Molecular docking studies of inhibitory activities of Phytochemicals in *Calotropis procera* against α -glucosidase hydrolase Sus B.

O. O., Adeboye¹*, S. A. Agboluaje², & O. F. Akinyele³,

 $Corresponding \ author \ email: \ \underline{moadeb5848@yahoo.com} \ or \ \underline{adeboyeomolara@eacoed.edu.ng}$

Received 11 December 2020; accepted 28 January 2021, published online 30 March 2021

Abstract

The use of synthetic drugs is associated with various side effects and it is important to look for other drugs from medicinal plants. Therefore, this study aimed at assessing the inhibitory activities of *Calotropis procera* leaf against α -glucosidase hydrolase Sus B and it's possible mode of inhibiting this enzyme through molecular docking studies. From the molecular docking analysis, the results shows that out of the thirty six (36) screened phytochemicals, only twenty six (26) fall between the recommended hit value of inhibition constant of (0.1-1.0 μ M) where their inhibition constant range from (0.01-0.59 μ M) after docking with target receptor α -glucosidase hydrolase SusB (PDB ID: 2ZQ0) using Pyrx-vitual screening tools (Autodock tool, Autodock vina and Open babel). Visualizing was done using Pymol and Biosvia discovery studio(2019). Considering the other analysis done, Drug likeness of Lipinski rule of five, only six(6): Hesperidine (3), Calotroposide (3), Calotropin (3), Ascleposide (4), Proceroside (4) and Voruschairin (3) out of the potent twenty six (26) contravene more than 2 of the Lipinski rules of five, therefore other twenty (20) compounds can be considered for processing into potent drugs.

Keywords: α-glucosidase hydrolase SusB, Calotropis procera, phytochemicals, molecular docking, drug likeness.

Introduction

Alpha-glucosidase belongs to oral medications class use for the treatment of type 2 diabetes to decrease the absorption of carbohydrates in the intestine. The consequence is a moderate and lower rise in postprandial blood level of glucose. Carbohydrates must be broken down to smaller sugar particles by natural enzymes like alpha glucosidase before the absorption. The inhibitory activities of synthetic alpha-glucosidase (acarbose and miglitol) responsible carbohydrates absorption from the digestive tract, which results in lowering the after meal glucose levels. Miglitol is a derivative of deoxynojirimycin and oral alpha-glucosidase which slows down the absorption of ingested carbohydrates. It also has the ability to enhance the glycemic control in type 2 diabetes mellitus [1]. The promising therapeutic potential of glucosidase inhibitors in the treatment of ailments such as diabetes, lysosomal storage diseases, metastatic cancer and human immunodeficiency virus (HIV) infection are currently attracting attention De Melo et al, [2] reported that the side effects and potency of alpha-glucosidase inhibitors make them not likely to be an anchor of diabetic therapy [3]. According to [4] [5] the alpha-glucosidase inhibitors are not recommended to be used as antidiabetic, because glucosidases are postulated to be a powerful therapeutic target. In recent time, medicinal

plants have become an originator of new bioactive molecules and biologically energetic natural substances which have been observed to be potent against diseases and their effects. Calotropis procera belongs to the family of Apocynaceae grows throughout the tropical and lightly hot temperate climates is a moderate with dark green wild shrub and spread fleshy leaves. It is use traditionally for curing various disorders since ancient times most especially the latex is used for curing leprosy, inflammation, eczema [6], diarrheal [7] and bronchial asthma. Nadeem, [6] reported that the flowers, fresh roots and leaves of calotropis procera were used as a tonic and appetizer, toothbrush to cure toothache, antidote for snake bite, rheumatic disorder, viral infection, injuries caused by burn, diarrhea, body pain, cure iaundice and catarrh. They have hepatoprotective activity, anti-fertility, antimalarial, anthelmintic and antioxidant activity as well as antiulcer activity.

There is no better understanding and knowledge about the scientific basis of the traditional usefulness of calotropis procera leaves, so it is necessary we evaluate the potency of dietary enzymes like α -glucosidase through molecular docking studies of C. procera leaves and explain possible interactions among compounds and active sites of the enzymes and as well offer logical

¹Department of Chemistry, Emmanuel Alayande College of Education, Oyo

²Ekiti State University, affiliated with Emmanuel Alayande College of Education, Oyo

³Department of Chemistry, Obafemi Awolowo University, Ile- Ife

basis for every possible enzyme inhibition mechanism by the mixture of compounds [6].

Several methods have been employed by the researchers in finding potential drugs for curing various diseases which involves long time laboratory works, too much capital and energy but this present work is focusing the use of one of the tools of Computer aided drug design (CADD) called molecular docking in discovering the potent among phytochemicals isolated from *Calotropis procera*.

Molecular Docking is a computational method mostly used to assist in understanding drug–receptor interaction [8] and forecasting ligands to macromolecular receptors binding mode, it is fast and effective with low cost compare to the traditional method of Drug Discovery. Therefore, this research was designed to evaluate α -glucosidase SusB (PDB ID: 2zq0) inhibitory efficacy along with profiling of potency of phytochemicals isolated from *Calotropis Procera* in order to give scientific evidence for their conventional uses.

Computational Methodology

Ligands preparation

Thirty-six (36) isolated phytochemicals from *Calotropis* procera were used against the target receptor (α-glucosidase hydrolase Sus B). The ligand molecules were copied from a drug database called PubChem (https://pubchem.ncbi.nlm.nih.gov/),

an open chemistry database, and a drug bank consisting of substance, compound, and bioassay [9]. The ligand molecules are; 3-0-rutinoside of kaempferol, Quercetin-3-O-rutinoside, Quercetin-3-rutinoside, Cardenolide. Hesperidine, α-amyrin, Taraxasterol. Ursharin, Germanicyl, Calactin, β-amyrin, β-sistosterol, Calotropin, Calotroposide, Ascleposide, Proceroside, Voruscharin, Stigmasterol, Lupeol, Uzarigenin, Frugoside, Rutin, Uscharidin, Chlorogenic(-)-Epicatechin, Acarbose, Ergosterol, Gallic acid, Epicatechin, Ferulic acid, Vanillic acid, p-coumaric acid, Glucosamine, L-Rhamnose, Arabinose, αrhamnose [10]. They served as the ligand molecules used determine the potency against α-glucosidase hydrolase Sus B, They were converted to 3-dimensional (3D) structures in (.pdb format) for the efficient virtual screening exercise employing SMILES Online Translator (https://cactus.nci.nih.gov/translate) then later minimized to acquire lowest energy and most stable conformer before docking.

Target Receptor Preparation

Crystal structure of α -glucosidase hydrolase SusB (**Pdb ID: 2zq0**) (Figure 1) was recovered from protein data bank RCSB (http://www.rcsb.org/pdb). α -glucosidase

hydrolase SusB occuring in the utilization of starch in the system of Bacteroides thetaiotaomicron in human.

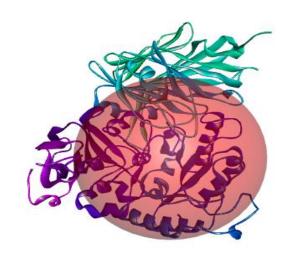


Figure I: Crystal structure of α-glucosidase hydrolase SusB (**PDB ID: 2zq0**)

Determination of (2zq0) Active Sites

All amino acids in the active site, Binding pocket and ligands interactions of α -glucosidase hydrolase SusB were determined with (Uniprot) (www.uniprot.org) and Discovery Studio (2019). The acquired data were contrast and justified with the reported experimental data for α -glucosidase hydrolase SusB complexed with Acarbose ligand [11].

Molecular Docking simulations

Before docking, all atoms and complexes including water molecules attached with the protein (2ZQ0) were detached using Biovia Discovery Studio 4.5, Pyrxvirtual screening tool (Autodock Vina and Open babel,) was employed for the docking process, the acquired grid size are 101.87,109.06, 110.39 for x, y and z axes respectively and grid center are 42.73 x 51.33x 31.76Å with 1.000 Å spacing, then the docking scores and other calculations were carried out using AutoDock Vina (MGL tools- 1.5.6), PyMOL Console Edu and Biovia Discovery studio 4.5.

Drug-like Properties Assessment

The dug-like features of the phytochemicals and SD under study were assessed using Molinspiration online tool (http://molinspiration.com/) while Lipinski's rule of five was employed.

Results and discussion Molecular docking analysis Molecular docking is one of the most important Computer-aided drug designs (CADD) tools used for virtual screening of small molecules at the initial stage of drug discovery. It shows and helps to understand the interactions between a ligand molecule and receptor macromolecule (protein), to establish the right positioning of a ligand and small molecule around the binding site of the target receptor, and to assess how effective the molecules can bind to the target receptor [8], [12] [13]. The Crystal structure of α -glucosidase hydrolase SusB (PDB ID: 2ZQ0) was used as the target receptor in the docking exercise. Thirty-six (36) phytochemicals from Calotropis procera (ligands) and were docked with the target receptor (2ZO0). As shown in (Table 1), the binding energies of the docked ligands against 2ZQ0 target receptor range from -11.0 Kcal/mol and -5.0 Kcal/mol. A reasonable number of ligands among the docked phytochemicals such as 3-0rutinoside of kaempferol (-11 Kcal/mol), Quercetin-3-O-rutinoside(-10.9Kcal/mol), Quercetin-3-rutinoside (-10.9Kcal/mol), Cardenolide(-10.2kcal/mol), Hesperidine (-10.1)Kcal/mol), α -amyrin(-10.1

kcal/mol), Taraxasterol (-10.0 kcal/mol), Ursharin (-10.0 Kcal/mol), Germanicyl (-9.8 Kcal/mol), Calactin (-9.8 kcal/mol) among others have better binding affinities. Moreover, the binding affinity of the ligands with the docking score is used in calculating the inhibition constant value (K_i) (Equation 1) [14] [15]. Also, the lower the Ki value (which is expected to be in the micromolar range for a hit or lead and not more than 10nM for a drug), the more the potency (inhibition efficiency) [16] [17] [18]. As seen in (Table 1), the Ki (Equation 1) value of the docked ligands ranges from 0.01µM and 216.99µM. However, only twenty-six (26) of the docked ligands have inhibition constant values that fall within the recommended range of 0.1µM and 1.0µM [16] [17] [18], and are considered as Hit compounds to be subjected for further analysis.

$$Ki = e^{\left[\frac{-\Delta G}{RT}\right]} - - - - - (Equation 1)$$

 $R = Gas\ constant\ (1.987 \times 10 - 3\ kcal/K-mol);$

T = 298.15 (Absolute Temperature);

ki = Inhibition constant

Table I: The Binding affinity, H-bond interaction, Electrostatic/hydrophobic interactions, 2ZQ0 amino acids forming H-bond with ligands and inhibition constant of the interaction of phytochemicals with α -glucosidase hydrolase SusB.

Ligands	Binding Affinity (ΔG), kcal/mol	2ZQ0 Receptor amino acids forming H-bond with ligands (H-Bond Distance, Å)	Electrostatic/ Hydrophobic Interactions	Inhibition Constant (Ki), µM
3-0-rutinoside of kaempferol	-11	Tyr533(2.22Å)Glu532(2.13 Å)Glu439(2.02Å),Pro215(2.22Å)	Phe536,Lys467, His437,Trp400, Trp341,Trp397	0.01
Quercetin-3-O- rutinoside	-10.9	Ser535(2.02Å)Glu508(3.64Å)	Glu532,Val471, Trp400,Trp341, Phe401	0.01
Quercetin-3- rutinoside	-10.9	Thr627(2.73 Å),His123(2.38Å),Glu117(2.46Å)	Arg121,Asp149,	0.01

			Asp29.	
Cardenolide	-10.2	Thr214(2.25 Å),Arg529(2.37 Å)	Val471,Phe401,	0.09
			Phe536,Trp400,	
			Tyr533,Glu532	
Hesperidine	-10.1	Glu398(2.15 Å),Glu391(2.07 Å),	Val471,Glu439,	0.04
		Trp397(2.81 Å),Ala342(1.93,2.81 Å)	Ser468,His437,	
			Trp400,Gly337	
α-amyrin	-10.1	Nil	Phe536, Val471, Phe401, Trp400, Trp341	0.04
Taraxasterol	-10.0	Nil	Tyr341,Tyr533,	0.05
			Val471, Trp400,	
			Phe536,Phe401	
Ursharin	-10.0	Arg121(2.77 Å),Asp149(2.16 Å),	Glu119,His123	0.05
		Lys118(2.99 Å)		
Germanicyl	-9.8	Lys365(2.13 Å)	Val471,Phe536,	0.07
			Phe401,Gly339,	
			Trp400	
Calactin	-9.7	Thr627(2.36 Å),Glu119(2.41 Å)	Asp149,Ala302	0.08
β-amyrin	-9.6	Nil	Phe401,Phe536,	0.09
			Trp400,Trp341	
			Val471	
β-sistosterol	-9.6	Nil	Val471,Phe536,	0.09
			Phe401,Tyr533,	
			Pro215,Trp397,	
			Trp341,Trp400	
			His507	
Proceragenin	-9.6	Thr627(2.31,2.39Å),	Arg121,Arg304,	0.09

		Arg304(2.38 Å)	Ala302, Thr627	
Calotropin	-9.3	Nil	Glu119,Arg304	0.15
Calotroposide	-9.3	Ala342(2.80 Å)	Val471,Phe401,	0.15
			Trp400,Pro215,	
			Phe536,Trp341,	
			Ser340,Ile335	
Ascleposide	-9.2	Glu398(3.04 Å),Trp397(2.80 Å),	Phe536,Phe401,	0.18
		Ser217(2.07 Å)	Trp400,Trp341,	
			Ser340,Ala342	
Proceroside	-9.2	Asn628(3.04,3.09Å),Asn308(Å),	Ala302	0.18
		Asn299(Å),Arg304(Å),		
		Ala302(Å)		
Voruscharin	-9.2	Nil	Val471,Phe536,	0.18
			Phe401,Trp400,	
			Trp341	
Stigmasterol	-9.1	Nil	Phe401,Phe536,	0.21
			Tyr533,Trp341,	
			Trp400,Tyr533	
Lupeol	-8.9	Nil	Phe536,Phe401,	0.30
			Val471,Trp400,	
			Ile335,Trp341	
Uzarigenin	-8.8	Glu194(Å)	Phe401,Phe536,	0.36
			Val471,Gly337,	
Frugoside	-8.7	Ser217(2.10 Å)	Phe401,Trp400,	0.42
			Phe536,	
Rutin	-8.6	Tyr629(),Asn628(2.53 Å),	Asn122,Ala302,	0.50
		His(2.14,2.23 Å),	Tyr629	
		Arg121(2.80 Å),Asn299(2.52 Å)		

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Uscharidin	-8.6	Trp400(2.70 Å),Ala342(2.21 Å), Phe401,Phe536		0.50
		Trp341(2.16 Å)		
Chlorogenic	-8.5	Asn31(3.0 Å), His123(1.96,2.80Å) Asn148(2.47 Å), Asp149(2.47 Å)		
(-)-Epicatechin	-8.3	Nil	Val471,Glu439,	0.59
			Ser217	
Ergosterol	-7.8	Lys326,Gly384	Lys645,Leu622,	1.93
			Ala621,Pro324,	
			Val113	
Ferulic acid	-6.9	His507, Glu391,	Glu439,Glu508,	8.80
		Ser217	Glu532,Trp331	
P-coumaric	-6.2	Nil	His437,Trp400,	28.65
acid			Val471,Lys467	
Gallic acid	-6.2	His123(2.46,1.92 Å),Asn148(2.77 Å),Arg121(2.23 Å)	Asp149	28.65
Vanillic acid	-6.0	Asn148(2.59,2.83 Å),His123(2.10 Å)	Asp149	40.15
Glucosamine	-5.9	Glu391(2.16 Å),His507(2.58,2.18 Å),Glu532(2.14,2.19 Å),Glu194(2.70 Å),Glu526(2.48 Å)	Glu439,Trp331	47.53
L-Rhamnose	-5.8	His123(2.03 Å)Asn148(2.27 Å),Asp149(1.95,1.94 Å)	Nil	56.27
D-arabinose	-5.1	Asn308(2.51 Å),His123(3.00 Å),Asp149(2.98 Å)	Asn308	183.30
α-rhamnose	-5.0	Asp149(2.26 Å),Arg304(2.35 Å)	Asp149	216.99

Drug-likeness Analysis of the selected phytochemicals

Drug-likeness of prospective active compounds from the plant is essential in drug discovery, as proposed by Lipinski, an effective oral therapeutic drug must obey the 'rule of five' (RO5) with not more than one (1) violation, this is because an oral bioavailability drug must possess molecular weight (MW) \leq 500Da,

hydrogen bond donor (HBD) \leq 5, hydrogen bond acceptor (HBAs) \leq 10 and log P (octanolwater partition coefficient) \leq 5 [19]. These descriptors of oral bioavailability are important as they predict the permeability and absorption of such drugs across biological membranes such as epithelium cell, partition coefficient value (LogP) is especially important in predicting intestinal absorption of such drug. As reported by [16] [17] [18] Bohacek 1996; Hughes 2011 and Stevens, 2014, the inhibition constant value (K_i) is expected to be in the micromolar range (μ M) for a hit or lead candidate and not more than 10nM for a drug candidate, therefore, considering all the ligands (Table 1) in order of

binding affinities and inhibition constants, only those that qualified as hit or lead compounds with inhibition constant value within the range of 0.1uM and 1.0uM [16] [17] [18] were selected for drug-likeness analysis Molinspiration online (http://www.molinspiration.com/).Notably, of all the hits selected for drug-likeness analysis, only six(6): Hesperidine (3),Calotroposide (4), Proceroside (3), Calotropin(3), Ascleposide (4) and Voruschairin (3) out of the potent twenty six (26) (Table II) violated more than 2 of the Lipinski rule of five, therefore other twenty(20) phytochemicals can be considered for further processing into potential therapeutic agent.

Table II: Drug Likeness properties of the best phytochemicals

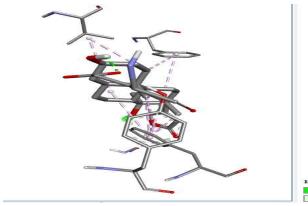
Compounds	Heavy atoms (HA)	Molecular Weight (MW)	RO5 violations	Hydrogen bond donor (HBD)	Hydrogen bond acceptor (HBA)	miLog P
Cardenolide	38	532.7	1	0	2	8.37
Hesperidine	43	610.5	3	8	15	-0.55
α-amyrin	31	426.73	1	1	1	8.08
Taraxasterol	31	426.73	1	1	1	8.10
Uscharin	40	587.72	1	2	9	2.09
Germanicyl	33	454.7	1	0	2	8.37
Calactin	38	532.62	1	3	9	1.15
β-amyrin	31	426.72	1	1	1	8.02
β-sistosterol	45	414.72	1	1	1	8.62
Proceragenin	34	470.7	0	2	4	4.60
Calotropin	38	532.63	1	3	9	1.15
Calotroposide	84	1189.4	3	3	21	5.82
Ascleposide	37	520.7	1	4	8	1.77
Proceroside	39	584.6	1	4	10	0.23
Voruscharin	41	589.7	1	3	9	2.01
Stigmasterol	30	412.70	1	1	1	7.87

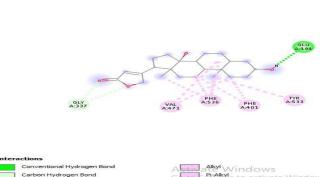
Lupeol	31	426.73	1	1	1	8.29
Uzarigenin	27	374.5	0	2	4	2.47
Frugoside	38	536.7	1	5	9	0.60
Rutin	43	610.5	3	10	16	-1.06
Uscharidin	38	589.75	1	3	9	2.01
Chlorogenic	6	354.31	1	6	9	-0.45
(-)-Epicatechin	31	426.7	1	1	1	8.29
3-0-rutinoside of kaempferol	42	594.5	3	9	15	-0.57
Quercetin-3-O- rutinoside	43	610.52	3	10	16	-1.06
Isorhamnetin-3-O- robinobioside	44	624.55	3	9	15	-0.57

Binding mode and Molecular Interactions of the best Hit compound

The binding mode and molecular interactions involved in the binding of ligands to the active site of the target receptors are very crucial in the lead optimization stage of drug discovery. It aids in improving the potency and efficacy of the selected hit compounds. Notably, all analyses performed so far on the phytochemicals from *calotropis procera*, proceragenin and uzarigenin (Figure II) showed outstanding (A)

results out of the other twenty (20) owing to their excellent binding affinities, inhibition constant and drug-likeness properties. However, since the two emerged as the best Hit compound with better binding affinities and inhibition efficiency, its binding mode and molecular interactions as shown in Table II, the interaction of proceragenin and uzarigenin with the active site of the target alpha glucosidase hydrolase Sus B shows in Figure II.





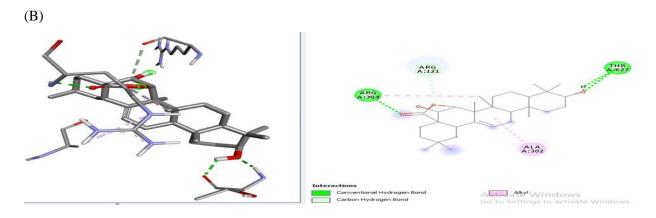


Figure II: Uzarigenin (A) and Proceragenin (B) interactions α-glucosidase hydrolase SusB

Conclusion

This work evaluates phytochemicals isolated from Calotropis procera against α-glucosidase hydrolase SusB using molecular docking studies. From the molecular docking analysis, the results shows that out of the thirty six (36) screened phytochemicals, only twenty six (26) fall between the recommended hit value of inhibition constant of (0.1-1.0 µM) where their inhibition constant range from (0.01-0.59 μM) after been docked with target receptor α-glucosidase hydrolase SusB(PDB ID: 2ZO0) using Pyrx-vitual screening tools (Autodock tool, Autodock vina and Open babel). Visualizing was done using Pymol and Biosvia discovery studio (2019). Considering the other analysis done, Drug likeness of Lipinski rule of five, only six(6):

References

- [1] Servey, J. T. (2007) Alpha-glucosidase inhibitors may reduce the risk of type 2 diabetes. Am Fam Physician; 76: 374-5.
- [2] De Melo E. B., Da Silveira G. A., Carvalho (2006) I. - and -Glucosidase inhibitors: chemical structure and biological activity. Tetrahedron; 62: 10277-02.
- [3] Van de Laar F.A., Lucassen PL, Akkermans R. P., Van de Lisdonk E. H., Rutten G.E., Van Weel. (2005) Alphaglucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. Diabetes Care; 28: 154-63.

Hesperidine(3),Calotroposide(3),Calotropin(3),Asc leposide(4),Proceroside(4) and Voruschairin(3) out of the potent twenty six(26) contravene more than 2 of the Lipinski rule of five, Therefore other twenty (20) compounds can be considered for processing into potent drugs.

However, the significance of performing other analysis such as bioactivities, ADMET properties and active site of the target receptor, Hit/Lead optimization, Molecular Dynamics, Meta-Dynamics (free energy calculation), and *In vivo* biochemical assay to further validate our outcome is highly recognized, but time constraint and some other factors restricted our scope.

- [4] Ahmad, M. B., Gwarzo, M. Y. and Anwar, S. (2015), Antioxidative and anti-hyperglycaemic effect of calotropis procera in alloxan induced diabetic rats. Journal of medicinal plant research February 2016 DOI: 10.5897/JMPR2014.5704
- [5] Mutiu Idowu Kazeem,, Ayuva Mercy Mayaki, Bimpe Folashade Ogungbe and Anthony Babajide Ojekale (2014), Invitro Studies on Calotropis procera Leaf Extracts as Inhibitors of Key Enzymes Linked to Diabetes Mellitus, Iranian Journal of Pharmaceutical Research (2016), 15 (Special issue): 37-44
- [6] Nadeem Muhammad, (2019), Calotropis procera: UHPLC-QTOF-MS/MS based profiling of bioactives, antioxidant

- and anti-diabetic potential of leaf extracts and an insight into molecular docking. Journal of Food Measurement Characterization https://doi.org/10.1007/s11694-019-
- [7] Suresh Kumar, S., Dewan, H. Sangraula, Kumar, V. L. (2001), Anti-diarrhoeal activity of the latex of Calotropis procera, journal of ethnopharmacology 76 (2001) 115–118

00243-z

- [8] Lyne, P. D. (2002) Structure-based virtual screening: an overview. Drug Discov Today 7:1047-1055
- [9] Kim, S. (2016), "PubChem substance and compound databases," *Nucleic Acids Res.*, vol. 44, no. D1, pp. D1202–D1213, doi: 10.1093/nar/gkv951.
- [10] Gaurav Parihar, Neelam Balekar (2016), Calotropis procera: A phytochemical and pharmacological review, TJPS 40 (3): 115-131 http://www.tips.pharm.chula.ac.th
- [11] Momoyo, K, Masayuki O, Fumiko T,Haruhide M,YuKitago,Nobuhisa W, Atsuo K,Isao T, and Min Ya (2008), Structural and Functional Analysis of a Glycoside Hydrolase Family 97 Enzyme from Bacteroides the taiota omicron, The Journal Of Biological Chemistry Vol. 283, NO. 52, pp. 36328-36337
- [12] Taylor, R. D., Jewsbury, P. J. and. Essex, J. W. (2002), "A review of protein-small molecule docking methods," *Journal of Computer-Aided Molecular Design*, 16 (3),pp. 151–166, doi:10.1023/a:1020155510718
- [13] Congreve, M. Murray, C.W. and Blundell T. L. (2005), Structural biology and drug discovery. Drug Discov Today. Jul 1;10(13):895-907. doi: 10.1016/S1359-6446(05)03484-7. PMID: 15993809.
- [14] Hopkins A. L, Keserü G. M;, Leeson P. D. (2014) The role of ligand efficiency metrics in drug discovery. Nat Rev Drug Discov 13(2): 105-121. https://doi.org/10.1038/nrd4163
- [15] Zhu, J. Adli, M. Zou, J. Y. Verstappen, G. Coyne, M. Zhang, X. Durham, T.

- Miri, M. Deshpande, V. De Jager, P. L., Bennett, D. A., Houmard, J. A., Muoio, D. M., Onder, T.T., Camahort, R., Cowan, C. A., Meissner, A., Epstein, C. B., Shoresh, N., and Bernstein, B. E. Genome-wide chromatin state transitions associated with developmental and environmental cues. Cell. 2013 Jan 31;152(3):642-54. doi: 10.1016/j.cell.2012.12.033. Epub 2013 Jan 17. PMID: 23333102; PMCID: PMC3563935.
- [16] Bohacek, R. S, McMartin C, Guida WC. The art and practice of structure-based drug design: a molecular modeling perspective. Med Res Rev. 1996 Jan;16(1):3-50. doi: 10.1002/(SICI)1098-1128(199601)16:1<3: AID-MED1>3.0.CO;2-6. PMID: 8788213.
- [17] Hughes, J. P., Rees S, Kalindjian S. B., Philpott K.L. (2011) Principles of early drug. Br J Pharmacol 162: 1239-1249. https://doi.org/10.1111/j.1476-5381.2010.01127.x
- [18] Stevens, E. (2014) "Medicianl Chemistry: The Modern Drug Discovery Process", Pearson Education, Inc., Boston..
- [19] Lipinski, C. A. (2004) Lead profiling Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today*.;1(4):337-341. doi: 10.1016/j.ddtec.2004.11.007