by donating hydrogen atoms or electrons to free radicals and chelating metal ions.

Oxidative stress plays a central role in the pathogenesis of metabolic syndrome [28, 29]. Excessive generation of reactive oxygen species leads to cellular and mitochondrial dysfunction, insulin resistance, lipid peroxidation, and chronic low-grade inflammation, all of which contribute to metabolic derangements. Therefore, the potent antioxidant activity exhibited by FRENL in this study suggests that it may confer protective effects against oxidative stress-mediated metabolic dysfunctions.

Hyperglycaemia, one of the components of metabolic syndrome, predisposes an individual to diabetes and its complications. Insulin is an important hormone that regulates the metabolism of biomolecules, including carbohydrates, fats, and proteins. Any defect in the secretion or action of insulin negatively affects the metabolism of these macromolecules. One major event that leads to a sharp increase in blood glucose i.e., postprandial hyperglycaemia is the rapid absorption of glucose in the intestine due to the activity of carbohydrate-metabolizing enzymes, α-amylase, and α-glucosidase. These enzymes aid in the hydrolysis of polysaccharides such as starch into monosaccharides, notably glucose [30]. Therefore, one of the important therapeutic approaches to decrease postprandial hyperglycaemia is to retard the absorption of glucose through inhibition of these carbohydrate-hydrolyzing enzymes. Currently, medications such as acarbose and voglibose inhibit the activities of α-amylase and α- glucosidase. These drugs, however, are not free from adverse side effects, such as diarrheoa, nausea, abdominal pain, emesis, and flatulence. These side effects are believed to be a result of over-inhibition of pancreatic α-amylase, which subjects the undigested carbohydrates to fermentation by the action of gut flora [30]. Consequently, natural compounds, such as those derived from plants with mild inhibitory action against α-amylase and hence, fewer side effects, have been utilized in blunting postprandial hyperglycaemia [30].

This study shows that FRENL exhibited concentration-dependent inhibitory effects on both α-amylase and α-glucosidase enzymes. This high inhibitory activity may be a result of the high flavonoid and phenolic content of FRENL, as these phytochemicals have been reported to play an important role in modulating glucosidase and amylase activities [30]. FRENL had IC_{50} values of 90.46 and 65.47 µg/ml for α-amylase and α-glucosidase, respectively. These values were observed to be higher than those of the standard drug acarbose, which were 73.50 and 50.93 µg/ml for α-amylase and α-glucosidase, respectively. The implication of this observation is that FRENL is not a potent inhibitor of both α-amylase and α-glucosidase like acarbose. Therefore, it is plausible to suggest that FRENL may induce fewer side effects compared to acarbose, although further *in vivo* and clinical investigations

are necessary to substantiate this claim. This study has shown that one of the possible antimetabolic syndrome actions of FRENL is via inhibition of these carbohydrate-metabolising enzymes. Hence, FRENL could play a key role in the management of metabolic syndrome.

Obesity or excessive weight is another component of metabolic syndrome, and it is a culprit in a number of chronic diseases such as hypertension, type 2 diabetes, reproductive cancer, heart diseases, myocardial infarction and cerebrovascular accident [31]. Obesity is believed to be caused by an excessive calorie intake. Hence, an important strategy for the development of anti-obesity drugs is to search for ways to decrease the digestion and absorption of high-calorie compounds such as lipids [31]. The enzyme, pancreatic lipase, which aids in the digestion and subsequent absorption of dietary lipids, is therefore an important target for the development of anti-obesity drugs [32]. Pancreatic lipase accounts for 50–70% hydrolysis of total dietary fats and is secreted by the pancreas into the duodenum [32]. It breaks down triglycerides into fatty acids and glycerol. These fatty acids are subsequently wrapped up in micelles and absorbed in the small intestines and enter into peripheral circulation. If the hydrolysis of triglycerides is obstructed, there will be a decreased utilization of dietary fat. Therefore, any compound that inhibits lipase activity reduces the absorption of fat.
Currently, orlistat, a drug whose mechanism of action is through inhibition of pancreatic lipase, is the only drug approved by the FDA and in use for the management of obesity. Orlistat is capable of reducing the absorption of dietary fat by approximately 30% [33]. In addition to its effect on obesity, orlistat is also beneficial in controlling the other components of metabolic syndrome, such as blood pressure, insulin resistance and oral glucose tolerance. Despite all these beneficial effects, the use of orlistat is associated with side effects as flatulence, abdominal cramping, diarrheoa, and oily spotting, just like other lipase inhibitors [33]. All these side effects of orlistat affect the quality of life of obese patients taking the drug. Hence, it has become necessary to search for novel inhibitors that are associated with fewer or no side effects.

In this study, FRENL exhibited concentration-dependent inhibitory effects on pancreatic lipase. It had an IC_{50} of 109.20 µg/ml, which was smaller than that of the standard drug orlistat (119.50 µg/ml). The implication of this is that FRENL may be more potent than orlistat in inhibiting the activity of lipase. Hence, the same side effects produced by orlistat might be observed with FRENL.
High blood pressure is another component of metabolic syndrome, causing several serious diseases such as heart failure, kidney failure and stroke [34]. A number of management options exist for hypertensive patients, ranging from diet adjustment, lifestyle modifications, use of herbs and chemotherapy. The chemotherapeutic option involves the use of calcium channel blockers, such as nifedipine, diuretics, such as moduretic, β-blockers, such as propranolol, and ACE inhibitors, such as lisinopril [34]. ACE is an enzyme that catalyses the conversion of angiotensin I (inactive) to angiotensin II, which is a very potent vasoconstrictor and metabolizes bradykinin. An increased ACE activity results in an increased concentration of angiotensin II and decreased concentration of bradykinin, thereby leading to hypertension [35]. As such, compounds that can inhibit the action of ACE can serve as therapeutic agents to manage hypertension. Medicinal plants contain important phytochemicals capable of inhibiting the action of ACE [35].

In this study, FRENL was found to exhibit ACE inhibitory activity comparable to that of lisinopril. Plant-based ACE inhibitors have therapeutic potential in treating hypertension and

other anomalies associated with diabetes. Most ACE inhibitory plant metabolites phenolics, flavonoids, terpenoids, and alkaloids, which were present in FRENL. Particularly, flavonoids. This extract, therefore, may be useful in ameliorating the hypertension component of metabolic syndrome.

GC-MS analysis revealed two important flavonoids, kaempferol and quercetin. Molecular docking of these compounds against the four key targets (alpha-amylase, alpha-glucosidase, lipase, and ACE) in the pathophysiology of metabolic syndrome showed strong affinity between the compounds and the molecular targets. The possible inhibition of alpha-amylase and alpha-glucosidase by FRENL could be useful in blunting post-prandial hyperglycaemia. Inhibition of lipase is a useful way to reduce obesity by preventing the breakdown of triglycerides, while the inhibition of ACE ameliorates hypertension.

5. Conclusion

This study showed the anti-hyperglycaemic, anti-obesity and anti-hypertensive potentials of the leaves of *Newbouldia laevis*. This validates the traditional use of this plant in the management of metabolic syndrome. The findings of this study also suggest that the flavonoid-rich extract of *Newbouldia laevis* leaves contains bioactive compounds with multi-target potential (hyperglycaemia, obesity, and hypertension). Further *in vivo* and clinical studies are recommended to validate these effects and explore therapeutic applications in metabolic syndrome management.

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7. Statements on compliance with ethical standards and standards of research involving animals

Not applicable

8. Disclosure and conflict of interest

The authors declare that they have no conflicts of interest.

References

[1] A. Alembagheri, H. Hajimehdipoor, R. Choopani, and S. Esmaeili, "The role of selected medicinal plants from Iranian traditional medicine for the treatment of fatigue in metabolic syndrome," *Traditional Medicine Research,* vol. 8, no. 4, pp. 23-30. 2023.

[2] E. L. Chávez-Delgado, D. A. Jacobo-Velázquez, and O. Essential, "Recent advances on their dual role as food preservatives and nutraceuticals against the metabolic syndrome," *Foods*, vol. 12, no. 1, pp. 1079-1085, 2023.

[3] S. Dobhal, M. F. Singh, S. Setya, and S. Bisht, "Comparative assessment of the effect of lemongrass (*cymbopogon citratus*) ethanolic extract, aqueous extract and essential oil in high fat diet and fructose induced metabolic syndrome in rats," *Indian Journal of Pharmaceutical Education and Research,* vol. 56, no. 2s, pp. s281–s293, 2022.

[4] E. Hameed and L. Al-Ameri, "Surrogates markers of insulin resistance," *Al- Kindy Colledge Medical Journal*, vol. 18, pp. 1–2, 2022.

[5] G. Nigussie, "A review on traditionally used medicinal plants for scabies therapy in Ethiopia," *Advanced Traditional Medicine*, vol. 21, no 2, pp. 199–208, 2021.

[6] M. A. Tola, F. Ibrahim, H. Melak, T. Tafesse, M. Alemayehu , and G. Nigussie, "Traditional

herbal remedies in the management of metabolic disorders in Ethiopia: a systematic review of ethnobotanical studies and pharmacological activities," *Evidence-Based Complementary and Alternative Medicine*, vol. 2023, no. 1, pp. 1-15, 2023.

[7] O. Adeniyi, L. Washington, C. J. Glenn, S. G. Franklin, A. Scott, and M. Aung, "The use of complementary and alternative medicine among hypertensive and type 2 diabetic patients in Western Jamaica: a mixed methods study," *Public Library of Science ONE*, vol. 16, no. 2, e0245163, 2021.

[8] E. Nasiri, F. Jafari, and R. Nasiri, "Evaluation of attitude and use of complementary and alternative medicine in doctoral graduates of Mazandaran university of medical science," *Journal of Complementary Medicine*, vol. 13, no.1, pp. 52–59, 2023.

[9] M. H. Fadhel and A. F. Hassan, "Protective effect of omega-7 against doxorubicin-induced Cardiotoxicity in Male Rats," *Iraqi Journal of Pharmaceutical Science*, vol. 32, no. 3, pp. 35-40, 2023.

[10] F. Nainu, A. Frediansyah, S. Mamada, A. Permana, M. Salampe, and D. Chandran, "Natural products targeting inflammation-related metabolic disorders. A comprehensive review," *Heliyon*, vol. 9, no. 6, e16919, 2023.

[11] F. S. Youssef, E. Ovidi, and M. Rai, "Editorial: natural product-based drugs that control obesity and other disorders that trigger and provoke inflammation," *Frontiers in Pharmacology*, vol. 2022, no. 13, 891496, 2022.

[12] S. Sabarathinam, S. K. R. Chandra, and T. Mahalingam Vijayakumar, "Necessity of herbal medicine in the management of metabolic syndrome," in Lifestyle-related diseases and metabolic syndrome (London, UK: IntechOpen). doi:10. 5772/intechopen.105199, 2022.

[13] A. Murtala and A. Akindele, "Anticonvulsant, Muscle Relaxant and In-vitro Antioxidant Activities of Hydro-ethanol Leaf Extract of *Newbouldia laevis* Seem. (Bignoniaceae) in Mice," *University of Lagos Journal of Basic Medical Science*, vol. 6, no. 1 & 2, pp 1-15, 2021.

[14] Y. F. J. Chu Sun, X. Wu, and R. H. Liu, "Antioxidant and antiproliferative activities of common vegetables," *Journal of Agriculture and Food Chemistry*, vol. 50, no. 2002, pp. 6910–6916, 2002.

[15] J. B. Harborne, "Phytochemical Methods. A Guide to Modern Technology of Plant Analysis," 3rd Edn. Chapman and Hall, New York. pp. 88-185. 1998.

[16] G. E. Trease, and M. C. Evans, "Pharmacognosy". 13th Edn. Bailiere, Tindall. pp. 200- 775, 2002.

[17] I. G. Munteanu and C. Apetrei, "Analytiical methods used in determining antioxidant activity: A review. *International Journal of Molecular Science,* vol. 22, pp. 3380, 2021.

[18] O. Ishola, I.E. Ojo, E. T. Babatunde, I. A. Temitayo, T. O. Fabiyi, and A. A. Adeyanju, "Determination of Antioxidant Capacity in Aqueous Extracts of Corymbia citriodora using DPPH, ABTS, FRAP, TPC and Hydrogen Peroxide Assays" *Asian Journal of Research in Biochemistry*, vol. 15, no. 3, pp. 171–178, 2025.

[19] M. Huang, Q. Xiao, Y. Li, M. Ahmad, J. Tang, Q. Liao, and C. Tan "IInhibition of alpha-amylase activity by quercetin via multi-spectroscopic and molecular docking approaches" *Food Bioscience*, vol. 61, pp. 104951, 2024.

[20] Z. Zumaider, N. Asmilia, S. Saudah and M. Husnah, "*In vitro* alpha-glucosidase inhibitory effect of Etlingera elatior ethanol extract growing in Gayo Highland, Aceh Province, Indonesia," *F1000Research*, vol. 2024, no. 13, pp. 489, 2024.

[21] J. Y. Jeong, Y. H. Jo, K. Y. Lee, S. G. Do, B. Y. Hwang, and M. K. Lee, "Optimization of pancreatic lipase inhibition by *Cudrania tricuspidata* fruits using response surface methodology," *Bioorganic and Medicinal Chemistry Letters*, vol. 24, no. 10, pp. 2329–2333, 2014.

[22] X. Y. Zu, Y. N. Zhao, Y. Liang, Y. Q. Li, C. Y. Wang, X. Z. Zhao, and H. Wang "Research on the screening and inhibition angiotensin converting enzyme (ACE) inhibitory peptides from tuna dark muscle," *Food Bioscience*, vol. 59, pp. 103956, 2024.

[23] W.L. DeLano, "The PyMOL Molecular Graphics System", Delano Scientific, San Carlos. 2002.

[24] O. Trott and A. J. Olson, "AutoDock Vina : Improving the Speed and Accuracy of Docking with a New Scoring Function , Efficient Optimization , and Multithreading," *Journal of Computational Chemistry*, vol. 31, 455–461, 2010.

[25] MOE (The Molecular Operating Environment), Chemical Computing Group Inc. Available from: http://www.chemcomp.com., 2015.

[26] K. Madan, S. Paliwal and S. Sharma, "Metabolic Syndrome: The Constellation of Co-morbidities, A Global Threat," *Endocrine, Metabolic and Immune Disorders- Drug Targets*, vol. 23, no. 2023, pp. 1491-1504, 2023.

[27] Y. Zhou and J. Gao, "Association between metabolic score for insulin resistance and cardiovascular disease mortality in patients with rheumatoid arthritis: evidence from the NHANES 1999-2018," *Frontiers in Endocrinolology* (Lausanne), vol. 15, no. 2024, pp.1444800, 2024.

[28] M. Alemany, "The Metabolic Syndrome, a Human Disease," *International Journal of Molecular Science*, vol. 25, no. 2024, pp. 2251, 2024.

[29]| X. Ma, B. Ji, W. Du, and S. Shi, "METS-IR, a Novel Simple Insulin Resistance Index, is Associated with NAFLD in Patients with Type 2 Diabetes Mellitus," *Diabetes, Metabolic Syndrome and Obesity*, vol. 17, no. 2024, pp. 3481-3490, 2024.

[30] J. M. Brimson, M. I. Prasanth, K. K. Kumaree, P. Thitilertdecha, D. S. Malar, T. Tencomnao, and A. Prasansuklab, "Tea Plant (*Camellia sinensis)*: A Current Update on Use in Diabetes, Obesity, and Cardiovascular Disease," *Nutrients*, vol. 2023, no. 15, pp. 37, 2023.

[31] V. Sukhikh, A. Larichev, T. Ivanova, S. Prosekov, and A. Dmitrieva, "Isolation of the main biologically active substances and phytochemical analysis of *Ginkgo biloba* callus culture extracts" *Molecules,* vol. 28, no. 4, pp. 1560, 2023.

[32] C. M. Claro-Cala , O. F. Jim´enez-Altay, S. Zagmutt, and R. Rodriguez-Rodriguez, "Molecular mechanisms underlying the effects of olive oil triterpenic acids in obesity and related diseases," *Nutrients, vol.* 14, no. 8, pp. 1606, 2022.

[33] M. M. Bayliak, T. R. Dmytriv, A. V. Melnychuk, N. V. Strilets, K. B. Storey, and V. I. Lushchak, "Chamomile as a potential remedy for obesity and metabolic syndrome,"*Experimental and Clinical Sciences Journal,* vol. 20, pp. 1261–1286, 2022.

[34] G. Derosa, A. D'angelo, P. Maffioli, L. Cucinella, and R. E. Nappi, "The Use of *Nigella sativa* in Cardiometabolic Diseases," *Biomedicines*, vol. 2024, no. 12, pp. 405, 2024.

[35] N. Iftikhar, A. I. Hussain, T. Fatima, B. Alsuwayt, and A. K. Althaiban, "Bioactivity-Guided Isolation and Antihypertensive Activity of *Citrullus colocynthis* Polyphenols in Rats with Genetic Model of Hypertension," *Medicina*, vol. 59, no. 1, pp. 1880, 2023.