Nig. J. Biotech. Vol. 41(2): 84-103 (Dec. 2024)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: **https://dx.doi.org/10.4314/njb.v41i2.8**

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Bioinformatic Analysis of Some Natural Antihypertensive Compounds from Medicinal Plants as Promising Inhibitory Agents Against Angiotensin-Converting Enzyme

Fawibe, O.O.[1*], Agunbiade, F. [1], Chukwurah, P.N.[2], Akinyemi, O.F. [1], Obiwole E.T.[1], Ilem, D.O.[3], Bamigboye, T.O.[1]

[1]Department of Pure and Applied Botany, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria
[2]Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria
[3]Department of Horticulture, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria

Abstract

Angiotensin Converting Enzyme (ACE) inhibition has been a promising avenue for anti-hypertensive drug development. Our study investigated the inhibitory potential of bioactive compounds derived from six medicinal plants (*Allium sativum* L., *Zingiber officinale* Roscoe, *Acalypha godseffiana* Mast., *Moringa oleifera* Lam., *Vernonia amygdalina* Delile, and *Rauvolfia vomitoria* Afzel.) against ACE using *in silico* methods. Thirty-one (31) bioactive compounds were screened while Ramipril, and Enalapril were employed as control drugs. 3D structures and canonical Simplified Molecular Input Line Entry System (SMILES) of the bioactive compounds and control drugs were obtained from the PubChem online server. Drug-likeness assessment of the bioactive compounds and protein-ligand docking of successful compounds were conducted using SwissADME online server and AutoDock Vina software. ADMET (absorption, distribution, metabolism, excretion, toxicity) analysis was also done to evaluate the suitability of the hit ligands for further drug development. Of the 31 compounds screened, 17 passed at least four of the five standard rules of drug-likeness determination, while the control drugs (Ramipril and Enalapril) failed one of the rules. Ajmaline, Apigenin, Quercetin, Cryptolepine, Luteolin, Hydroxyvernolide, Kaempferol and Vernodalol had higher binding energies of -9.6 kcal/mol, -8.7 kcal/mol, -8.5 kcal/mol, -8.4 kcal/mol, -8.4 kcal/mol, -8.3 kcal/mol, -8.3 kcal/mol and -7.8 kcal/mol, respectively than Ramipril and Enalapril (-7.6 kcal/mol, and -7.5 kcal/mol). The higher binding energies and the stability of their binding interactions denote these hit ligands as potential antihypertensive drugs targeting ACE. However, wet lab experimental investigation is necessary to validate the inhibitory activity of these compounds and elucidate their mechanisms of action.

Keywords: Phytocompounds, binding affinity, hypertension, target protein, medicinal plants
*Corresponding Author's email: fawibeoo@funaab.edu.ng

Introduction

Hypertension, commonly known as high blood pressure is one of the most prevalent diseases that affect human beings, extending widely to both developed and developing countries. It is referred to as the silent killer because it is asymptomatic in its early stages; hence,

contributed to nearly 9.4 million deaths annually (Forouzanfar et al., 2017). Hypertension is at the forefront of the factors that predispose people to various cardiovascular diseases, including stroke and heart attack (Mills et al., 2016). The World Health Organization, in 2019, put the statistics of people affected by hypertension globally at around 1.13 billion which represented about 15% of the human population. Also, 1.28 billion adults between the age class of 30-79 years have hypertension globally (Farhadi et al., 2023).

Angiotensin Converting Enzyme (ACE) has long been recognized as a prospective target for antihypertensive drug development due to its central role in regulating blood pressure and cardiovascular function. ACE plays a critical role in the operations of the "renin-angiotensin" physiological system that regulates blood pressure (Hafiz et al., 2023). The enzyme converts angiotensin I to active angiotensin II (Wong, 2016), and the active angiotensin II causes hypertension via narrowing of the blood vessels. Hence, the suppression of ACE is considered an essential approach to regulating hypertension (Atkinson and Robertson, 1979). ACE inhibitors (Ramipril and Enalapril), a class of drugs that block the activity of ACE have been widely used and proven effective in managing hypertension. However, the use of these drugs has been linked with side effects such as nausea, hyperkalemia, headache, cough, disturbances in taste, dry cough, skin rashes or erythema, taste turbulences, and the modifications in serum lipid metabolism (Sharma et al., 2016).
Therefore, there is a need for a more effective, affordable, and safer alternative natural antihypertensive drugs.

Traditional medicine utilized medicinal plants for the treatment of various ailments including hypertension. These medicinal plants are rich in diverse biologically potent compounds including those that exhibit potential antihypertensive properties (Patten et al., 2016). Harnessing the potential of plant chemical compounds for drug discovery is promising due to their bioavailability, relatively low toxicity, and traditional use (Chaachouay and Zidane, 2024).
Allium sativum L. (Garlic) is a member of the onion family and it contains sulfur compound allicin, which has been linked to various health benefits. Studies have suggested potential roles of the plant in bringing down cholesterol levels and blood pressure, as well as improving the functioning of the heart.
Zingiber officinale Roscoe (Ginger) is a rhizomatous plant whose roots are useful as both medicine and spice. A major bioactive compound found in the plant is gingerol, which imparts ginger's characteristic pungent flavor and contributes to the plant's medicinal value. For a very long time, traditional medicine has used ginger to improve food digestion and reduce nausea, vomiting, and inflammation. Shalaby et al. (2023) reported that ginger could be used to manage hypertension and help relieve muscle pain and menstrual discomfort.
Acalypha godseffiana Mast. (Copperleaf Plant), is a tropical evergreen shrub known for its colorful foliage. It has potential medicinal properties due to its rich pigmentation. Compounds present in the leaves may have antioxidant and antihypertensive properties, although further research is needed to fully understand their therapeutic potential (Asekunowo et al., 2019).
Moringa oleifera Lam. (African Moringa or Drumstick tree), is a fast-growing tree native to parts of Asia and Africa. Its leaves are rich in vitamins, minerals, and protein, making them a valuable dietary supplement. Karima et al. (2023) reported that Moringa contains antioxidant, anti-inflammatory, and cholesterol-lowering properties, as well as potential benefits for blood sugar control and wound healing.
Vernonia amygdalina Delile (Bitter Leaf) is a shrub known for its bitter taste and use in traditional medicine. Its medicinal value has been attributed to its rich content of bioactive compounds, particularly flavonoids and alkaloids. Bitter leaf has been used to treat various ailments, including malaria, diabetes, and gastrointestinal disorders. Studies have shown that extracts from bitter leaf possess anti-malarial, anti-diabetic, and antioxidant properties (Ugbogu et al., 2021).
Rauvolfia vomitoria Afzel. (Rauwolfia or Poison devil's pepper) is a flowering shrub known for its medicinal properties. The extract of the plant has been commonly prescribed by herb sellers and traditional healers for the treatment of high blood pressure and anxiety (Eluwa et al., 2010).
With the advancement of computational techniques, in silico analysis has gained prominence as a cost-effective and efficient approach to screening and predicting the interactions between bioactive compounds and specific

target proteins. It can be used to discover the binding affinities of bioactive compounds in plants to the target protein (Angiotensin Converting Enzyme) and to provide knowledge about their inhibitory abilities. Therefore, this study aimed at (i) determining some bioactive compounds with antihypertensive properties present in the selected plants using a computational approach (ii) investigating the inhibitory activities of some selected bioactive compounds against Angiotensin Converting Enzyme (ACE).

Materials and Methods

Ligands selection

This study involved six medicinal plants with reported therapeutic effects on hypertension. These included: *Allium sativum* L.*, Zingiber officinale* Roscoe*, Acalypha godseffiana* Mast.*, Moringa oleifera* Lam.*, Vernonia amygdalina* Delile*, Rauvolfia vomitoria* Afzel (Table 1). Thirty-one (31) bioactive compounds present in these plants were selected based on the reported antihypertensive properties they possessed. In addition, two commonly used drugs for the treatment of hypertension (Ramipril and Enalapril) were used as the control drugs. The PubChem identification number (Pub ID) and the Canonical smiles of the plant bioactive compounds and those of the control drugs were retrieved from a chemical repository server (PubChem web).

Table 1: Publication ID and canonical smile of bioactive compounds selected from medicinal plants and the control drugs

S/N	PLANT	COMPOUNDS	PUB ID	CANONICAL SMILE	CITATION
1	Allium sativum L.	Alliin	87310	C=CCS(=O)CC(C(=O)O)N	
		Allicin	65036	C=CCSS(=O)CC=C	El-Saber et al., 2020
		E-Ajoene	5386591	C=CCSSC=CCS(=O)CC=C	
		Z-Ajoene	9881148	C=CCSSC=CCS(=O)CC=C	
		2-Vinyl-4H-1,3-dithiin	133337	C=CC1SCC=CS1	
		Diallyl sulfide (DAS)	11617	C=CCSCC=C	
		Diallyl disulfide (DADS)	16590	C=CCSSCC=C	
		Diallyl trisulfide (DATS)	16315	C=CCSSSCC=C	
		Allyl methyl sulfide (AMS)	66282	CSCC=C	
		Quercetin	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	
		Kaempferol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	
		Apigenin	5280443	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	
2	Zingiber officinale Roscoe	Zingerone	31211	CC(=O)CCC1=CC(=C(C=C1)O)OC	
		Gingerenone-A	5281775	COC1=C(C=CC(=C1)CCC=CC(=O)CCC2=CC(=C(C=C2)O)OC)O	Shalaby et al., 2023
		6-Dehydrogingerdione	9796015	CCCCCC(=O)CC(=O)C=CC1=CC(=C(C=C1)O)OC	
		Zingiberene	92776	CC1=CCC(C=C1)C(C)CCC=C(C)C	
3	Acalypha godseffiana Mast.	Lupeol	259846	CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)C	Asekunowo et al., 2019
		Betulinic acid	64971	CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)C(=O)O	
		Caffeic acid	689043	C1=CC(=C(C=C1C=CC(=O)O)O)O	
4	Moringa oleifera Lam.	Chlorogenic acid	1794427	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	Karima et al., 2023
		β-Sitosterol	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
		Stigmasterol	5280794	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	
		Campesterol	173183	CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C	
		Kaempferol-3-O-rutinoside	5318767	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC=C(C=C5)O)O)O)O)O)O)O	

5	Vernonia amygdalina Delile	Luteolin	5280445	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O	Ugbogu et al., 2021
		Hydroxyvernolide	5281472	C=C1C2C(CC34C(O3)CCC(=CC2OC1=O)COC4O)OC(=O)C(=C)CO	
		Cryptolepine	82143	CN1C2=CC=CC=C2C=C3C1=C4C=CC=CC4=N3	
		Vernodalol	442318	COC(=O)C(=C)C1C(CC2(COC(=O)C(=C)C2C1O)C=C)OC(=O)C(=C)CO	
		4-methylumbelliferone	5280567	CC1=CC(=O)OC2=C1C=CC(=C2)O	
6	Rauvolfia vomitoria Afzel.	Serpentine	73391	CC1C2C[N+]3=C(CC2C(=CO1)C(=O)OC)C4=C(C=C3)C5=CC=CC=C5N4	Eluwa et al., 2010
		Ajmaline	6100671	CCC1C2CC3C4C5(CC(C2C5O)N3C1O)C6=CC=CC=C6N4C	
	Control Drugs (ACE Inhibitors)	Ramipril	5362129	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2C3CCCC3CC2C(=O)O	
		Enalapril	5388962	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2CCCC2C(=O)O	

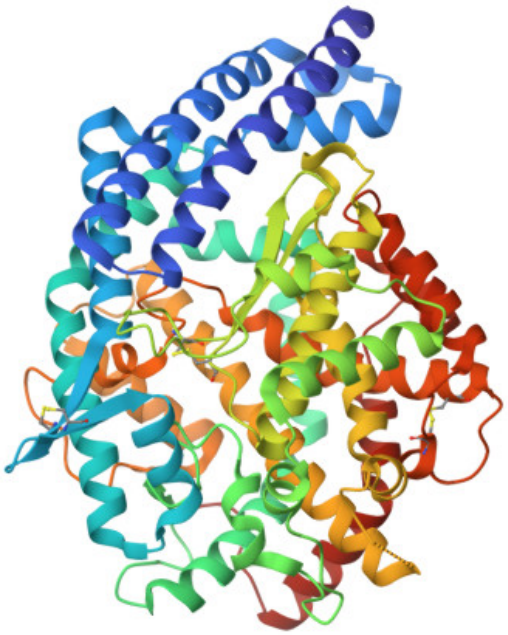


Fig 1: Three-dimensional (3D) crystallographic structure of Angiotensin Converting Enzyme (Adopted from RCSB)

Target protein selection and preparation

The three-dimensional (3D) crystallographic structure of ACE protein (Figure 1) was downloaded from the Research Collaboratory of Structural Bioinformatics (RCSB) protein databank (www.rcsb.org). The target protein was cleaned and prepared by removing water, adding hydrogens, assigning Gasteiger-Huckel charges, and was separated from co-crystallized ligands using UCSF-Chimera (Pettersen et al., 2004). The protein was subsequently minimized in preparation for molecular docking analysis.

Drug-likeness screening of bioactive compounds and the control drugs

The thirty-one bioactive compounds under study as well as the control drugs were screened for drug-likeness using SwissADME (http://swissadme.ch/) following the process of Daina et al. (2017). The molecular properties and rule of five were used to select bioactive compounds and control drugs with novel drug-like properties. The rule of five used for the assessment included: Lipinski's (Lipinski, 2016; Lipinski 2008; Lipinski et al., 2001), Ghose's (Bickerton et al., 2012), Veber's (Veber et al., 2002), Egan's (Egan et al., 2000), and Muegge's (Muegge et al., 2001) rules.

Ligand optimization and molecular docking

Ligand optimization and molecular docking analyses were performed following the method of Trott and Olson (2010). Briefly, the 3D structures of the downloaded ligands were first uploaded into PyRx's Open Babel software, followed by the optimization of the ligands to their lowest energy state using the Merck molecular Force Field (MMFF94). The AutoDock ligand format (PDBQT) was subsequently applied to the ligands and the PDBQT files of the target protein were generated using the PyRx software. Moreover, docking of ligands and the protein receptors was done using AutoDock Vina. The protein's active site was adjusted using the grid box with the following dimensions: size (x: 109.4783, y: 119.6853, z: 118.3517 angstroms), center dimension (x: 9.8163, y: -7.8331, z: -23.8252). The molecular docking process made use of the exhaustiveness of 8. The molecular docking of each

ligand and protein yielded the binding energy in kcal/mol. The PyRx software was used to determine the binding affinities of the bioactive compounds and control drugs against ACE. The docked ligands and the protein were converted from their PDBQT format to PDB files and saved for visualization.

Molecular interaction analysis

Using PyMOL molecular graphics, the ligands, and target protein were examined to create protein-ligand complexes, which were stored in PDB format (Delano, 2005). Images of the complexes were also saved. To ascertain their molecular interactions, the complexes were uploaded to the web server (https://proteins.plus) (Stierand et al., 2006) and protein-ligand interaction profiler (https://projects.biotec.tudresden.de/plip-web/plip) (Salentin et al., 2015).

Bioactivity and pharmacokinetics property prediction

Molinspiration web server (https://www.molinspiration.com) was used to ascertain the bioactivity of the compounds (Khan et al., 2017). The activity score for the GPCR ligand, nuclear receptor ligand, modulator, kinase inhibitor, protease inhibitor, ion channel, and enzyme inhibitor of ligands was determined using the online server. Bioactive compounds with activity scores more than zero (>0) are deemed active, while those with activity scores within the range of -5.0 to 0.0 exhibit moderate levels of activity. However, bioactive compounds are regarded as inactive if their activity score is less than -5.0 (< -5.0) according to Khan et al. (2017).

The ADMETlab online tool (https://admetmesh.scbdd.com/service/evaluation/cal) was used to determine the absorption, distribution, metabolism, excretion, and toxicity (ADMET) and pharmacokinetic properties of the ligands (Cheng et al., 2012; Dong et al., 2021).

Results

Drug-likeness Screening

Thirteen (13) out of the thirty-one (31) bioactive compounds from the six medicinal plants investigated passed all five rules (Lipinski's, Ghose's, Veber's, Egan's, and Muegge's) as presented in Table 2. Moreover, four (4) of the bioactive compounds and the two control drugs breached only one of the five rules. Notably, fourteen (14) bioactive compounds violated more than one of the five rules as shown in Table 2. Therefore, a total of fourteen (14) bioactive compounds with more than one violation were considered to have failed the drug-likeness screening and were exempted from molecular docking analysis with ACE (Table 2).

Molecular docking and interaction of ligands and target protein

The molecular docking results showed that out of the total seventeen (17) that passed the drug-likeness screening test, eight (8) bioactive compounds from four (4) medicinal plants had higher binding affinity against the target protein (ACE) compared with the control drugs (Table 3). Ajmaline (-9.6 kcal/mol), Apigenin (-8.7 kcal/mol), Quercetin (-8.5 kcal/mol), Cryptolepine (-8.4 kcal/mol), Luteolin (-8.4 kcal/mol), Hydroxyvernolide (-8.3 kcal/mol), Kaempferol (-8.3 kcal/mol), and Vernodalol (-7.8 kcal/mol) while Enalapril and Ramipril had binding energies of -7.5 kcal/mol and -7.6 kcal/mol, respectively (Table 3). However, the interactions between the bioactive compounds and the residues present at the active site of the protein are illustrated in Figures 2, 3, and 4.

Ajmaline established one hydrogen bond with Arg88 and interacted hydrophobically with Asp1, Arg2, Val3, Tyr26, and Ile52. Apigenin established two hydrogen bonds with Asn30 and Thr56. It also interacted hydrophobically with Asp1, Arg2, Val3, Tyr26, and Ala27. Cryptolepine established no hydrogen bond but interacted hydrophobically with Asp1, Val3, Trp23, Tyr26, and Ala27. Enalapril had four hydrogen bonds with Asn249, Ser262, Thr266 and Lys413 and interacted hydrophobically with Tyr251, Asp264, Glu340, Leu397, Phe410 and Lys413. Hydroxyvernolide established five hydrogen bonds with Ser248, Asn249, Thr265, Thr266 and Lys413 and interacted hydrophobically with Ala134, Thr135, Tyr251 and Asn338. Kaempferol had two hydrogen bonds with Asn30 and Tyr324 and interacted hydrophobically with Asp1, Arg2, Val3, Trp23, Tyr26 Ala27, Ile52 and Tyr324 residues. Luteolin established four hydrogen bonds with Asn446, Gln447, Tyr583, and Asn584 and interacted hydrophobically with Gln447 and Leu454. Quercetin had two hydrogen bonds with Asn446, and Gln447 but had no residue interaction. Ramipril established no hydrogen bond but interacted

hydrophobically with Trp450, Leu454, and Tyr583. Vernodalol had one hydrogen bond with Asp1 and had no interaction. The 2D structures of the bioactive compounds with higher binding affinity and that of the control drugs are shown in Figure 5.

Table 2: Drug-likeness results of bioactive compounds and control drugs using Swissadme

S/N	Bioactive compounds	Formula	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations
1	E-Ajoene	C9H14OS3	0	0	0	0	0
2	Z-Ajoene	C9H14OS3	0	0	0	0	0
3	Quercetin	C15H10O7	0	0	0	0	0
4	Kaempferol	C15H10O6	0	0	0	0	0
5	Apigenin	C15H10O5	0	0	0	0	0
6	Gingerenone-A	C21H24O5	0	0	0	0	0
7	6-Dehydrogingerdione	C17H22O4	0	0	0	0	0
8	Luteolin	C15H10O6	0	0	0	0	0
9	Hydroxyvernolide	C19H22O8	0	0	0	0	0
10	Cryptolepine	C16H12N2	0	0	0	0	0
11	Vernodalol	C20H24O8	0	0	0	0	0
12	Serpentine	C21H21N2O3+	0	0	0	0	0
13	Ajmaline	C20H26N2O2	0	0	0	0	0
14	Alliin	C6H11NO3S	0	0	0	0	2
15	Zingerone	C11H14O3	0	0	0	0	1
16	Caffeic acid	C9H8O4	0	0	0	0	1
17	4-methylumbelliferone	C10H8O3	0	0	0	0	1
18	Ramipril	C23H32N2O5	0	0	1	0	0
19	Enalapril	C20H28N2O5	0	0	1	0	0
20	Allicin	C6H10OS2	0	1	0	0	1
21	Diallyl trisulfide (DATS)	C6H10S3	0	1	0	0	1
22	2-Vinyl-4H-1,3-dithiin	C6H8S2	0	2	0	0	1
23	Diallyl disulfide (DADS)	C6H10S2	0	2	0	0	1
24	Zingiberene	C15H24	1	0	0	0	2
25	Diallyl sulfide (DAS)	C6H10S	0	3	0	0	2
26	Allyl methyl sulfide (AMS)	C4H8S	0	3	0	0	3
27	Campesterol	C28H48O	1	2	0	1	2
28	Lupeol	C30H50O	1	3	0	1	2
29	Betulinic acid	C30H48O3	1	3	0	1	1
30	Sitosterol	C29H50O	1	3	0	1	2
31	Stigmasterol	C29H48O	1	3	0	1	2

32	Chlorogenic acid	C16H18O9	1	1	1	1	2
33	Kaempferol-3-O-rutinoside	C27H30O15	3	4	1	1	3

Table 3: Binding energy and residue interaction of bioactive compounds and control drugs at the protein active site

S/N	Plant source	Molecule	Binding energy (kcal/mol)	Number of hydrogen bond (s) formed	Residues involved in hydrogen bond formation (Å)	Residues involved in hydrophobic interaction (Å)	Residues involved in π-stacking (Å)	Residues involved in π-cation interaction (Å)
1	Allium sativum L.	Apigenin	-8.7	6	Asn30 (2.02), Thr56 (2.05)	Asp1 (3.53), Arg2(3.75), Val3(3.80), Tyr26(3.52, 3.56), Ala27(3.68)		
		Kaempferol	-8.3	2	Asn30(2.07), Tyr324(2.69)	Asp1(3.53), Arg2(3.66), Val3(3.70), Trp23(3.59), Tyr26(3.58), Ala27(3.73), Ile52(3.79) Tyr324(3.79)		
2	Rauvolfia vomitoria Afzel.	Ajmaline	-9.6	2	Arg88(2.42, 2.77)	Asp1(3.52), Arg2(3.65), Val3(3.72), Tyr26(3.50, 3.70, 3.68), Ile52(3.43)		
3	Vernonia amygdalina	Cryptolepine	-8.4	0		Asp1(3.58), Val3(3.64), Trp23(3.76), Tyr26(3.69), Ala27(3.51)		
		Hydroxyvernolide	-8.3	5	Ser248(2.77), Asn249(3.05), Thr265(2.34), Thr266(2.53), Lys413(2.63)	Ala134(3.91), Thr135(3.32), Tyr251(3.44, 3.70), Asn338(3.76)		
		Luteolin	-8.4	4	Asn446(2.19), Gln447(2.35), Tyr583(3.45), Asn584(3.40)	Gln447(3.37, 3.82), Leu454(3.84, 3.78)		
		Vernodalol	-7.8	1	Asp1(3.29)			
4	Zingiber officinale Roscoe	Quercetin	-8.5	2	Asn446(2.20), Gln447(2.55)			
	Control Drugs	Enalapril	-7.5	4	Asn249(3.56), Ser262(2.65),	Tyr251(3.95, 3.35), Asp264(3.74), Glu340(3.44),		

			Thr266(2.20), Lys413(2.93)	Leu397(3.77), Phe410(3.55), Lys413(3.65, 3.93)
Ramipril	-7.6	0		Trp450(3.63), Leu454(3.62, 3.68), Tyr583(3.58, 3.75)

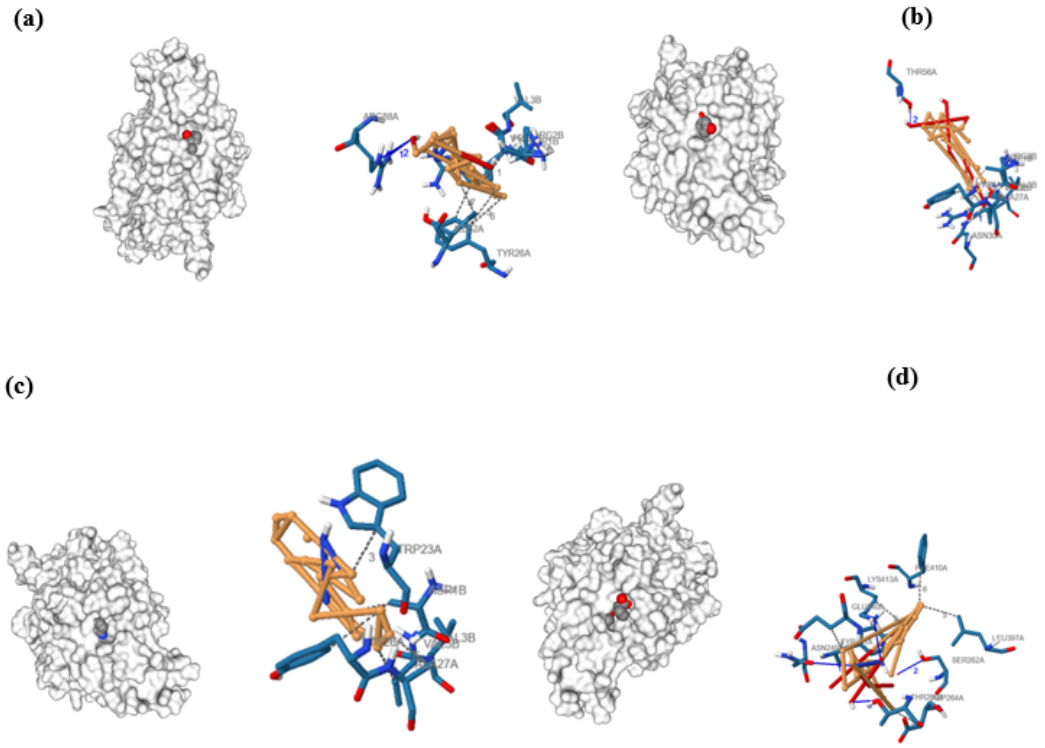


Fig 2: Binding configuration of Ajmaline (a) and Apigenin (b) Cryptolepine (c) and Enalapril (d) in the ACE active site as obtained from molecular docking analysis. Blue dashed line, green and grey dotted lines represent hydrogen bond, Pi stacking and hydrophobic interaction, respectively.

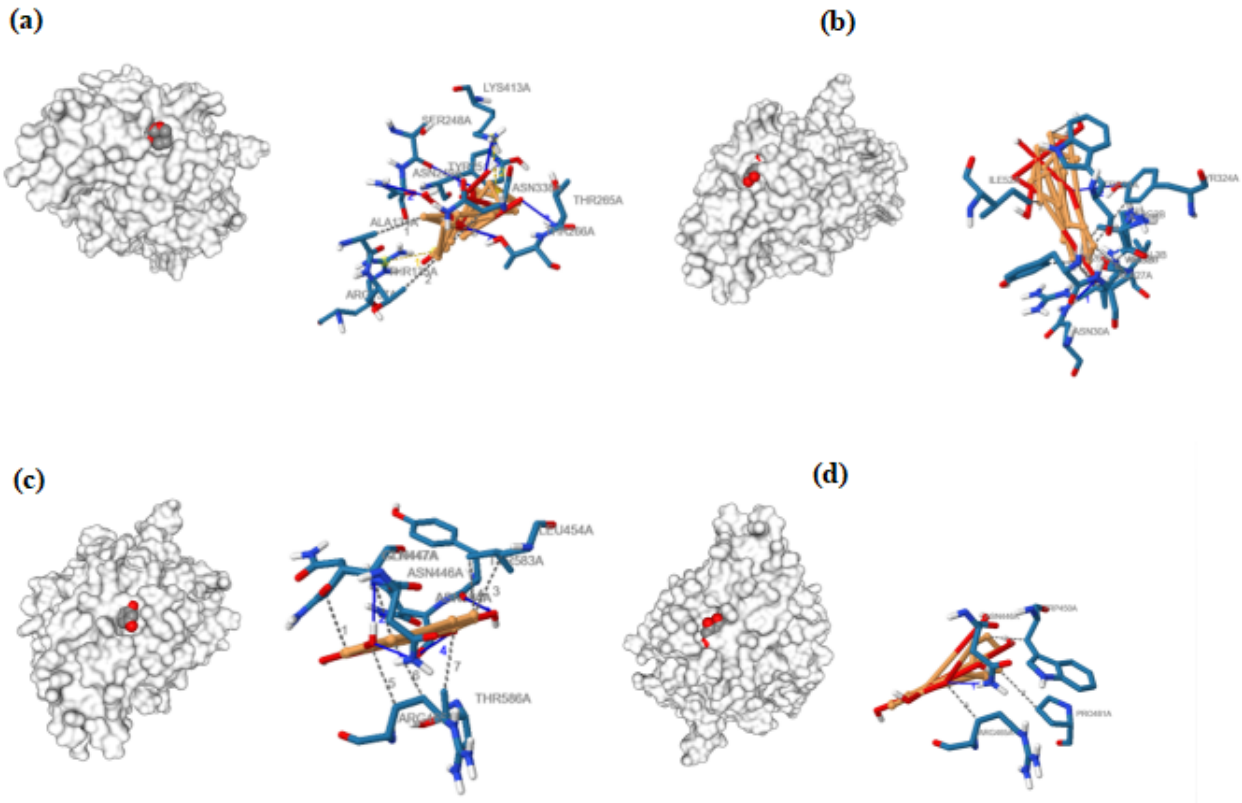


Fig 3: The binding configuration of Hydroxyvernolide (a) and Kaempferol (b) Luteolin (c) and Quercetin (d) in the ACE active site as obtained from molecular docking analysis. Blue dashed line, green and grey dotted lines represent hydrogen bond, Pi stacking and hydrophobic interaction, respectively.

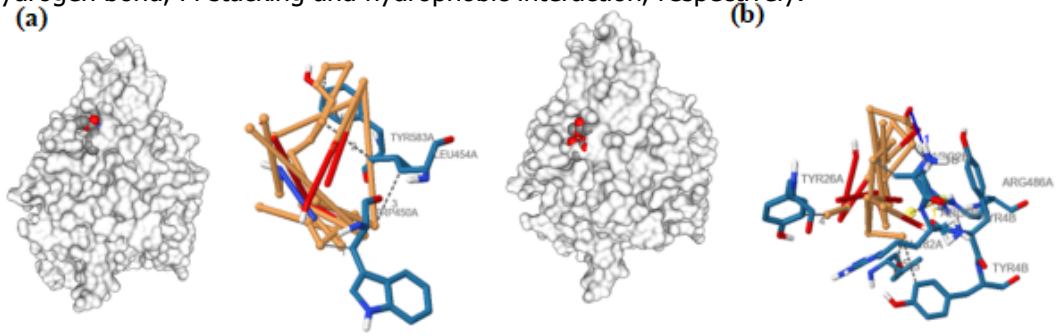
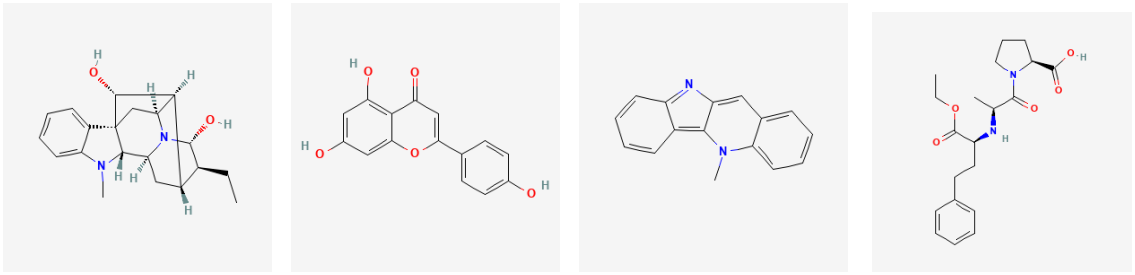
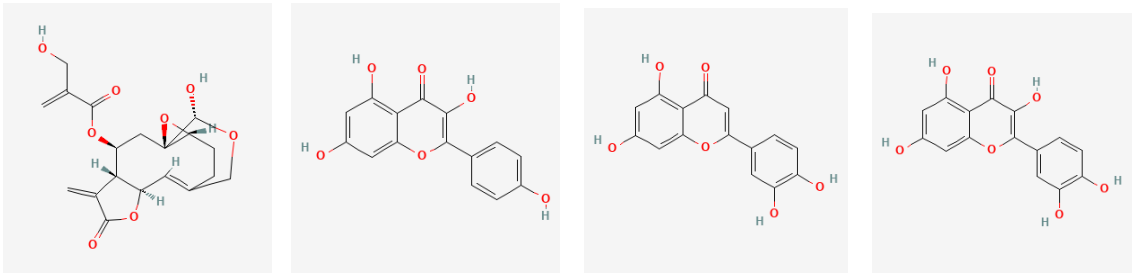


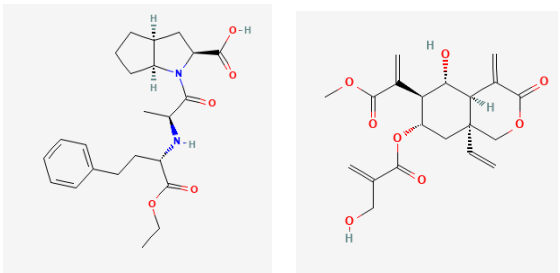
Fig 4: Binding configuration of Ramipril (a) and Vernodalol (b) in the ACE active site as obtained from molecular docking analysis. Blue dashed line, green and grey dotted lines represent hydrogen bond, Pi stacking and hydrophobic interaction, respectively.



Ajmaline Apigenin Cryptolepine Enalapril



Hydroxyvernolide Kaempferol Luteolin Quercetin



Ramipril Vernodalol

Fig 5: 2D structure of bioactive compounds and control drugs (Enalapril and Ramipril).

Predicted activity scores of bioactive compounds and the control drugs

The predicted bioactivity scores of the compounds and that of the control drugs are shown in Table 4. Apigenin, Quercetin, Luteolin, and Kaempferol had bioactivity scores between −5.0 and 0.0 for GPCR ligand, indicating that they were moderately active in binding the GPCR ligand; whereas Ajmaline, Cryptolepine, Hydroxyvernolide, Vernodalol, Ramipril, and Enalapril were actively binding GPCR ligand as indicated by their bioactivity scores (greater than 0.0 for GPCR ligand). The ion channel modulator bioactivity scores for Apigenin, Quercetin, Luteolin, Kaempferol and Vernodalol ranged between −5.0 and 0.0, suggesting moderate activity, while Ajmaline, Cryptolepine, Hydroxyvernolide, Ramipril, Enalapril had bioactivity scores greater than 0.0, indicating high activity.

Moreover, none of the bioactive compounds had a bioactive score less than -5.0, which indicated their moderate or active bindings to the ligands and inhibitors (Table 4).

Table 4: Predicted bioactivity score for bioactive compounds and control drugs

S/N	Compound name	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	Ajmaline	0.38	0.14	-0.24	0.16	0.16	0.04
2	Apigenin	-0.07	-0.09	0.18	0.34	-0.25	0.26
3	Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28
4	Cryptolepine	0.10	0.33	0.10	-0.24	-0.42	0.18
5	Luteolin	-0.02	-0.07	0.26	0.39	-0.22	0.28
6	Hydroxyvernolide	0.40	0.16	-0.11	1.02	0.46	0.91
7	Kaempferol	-0.10	-0.21	0.21	0.32	-0.27	0.26
8	Vernodalol	0.02	-0.05	-0.31	0.48	0.14	0.28
9	Ramipril	0.36	0.08	-0.36	-0.12	0.78	0.23
10	Enalapril	0.36	0.16	-0.30	-0.08	0.70	0.18

Table 5: Predicted ADMET screening results of bioactive compounds and control drugs

S/N. Class	Properties	Ajmaline	Apigenin	Cryptolepine	Enalapril	Hydroxyvernolide	Kaempferol	Luteolin	Quercetin	Ramipril	Vernodalol
1. Absorption	BBB	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
	Caco-2 permeability	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No	Yes
	Pgp-inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Pgp-Substrate	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	PPB	Yes	No	No	Yes	Yes	No	No	No	Yes	Yes
2. Distribution	Sub-cellular localization	Yes	No	No	Yes	Yes	No	No	No	Yes	Yes
3. Metabolism	CYP1A2 Inhibition	Yes	No	Yes	Yes	Yes	No	No	No	Yes	No
	CYP3A4 substrate	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP3A4 Inhibition	Yes	No	Yes	Yes	Yes	No	No	No	Yes	No
	CYP2C9 inhibition	Yes	No	No	Yes	Yes	No	No	No	Yes	Yes
	CYP2C9 substrate	Yes	Yes	No	No	Yes	No	No	No	No	Yes
	CYP2C19 inhibition	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP2D6 inhibition	Yes	No	Yes	Yes	Yes	No	No	No	Yes	Yes
	CYP2D6 substrate	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes
4. Toxicity	Acute oral Toxicity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Human hepatotoxicity	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes
	Ames mutagenicity	Yes	No	No	Yes	No	No	No	No	Yes	Yes
	Carcinogens	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes

BBB - blood-brain barrier, PPB - plasma protein binding, hERG - human ether-a-go-go.

Pharmacokinetics properties prediction

The ADMET properties of the bioactive compounds and that of the control drugs are expressed in Table 5. Except for Ajmaline, Cryptolepine, and Vernodalol, the hit ligands were predicted to penetrate the blood-brain barrier (BBB). Also, all hit ligands except Enalapril, Quercetin, and Ramipril had low absorption in the intestine through Caco-2 permeability. Apigenin, Cryptolepine, Enalapril, Hydroxyvernolide, Kaempferol, Luteolin, Quercetin, Ramipril, and Vernodalol were substrates of CYP3A4, while Ajmaline, Cryptolepine, Enalapril, Hydroxyvernolide, Ramipril might likely inhibit CYP3A4 (as some were predicted as substrates). Ajmaline, Apigenin, Hydroxyvernolide, and Vernodalol were found to be potential substrates of CYP2C9. For toxicity, Enalapril, and Ramipril were predicted not to cause hepatotoxicity in humans, whereas Cryptolepine and Hydroxyvernolide were predicted not to be carcinogenic.

Discussion

Hypertension remains a cause of premature death among youths and adults globally. However, medicinal plants such as *Allium sativum*, *Zingiber officinale*, *Acalypha godseffiana*, *Moringa oleifera*, *Vernonia amygdalina*, and *Rauvolfia vomitoria* have been reported to contain active ingredients capable of curing hypertension (Eluwa et al., 2010; El-Saber et al., 2020; Karima et al., 2023; Ugbogu et al., 2021). Our study shows the antihypertensive potential of thirty-one bioactive compounds from these six medicinal plants against a target protein Angiotensin Converting Enzyme (ACE).

Among the screened bioactive compounds, seventeen (17) and the two (2) control drugs showed potential to be used as oral drugs. This is as a result of their positive response and non-violation of the five drug-likeness screening rules (Lipinski, Egan, Veber, Muegge, and Ghose) (Egan et al., 2000; Muegge et al., 2001; Veber et al., 2002; Bickerton et al., 2012; and Lipinski, 2016). Moreover, the molecular docking analysis showed that eight bioactive compounds (Ajmaline, Apigenin, Quercetin, Cryptolepine, Luteolin, Hydroxyvernolide, Kaempferol, and Vernodalol) among the seventeen had stronger binding interaction with the target protein than the control drugs; hence, offer anti-hypertensive benefits by inhibiting the target's catalytic sites than the control drugs (Zeng et al., 2018).

Notably, Ajmaline derived from *R. vomitoria* had the strongest binding affinity of -9.6 kcal/mol against the target protein compared with other ligands and the control drugs (Table 4). This could be as a result of the high number of molecular interactions in the protein's binding pocket (David et al., 2018) which predicts the ligand-protein binding conformation as a docking score with negative value based on their shapes and electrostatic interactions. Scores with lower negative values indicate high binding affinity between ligand and protein.

Moreover, our study shows that the hit ligands interacted with key amino acid residues at the catalytic sites of ACE thereby suppressing the activity of the enzyme to convert angiotensin I to angiotensin II, and potentially prevents the narrowing of the blood vessels. This agrees with the findings of Sakar et al. (2019) that the inhibition of protein largely depends on the ability and quality of bonds between the amino acid residues and the ligand at the active site. Moreover, the compounds' ability to specifically interact with amino acid residues at ACE's active site could help eliminate toxicity (Sakar et al., 2019).

Bioactivity refers to the ability of a compound to interact with biological systems and produce a specific effect (Walubo, 2007; Khan et al., 2017). The bioactivity screening showed that all the bioactive compounds with higher binding energy than the control drugs had bioactivity scores greater than -5.0. This indicates that they were all moderately or actively binding to GPCR and nuclear receptor ligands, ion channel modulators, kinase inhibitors, and protease inhibitors. Notably, all the bioactive compounds and the control drugs were actively binding to enzyme inhibitors.

The investigated compounds possess the ability to traverse the blood-brain barrier (BBB), a selective filter that separates the brain from the bloodstream. However, some compounds exhibited therapeutic promise with low predicted intestinal absorption via Caco-2 permeability. This suggests limited oral

bioavailability, potentially hindering their efficacy. Some of the compounds, including Apigenin, Cryptolepine, Enalapril, Hydroxyvernolide, Kaempferol, Luteolin, Quercetin, Ramipril, and Vernodalol, were identified as potential substrates for the CYP3A4 enzyme, responsible for metabolizing numerous drugs and other substances (Dong et al., 2021). Ajmaline, Cryptolepine, Enalapril, and Hydroxyvernolide were also predicted to potentially inhibit CYP3A4, hence impacting the metabolism of co-administered drugs (Dong et al., 2021).

Additionally, Ajmaline, Apigenin, Hydroxyvernolide, and Vernodalol were found to be possible substrates for another enzyme, CYP2C9, suggesting its potential role in their metabolism. Enalapril and Ramipril displayed a low predicted risk of causing liver damage, suggesting good liver tolerability (Guo-Li et al., 2021). However, several compounds, including Ajmaline, Enalapril, Ramipril, and Vernodalol, exhibited signs of potential genotoxicity in the Ames test, a common mutagen identification tool. Notably, Cryptolepine and Hydroxyvernolide were predicted to be non-carcinogenic, hence, suggesting a potentially lower cancer risk.

Conclusion

The study revealed Apigenin and Kaempferol (*Allium sativum*), Ajmaline (*Rauvolfia vomitoria*), Cryptolepine, Hydroxyvernolide, Luteolin, and Vernodalol (*Vernonia amygdalina*), and Quercetin (*Zingiber officinale*) as bioactive compounds with higher binding affinity to the ACE binding pockets compared with the control drugs. Their high binding energies and safety profiles qualify them as a novel therapeutic compounds for hypertension drug development; however, wet lab experimental evaluation will be required for their validation.

References

Asekunowo, A.K., Ashafa, A.O.T., Okoh,O., Asekun, O.T. and Familoni, O.B. (2019). Phenolic constituents, antioxidant and hypoglycaemic potential of leaf extracts of *Acalypha godseffiana* from Eastern Nigeria: In vitro study. *Journal of Medicinal Plants for Economic Development,* 3(1): a36.

Atkinson A.B. and Robertson J.I. (1979). Captopril in the treatment of clinical hypertension and cardiac failure. *Lancet*, 2(8147): 836–9.

Bickerton, G.R., Paolini, G.V., Besnard, J., Muresan, S. and Hopkins, A.L. (2012). Quantifying the chemical beauty of drugs. *Nature chemistry,* 4(2):90-98.

Chaachouay, N. and Zidane, L. (2024). Plant-derived natural products: A source for drug discovery and development. *Drugs and Drug Candidates,* 3(1): 184-207.

Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P.W. and Tang, Y. (2012). admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J. Chem. Inf. Model,* 52: 3099-3105.

Daina, A., Michielin, O. and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.,* 7:42717.

David, T.1., Adelakun, N. S., Omotuyi, O. I., Metibemu, D. S., Ekun, O. E., Eniafe, G. O., Inyang, O. K., Adewumi, B., Enejoh, O. A., Owolabi, R. T. and Oribamis, E. I. (2018). Molecular docking analysis of phytoconstituents from Cannabis sativa with pfDHFR. *Bioinformation*, 14(9): 574-579.

Delano (2005). The PyMOL molecular graphics system, DeLano Scientific LLC, 400 Oyster Point Blvd., Suite 213, South San Francisco, CA 94080-1918.

Dong, J., Wang, N. N., Yao, Z. J., Zhang, L., Cheng, Y., Ouyang, D., Lu, A.P. and Cao, D.S. (2021). ADMETlab: a platform for systematic ADMET evaluation based some active compounds from selected African plants. *J Taibah Univ Med Sci.,* 16 (2): 162–76.

Egan, W.J., Merz, K.M. and Baldwin, J.J. (2000). Prediction of Drug Absorption Using Multivariate Statistics. *J. Med. Chem.*, 43:3867–3877.

El-Saber B.G., Magdy B.A., G Wasef L., Elewa, Y.H.A., A Al-Sagan A., Abd El-Hack, M.E., Taha, A.E., M Abd-

Elhakim, Y., Prasad, D.H. (2020). Chemical constituents and Pharmacological Activities of Garlic (Allium sativum L.): A review. *Nutrients,* 12 (3): 872.

Eluwa, M.A., Udoaffah, M.T., Vulley, M.B.G., Ekanem, T.B., Akpantah, A.O., Asuquo, O.A. and Ekong, M.B. (2010). Comparative study of teratogenic potentials of crude ethanolic root, bark and leaf extract of Rauwolfia vomitoria (apocynaceae) on the fetal heart. *North American Journal of Medicinal Sciences,* 1: 592-595.

Farhadi, F., Roqayeh Aliyari, Ebrahimi, H., Hashemi, H., Mohammad Hassan Emamian and Akbar Fotouhi (2023). Prevalence of uncontrolled hypertension and its associated factors in 50–74 years old Iranian adults: a population-based study, *BMC Cardiovasc Disord.,* 23(1):318.

Forouzanfar, M. H., Liu, P., Roth, G. A., et al. (2017). Global Burden of Hypertension and Systolic Blood Pressure of at Least 110 to 115 mm Hg, 1990-2015. *JAMA,* 317(2): 165-182.

Guo-Li, X., Zhen-Xing, W., Jia-Cai, Y., Li Fu, ZhiJiang, Y., Chang-Yu, H., Ming-Zhu, Y., Xian-Xiang, Z., Cheng-Kun, W.; Ai-Ping, L.; Xiang, C.; Ting-Jun, H. and Dong-Sheng, C. (2021). ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Res*., 49: 5-14.

Hafiz, A., Huma, K., Shabirul, H., Shameem, A., Namita, S. and Azhar, K. (2023). Angiotensin-Converting Enzyme and Hypertension: A systemic Analysis of Various ACE inhibitors, their side effects, and bioactive peptides as a putative therapy for hypertension. *J. Renin Angiotensin Aldosteone Syst.,* 2023:7890188.

Karima, G., Leila, R., Muhammad, S.R.R., Hafiza, M.M., M.Abbas, A. and Sook, C.C. (2023). *Moringa oleifera*: processing, phytochemical composition, and industrial application. South African Journal of Botany, 160:180-193

Khan, T., Dixit, S., Ahmad, R., Raza, S., Azad, I., Joshi, S. and Khan, A.R. (2017). Molecular docking, PASS analysis, bioactivity score prediction, synthesis, characterization and biological activity evaluation of a functionalized 2-butanone thiosemicarbazone ligand and its complexes. *J. Chem. Biol.,* 10(3): 91-104.

Lipinski, C. (2016). Rule of five in 2015 and beyond: Target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions. *Adv. Drug Deliv. Rev.,* 101: 34-41.

Lipinski, C.A. (2008). Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol. Toxicol. Methods*, 44: 235-249

Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev*., 46: 3-26.

Mills, K. T., Bundy, J. D., Kelly, T. N., et al. (2016). Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies from 90 Countries. *Circulation,* 134(6): 441-450.

Muegge, I., Heald, S.L. and Brittelli, D. (2001). Simple Selection Criteria for Drug-like Chemical Matter. *J. Med. Chem.,* 44: 1841-1846.

Patten, G.S., Abeywardena, M.Y. and Bennett, L.E. (2016). Inhibition of angiotensin converting enzyme, angiotensin II receptor blocking, and blood pressure lowering bioactivity across plant families. *Crit. Rev. Food Sci. Nutr.*, 56(2): 181-214

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C. and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *J. Comput. Chem.,* 25(13): 1605-12.

Salentin, S., Schreiber, S., Haupt, V. J., Adasme, M. F. and Schroeder, M. (2015). PLIP: fully automated proteinligand interaction profiler. *Nucleic acids research*. 43: 443-447.

Sakar, B., Islam, S. S., Ullah, M. A., Hossain, S, Prottoy M. N., Araf, Y. and Taniya, M. A. (2019). Computational assessment and pharmacological property breakdown of eight patented and candidate drugs against four intended targets in Alzheimer's disease. *Advances in Bioscience and Biotechnology,* 10(11): 405.

Shalaby, E.A., Shanab, S.M.M., Hafez, R.M. and El-Ansary, A.E. (2023). Chemical constituents and biological activities of different extracts from ginger plant (*Zingiber officinale*). *Chemical and Biological Technologies in Agriculture,* 10:1-14

Sharma, R.K., Espinoza-Moraga, M., Poblete, H., Douglas, R.G., Sturrock, E.D., Caballero, J. et al. (2016). The dynamic nonprime binding of sampatrilat to the C-domain of angiotensin-converting enzyme. *J. Chem. Inf. Model,* 56(12): 2486-2494.

Stierand, K., Maass, P. C. and Rarey, M. (2006). Molecular complexes at a glance: automated generation of two-dimensional complex diagrams. *Bioinformatics*, 22 (14): 1710-6.

Trott, O. and Olson, A.J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput. Chem.,* 31(2): 455–461.

Ugbogu, O.C., Emmanuel, O., Agi, G.O., Ibe, C., Ekweogu, C.N., Ude, V.C., Uche, M.E., Nnanna, R.O. and Ugbogu, E.A. (2021). A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). *Heliyon,* 7(11).

Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W. and Kopple, K.D. (2002). Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem.,* 45: 2615–2623.

Walubo, A. (2007). The role of cytochrome P450 in antiretroviral drug interactions. *Expert Opinion on Drug Metabolism & Toxicology*, 3(4): 583–598.

Wong, M.K.S. (2016). Angiotensin Converting Enzymes In: Handbook of Hormones. Comparative Endocrinology for Basic and Clinical Research, Pg: 263-265.

World Health Organization (2019). Non communicable diseases country profiles. Retrieved from https://www.who.int/nmh/countries/nga_en.pdf.

Zheng, M., Zhao, J., Chen, C., Fu, Z., Li, X., Liu, X., Ding, X., Tan, X., Li, F., Luo, X., Chen, K. and Jiang, H. (2018). Computational chemical biology and drug design: Facilitating protein structure, function, and modulation studies. *Med. Res. Rev,* 38(3): 914–950