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Research Article

Unveiling the Potential of *Dombeya buettneri* K.Schum As a Drug Agent: Molecular Docking of Compounds With Enzymes Linked to Diabetes and Neurodegenerative Disease

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Abstract: This study investigated the mechanisms of *Dombeya buettneri* (snowball leafy) extracts on diabetes, hypertension, and neurodegenerative diseases. It compared extracts obtained using steep water (a by-product of corn wet milling) and aqueous water. The extracts were analyzed for total phenol, total flavonoid content, antioxidant activities such as FRAP, ABTS, NO, DPPH, iron-induced lipid peroxidation assay, degradation of deoxyribose (Fenton's reaction), and enzymes [α-amylase, α-glucosidase, angiotensin-I-converting enzyme, acetylcholinesterase, and butyrylcholinesterase] inhibition effects through in-vitro assays. High-performance liquid chromatography was used to identify the compounds presence in the freeze- dried extracted samples while in-silico molecular interactions between the compounds and the enzymes of interest were investigated. Results revealed no significant differences ($p>0.05$) between extraction methods in the enzyme inhibition and antioxidant properties. High-performance liquid chromatography (HPLC) identified 15 major polyphenolic compounds, with rutin emerging as the lead compound. Molecular docking and dynamic simulations showed that rutin had the highest binding energy with disease-related proteins and excellent drug-likeness properties of 100% ADMET (absorption, distribution, metabolism, excretion, and toxicity) suitability. The study concludes that *Dombeya buettneri* leafy plant is a promising rutin-rich plant with potential therapeutic applications for type 2 diabetes and neurodegenerative diseases, regardless of the extraction method used.

Keywords: *Dombeya buettneri*, Medicinal Plants, Neuroprotection, Enzymes, Molecular Docking

Introduction

Radicals species such as superoxide, oxygen radical, hydroxyl, peroxyl radical, and nitric oxide have been found to worsen diabetes by oxidizing glucose glycating proteins non-enzymatically and degrading glycated proteins oxidatively, as well as to cause other complications related to neurotransmission One of the therapeutic approaches to treating diabetes is to reduce postprandial hyperglycemia (Hinnen, 2015) through inhibiting α-glucosidase and α-amylase enzymes in the digestive tract, thereby delaying carbohydrate digestion and prolonging overall carbohydrate digestion time resulting in a reduction in the postprandial plasma glucose rise (Giuntini *et al.*, 2022). Hypertension, or HBP, is one of the long-term complications of type-2 diabetes (Chiriacò *et al.*, 2019). The renin-angiotensin system (RAS) plays a crucial role in blood pressure regulation in humans where there is release of angiotensin II, a potent vasoconstrictor inhibiting this process is considered a therapeutic approach for HBP management (Adeloye *et al.*, 2021).

The purinergic pathway has been found to have impaired neurotransmission in untreated diabetes, resulting in harm to brain neurons (Nwanna *et al.*, 2019).

Type 1 or 2 diabetes occurs due to low or no insulin release, which affects the proper functioning of the central nervous system due to insufficient neurotransmitters such as acetylcholine (AchE) synthesis (Paul *et al.*, 2022). Hyperglycemia-induced neurodegenerative disorders of AChE and Butyrylcholinesterase (BChE) enzymes in a diabetic model have been confirmed in a previous study of (Nwanna *et al.*, 2019). Untreated diabetes can result in brain impairments due to its complex pathogenesis. Consequently, demand for natural products as alternatives to synthetic drugs, offering multifunctional benefits, affordability, accessibility, and minimal side effects.(Rajamani, 2014).

Dombeya buettneri K., a tropical plant from the Malvaceae family known as the snowball, is found in West and Central Africa. While previous studies have reported its potential to lower gastric acid secretion and blood pressure in animal models (Okwari *et al.*, 2000), its mechanisms of action and broader pharmacological potential remain largely unexplored.

This study advances current knowledge by investigating the effects of D. buettneri extracts obtained using solvents (aqueous water and steep water) on key in-vitro parameters, including antioxidant, anti-diabetic, and neuroprotective properties. Unlike previous research, this study integrates analysis using HPLC to identify the chemical compounds in the leafy extract while computational approaches [molecular docking, Molecular Dynamics (MD) simulations] and post-MD analyses, including ADMET profiling on the known compounds which were docked with our proteins of interest. These in-silico techniques provide deeper insights into the binding interactions between identified bioactive compounds and specific enzyme targets, elucidating their potential therapeutic mechanisms. By combining experimental and computational analyses, this study not only validates the plant's bioactivity but also offers novel scientific evidence supporting its potential for drug discovery particularly in managing type 2 diabetes and neurodegenerative diseases.

Materials and Methods

Sample Collection

Dombeya buettneri K. (DBK) was obtained from FUTA botanical garden Ondo State, Nigeria with location Latitudes 7° 17′ 0″ N -7° 19′ 0″ N and Longitudes 5° 7′ 0″ E -5° 9′ 0″ E in April, 2023 (Adebiyi *et al.*, 2020) . The plant identity was validated by Mr Omomoh of the Department of Forestry and Wood Technology of the same institution with herbarium number IFE-17690 granted and deposited in the herbarium library. The leaves were collected into a sterile plastic bags plucked, washed under running water, dried to constant weight for five days, blended and stored in a container at room temperature 25 °C.

Aqueous and Steep Water Extract Preparation

An extracted sample of DBK (50g) was dissolved in 700 ml of aqueous and steep water (obtained from fermented corn) separately. It was incubated for six hours with shaking, following filtering using cheesecloth which was centrifuged at 1000 rpm for 10 minutes while the supernatant was freeze-dried and stored until use.

The total phenol content was determined following a method of Singleton *et al.* (2003).

Total Phenol Content

Gallic acid was used as a control, and the total phenol content was calculated as the gallic acid equivalent.

Total Flavonoid Content

The total flavonoid content was determined according to the method by Meda *et al.* (2005), quercetin was used as standard equivalent.

Ferric Reducing Property

The ability of the plant extracts to reduce $FeCl_3$ solution as described by Oyaizu (1986) while the ferric-reducing antioxidant property was calculated as an ascorbic acid equivalent.

ABTS Scavenging Ability

The ABTS scavenging ability of the aqueous extracts was determined using the described method (Re *et al.*, 1999). While the trolox equivalent antioxidant capacity was calculated.

Nitric Oxide (NO) Scavenging Ability

The Griess reagent method, as outlined by Nwanna *et al.* (2013) was employed to conduct the nitric oxide scavenging assay.

DPPH Scavenging Ability

The ability of the extracts to scavenge free radicals against the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was evaluated following the method described by Gyamfi *et al.* (1999).

Degradation of Deoxyribose (Fenton's Reaction)

The method is used to determine extract inhibitory potential against decomposition of deoxyribose induced by Fe^{2+}/H_2O_2 based on the procedure outlined by Halliwell and Gutteridge (1981).

Enzymatic Inhibition Assays

α-Amylase Inhibition Assay

The α-amylase inhibition activity of the phenolic extract was carried out according to the method described by Nwanna *et al.* (2019).

α-Glucosidase Inhibition Assay

The Apostolidis *et al.* (2007) method was used to determine α-glucosidase inhibition activity of the phenolic extract

Angiotensin-I-Converting Enzyme (ACE) Inhibition Assay

ACE inhibition was assayed by a spectrophotometric using the method of Cushman and Cheung (1971).

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Inhibition Assay

A modified colorimetric method of assessing AChE and BChE inhibition was used according to (Smith *et al.*, 1994).

Iron-Induced Lipid Peroxidation Assay

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al.* (1979).

Quantification and Characterization of Bioactive Compounds Using HPLC

Phenolic Compounds Extraction

Stage 1: 50 mg of the sample was extracted with 5 ml of 1M NaOH for 16 hours on a shaker at ambient temperature, as described by Provan *et al.* (1994). Extract was centrifuged (5000 x g), rinsed with water, centrifuged again all the supernatants were combined, placed in a disposable glass test tube and heated at 90 °C for 2 hrs to release the conjugated phenolics compounds as reported by Whitehead *et al.* (1983). The heated extract was cooled, titrated with 4 M HCl pH <2.0, diluted to 10 ml with deionized water, and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification, and the residue was extracted further in stage 2.

Stage 2: The residue from stage 1 (described above) was extracted with 5ml of 4 M NaOH, heated to 160°C in Teflon as described (Provan *et al.*, 1994). The mixture was filtered after cooling. Supernatant was collected, and the residue was washed with deionized water. The supernatant collected was adjusted to pH<2.0 with 4 M HCL, after which the filtrate was used in the purification (Provan *et al.*, 1994) along with standard purchased phenolics the Provan *et al.* (1994) method was used for downstream purification and identification of the types of polyphenols in the extract.

Drug-Likeness Pharmacokinetics Property Evaluation and Admet Properties Evaluation

The drug-likeness of the hit compounds were evaluated using the Lipinski's rule of five (Ro5), the parameters of this rule were calculated using the QikProp module of Maestro (Shafiq *et al.*, 2024.) while the ADMET profiles were evaluated using the same module.

Molecular Docking

Fifteen selected compounds from snowball leaves were docked with different proteins linked to diabetes, hypertension, and neurodegeneration. These enzymes were: α-amylase, α-glucosidase, ACE, AChE, and BChE. The Schrödinger Maestro software was used for the ligand and protein preparations. The proteins (PDB IDs: 3BAJ (α-amylase), 3W37 (α-glucosidase), 4APH (ACE), 4EY7 (AChE), and IP0M (BChE)) source from the RCSB protein data bank. The protein structure was prepared using Maestro protein preparation wizard and grid generation. The two-dimensional (2D) structures of the 15 selected compounds, as depicted in Figure 1, were drawn using Chemdraw. Subsequent optimization and 3D conversion was done using Avogadro software. Molecular docking was done using Glide XP (extra precision) in Maestro (Shoukat *et al.*, 2025). Molecular dynamics simulations were further conducted of the prioritized docked complexes.

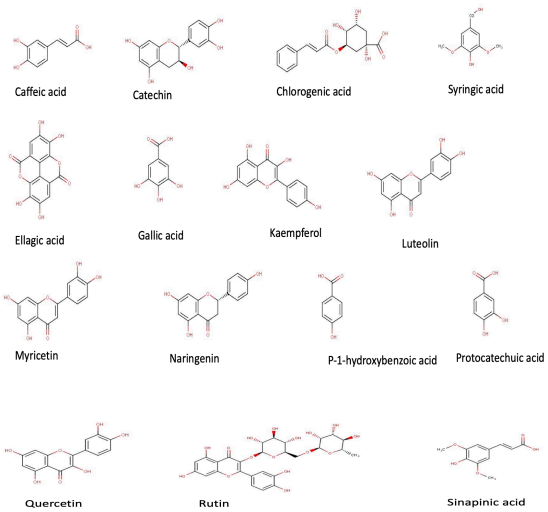

Fig. 1: 2D structures of the extract from snowball leaf

Molecular Dynamic Simulations (MDS)

MDS as a computational chemistry tool unveils biomolecular compounds structural dynamism and ligand-protein interactions. The information revealed by MDS has been helpful in interpreting the targeted compounds' structural and functional properties. The interpreted data was further assisted in the drug discovery and design processes described (Ugbaja *et al.*, 2022a; Song *et al.*, 2019). Finally, the molecular dynamics simulation was carried out for 120000 ps or 120 ns, and trajectories were used to analyze root mean square deviations (RMSD) root mean square fluctuations (RMSF) which measures the stability of the protein structures when bound with the ligand during the MD simulations timescale (Ugbaja *et al.*, 2022)

Thermodynamic Analysis

The binding energy computation analyses the endpoint landscape and provides additional essential insight into the interaction that occurs when a ligand binds with a protein to form complex. The spontaneity of reactions occurring at equilibrium states of constant pressure and temperature results in the negative Gibbs free energies (ΔG) of the system. The stability of the ligand-protein complex is relatively proportional to the magnitude of negative binding free energy (– ΔG) formed. The method described by Ugbaja *et al.* (2022a) was employed for the calculation of the binding free energies of Rutin-ACE, myricetin- AChE, rutin-α-amylase, rutin-BChE, and rutin-α-glucosidase complexes were carried out.

Molecular Mechanics and Generalized Born Surface Area (MM-GBSA) Calculation

The MM-GBSA module in Prime was employed to compute the binding free energy (Gbind) for the rutin with complexes ACE, AChE, BChE, α-glucosidase and α-amylase during MDS (Ugbaja *et al.*, 2022a).

Data Analysis

One-way Analysis of Variance (ANOVA) was used to analyze replicate results, followed by Turkey's post hoc test, with levels of significance accepted at $p<0.05$. All statistical analyses were carried out with Graph pad PRISM (V.5.0) While enzymes (proteins) of interest was carried out using an *in-silico* study (Ugbaja *et al.*, 2022).

Results and Discussion

In many developing countries, limited access to healthcare services due to high costs or geographical barriers leads communities to rely on indigenous plants for treating and managing disease conditions. For instance, in Ethiopia, approximately 80% to 90% of the population utilizes herbal medicine as their primary healthcare source. This widespread use of traditional medicine is often attributed to its accessibility, affordability, and cultural acceptance within these communities (Mutombo *et al.*, 2023). However, different solutions ranging from organic to inorganic are being employed to extract the active constituents from medicinal plants for their maxima activity. This study investigated the aqueous and steep water extracts from snowball as shown in Tables 1 and 2. The results reveal aqueous extract had higher total phenol content of (6.30mg GAE/g), while the steep extract had (4.30mg GAE/g) the same trend as observed for total flavonoid. This suggests that aqueous water seems to be a better extractant for this plant which means the chemical compounds in the plant are more soluble. It was reported that the higher the phenolic and flavonoid content of an extract, the better its antioxidant activity (Do *et al.*, 2014; Oboh *et al.*, 2016). A report from Nwanna *et al.*, 2019, also confirms that flavonoids are the most abundant polyphenols in human diets which come from compounds such as flavonoids, isoflavonoids, flavones, anthocyanins, catechin and isocathechin rather than from vitamin C, E and β-carotene (Marín *et al.*, 2004). It was noted that the *in-vitro* antioxidant potential of this study plant chemicals from the samples reduced FRAP, DPPH, NO and OH radicals as shown in Table 3 while Fig. 2 is the concentration dependent graph where the IC_{50} Table 3 was generated. This antioxidant activity is attributed to the presence of enriched bioactive compounds in the plant. Table 4 presents the EC_{50} values of aqueous and steep extracts from *Dombeya buettneri* for carbohydrate hydrolyzing enzymes (mg/mL), hypertension, and neuroprotective enzymes (Âµg/mL). This present study further identified the specific chemical compounds from the plant freeze-dried extract with HPLC while the analysis is presented in Table 5 and while the chromatogram picture could be requested from the author. The HPLC reveal that there are more flavonoids than phenols in the snowball plant. Various studies have confirmed that these bioactive compounds possess multiple pharmacological properties, including anti-inflammatory, antibacterial, anti-ulcer, anti-cholesterol, anti-hemolytic, allelopathic, antioxidant, and anticancer activities (Nwanna *et al.*, 2019; Oboh *et al.*, 2016).

Table 1: Total phenol and flavonoid content in the aqueous and Steep water extracts from *Dombeya buettneri* plant

Parameter	Aqueous Extract	Steep Water Extract
Total Flavonoid (mgQE/g)	10.21 ± 2.80^{a}	9.75 ± 2.89^{a}
Total Phenol (mgGAE/g)	6.30 ± 1.84^{a}	4.16 ± 0.21^{b}

Data represents n=3. Values with the same letter along the same row are not significantly different (p<0.05).

Table 2: ABTS and Ferric reducing property in the aqueous and Steep water extracts from *Dombeya buettneri* plant

Parameter	Aqueous Extract	Steep Water Extract
ABTS (mmolTEAC/µM)	0.33 ± 0.20^{a}	0.33 ± 0.03^{a}

Data represents n=3. Values with the same letter along the same row are not significantly different (p<0.05)

Table 3: IC_{50} of the antioxidant ability of DPPH, LPO, NO of the aqueous and Steep water extracts from *Dombeya buettneri* plant in (mg/mL)

Parameter	Aqueous Extract	Steep Water Extract
DPPH	8.13 ± 0.11^{a}	8.12 ± 0.12^{a}
LPO	8.46 ± 0.20^{a}	9.24 ± 0.17^{a}
NO	0.08 ± 0.01^{a}	0.08 ± 0.01^{a}

Data represents n=3. Values with the same letter along the same row are not significantly different (p<0.05).

Oxidative stress, driven by free radical damage to cellular components, contributes to age-related pathologies such as brain dysfunction, cancer, and cardiovascular disease. (Akbari *et al.*, 2022). Inhibting free radicals from with antioxidants from natural plant products could prevent free radical-induced degenerative diseases (Martemucci *et al.*, 2022). Additionally, studies indicate that uncontrolled hyperglycemia contributes to

the generation of free radicals, which can dysregulate key enzymes, including α-amylase, α-glucosidase, angiotensin-converting enzyme (ACE), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE), when left unmanaged (Nwanna *et al.*, 2019). Antioxidants from plant products can inhibit or reduce radicals generated during oxidation, a mechanism that has been explored as a therapeutic strategy for managing postprandial hyperglycemia (Nwanna *et al.*, 2019) and in the modulation of neuronal enzymes in neurodegeneration or Alzheimer- like symptoms (Ramli *et al.*, 2020).

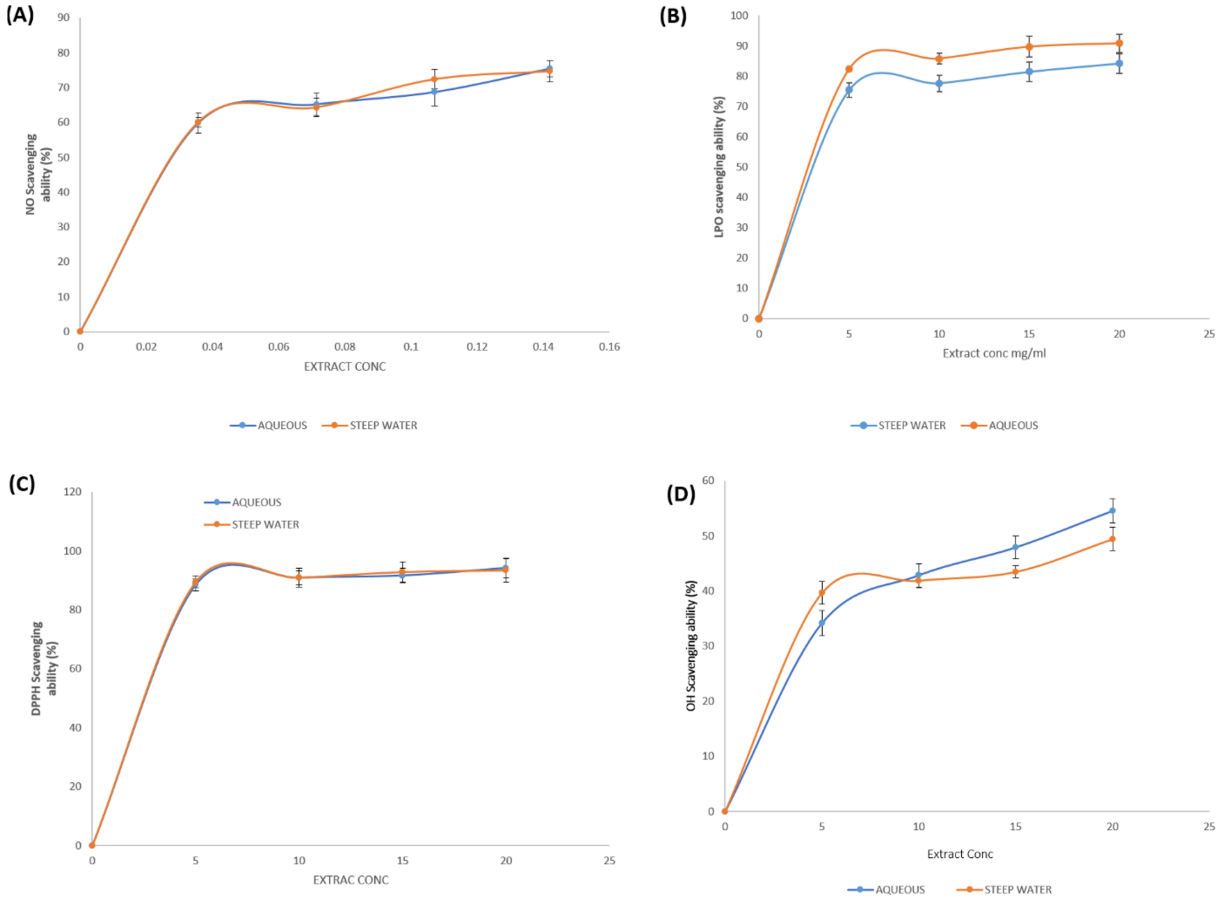

Fig. 2: (A) Nitric oxide (NO) scavenging ability; (B) Lipid peroxidation scavenging ability; (C) 1,1-diphenyl–2 picrylhydrazyl (DPPH) free radical scavenging ability; and (D) Hydroxyl (OH) radical scavenging ability of *Dombeya buettneri* obtained through both steeping and aqueous methods.

Table 4: EC_{50} values of aqueous and steep extracts from *Dombeya buettneri* on carbohydrate hydrolyzing enzymes (mg/mL), hypertension and neuroprotective enzymes (µg/mL)

Parameter	Aqueous Extract	Steep Water Extract
α-glucosidase	8.10±0.03[a]	7.34±0.03[a]
α -amylase	6.23±0.03[a]	4.94±0.03[a]
ACE	3.81±0.01[a]	3.19±0.10[a]
AChE	3.51±0.13[a]	3.60±0.10[a]
BChE	4.84±0.09[a]	4.88±0.07[a]

Values with the same letter along the same row are not significantly different ($p<0.05$).

Fig. 3 illustrates the antioxidant activities of aqueous and steep water extracts from *Dombeya buettneri*, assessed through four assays: (A) nitric oxide (NO) scavenging, (B) lipid peroxidation inhibition, (C) DPPH radical scavenging, and (D) hydroxyl (OH) radical scavenging.

Table 5: Polyphenolic compounds (mg/100g) in Snowball leaf

Phenolic	Concentration (mg/100g)
Protocatechuric acid	17.12
Gallic acid	5.67
Catechin	1.28
p-hydroxybenzoic acid	11.57
Caffeic acid	20.59
Syringic acid	4.83
Naringenin	2.32
Sinapinic acid	9.62
Kaempferol	33.06
Ellagic acid	5.63
Quercetin	57.31
Myricetin	1.13
Chlorogenic acid	20.43
Rutin	12.51
Ferulic acid	20.58
Luteolin	4.58

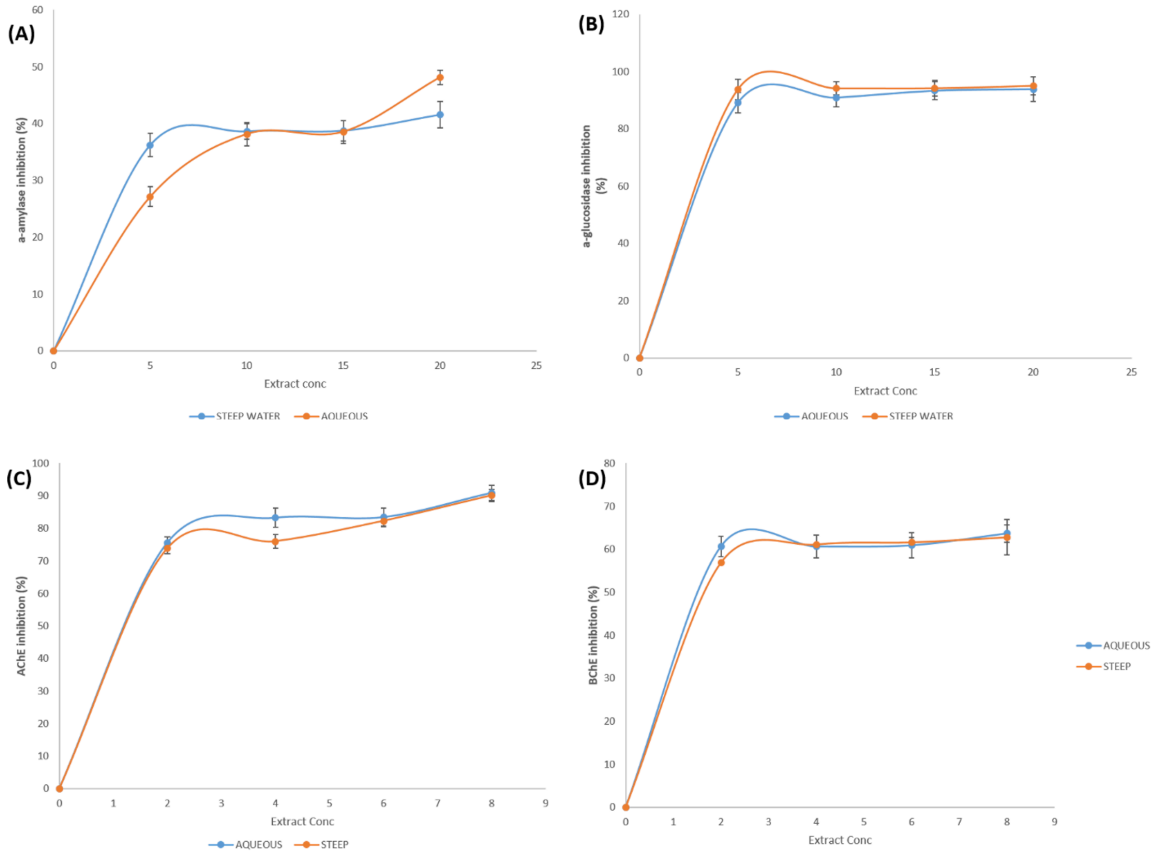

Fig. 3: The antioxidant activities of aqueous and steep water extracts from *Dombeya buettneri*, as determined by; (A) nitric oxide (NO) scavenging ability; (B) lipid peroxidation scavenging ability; (C) 1,1-diphenyl–2 picrylhydrazyl (DPPH) radical scavenging ability, and (D) hydroxyl (OH) free radical scavenging ability.

Molecular Docking

Molecular docking as a computational chemistry method has been helpful in predicting the different conformations, poses, and scoring of ligands in receptor complexes. The docking scores depict how tight the conformation of each ligand is bound at the receptor's active site. Conformations or orientations with higher negative binding scores in kcal/mol suggest better binding energies. The docking results, as depicted in Table 6, show that rutin predominantly maintained higher docking scores with all the proteins except AChE where myricetin exhibited a score of -12.935kcal/mol. Rutin exhibited the highest docking score of -13.062 kcal/mol with BChE. The high binding energies exhibited by α-amylase-rutin (-10.855), α-glucosidase-rutin (10.130), ACE-rutin (-12.033), AChE-rutin` (-10.598), and AChE-rutin (-13.062) kcal/mol, respectively, suggests that rutin could be an effective treatment for diabetes, hypertension, and neurodegenerative diseases. Rutin (3,3,4′5,7-pentahydroxyflavone-3-rhamnoglucoside) as a flavonol extract from plants such as snowball. Chemical components of rutin included disaccharide rutinose, glycoside, and flavonolic aglycone quercetin (Ganeshpurkar and Saluja, 2017). To substantiate rutin's ability to bind to and inhibit these enzymes, we conducted MDS, a binding free energy analysis (Table 7), and an examination of the energy contributions made by each amino acid when forming a complex with rutin.

Table 6: Molecular Docking Results of compound with five enzymes

Compounds	Docking Scores (kcal/mol)				
	α-amylase	α-glucosidase	ACE	AChE	BChE
Rutin	-10.855	-13.062	-12.033	-10.598	-10.130
Gallic	-5.458	-8.167	-5.435	-8.399	-7.764
Protocatechin	-4.232	-7.887	-5.345	-8.096	-7.177
Myricetin	-9.203	-7.753	-9.078	-12.935	-9.639
Catechin	-6.731	-7.502	-7.769	-11.232	-7.699
Quercetin	-7.592	-7.323	-8.338	-11.722	-9.360
Chlorogenic	-7.445	-7.179	-5.326	-10.440	-4.446
Caffeic	-5.301	-7.018	-6.587	-7.582	-6.605
Luteolin	-7.445	-6.710	-6.943	-11.434	-8.215
Naringeni	-5.916	-6.099	-5.986	-10.513	-6.668
Kaemferol	-5.930	-5.691	-6.915	-10.528	-7.025
P-hydroxylbenzene	-3.560	-5.655	-4.252	-6.798	-5.483
Sinapinic	-5.894	-5.482	-5.593	-7.172	-6.321
Syringic	-4.089	-5.363	-5.096	-7.525	-5.752
Ellagic	-7.994	-5.153	-7.884	-12.136	-8.373

Binding Free Energy Docked Complexes

The calculated lowest binding free energies of -65.495 kcal/mol for α-glucosidase and -53.104 kcal/mol for α-amylase with rutin demonstrate that rutin has a strong affinity to bind with these enzymes' active sites, indicating its usefulness as an anti-diabetic compound. Conversely, the low van der Waals (-8.508 kcal/mol) and binding free energies (-0.658 kcal/mol) of ACE with rutin predicted that rutin atoms do not bind strongly with the enzyme's active site, indicating that it is unlikely to be an effective anti-hypertensive compound. Additionally, the results of the MMGBSA energy analysis (Table 7) align with the experimentally determined IC_{50} values of the extract with the enzymes. Rutin highest binding free energy of -65.495 kcal/mol determined in silico for α-glucosidase corresponds to an experimental IC_{50} value of 0.038μM. The lowest in silico binding free energy of rutin with angiotensin-1-converting enzyme corresponds to an experimental IC_{50} value of 64μM, as depicted in Table 7.

Table 7: MMGBSA binding free energy contributions of rutin in complex with ACE, AChE, BChE, α-glucosidase and α-amylase

Complexes	ΔG_{vdw}	ΔE_{ele}	ΔG_{sol}	ΔE_{gas}	ΔE_{bind}	Exp IC_{50} value
ACE	-8.508	-111.731	71.623	-72.281	-0.658	64μM
AChE	-36.960	-58.580	47.756	-95.540	-47.783	9.96μM
BChE	-62.640	-41.148	54.077	-103.788	-49.711	29μM
α-glucosidase	-46.360	-100.353	81.218	-146.712	-65.495	0.038μM
α-amylase	-45.447	-73.268	65.611	-118.715	-53.104	0.048μM

Receptor-Ligand Interactions (Snapshot Analysis)

Structural characteristics of compounds affect the type of contact they form with the amino acid residues of the enzymes (Kumar *et al.*, 2020). Fig. 4 displays the snapshots of the examined complexes collected at 60 000 picoseconds of the 120 000 MD simulations; it also shows binding cavity residues of the ligands that interact with the enzymes within 5.0 Å. The attractions comprise mostly of Van der Waals forces, a-a stacking and hydrogen bonds. The function chemical bonds perform in ligand-receptor interaction is particularly significant in the stability of biomolecular complexes (Ugbaja *et al.*, 2022a).

Additionally, the ADMET properties shows that Rutin to be the hit compound from QplogKhsab (1.324) and Human oral absorption (100 %) results (Table 8), while the percentage of hydrogen bond occupancy tenancy analysis was done on the complexes to determine the residues responsible for the stability of the complexes, as indicated in Table 9. All the investigated complexes demonstrated various hydrogen bond (DonorH) percentage occupancy, with rutin having the greatest percentage hydrogen occupancy of 98.81% with a-glucosidase. All complexes demonstrated a varied percentage of hydrogen bond occupancy with the proteins, as indicated in Table 8. The rutin-a-glucosidase complex, GLU463 & HIS457 formed the greatest percentage occupancy of 98.81%.

The greater percentage occupancy suggests that the ligand binds with the receptor and develops a strong and durable binding. Increased percentage occupancy also signifies robust bonding of ligand-receptor at the active site. This Greater percentage occupancy implies a strong inhibitory potential of rutin in the therapy of the targeted condition (Bertalan *et al.*, 2021).

Table 8: ADMET properties of the hit compounds

S/N	Compounds	QplogKhsa[b]	%HumanOralAbsorption[c]	QPlogHERG[d]
1	Caffeic-Acid	-0.83	63.614	-2.596
2	Catechin	-0.085	58.492	-5.223
3	Chlorogenic Acid	-0.96	33.493	-5.047
4	Ellagic Acid	-1.542	43.387	-2.645
5	Ferulic Acid	-0.619	75.686	-2.714
6	Galic Acid	-0.818	60.041	-2.168
7	Kaempferol	-0.52	67.543	-4.334
8	Luteolin	-0.614	62.092	-3.587
9	Myricetin	-0.405	45.589	-4.695
10	Naringenin	-0.463	74.453	-3.105
11	P-Hydroxybenzoic Acid	-0.852	74.185	-2.004
12	ProtocatechuicAcid	-0.837	67.101	-2.085
13	Quercetin	-0.459	61.013	-4.796
14	Rutin	-1.324	100	-5.497
15	Sinapinic Acid	-0.508	65.287	-4.139
16	Syringic Acid	-0.565	67.833	-3.946

[a]*Prediction of human oral absorption on 0 to 100% scale (Range: <25% is poor; > 80% is high)*

Table 9: Percentage of Occupancy, Interactions and Average Distance of rutin with enzymes

Complexes	Acceptor	DonorH	Donor	Frames	Percentage Occupancy	Average Distance
Rutin-α-glucosidase	GLU463@0E2	HIP457@HE2	HIP457@NE2	134855	98.81	2.7074
	GLU463@OE1	HIP467@HD1	HIP467@ND1	133503	97.82	2.7265
	ASP383@OD1	HIP457@HD1	HIP457@ND1	132724	97.25	2.7394
Rutin-AChE	ASP395@OD2	TYR373@HH	TYR373@OH	123755	98.19	2.6713
	ASP172@OD1	ARG149@HH21	ARG149@NH2	123325	97.85	2.7620
	GLU325@OE2	SER226@HG	SER226@OG	122665	97.32	2.6381
Rutin-BChE	ASP390@OD1	TYR370@HH	TYR370@OH	128964	96.71	2.7294
	GLU454@OE2	SER421@HG	SER421@OG	128650	96.47	2.7071
	GLU322@OE1	HID433@HD1	HID433@ND1	128420	96.30	2.6866
Rutin-α-amylase	GLU_384@OE1	SER_340@H	SER_340@N	129662	97.81	2.7831
	ASN_398@OD1	THR_10@HG1	THR_10@OG1	129656	97.80	2.7067
	TRP_18@O	SER_72@HG	SER_72@OG	128471	96.91	2.6896
Rutin-ACE	GLU372@OE1	HIP344@HE2	HIP344@NE2	128964	90.71	2.7294
	VAL251@O	TYR211@HH	TYR211@OH	128650	90.47	2.7071
	ASP102@O	THR106@HG1	THR106@OG1	128420	90.30	2.6866

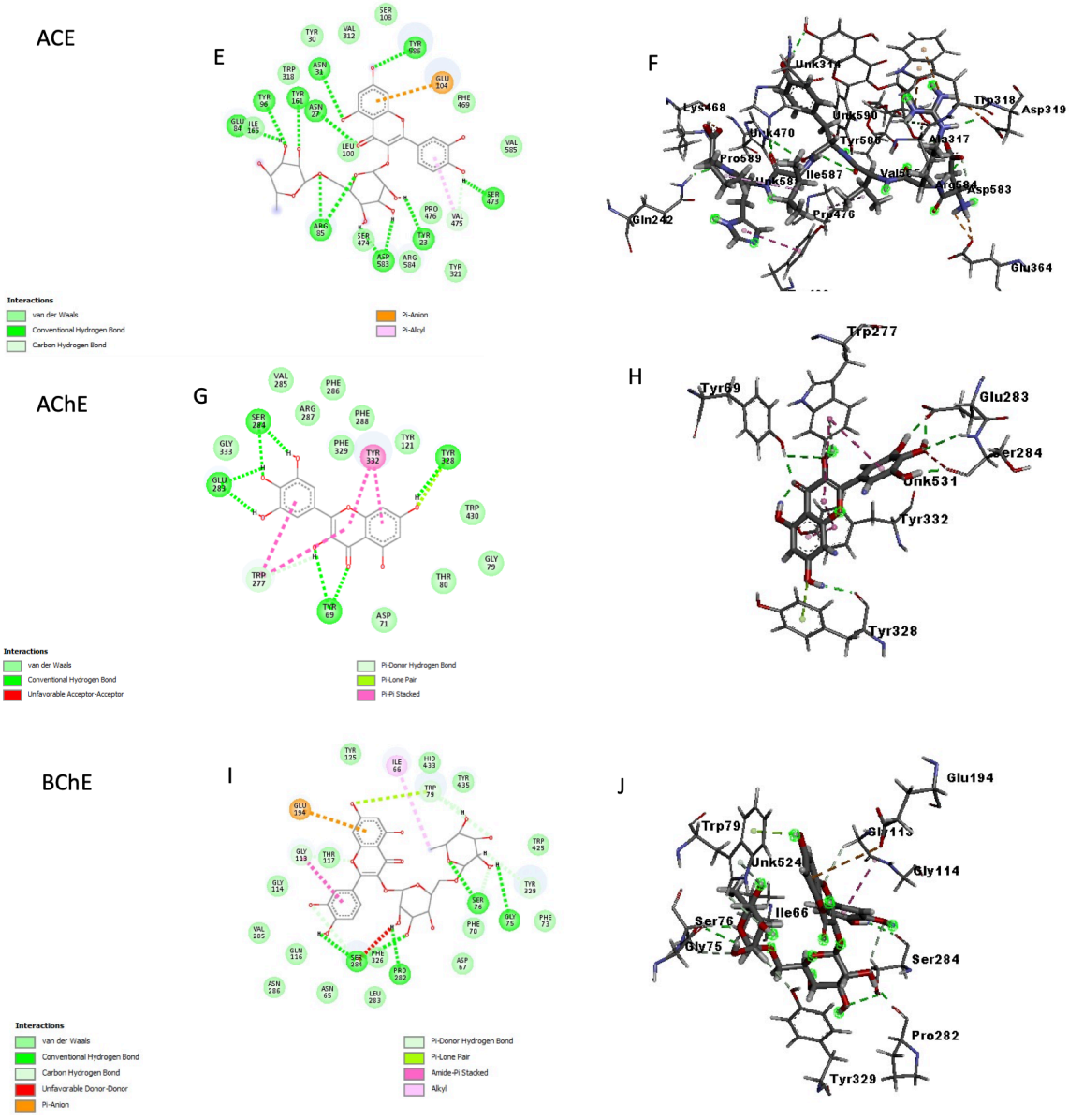

Fig. 4: 2D and 3D diagrams of snapshots at 60 000 Picoseconds MD simulations

The calculations of RMSD and RMSF of the five complexes were carried out to examine the stiffness of rutin while in complex with α-glucosidase, α-amylase, acetylcholinesterase, butylcholinestrase and angiotensin-1-converting enzyme. Figure 5B displays the RMSD of the α-glucosidase-rutin complex and apoprotein. The apoprotein and the α-glucosidase-rutin complex revealed larger RMSD values, indicative of amino acid residues wandering relative to the Cα molecules framework, as predicted. Nonetheless, both systems retained stability after 30 000 picoseconds of the simulation. The root means square deviation of less than 2.2 Å profile was maintained, which shows stable status of the simulated systems. Accordingly, RMSF of α-glucosidase-rutin complex and apoprotein, as illustrated in Figure 5A, assessed the flexibility of the amino acid residues when associated with the ligand.

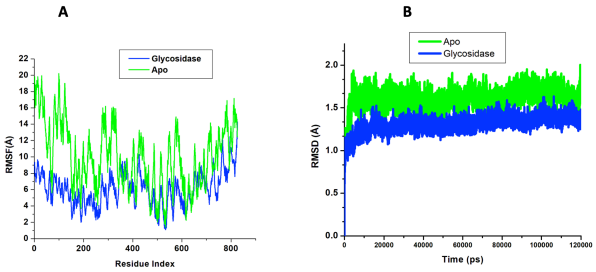

Fig. 5: Diagrams of RMSF (A) and RMSD (B) for rutin and a-glucosidase complex

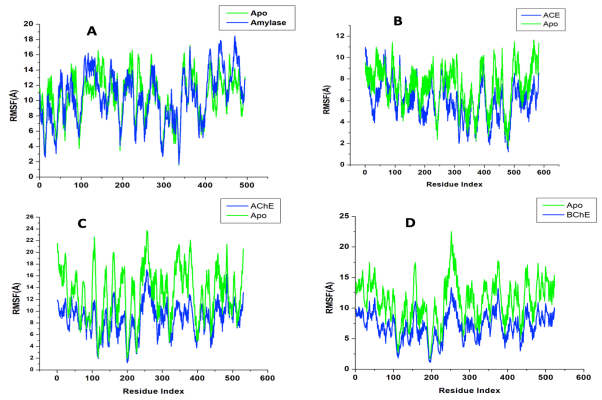

Fig. 6: Diagram of RMSF for apoprotein and a-amylase (A), ACE (B), AChE (C) and BChE (D) complexes

The average root-mean-square variations of the complexes were studied to understand the flexibility level across the amino acid residues of the systems apoproteins and α-glucosidase-rutin complex system Diagrams of RMSF (A) and RMSD (B) for rutin and α-glucosidase complex The α-glucosidase-rutin complex maintained a continuously lower RMSF value of 957Å and 196Å for apoproteins, showing that rutin binding drastically decreased the flexibility of the protein's amino acids compared to the apoprotein at 185 residue index Similar patterns were seen for rutin-AChE (Figure 6C), and rutin-BChE (Figure 6D) complexes except for rutin-ACE (Figure 6B) and α-amylase -rutin (Figure 6A) complexes where certain of the complex amino acid residues displayed larger RMSF values were 1816Å, and 165 Å for apoprotein This shows that in both rutin-ACE and rutin-α-amylase complexes, the ligands could not bind correctly, notably in the rutin-α-amylase complex

The RMSD of a-amylase (Fig. 7A), ACE (Fig. 7B), AChE (Fig. 7C) and BChE (Fig. 7D) complexes displayed initial higher RMSD values like the apoprotein and a-glucosidase-rutin complex but maintained stability after 60 000 picoseconds to the end of the 120 000 MD simulations.

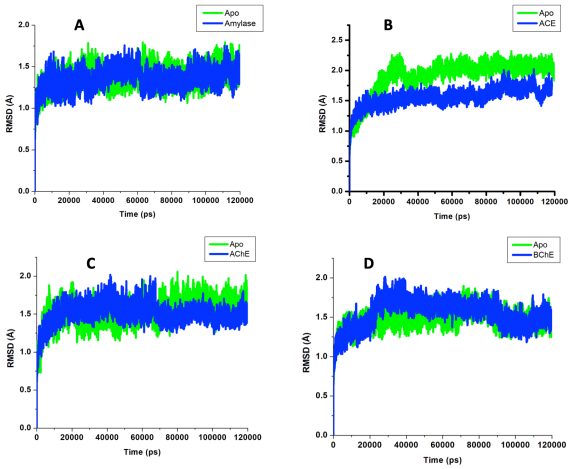

Fig. 7: Diagram of RMSD for apoprotein with a-amylase (A), ACE (B), AChE (C) and BChE (D) complexes

Discussions

In many countries, various extraction methods, including aqueous water, steep water, and organic solvents such as ethanol, are employed to isolate active constituents from medicinal plants. Scientific studies are being conducted to evaluate the therapeutic potential of these extracts and validate their efficacy against different disease conditions, as traditionally utilized in folklore medicine. Steep water is commonly used in some parts of Africa to prepare herbal infusions; however, its effectiveness compared to aqueous water remains scientifically unverified and requires further investigation. The results of HPLC in Table 1 reveal different chemical compounds in the plant extract. Further, in-vitro antioxidant investigation as shown in Tables 2-5, and Figs. 2-3 reveals that the aqueous extract had a higher total phenol content of (6.30mg GAE/g). In contrast, the steep extract had (4.30mg GAE/g) and the same trend was observed for total flavonoid. This suggests that water seems to be a better medium for extracting chemical molecules from this plant which means they are mostly hydrophilic from their functional groups such as -OH, -COOH, and -NH_2. It has been reported that the higher the phenolic and flavonoid

content of plant products extract, the better its antioxidant activity (Do *et al.*, 2014; Oboh *et al.*, 2016), because of its higher polarity also suggested that most plant foods as alternate antioxidant vitamins.

Oxidative stress resulting from the toxic effects of free radicals on tissues caused disease conditions but inhibition of such free radicals has become a central focus for research efforts designed to prevent or ameliorate tissue injury, and many studies have confirmed that antioxidants from natural plant products could prevent free radical-induced degenerative diseases (Akbari *et al.*, 2022; Martemucci *et al.*, 2022). Moreso, antioxidants from plant products could inhibit, reduce or chelate the radicals produced during the oxidation process (Nwanna *et al.*, 2013; Nwanna *et al.*, 2019) this process has reduced postprandial hyperglycemia complications modulation of neuronal enzymes in neurodegeneration or Alzheimer like symptoms It was observed that the in-vitro antioxidant potential of this study plant reduced radical species such as FRAP, DPPH, NO, OH, ABTS investigated.

The computation study analyses using different software and modelling to depicts the interactions of the bioactive compounds identified with our enzymes of interest show that structural characteristics of compounds affect the type of contact they form with the amino acid residues of the enzymes (Ramli *et al.*, 2020). This ligand-receptor interaction is particularly significant in the stability of biomolecular complexes (Ugbaja *et al.*, 2022a). Rutin from all the compounds had the highest binding affinity. All complexes demonstrated varied percentages of hydrogen bond occupancy with the proteins, as indicated in Table 8. The rutin-a-glucosidase complex, GLU463 & HIS457 formed the greatest percentage occupancy of 98.81% in Table 9. The greater percentage of occupancy suggests that the ligand binds with the receptor and develops a strong and durable binding. Increased percentage occupancy also signifies robust bonding of ligand-receptors at the active site. This greater percentage occupancy implies a strong inhibitory potential of rutin in the therapy of the targeted condition (Ganeshpurkar and Saluja, 2017).

The computational analyses (Tables 6-9) coupled with ADMET analysis found rutin the best candidate to serve as an oral drug and were evaluated based on the parameters of the rule of five (Ro5), which involves analyzing several physicochemical properties of the compounds. The results of the drug-likeness evaluation, which are presented in Tables 8 and 9, revealed that most of the compounds in this plant have value for certain parameters that are within the threshold of a drug-like compound. Specifically, rutin was found to be drug-like with no violation of the Ro5. Therefore, our findings suggest snowball plant is rutin-rich and which could serve as a promising medicinal agent for the treatment/management of metabolic disorders.

Conclusion

This study highlights bioactive compounds in *Dombeya buettneri* snowball plant regardless of the extraction solvent used. Notably, rutin was found to be abundant in the plant, reinforcing its potential as a therapeutic agent and supporting its traditional medicinal use. However, several limitations should be considered when interpreting these findings. Firstly, while in-silico predictions provide valuable insights, they are based on computational models and simulations, which may not fully reflect actual biological responses. Further experimental study is needed to validate these findings and establish the plant pharmacological efficacy.

Authors Contributions

Esther Emem Nwanna: Conceptualization, Methodology, Software, Validation, Resources, Writing - Original Draft.

Samuel Chima Ugbaja: Conceptualization, Methodology, Software, Validation, Resources, Writing - Original Draft.

Fisayo Eunice Adeyeye: Conceptualization, Methodology, Validation, Data Curation, Data Interpretation, Writing - Original Draft, Writing - Review & Editing.

Onyekachi Paschal Ezeoru: Conceptualization, Methodology, Software, Validation, Resources, Writing - Original Draft.

Awais Ali: Data Curation, Writing - Review & Editing.

Augustina Akinsanmi: Data Curation, Writing - Review & Editing.

Hezekiel M. Kumalo: Validation, Data Interpretation.

Rene Khan: Validation, Data Interpretation.

Emmanuel Okello: Validation, Data Interpretation.

Data Availability Statement

All data and Database are available on request to the authors.

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