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In Silico Profiling of Pharmacokinetic Properties and Inhibitory Potentials of *Chromolaena odorata* aqueous leaf extract against Type-2 Diabetes Protein Targets, Neprilysin and Alpha Amylase

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ABSTRACT

Chronic type 2 diabetes interferes with the body's ability to use insulin as intended. Two protein targets, neprilysin and alpha amylase have been reported to contribute to the emergence of type 2 diabetes. Type 2 diabetes medications are ineffective and have unfavorable side effects. The objective of this study was to discover novel chemical agents of *Chromolaena odorata* aqueous leaf extract that can precisely inhibit the proteins, neprilysin and alpha amylase in type 2 diabetes to restrict the proteins' actions and so covertly induce anti-diabetic effects. Using GC-MS analysis, the phytoconstituents of *Chromolaena odorata's* aqueous leaf extract were identified. Ten (10) of the twenty-one (21) identified compounds complied with Lipinski rule of five. Swiss-ADME (Absorption, Distribution, Metabolism & Excretion) was used to analyze the pharmacokinetic properties of the compounds that did not violate the Lipinski rule. ADME analysis revealed that the selected compounds displayed suitable pharmacokinetic properties. Molecular docking was employed using PyRx and Biovia Discovery Studio software to predict potential selected bioactive antagonists and non-covalent interactions between the selected ligands, standard drugs and the target proteins. The findings from this study revealed that the identified ligand (carbamic acid) had the highest binding affinity against the target protein neprilysin and alpha amylase when compared to the standard drug, and it also has the potential to advance into pre-clinical and clinical research for future development as an antidiabetic medication.

Keywords: Diabetes, *Chromolaena odorata*, Neprilysin, Alpha-amylase, Molecular docking

INTRODUCTION

Diabetes mellitus has more than doubled in global prevalence over the last three decades, making it one of the most serious public health issues confronting all countries. Children, adolescents, and younger adults are increasingly being diagnosed with type 2 diabetes mellitus (T2DM) and prediabetes (Chen et al., 2012). The reasons for the type 2 diabetes mellitus epidemic are rooted in a complex network of genetic and epigenetic processes that interact within a sociocultural framework that shapes behavior and influences the environment (Chen et al., 2012). Diabetes mellitus is a chronic disorder of glucose metabolism with serious clinical consequences. The multi-system complications of diabetes include microvascular (retinopathy, nephropathy, neuropathy) and macro-vascular (ischaemic heart disease, stroke, peripheral vascular disease) endpoints. The prevalence of diabetes has been rising in the last few decades, fueled by the global rise in the

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prevalence of obesity. The premature morbidity, mortality, reduced life expectancy and financial and other costs to the patient with diabetes, their careers and the health service, make it an important public health condition (Forouhi and Wareham, 2010). Many essential enzymatic checkpoints work to regulate hepatic glycolysis (glucokinase), glycogen synthesis (glycogen synthetase kinase 3), glycogenolysis (phosphorylase), gluconeogenesis (phosphoenolpyruvate Carboxykinase, fructose-1,6-bisphosphate), or specific pathway steps are accessible (Gupta et al., 2021).

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization. The setting up of the input structures for the docking is just as important as the docking itself, and analyzing the results of stochastic search methods can sometimes be unclear (Morris and Lim-Wilby, 2008).

Plants' therapeutic properties are found in phytochemicals like alkaloids, tannins, flavonoids, and other phenolic compounds, which have a specific physiological effect on the human body. In nutraceutical and medication research, a systematic search for valuable bio-activities from medicinal plants is currently regarded as a sensible method (Afolabi et al., 2007). *Chromolaena odorata* (Siam weed) is one of the oldest cultivated plants by man and it is commonly known as Siam weed (Chakraborty et al., 2011). All parts of the plant are known to be actively used in traditional medicine. The floral portion of the plant is known to be biochemically composed of constituents that are actively used in the treatment of ulcers and dysentery. Several reports on *C. odorata* indicate that the plant has several biochemical activities which express the anti-oxidative, anti-inflammatory, anticancer, anti-diabetic, antimicrobial and anti-hemorrhagic properties of the plant (Sirinthipaporn and Jiraungkoorskul, 2017).

Neutral endopeptidase neprilysin enhances the bioavailability of substance P, bradykinin, and natriuretic peptides, which have natriuretic, vasodilatory, and anti-proliferative properties. When these actions work together, a potent ventricular unloading and anti-hypertensive response is likely to result. The angiotensin II-receptor neprilysin inhibitor LCZ696 (Valsartan/sacubitril) is the first of its kind. In addition to being a novel medicine for the treatment of heart failure, LCZ696 is also anticipated to be an effective anti-hypertensive and could have a preference for systolic pressure (Bavishi et al., 2015).

By dissolving larger polysaccharide molecules into simpler ones like glucose and maltose, alpha-amylase, a calcium metalloenzyme, facilitates digestion. The enzyme also raises blood glucose levels and postprandial hyperglycemia. A well-known pharmacological target for the management of postprandial blood glucose increases is α-amylase. Researchers have expressed interest in creating potent alpha-amylase inhibitor compounds as a result of the discovery that many enzymatic inhibitors, including acarbose, miglitol, and voglibose, are successful in targeting this enzyme (Kaur et al., 2021). For the proper management of DM, anti-diabetic therapeutic drug screening is crucial. With the aid of *In-silico* molecular docking tools, numerous researchers have worked on the extraction, isolation, and characterization of extracts and bio-active fractions from medicinal plants. They have also established profiles and data on the interactions of active components against various targets and DM enzymes (Levitt, 2008).

A severe worldwide health issue, type 2 diabetes mellitus (T2DM) is a long-term, intricate, and multivariate metabolic condition. In addition to the already recognized negative effects for the

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present preclinical treatments against T2DM, the side effects of known medications and the lack of long-term safety evidence have substantially prompted the urgent investigation of novel therapeutic and preventative measures against this disease (Xu et al., 2014). The behavior of tiny molecules in a target protein's binding site is investigated via molecular docking methods. Molecular docking is utilized more frequently as a method in drug development as more protein structures are discovered experimentally using X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. For proteins whose structures are unknown, docking against homology-modeled targets also becomes feasible. For subsequent lead optimization procedures, the druggability of the compounds and their specificity against a certain target can be determined using docking techniques. Programs for molecular docking run a search method in which the ligand's conformation is assessed repeatedly until convergence to the minimal energy is attained.(Pagadala et al., 2017). To evaluate the pharmacokinetics and inhibitory potentials of ligands of Chromolaena odorata aqueous leaf extract against neprilysin and alpha-amylase in type 2 diabetes mellitus: An In silico study.

METHODOLOGY

Collection of plant and identification

Plant was collected from Badagry West Local Council Development Area, Owode Apa, Irosu village around August (Rainy season) and identified at Lagos State University Ojo, Faculty of Science, Department of Botany.

Aqueous extract preparation

The Chromolaena odorata leaves were rinsed, then 80g of the leaf was weighed and mixed with 100ml of distilled water to create the aqueous extract. The C. odorata aqueous extract was heated for two hours at 70°C in a water bath. Using Whatman no. 1 filter paper, the extract was collected through filtering. To obtain a clear solution, the filtrates were then centrifuged at 5000 rpm for 5 minutes (supernatant). For later usage, these filtrates were kept in the refrigerator.

Gas chromatography-mass spectrometry analysis

Using GC-MS QP2010, the phytochemicals in chromolaena odorata were characterized. AQP2010 gas chromatography with Thermal Desorption System, TD 20 paired with Mass Spectroscopy was used to identify the phytochemicals in the sample. It was 70eV for the ionization voltage. A Restek column was used for gas chromatography in the temperature programming mode (0.25 mm, 60m, XTI-5). The initial column temperature was 80°C for 1 minute, followed by a linear increase of 70°C min-1 to 220°C, held for 3 minutes, and then a linear increase of 10°C min-1 to 290°C for 10 minutes. The GC-MS interface was kept at 290°C, while the temperature of the injection port was 290°C. A glass-only injector operating in split mode was used to introduce the sample (Owolabi et al., 2010).

Protein preparation

The 3D crystal structures of neprilysin and alpha-amylase, respectively designated by the RCSB PDB codes 1R1H and 1MXD, were downloaded from the Protein Data Bank at https://www.rcsb.org. Using the Biovia Discovery Studio, the proteins were prepared for docking. Hetatoms and water molecules were removed then polar hydrogen atoms were added.

Ligand preparation

From PubChem, CID:10352608, CID:56505, CID:3814016, CID:1111031, CID:227, CID:567912, CID:5368213, CID:326617 ligands were imported (nih.gov) universal force field was used to

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minimize energy. To assist the docking software in locating the bioactive conformer from the local minima, energy reduction is carried out.

Molecular docking

For molecular docking studies, AutoDock Vina was employed. Gradient-based conformational search is used in Auto Vina docking. Pentacosane, 5-Vinylpyrazole, 3-Bromo(propoxyl), carbamic acid, 1,7-octadiyne, 1-hydroxy-2-indolinone, phenylester, as well as sacubitril and acarbose, which are standard medications for neprilysin and alpha-amylase, respectively, and the co-crystallized ligands of the target proteins The study was based on binding free energies and root mean square deviation (RMSD) values, and the ligand molecules were then sorted in the order of increasing docking energies. Each ligand underwent a docking experiment consisting of 100 stimulations. lowest energy that each binding energy can represent (Cheng et al., 2012).

ADME Properties

The discovery and development of pharmaceuticals, insecticides, food additives, consumer goods, and industrial chemicals depend heavily on the understanding of absorption, distribution, metabolism, excretion (ADME) qualities. When assessing environmental and human hazards, this knowledge is extremely helpful. The availability of good quality data is the most important rate-limiting phase in the chemical safety assessment workflow. The term "admeSAR" refers to a database of ADME structure-activity relationships. It is an open-source database that collects, organizes, and manages data on available ADME-associated features from the published literature. It is text and structure searchable and updated regularly. A thoroughly curated set of over 210,000 ADME annotated data points for over 96,000 distinct chemicals with 45 types of ADME-associated proteins, species, or animals are available in ADME SAR (Cheng et al., 2012). Using their canonical smile notation in the Swiss ADME web-based tool, ligands' ADME (absorption, distribution, metabolism, excretion) properties were determined.

Protein-ligand interaction

All activities in living things are based on molecular recognition, which is the interaction of different small molecules or biological macromolecules with one another or with a high degree of specificity and affinity to form a particular complex. An important group of biological macromolecules called proteins carry out their tasks by attaching to other molecules or to other proteins. Therefore, a thorough comprehension of protein-ligand interactions is essential to comprehending biology at the molecular level. Additionally, understanding the mechanisms underlying protein-ligand recognition and binding can help with drug discovery, design, and development. First, the physicochemical mechanisms underpinning protein-ligand binding are introduced and rationalized in this study (Cheng et al., 2012). Biovia Discovery Studio automatically created the 2D and 3D schematics of the protein-ligand complexes when we complexed the ligands, carbamic acid with neprilysin and alpha-amylase, respectively.

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RESULTS

TABLE 1: **Lipophilicity Properties Of *Chromolaena Odorata* Identified Chemical Compounds**

	Phenyl Ester ID:10352608	5-Vinylpyrazole ID:56505	4-Methyl-2-Nitrophenyl ID:3814016	Benzene-3-Bromo ID: 1111031	Carbamic acid ID:277	Pentacosane ID:567912	Cyclooctene,4-methylene-6-(1-propenyldene)- ID:5368213	1-hydroxy-3H-indol-2-one ID:326617
Log p $_{o/w}$ (LOGP)	2.46	1.00	0.98	3.51	0.20	-0.17	2.94	1.33
Log p $_{o/w}$ (XLOGP)	2.37	1.03	1.49	4.88	-0.78	-0.28	2.60	0.46
Log P $_{0/w}$ (WLOGP)	2.24	0.94	1.00	4.28	-0.38	-0.56	3.77	0.58
Log P $_{o/w}$ (MLOGP)	2.23	0.25	0.59	3.82	-1.60	1.11	3.65	1.07
Log P $_{o/w}$ (SILICOS-IT)	2.69	1.73	1.36	4.68	-0.68	0.70	3.52	0.80
Consensus Log $P_{0/w}$	2.40	0.99	0.54	4.23	-0.65	-0.17	3.30	0.85

The Table 1 above reveals the lipophilicity properties of *Chromolaena Odorata* identified chemical compounds in which Benzene-3-bromo has the highest Log Po/w(XLOGP), Log Po/w(WLOGP) and Log P(MLOGP) Values.

Table 2: Pharmacokinetics Of *Chromolaena Odorata* Identified Chemical Compounds

	Phenyl Ester ID:10352608	5-Vinyl-Pyrazole ID:565051	4-Methyl-2-Nitrophenyl ID:3814016	Benzene-3-Bromo ID: 1111031	Carbamic acid ID: 277	Pentacosane ID:567912	Cyclooctene,4-methylene-6-(1-propenyldene) ID:5368213	1-hydroxy-3H-indol-2-one ID:326617
GI absorption	High	High	High	High	high	High	Low	High
BBB permeant	Yes	Yes	No	Yes	No	No	Yes	Yes
P-gb substrate	No	No	No	No	No	Yes	No	No
CYP1A2 inhibitor	Yes	No	No	Yes	No	No	No	No
CYP2C19 inhibitor	No	No	No	Yes	No	No	No	No
CYP2C9 inhibitor	No	No	No	Yes	No	No	No	No
CYP2D6 inhibitor	No	No	No	yes	No	No	No	No
CYP34A inhibitor	No	No	No	No	No	No	No	No
Log K_p (skin permeation)	-5.80 cm/s	-6.14 cm/s	-5.92 cm/s	-4.79 cm/s	-7.23 cm/s	-8.62 cm/s	-5.43 cm/s	High

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Table 2 shows the pharmacokinetics of *Chromolaena Odorata* chemical compounds. Cyclooctene,4-methylene-6(1-propenyldene) is the only compound with low GI absorption. Also pentacosane has a P-gb substrate and only benzene-3-bromo has CYP2C9 and CYP2D6 inhibitors. None of the compounds have CPY34A inhibitor.

Table 3: Drug Likeness of *Chromolaena odorata* Identified Chemical Compound

Rules	**Phenyl Ester ID:10352608**	**5-Vinyl-Pyrazole ID:565051**	**4-Methyl-2-Nitrophenyl ID:3814016**	**Benzene-3-Bromo ID: 1111031**	**Carbamic acid ID: 277**	**Pentacosane ID:567912**	**Cyclooctene,4-methylene-6-(1-propenyldene)- ID: 5368213**	**1-hydroxy-3H-indol-2-one ID:326617**
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose	Yes	No; 3 violations: MW<160, MR<40,# atoms<20	Yes	Yes	No; 3 violations: MW<160, MR<40,# atoms<20	Yes	Yes	No; 2 violations : MW<160 , #atoms<20
Veber	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Egan	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Muegge	No; 1 violation: W<200	No; 1 violation: MW<200	NO:1 violation M/W<200	yes	No; 2 violations: MW<200, #C<5	Yes	No; 2 violations : MW<200 , Heteroatoms<2	No; 1 violation: MW<200
Bioavalability score	0.55	0.55	0.55	0.55	0.85	0.55	0.55	0.55

Table 3 below shows the druglikeness of *Chromolaena odorata* chemical compounds. All of the compounds complied with Lipinski rule of five and Egan rule with no violation while 5-vinylpyrazole, carbamic acid and 1-hydroxy-3H-indol-2-one violated Ghose rule contrary to other compounds. None of the compounds violate the Veber rule. Also, benzene-3-bromo and pentacosane are the only compounds without violation to Muegge rule.

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Table 4: Medical Chemistry of *Chromolaena odorata*

	Phenyl Ester ID:10352608	5-Vinyl-Pyrazole ID:565051	4-Methyl-2-Nitrophenyl ID:3814016	Benzene-3-Bromo ID: 1111031	Carbamic acid ID: 277	PENTACOSANE ID:567912	Cyclooctene, 4-methylene-6-(1-propenyldene)- ID: 5368213	1-hydroxy-3H-indol-2-one ID:326617
Pains	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	1 alert phenol ester	0 alert	3 alerts: hydrazine nitro group oxygen-nitrogen single bond	1 alerts: alkyl halide	0 alert	0 alert	1 alert: isolated_alkene	1 alert: hydroxamic_acid
Leadlikeness	No; 1violatons: MW<250,	No; 1 violation: MW<250	No:1 violations: MW<250	No; 1 violations: XLOGP3> 3.5	No; 1 violation: MW<250	No; 1 violation: MW>350	No; 1 violation: MW<250	No; 1 violation: MW<250
Synthetic Accessibility	1.73	1.62	2.14	2.39	1.00	0 alert	3.35	1.59

Table 4 revealed the medical chemistry of *Chromolaena odorata* identified chemical compounds. All compounds have zero alert of pains. 4-methyl-2-nitrophenyl has 3 alert of hydrazine nitro group,oxygen-nitrogen single bond in their break. All compounds have 1 violation in lead likeness. Cyclooctene,4-methylene-6 (1-propenyldene has the highest number of synthetic accessibility.

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Table 5: Physiochemical properties of *Chromolaena odorata* bioactive compounds

	Phenyl Ester ID:10352608	5-Vinyl-Pyrazole ID:56505	4-Methyl-2-Nitrophenyl ID:3814016	Benzene-3-Bromo ID: 1111031	Carbamic Acid ID: 277	Pentacosane ID:567912	Cyclooctene, 4-methylene-6-(1-propenyldene)-ID:5368213	1-hydroxy-3H-indol-2-one ID:326617
Formula	$C_{11}H_{14}O_3$	$C_5H_6N_2$	$C_7H_9N_3O_2$	$C_{16}H_{17}BrO_2$	CH_3NO_2	$C_{18}H_{36}N_2O_5$	$C_{12}H_{16}$	$C_8H_7NO_2$
Molecular Weight	194.23g/mol	94.11g/mol	167.17g/mol	321.21g/mol	61.04g/mol	360.49g/mol	160.26 g/mol	149.15g/mol
No. Of Heavy Atoms	17	7	12	19	4	25	12	11
No.OfAromatic Heavy Atoms	14	5	6	12	0	0	0	6
FractionCsp3	0.36	0.00	0.14	0.25	0.00	1.00	0.42	0.12
No.Of Rotatable Bonds	3	1	2	7	0	0	0	0
No.H-Bond Acceptors	3	1	3	2	2	7	0	2
No.H-Bond Donors	0	1	2	0	2	0	0	1
Molecular Refractivity	54.36	28.68	47.44	81.40	11.79	103.46	54.97	42.56
TPSA	35.53A	28.68A	83.87A	18.46A	63.32A	52.63A	0.00A	40.54A

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Table 5 shows the physiochemical properties of *Chromolaena odorata* chemical compounds. Carbamic acid had the lowest molecular weight whereas pentacosane had the highest number of heavy atoms. Carbamic acid, pentacosane and cyclooctene,4-methylene-6 (-1-propenyldene) do not have aromatic heavy atoms. Carbamic acid and 5-vinylpyrazole do not have fraction Csp3. carbamic acid, pentacosane, cyclooctene,4-methylene-6(-1_propenyldene) and 1-hydroxy-3H-indol-2-one does not have rotable bonds while phenyl-ester, benzene-3-bromo, pentacosane and cyclooctene,4-methylene-6(-1_propenyldene) have no hydrogen bond donors. All compounds except cyclooctene,4-methylene-6 (-1_propenyldene) have hydrogen bond acceptors.

Table 6: Water solubility of identified chemical compounds

	Phenyl Ester ID:10352608	**5-Vinyl-Pyrazole ID:565051**	**4-Methyl-2-Nitrophenyl ID:3814016**	**Benzene-3-Bromo ID: 1111031**	**Carbamic acid ID: 277**	**Pentacosane ID:567912**	**Cyclooctene,4-methylene -6-(1-propenyldene)-**	**1-hydroxy-3H-indol-2-one ID:326617**
Log S (ESOL)	-2.66	-1.53	-2.05	-4.91	-0.27	-1.97	-2.47	-1.46
Solubility	4.28e-01 mg/ml; 2.21e-03mol/l	2.75e+00 mg/ml;2.92e-02 mol/l	1.48e+00 mg/ml;8.85e-03 mol/l	3.94e-03 mg/ml; 1.2e-05 mol/l	1.14e+02 mg/ml ; 1.87e+00 mol/l	3.88e+00 mg/ml ; 1.08e-02 mol/l	5.41e-01 mg/ml ; 3.38e-03 mol/l	5.19e+00 mg/ml ; 3.48e-02 mol/l
Class	Soluble	Very soluble	Soluble	Moderately soluble	highly soluble	Very soluble	Soluble	Very soluble
Log S(All)	-2.76	-1.22	-1.85	-5.00	-0.07	-0.48	-2.25	-0.88
Solubility	3.40e-01mg/ml; 1.75e-03 mol/l	5.64e+00 mg/ml; 5.99e-02 mol/l	8.89e-02 mg/m; 1.50e-04 mol/l	2.31e-01mg/ml; 1.38e-03mol/l	5.18e+01 mg/ml ; 8.48e-01 mol/l	1.19e+02 mg/ml ; 3.31e-01 mol/l	9.03e-01 mg/ml ; 5.63e-03 mol/l	1.97e+01 mg/ml ; 1.32e-01 mol/l
Class	Soluble	Very soluble	Soluble	Poorly soluble	Very Soluble	Very soluble	Soluble	Very soluble
Log S (SILICOS-IT)	-3.38	-1.59	-3.48	-1.85	1.28	-2.22	-2.47	-1.58
Solubility	8.12e-02m/ml; 4.18e-04 mol/l	2.41e+00 mg/ml; 2.56e-02 mol/l	8.89e-02 mg/ml; 3.35e-04 mol/l	2.36e-00 mg/ml; 1.41e-05 mol/l	1.16e+03 mg/ml ; 1.89e+01 mol/l	2.19e+00 mg/ml ; 6.07e-03 mol/l	5.43e-01 mg/ml ; 3.39e-03 mol/l	3.93e+00 mg/ml ; 2.64e-02 mol/l
class	Soluble	Soluble	Soluble	soluble	Soluble	Soluble	Soluble	Soluble

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Table 6 revealed the water solubility of C*hromolaena odorata* chemical compounds. Benzene-3-bromo had the best LogS (ESOL) value. Carbamic acid is highly soluble in class while benzene-3-bromo is poorly soluble in class (II).

Table 7: Binding energies of ligands complexed with neprilysin and alpha-amylase

Ligands	**Neprilysin (Kcal /mol)**	**Alpha-amylase (Kcal /mol)**
2,4-hexadiyne	-4.8	-4.4
Benzenesulfonic acid	-3.6	--
Benzene, (3-bromopropoxy)	-6.3	-6.3
Cyclooctene,4-methylene-6-(1 propenylidene)	-6.4	-4.9
Cyclonona-1,2,6-triene	-6.2	-3.3
Cyclohexane,1-ethylene-3-methylene-5-(1-propenylidene)	-5.7	-5.8
Pentacosane	-7.1	-6.0
5-vinyl-pyrazole	-4.6	-4.9
1,7-octadiyne	-7.2	__
Carbamic acid	-7.7	-7.6
Phenylester	-7.1	-5.9
1-hydroxy-2-indolinone	-6	-6.2
**2-acetamido-2-deoxy-beta-D-glucopyranose (co-crystaline ligand for neprilysin)	-6.5	-
**Alpha -D-glucopyranose (co-crystaline ligand for alpha amylase)	-	-5.9
*Sacubitril (drug)	-7.6	__
*Acarbose (drug)	-	-7.6

* indicates the drug
** indicates the co-crystallized ligands

Table 8: Top 5 Ligands with the best binding affinity for neprilysin

Ligand	**Binding Affinity** (Kcal /Mol)
Carbamic Acid	-7.7
*Sacubitril	-7.6
Phenyl Ester	-7.2
1,7-Octadiyne	-7.2
Pentacosane	-7.1

* indicates the drug
** indicates the co-crystallized ligands

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Table 9: Top 5 ligands with the best binding affinity for alpha-amylase

Ligand	**Binding Affinity** (Kcal /Mol)
CARBAMIC ACID	-7.6
*ACARBOSE	-7.5
BENZENE, (3-BROMOPROPOXY)	-6.3
PENTACOSANE	-6.0
**ALPHA -D-GLUCOPYRANOSE	-5.9

* indicates the drug
** indicates the co-crystallized ligands

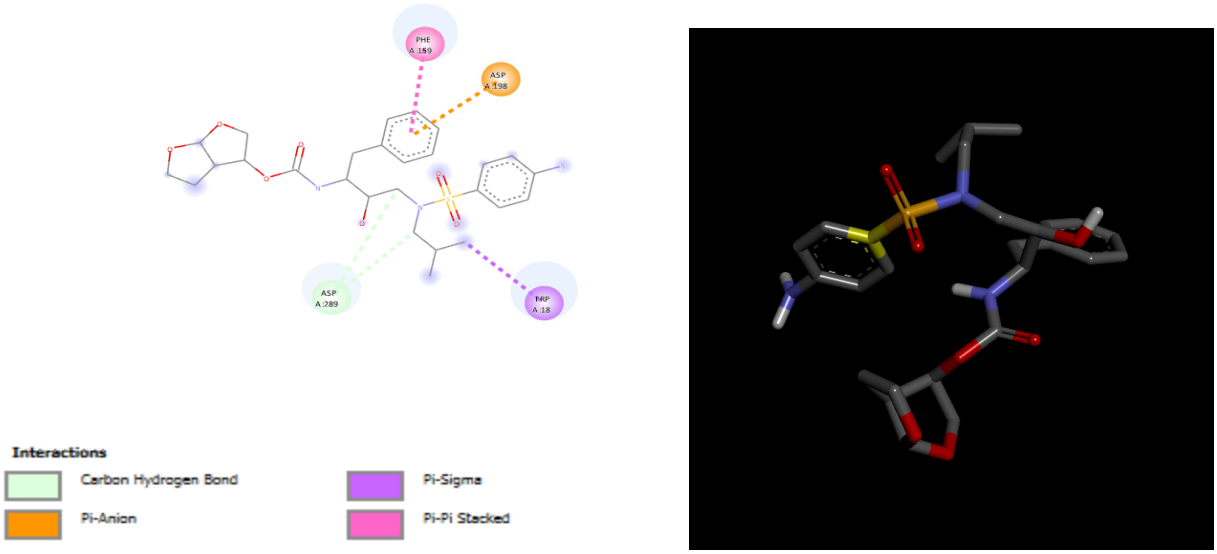

Figure 1: 2D and 3D Interaction of Neprisylin - Carbamic Acid

2D and 3D protein-ligand complex interaction

Figure 1 revealed the presence of one pi-sigma bond ,one pi-anion bond and one pi-pi stacked linkage in the interaction between neprilysin and carbamic acid which shows a strong interaction.

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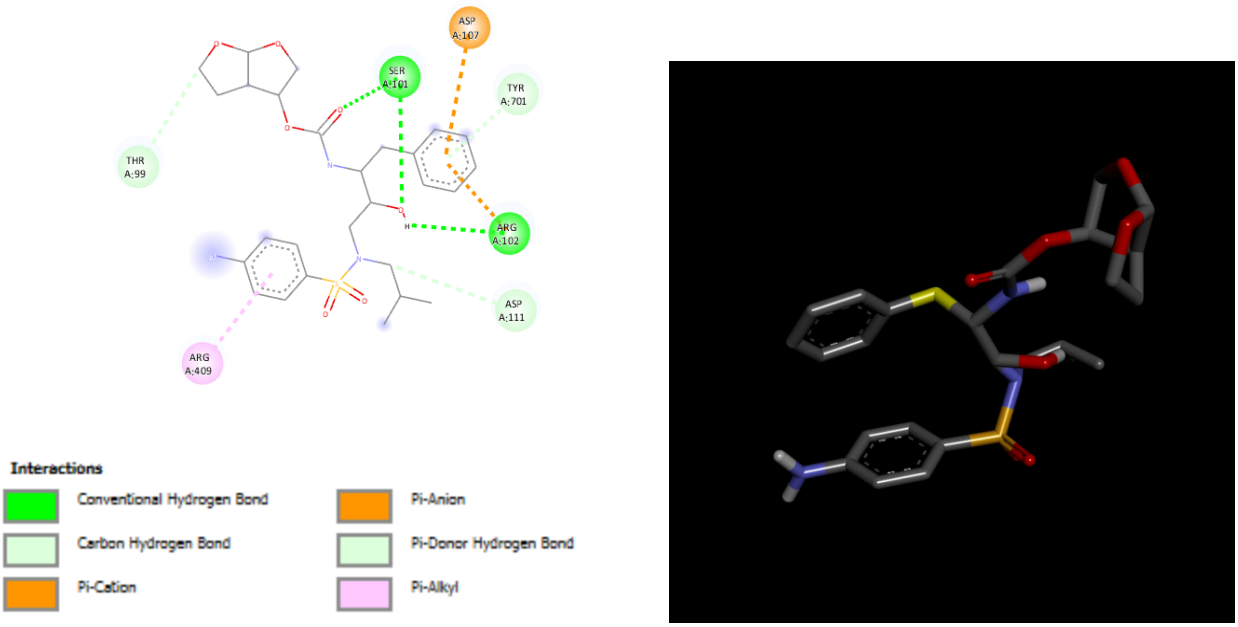

Figure 2: 2D and 3D Interaction of Alpha Amylase -Carbamic Acid Complex

Figure 2 revealed the presence of two conventional hydrogen bonds ,two pi-donor hydrogen bonds, one pi-cation, one pi-anion ,one carbon hydrogen bond and one pi-alkyl bond in the interaction between alpha-amylase and carbamic acid which shows a non-covalent intreraction.

Discussion

Due to its ability to speed up the rate of drug discovery, manufacture, and screening utilizing computer-based program approaches, *in-silico* studies have gained increased attention in biochemistry and other experimental disciplines (Colquitt et al., 2011). The rule of five (RO5), also referred to as Lipinski's rule of five, Pfizer's rule of five, or simply the rule of five, is a general guideline used to assess how similar a chemical compound is to an existing drug or to ascertain whether it possesses chemical and physical characteristics that would make it likely to be an orally active drug in humans (Ekins et al., 2007). Gas chromatography mass spectrometry (GC-MS) was used in a quantitative investigation to assess the phytochemical composition of *chromolaena odorata* (GCMS).

The analysis of the physiochemical properties of *Chromolaena odorata* revealed ligands like Carbamic acid, phenyl ester, pentacosane, cyclooctene,4-methylene-6(-1_propenyldene) and 1-hydroxy-3H-indol-2-one, cyclooctene, 4-methylene-6 (-1_propenyldene). Some of the constsituens of *Chrmonelaena Odorata* have been known to exhibit therapeutic potentials (antioxidant, anti-infammatory and anticancer activities) (Aziz et al., 2020). It was also revealed that Carbamic acid had the lowest molecular weight, pentacosane has the highest number of heavy atoms, carbamic acid, pentacosane and cyclooctene,4-methylene-6(-1_propenyldene) do not have aromatic heavy atoms. carbamic acid and 5-vinylpyrazole do not have fraction Csp3. carbamic acid, pentacosane, cyclooctene,4-methylene-6(-1_propenyldene) and 1-hydroxy-3H-indol-2-one does not have rotable bonds while phenyl-ester, benzene-3-bromo, pentacosane and cyclooctene,4-

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methylene-6(-1_propenyldene) have no hydrogen bond donors. All compounds except cyclooctene, 4-methylene-6 (-1_propenyldene) have hydrogen bond acceptors. The water solubility properties of *Chromolaena odorata* vary from poorly soluble to highly soluble. However most of the ligands were soluble, this is in line with Yusuf *et al*., 2021 who revealed that most bioactive components of *Chromonela Odoarta* were highly soluble.

The ADME properties of *C. odorata* aqueous leaf extract were evaluated after screening the identified ligands from GCMS analysis with Lipinski rule of five. The drug likeness properties revealed that all of the compounds complied with Lipinski rule of five and Egan rule with no violation. 5-vinylpyrazole, carbamic acid and 1-hydroxy-3H-indol-2-one violated Ghose rule contrary to other compounds. None of the compounds violate Veber rule while benzene-3-bromo and pentacosane are the only compounds without violation to Muegge rule. The pharmacokinetics of *Chromolaena Odorata* identified chemical compounds, Cyclooctene,4-methylene-6 (1-propenyldene) which is the only compound with low GI absorption. Only pentacosane has P-gb substrate, and only benzene-3-bromo has CYP2C9 and CYP2D6 inhibitors. None of the compounds have CPY34A inhibitor. The lipophilicity properties of *Chromolaena Odorata* identified chemical compounds were displayed in Table with benzene-3-bromo having the highest Log Po/w (XLOGP), Log Po/w(WLOGP) and Log P(MLOGP) Values.

Neprilysin is a widely expressed peptidase with broad substrate specificity that preferentially hydrolyses oligopeptide substrates, many of which regulate the cardiovascular, nervous and immune systems. Emerging evidence suggests that neprilysin also hydrolyses peptides that play an important role in glucose metabolism. Neprilysin inhibitors have been used for decades to treat acute diarrhoea and have also been studied for their blood pressure-lowering, natriuretic and analgesic properties (Roques et al., 1993). In both humans and animals, they have also been shown to improve insulin sensitivity, beta cell function and glucose tolerance in diabetic and obese states (kristensen et al., 2016). Alpha-amylase is a key enzyme involved in the conversion of starch to glucose, raising blood glucose levels. An essential tactic in the management of blood glucose levels in diabetes is the inhibition of neprilysin and alpha-amylase, which are involved in the digestion of carbohydrates. This can considerably lower the postprandial increase of blood glucose (Esser and Zraika, 2019).

Protein-ligand complex interaction studies are important for understanding the mechanisms of biological regulation and they also provide the theoretical basis for the design and discovery of new drug targets. The 2D and 3D protein-ligand complex interaction of neprilysin and carbamic acid revealed the presence of three pi-sigma bond, three pi-anion bond and one pi-pi stacked in the interaction between neprilysin and carbamic acid. The interaction between alpha amylase and carbamic acid revealed the presence of two conventional hydrogen bonds ,two pi-donor hydrogen bonds, one pi-cation, one carbon hydrogen bond and one pi-alkyl bond in the interaction between alpha-amylase and carbamic acid. The interactions between the ligands and amino acids in the active site of the protein can be further studied to enhance the binding properties and selectivity of the ligands to the proteins. This interaction is vital for the development of a drug for the treatmet and management of diabetes, this is in line with Jhong et al., 2015 that suggested that curcumin inhibited alpha amylase.

The binding affinity results showed that for neprilysin, the highest binding energy was observed with Carbamic acid followed by the reference drug (sacubitril) when compared with other ligands including the co-crystalized ligand (2-acetamido-2-deoxy-beta-D-glucopyranose). Therefore, for

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both enzymes (Neprilysin and alpha amylase) carbamic acid had the greatest binding affinity for both compared to he standard drugs.

Conclusion

The findings from this study suggests that carbamic acid demonstrated antidiabetic activity by inhibiting neprilysin and Alpha-amylase via molecular docking, and it has the potential to advance into pre-clinical and clinical research for future development as an antidiabetic medication.

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