Original Research Article

In silico study of ornithine decarboxylase and HSP-90 gene in the anti-trypanosomal activities of *Annona muricata* Annonaceae

ABSTRACT

Aims: Trypanosomosis is one of the neglected tropical diseases of Sub-Saharan Africa caused by the numerous species and sub-species of the protozoan genus *Trypanosoma*. Soursop, also known as *Annona muricata* (Annonaceae), is a prevalent tropical plant species renowned for its numerous medicinal properties, including the treatment of protozoan infections. The fundamental mechanism of anti-trypanosomal effects of *A. muricata* was investigated using ornithine decarboxylase and HSP-90, which are validated potential drug targets.

Place and Duration of Study: Animal Parasitology and Microbiology Research Unit, Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria, between March and October 2022.

Methodology: The interaction of compounds previously characterized from *A. muricata* was investigated against Ornithine decarboxylase and HSP-90 genes of *Trypanosoma brucei brucei* using Autodock Vina.

Results: Based on their binding affinity and binding free energy, eight compounds (rutin, kaempferol 3-O-rutinoside, annomuricinA, murihexocin B, muricatocin A, acetogenin, asimilobine, and gigantetrocin A) out of the 160 compounds screened were found to be effective against *Trypanosoma brucei brucei*. The hit molecules were further screened for ADME profiles. Acetogenin and asimilobine were considered the ideal drug candidates because they showed moderation for ADME properties and obeyed Lipinski's rule of five.

Conclusion: This study confirmed the use of *Annona muricata* in the treatment of trypanosomosis and the probable compounds responsible for its antitrypanosomal effect are acetogenin and asimilobine which acts at the HSP-90 genes of the parasite *Trypanosoma brucei brucei.*

Keywords: Annona muricata; antitrypanosomal activities; ornithine decarboxylase; HSP-90; Trypanosoma brucei brucei; binding affinity; rigid docking.

1. INTRODUCTION

In tropical Africa, animal trypanosomiasis has a catastrophic economic impact and poses a significant barrier to livestock production [1]. It is a parasitic infection caused by haemoprotozoa belonging to the Trypanosoma genus and the Trypanosomatidae family,

which proliferate in the blood, lymphatic vessels, and tissues such as cardiac muscles and the central nervous system. Although not contagious (with the exception of dourine, a form of equine venereal trypanosomiasis), it is severe, inoculable, fatal, and zoonotic. The transmission of trypanosomiasis is by a variety of hematophagous insects, primarily Glossina species, often known as tsetse flies [2].

Controlling the intermediate host and administering chemotherapy have been the primary methods for combating this disease. The available pharmaceuticals have adverse effects, including toxicity, cost, and scarcity. Consequently, it is necessary to search for alternate drug sources. Plants are well-known sources of secondary metabolites, which have proved over the years to be effective in the treatment of numerous animal and human diseases.

Soursop (*Annona muricata* L.), also known as Graviola or guanabana, is an extensively cultivated edible tropical fruit tree [3]. The economic importance of soursop fruit in the production of beverages, candies, and sherbets is widely acknowledged [4]. Extensive chemical research on the leaves and seeds of this plant has led to the isolation of a large number of bioactive compounds with intriguing biological properties, such as anticancer, cytotoxic, antiparasitic, and pesticidal activities.

Throughout the globe, medicinal plants have been used to treat and prevent a wide range of diseases, particularly in underdeveloped countries where infectious diseases are prevalent and there are insufficient modern health facilities and services [5]. Historically, various parts of the *A. muricata* tree have been used to treat a variety of human diseases and ailments. In the past, the fruit was used to cure a wide range of conditions, including arthritis pain, rheumatism, dysentery, diarrhea, fever, parasites, and skin rashes. The leaves are effective at treating diabetes, incontinence, rheumatism, and headaches [6]. “Seeds were used as an antihelmintic against a variety of parasites and worms, both internal and external” [7].

“Ornithine decarboxylase (ODC) catalyzes the ornithine decarboxylation to putrescine. This is a crucial phase in the biosynthesis of polyamines in Trypanosoma brucei. These polyamines are essential for the growth and proliferation of microbial cells. Therefore, the ODC enzyme is the ideal target for treating the protozoan parasite that causes African sleeping sickness, *T. brucei*. ODC is a 5-pyridoxal phosphate (PLP)-dependent, obligate homodimer enzyme with two identical active sites at the dimer interface, consisting of the beta or alpha barrel domain from one subunit and the beta-sheet domain from the other” [8].

For survival in the insect vector and mammalian host, the trypanosome requires heat shock proteins. Heat shock protein 90 (HSP90) serves a crucial role in the cellular stress response. The inhibition of its interactions with chaperones and co-chaperones has been investigated as a potential therapeutic target for a variety of diseases.
“The HSP90 apparatus is essential for environmental sensing and life cycle regulation in Trypanosoma and Leishmania” [9]; [10]. There have been publications of in silico analyses of the HSP90/HSPC family of intracellular kinetoplastid parasites [11], [12], [13], [14], [15].

2. METHODOLOGY

2.1 Preparation of natural compounds

Compounds previously characterized from *Annona muricata* were retrieved from a variety of published works [16], [17] and drawn using the ChemAxon suite (https://www.chemaxon.com). Using the ligprep panel on Maestro 11.5 and the OPLS3 force field at pH 7.0 +/- 2.0, the compounds were generated. Desalt and generate tautomers were selected in the ligprep tab. The stereoisomer computation was set to produce a maximum of 32 per ligand. The output format was not changed from maestro.

2.2 Protein preparation

The crystal structures of ornithine decarboxylase (PDB ID-1NJJ) and HSP90 of T. brucei (PDI ID-3OPD) were obtained from the protein data bank (PDB) and uploaded to the Maestro (11.8) workstation. “The downloaded protein was prepared via protein preparation wizard of Schrodinger suite. In preprocessing of the protein, bond orders were assigned, waters were deleted from 5.0 Å from het groups, het states was set at pH 7.0 +/- 2.0” [18]. “Hydrogen bond was added, ions were removed. In the refine tab, H-bond network was optimized using PROPKA; water molecules with less than 3 H-bonds to non-waters were removed” [19]. The retrained minimization was carried out using OPLS3 force field with RMSD at 0.30 Å. Using the protein preparation program, the resulting three-dimensional crystal structure was created.

2.3 Molecular Docking (Rigid Docking)

“The prepared compounds were docked into the active site of the protein crystals using high throughput virtual screening (HTVS) with flexible ligand sampling, followed by extra precision (XP) with none (refine only) ligand sampling. Using a model energy score (e-model) that incorporates glide score, the non-bonded interaction energy, and the excess internal energy of the generated ligand conformation, the optimal docked structure for each ligand was determined. The procedure was carried out by considering the flexibility of the ligand such that all rotational bonds were set free and the estimated binding energies for the best pose were recorded” [20].

2,4 ADME predictions

Using Qikprop [18], the Absorption, Distribution, Metabolism, and Excretion (ADME) and molecular properties of the principal compounds were predicted. This evaluates the acceptability of hit compounds based on Lipinski's rule of five, provides ranges for comparing the properties of a particular molecule to those of 95% of known drugs, and retrieves the most similar drugs available.

3. RESULTS AND DISCUSSION

3.1 Docking analysis

In the present study 160 compounds from *Annona muricata* were screened, and compounds with high docking score were picked. About five compounds each have shown high binding affinity with ornithine decarboxylase and HSP-90 proteins of *Trypanosoma brucei brucei*. Molecular docking result ant the interacting residues of the respective ligand-protein complex of the investigated compounds are presented in Table 1 and 2. Rutin (-8.069kcal/mol) and Kaempferol 3-O-rutinoside (-7.867kcal/mol) showed highest binding affinity for ornithine decarboxylase (Table 1). Kaempferol 3-O-rutinoside and rutin has docking scores of -12.469 kcal/mol and -12.135kcal/mol respectively with HSP-90 (Table 2). From this study, Muricatocin A showed the least binding affinity with a docking score of -6.778kcal/mol with ornithine decarboxylase while Gigantetrocin A showed the least binding affinity with a docking score of -10.283kcal/mol with HSP-90.

Table 1: Docking score of lead compounds of *Annona muricata* and interacting residues of ornithine decarboxylase

.

S/N	Compound name	Docking score	Interacting residues	Number of H-bonds	Salt bridge	Pi cation
1	rutin	-8.069	ARG337, GLU384, ARG242, SER282, THR285, THR21	8	-	1
2.	kaempferol 3-O-rutinoside	-7.867	ARG242, ASP47, ARG22, THR285, ASP385, GLU384	8	-	-
3.	Annomuricin A	-7.086	ARG242, ARG22, THR285, ASP383, GLU384, PRO340	7	-	-
4.	murihexocin B	-6.812	GLU384, LEU339, PRO340, ARG342, GLU343	5		
5.	muricatocin A	-6.778	GLU343, PRO340, LEU381, ARG342	4		

Table 2: Docking score of lead compounds of *Annona muricata* and interacting residues of HSP-90

S/N	Compound name	Docking score	Interacting residues	Number of H-bonds	Salt bridge	Pi cation
1.	Kaempferol 3-O-rutinoside	-12.469	ASN91, TYR124, ASP39, LYS43, ASP78	5	-	-
2.	Rutin	-12.135	LYS43, ASP39, ASP78	3	-	-
3.	Acetogenin	-11.133	ASN91, TYR124, GLY120, ASN139	4	-	-
4.	Asimilobine	-11.121	ASN91, TRP147	1	-	1

5.	Gigantetrocin A	-10.283	ASP78, GLY82	2	-	-

The hit molecules were further accessed for interacting profiles with the protein targets. Figures 1-10 showed the 2D diagram of hit compounds illustrating their intermolecular interaction at the active sites of ornithine decarboxylase and HSP-90.

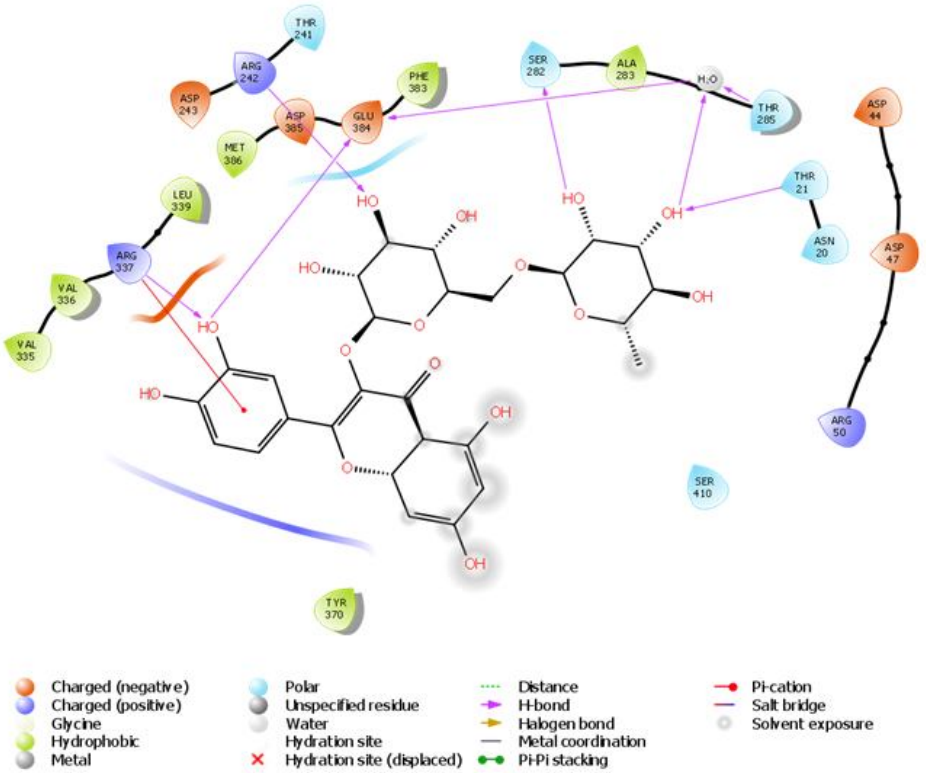

Fig. 1: Binding pose of rutin from *Annona muricata* with *T. brucei* ornithine decarboxylase showing critical amino acid interactive within the protein's active site in 2D

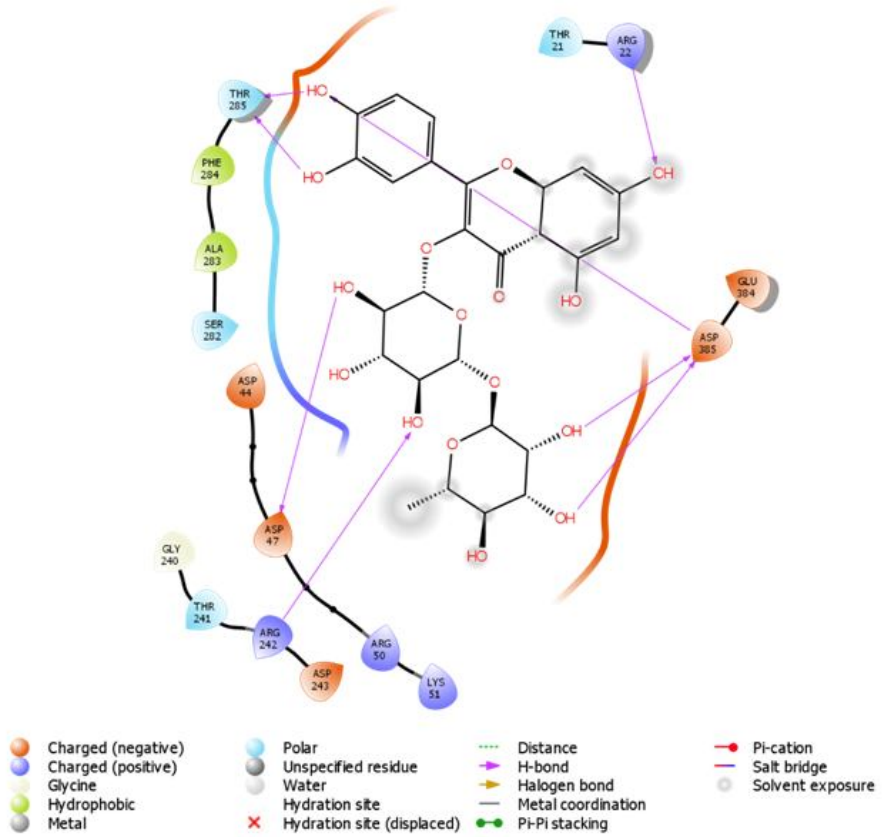

Fig. 2: Binding pose of kaempferol 3-O-rutinoside from *Annona muricata* with *T. brucei* ornithine decarboxylase showing critical amino acid interactive within the protein's active site in 2D

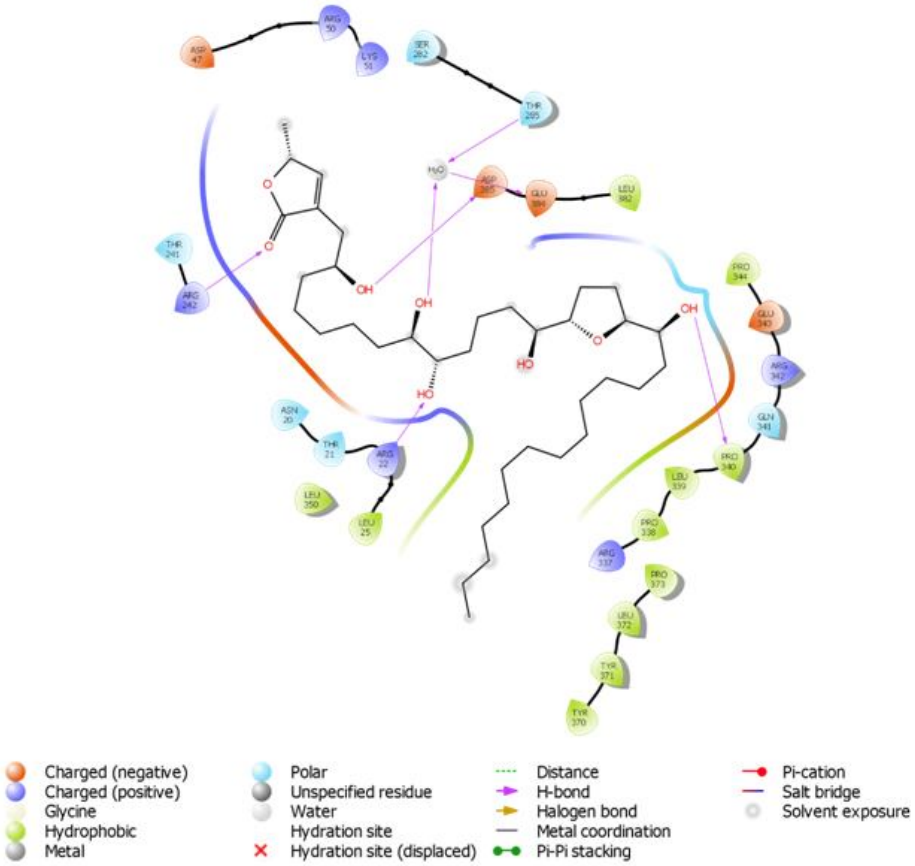

Fig. 3: Binding pose of Annomuricin A from *Annona muricata* with *T. brucei* ornithine decarboxylase showing critical amino acid interactive within the protein's active site in 2D

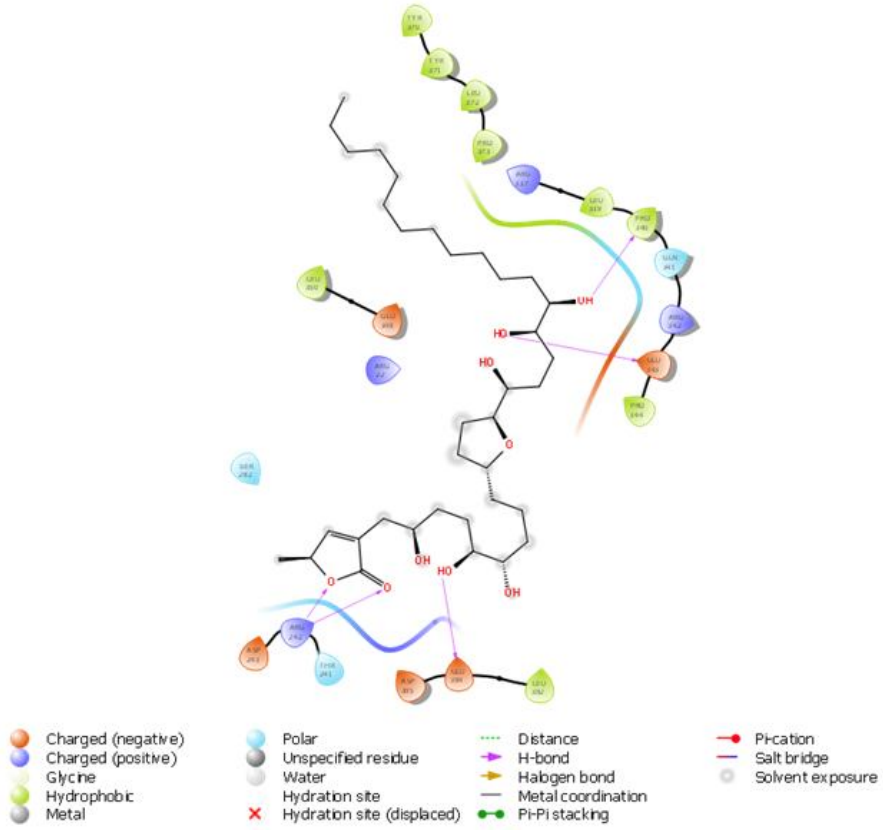

Fig. 4: Binding pose of murihexocin B from *Annona muricata* with *T. brucei* ornithine decarboxylase showing critical amino acid interactive within the protein's active site in 2D

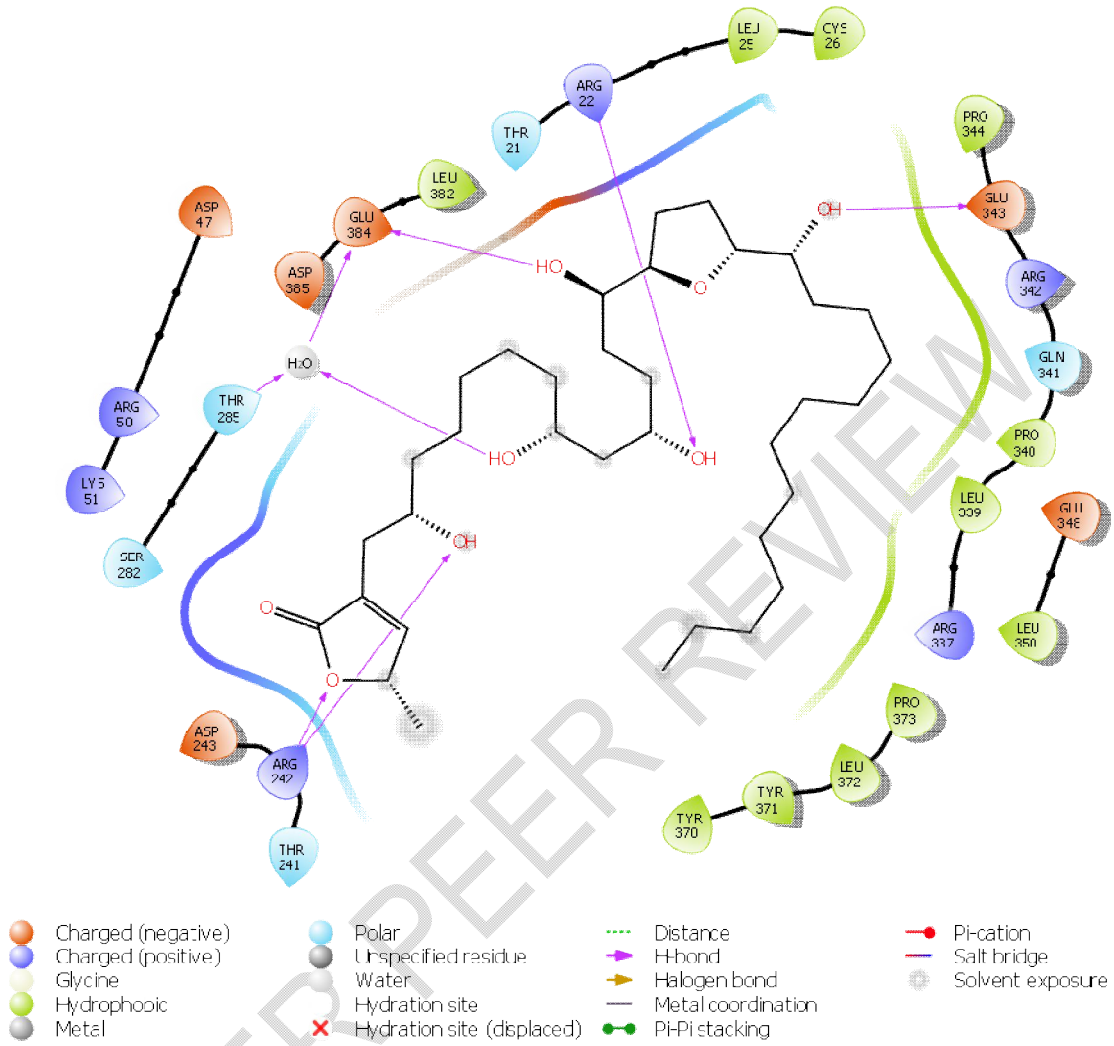

Fig. 5: Binding pose of muricatocin A from *Annona muricata* with *T. brucei* ornithine decarboxylase showing critical amino acid interactive within the protein's active site in 2D

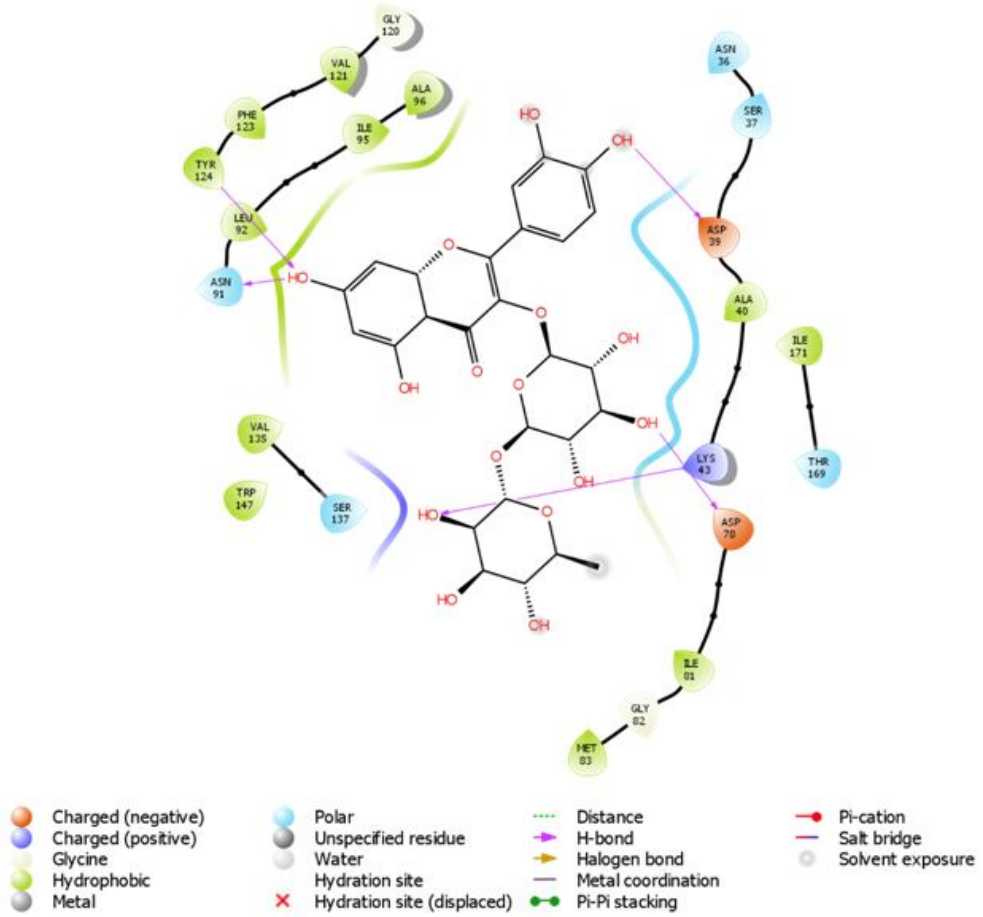

Fig. 6: Binding pose of Kaempferol 3-O-rutinoside from *Annona muricata* with *T. brucei* HSP-90 showing critical amino acid interactive within the protein's active site in 2D

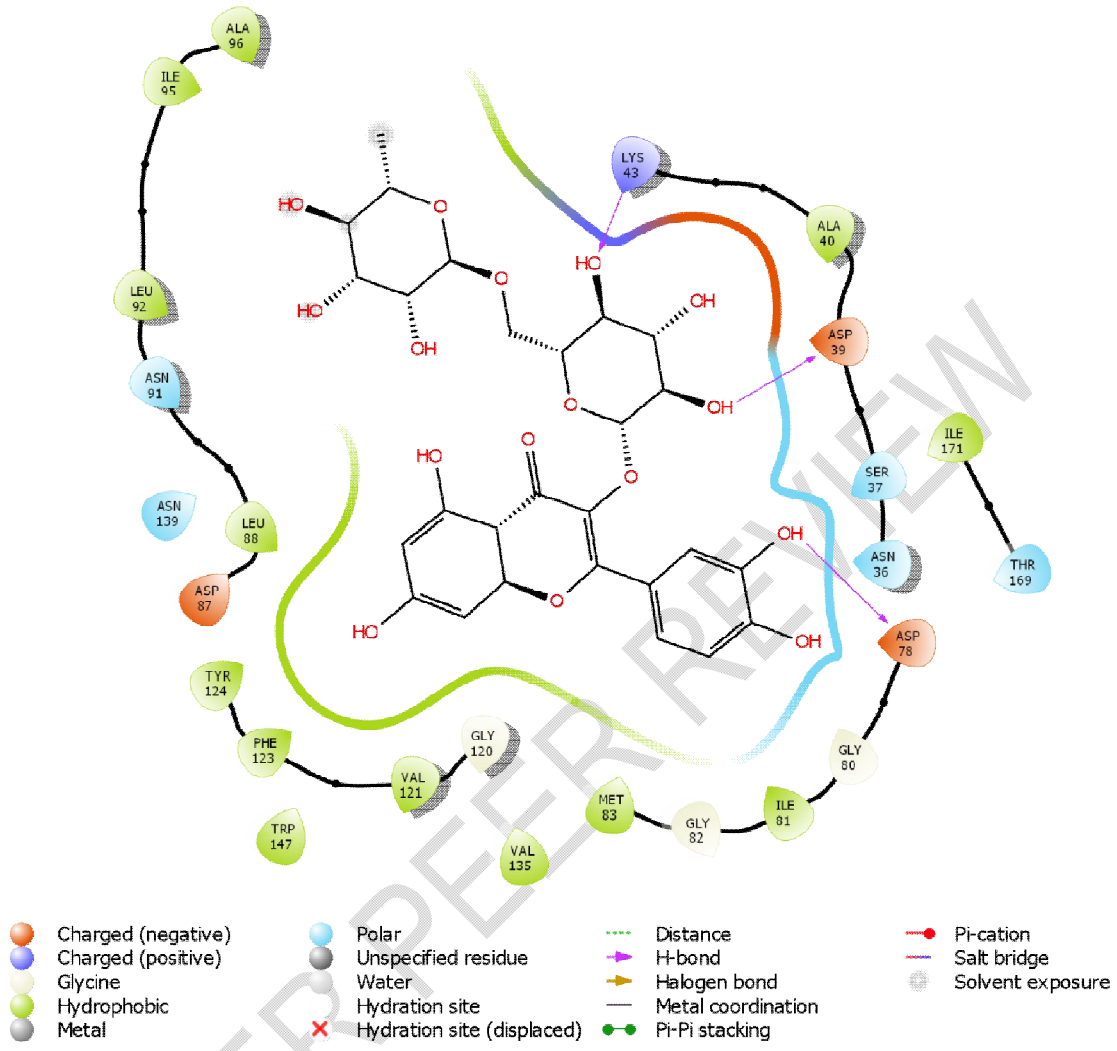

Fig. 7: Binding pose of rutin from *Annona muricata* with *T. brucei* HSP-90 showing critical amino acid interactive within the protein's active site in 2D

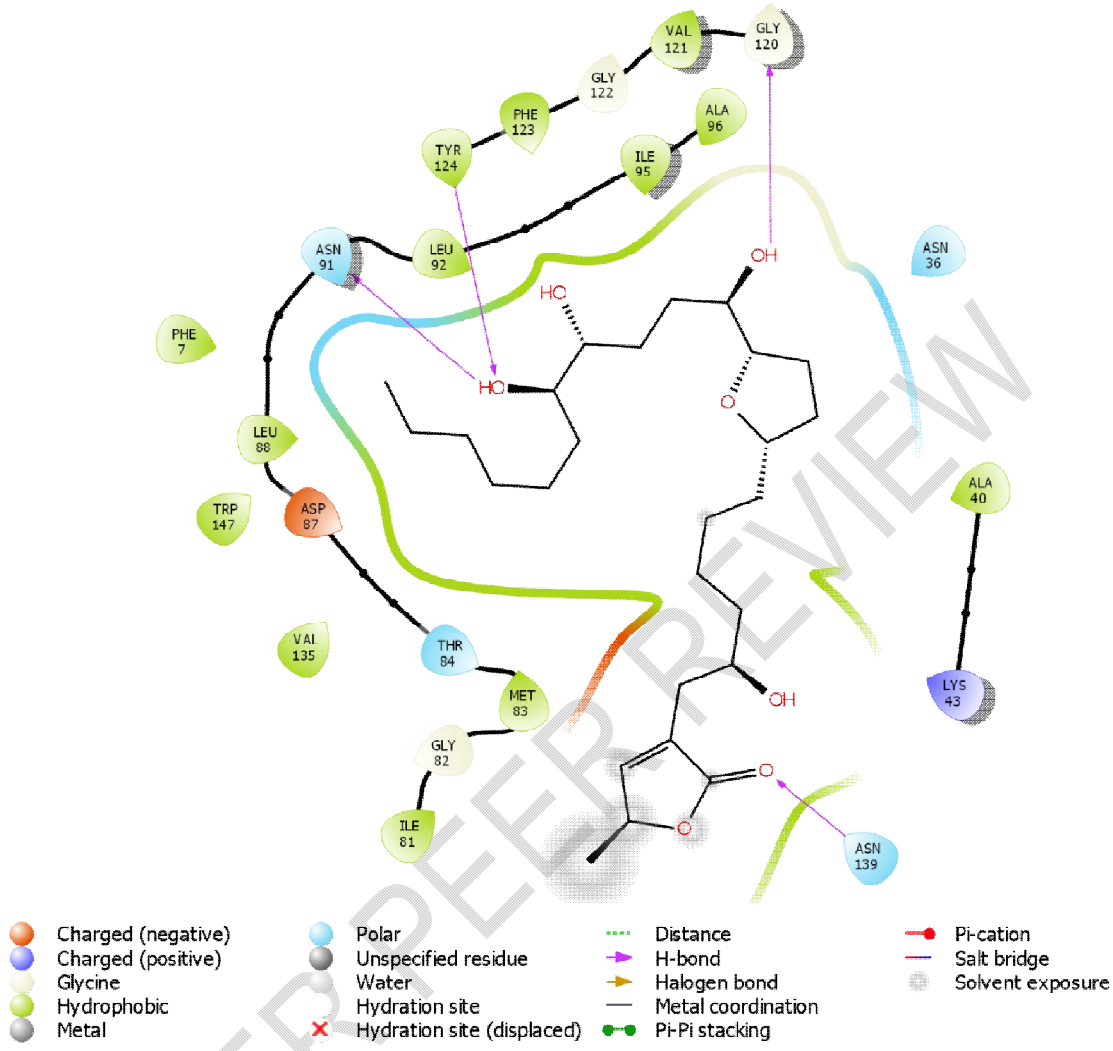

Fig. 8: Binding pose of Acetogenin from *Annona muricata* with *T. brucei* HSP-90 showing critical amino acid interactive within the protein's active site in 2D

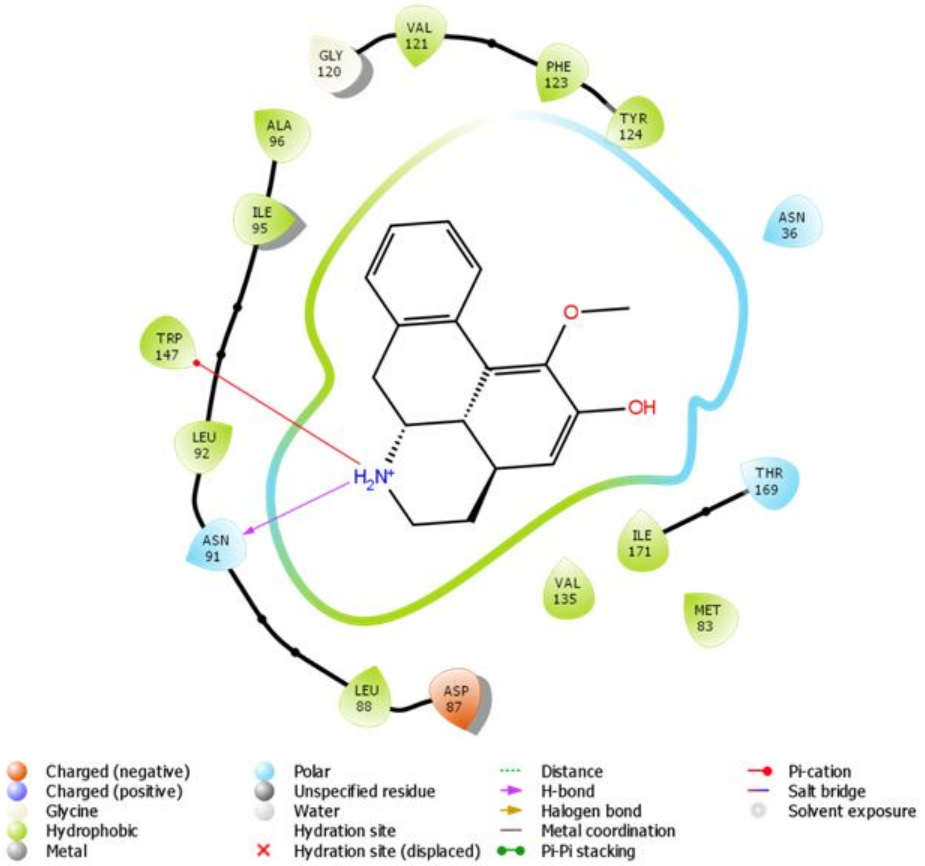

Fig. 9: Binding pose of Asimilobine from *Annona muricata* with *T. brucei* HSP-90 showing critical amino acid interactive within the protein's active site in 2D

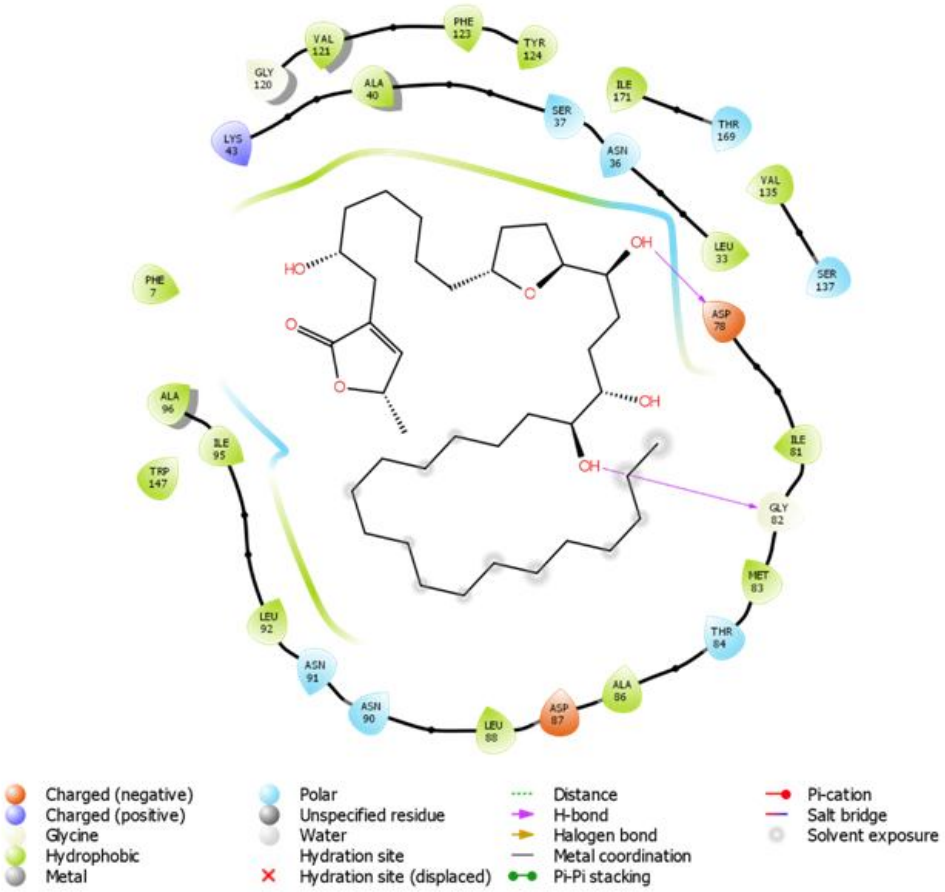

Fig. 10: Binding pose of Gigantetrocin A from *Annona muricata* with *T. brucei* HSP-90 showing critical amino acid interactive within the protein's active site in 2D

3.2 ADME predictions

The analysis of the eight hit compounds using Lipinski rule of five is presented in Table 3. Acetogenin and asimilobine passed the rule of five.

Table 3: Physicochemical properties of lead compounds.

S/N	Entry name	Molecular Weight	H-B Donor	H-B acceptor	QPlogPo/w	QPlogBB	PSA	Rule Of Five
1	rutin	612.54	9	20.8	-2.343	-4.061	267.856	3
2	Kaempferol 3-0-rutinoside	598.513	9	20.8	-2.788	-4.675	272.457	3
3	annomuricinA	626.913	5	13.2	5.864	-4.79	149.399	2

4	murihexocin B	628.885	6	14.9	4.512	-4.853	169.376	2
5	muricatocin A	612.886	5	13.2	5.219	-3.742	148.615	2
6	Acetogenin	470.645	4	11.5	3.293	-3.086	128.986	0
7	Asimilobine	269.343	2	3	2.652	0.327	39.204	0
8	Gigantetrocin A	638.967	4	11.5	7.689	-4.481	129.025	2

Note: [Recommended values]: PSA (Polar surface area) = 7 to 200; QlogBB between −3.0 and 1.0; QPlogPo/w (expected octanol/water partition coefficient) between −2 and 6.5; MW between 130 and 725.

4. DISCUSSION

"Ornithine decarboxylase (ODC) catalyzes the ornithine decarboxylation to putrescine. This is a crucial phase in the biosynthesis of polyamines in Trypanosoma brucei. These polyamines are essential for the growth and proliferation of microbial cells" [21]. They modulate gene expression and cell growth by interacting with RNA and proteins in the cell [22]. In *T. brucei*, polyamines are precursors for the synthesis of trypanothione, a trypanosomatid-specific thiol that is essential for redox regulation and defense against oxidative damage [23] and is associated with drug extrusion in the closely related trypanosomatid Leishmania [24]. Ornithine decarboxylation, catalyzed by ornithine decarboxylase, results in the production of putrescine, a precursor for the polyamines, which are essential for cell division. Consequently, targeting this enzyme has been shown to be effective in the treatment of trypanosomosis.

"To obtain accurate predictions of ligand conformation and orientation within a targeted binding site, docking studies were conducted" [25], [26], [27]. To determine the binding affinity of the compounds with the receptor, each compound was individually docked using the glide docking algorithm into the active site of ornithine decarboxylase and HSP-90 of *Trypanosoma brucei brucei*. [28], [29]. Glide docking employs hierarchical filters to identify the optimal ligand binding sites in the defined receptor grid space. Extra precision (XP) docking scores were reported for the docked compounds. The hit molecules showed a docking score ranging from -6.778 kcal/mol to -12.469 kcal/mol. The four top docked compounds which are kaempferol 3-O-rutinoside, rutin, acetogenin, and assimilobine had a docking score of -12.469 kcal/mol, -12.135kcal/mol, -11.133kcal/mol, and -11.121kcal/mol respectively. Rampogu *et al.,* [30] reported that "the docking score reflects the inhibitory activities of the ligand in the protein-ligand complex. Therefore this result proves that the lead compounds may have inhibitory activities".

The interacting residues of ornithine decarboxylase and HSP-90 with lead compounds were listed in Tables 1 and 2. Pro-340, Glu-384, Asp-385, and Asp-243 have been reported to make H-bonds with the inhibitors of ODC [31]. Our results interestingly revealed that some of the studied compounds isolated from *Annona muricata* (rutin, kaempferol-3-0-rutinoside, Annomuricin A, murihexocinB and muricatocin A) as proposed inhibitors of ornithine decarboxylase were involved in hydrogen bond interaction with GLU384, PRO 340, ARG242, THR285, and ARG342. Furthermore, water-mediated interactions have been shown to occur between the ligand and the residues Asn91, Gly122, Phe123, Asp78, and Asn36 at HSP90 binding site [32]. In this study also, Kaempferol 3-O-rutinoside, acetogenin,

asimilobine, and gigantetrocin-A as proposed inhibitors of HSP-90 formed hydrogen interaction with ASN91. “The importance of hydrogen bonds for their crucial role in evaluating the specificity of ligand binding has been reported” by Wade and Goodford [33].

The evaluation of the efficacy and toxicity of the new drug candidates is one of the most essential aspects of drug discovery. The introduction of ADME has considerably reduced the number of ineffective drug candidates in the early stages of drug development, allowing more resources to be allocated to promising drug candidates [34]. Matias et al. [35] state that “a number of methods and instruments exist to evaluate the physicochemical properties of a molecule that can influence its pharmacokinetic and pharmacodynamic properties. Relevant physicochemical and pharmacokinetic properties were conducted on the lead compounds identified in this study. Before a compound is designated a drug candidate, the Lipinski rule of five (ROF) is one of the necessary criteria”. They were: molecular weight in g/mol (acceptable range of 150–650), Octanol/water partition coefficient which is critical for estimation of absorption and distribution of drugs within the body (QlogP o/w, −2 to 6.5), Brain/blood partition coefficient which is used as a measure of access to the central nervous system (QPlogBB in ml blood/g brain, −3.0 to 1.2), hydrogen bond donor ≤5, hydrogen bond acceptor ≤10 [36]. [37] have demonstrated that the polar surface area (PSA) can accurately predict drug absorption. The polar surface area is the combined surface area of oxygen, nitrogen, and hydrogen atoms bonded to these electronegative atoms. PSA ranges between 7 to 200.
From our study, the pharmacokinetic properties of acetogenin and assimilobine obeyed the rule of five indicating their potential as drug-like candidates.

5. CONCLUSION

In the present study, acetogenins and asimilobine from *Annona muricata* seem to have better criteria than other tested compounds as inhibitors of HSP-90 of *Trypanosoma brucei brucei*. They possess excellent ADME attributes as potential drug candidates and showed good docking scores. In view of this, further, in vitro and in vivo biological investigations are needed to affirm their therapeutic effects in the management of trypanosomosis.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist

AUTHORS’ CONTRIBUTIONS

Authors IBO and IAA did the conceptualization, supervision and editing, Author OOE and COD did the methodology, reviewing, formal analysis, writing and editing. All authors read and approved the final manuscript.

REFERENCES

[1] Berresaw MK, Fetene GM. The Economics of Trypanosomiasis : Empirical Evidence on Its Impacts on Livestock Production and Welfare. October 2021. https://doi.org/10.22004/ag.econ.315259

[2] Ikenna E. Animal Trypanosomiasis in Africa : Aetiology And Epidemiology. 2008; 5, 811–815.
[3] Wele AY, Zhang C, Caux JP, Brouard JL, Pousset, Bodo B. “Annomuricatin C, a novel cyclohexapeptide from the seeds of *Annona muricata*,” ComptesRendusChimie, 2004; 7(10-11): 981–988.
[4] Adefegha SA, Oyeleye SI, Oboh G. Distribution of Phenolic Contents, Antidiabetic Potentials , Antihypertensive Properties , and Antioxidative Effects of Soursop (*Annona muricata* L .) Fruit Parts In Vitro. 2015.
[5] Zaidan MRS, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop Biomed 2005; 22(2): 165-170.
[6] De Souza EB, Benassi E, da Silva RR, Afonso S, Scarminio IS. Enhanced extraction yields and mobile phase separa- tions by solvent mixtures for the analysis of metabolites in *Annona muricata* L. Leaves. J Sep Sci. 2009; 32:4176–85. https://doi.org/10.1002/jssc.200900375
[7] Taylor L. Technical data report for graviola, *Annona muricata*, Vol. 10 Austin: Sage Press; 2002. p. 1–6.
[8] Hukkeri S, Ambrosio AB. In-silico Identification of Candidate Inhibitory Ligands against Ornithine Decarboxylase Enzyme for Human Sleeping Sickness Causing Trypanosoma brucei. J Biomol Res Ther. 2017; 6: 148. doi:10.4172/2167-7956.1000148.
[9] Wiesgigl M, Clos J. The heat shock protein 90 of Leishmania donovani. *Med. Microbiol. Immunol.* 2001; 190, 27–31. 10.1007/s004300100074
[10] Graefe SEB, Wiesgigl M, Gaworski I, Macdonald A, Clos J. Inhibition of HSP90 in trypanosoma cruzi induces a stress response but No stage differentiation. *Eukaryot. Cell*. 2002; 1: 936–943. 10.1128/EC.1.6.936-943.
[11] Shonhai A, Maier G, Przyborski AM, Blatch L. Intracellular Protozoan parasites of humans: The role of molecular chaperones in development and pathogenesis. *Protein Pept. Lett.* 2011; 18: 143–157. 10.2174/092986611794475002
[12] Roy N, Nageshan RK, Ranade S, Tatu U. Heat shock protein 90 from neglected protozoan parasites. *Biochim. Biophys. Acta*. 2012: 1823, 707–711. 10.1016/j.bbamcr.2011.12.003.
[13] Figueras MJ, Echeverria PC, Angel SO. Protozoan HSP90-heterocomplex: Molecular interaction network and biological significance. *Curr. Protein Pept. Sci.* 2014; 15, 245–255. 10.2174/1389203715666140331114233
[14] Urményi TP, Silva R, Rondinelli E. The heat shock proteins of Trypanosoma cruzi. *Subcell. Biochem.* 2014: 74, 119–135. 10.1007/978-94-007-7305-9_5
[15] Requena JM, Montalvo AM, Fraga J. Molecular chaperones of Leishmania: Central players in many stress-related and -unrelated physiological processes. *Biomed. Res. Int.* 2015. e301326. 10.1155/2015/301326
[16] Moghadamtousi SZ, Fadaeinasab M. Nikzad S, Mohan G, Ali HM, Kadir HA. *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *Int. J. Mol. Sci.* **2015**, *16*, 15625-15658. https://doi.org/10.3390/ijms160715625
[17] Coria-Téllez AV, Montalvo-Gónzalez E, Yahia EM, Obledo-Vázquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity, Arabian Journal of Chemistry, Volume 11, Issue 5, 2018, Pages 662-691, https://doi.org/10.1016/j.arabjc.2016.01.004.
[18] Release S. 2: QikProp, Schrödinger. 2018.
[19] Olsson MH, Sondergaard CR, Rostkowski M, Jensen JH (2011) PROPKA3: Consistent treatment of internal and surface residues in empirical p K a predictions. J Chem Theor Comput 7: 525-537.

[20] Elekofehinti OO, Ariyo EO, Akinjiyan MO, Olayeriju OS, Lawal AO, Adanlawo IG, Rocha JBT. Potential use of bitter lemon (Mormodica charantia) derived compounds as antidiabetics: in silico and in vivo studies. Pathophysiology 2018: 25, 327–333.

[21] Miller-Fleming L, Olin-Sandoval V, Campbell K, Ralser M. Remaining mysteries of molecular biology: the role of polyamines in the cell. J. Mol. Biol. 2015; 427: 3389–3406.
[22] Childs AC, Mehta DJ, Gerner EW. Polyaminedependent gene expression. Cell. Mol. Life Sci. 2003; 60, 1394–1406.
[23] Fairlamb AH, Blackburn P, Ulrich P, Chait BT, Cerami A. Trypanothione: a novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. Science. 1985; 227, 1485–1487.
[24] Mukhopadhyay R, Dey S, Xu N, Gage D, Lightbody J, Ouellette M, Rosen BP. Trypanothione overproduction and resistance to antimonials and arsenicals in Leishmania. Proc. Natl. Acad. Sci. USA. 1996; 93: 10383–10387.
[25] Kroemer RT. Structure-based drug design: Docking and scoring. Curr Prot Peptide Sci. 2007; 8: 312-328.
[26] Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011; 7: 146-157.
[27] Ramírez D, Caballero J. Is it reliable to use common molecular docking methods for comparing the binding affinities of enantiomer pairs for their protein target?. Internat J Molecul Sci. 2016; 17: 525.
[28] Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem. 2004; 47: 1739-1749.
[29] Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL. Glide: A new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem. 2004; 47: 1750-1759.
[30] Rampogu S, Park C, Son M, Baek A, Zeb A, Lee G. Lee KW. Modulation of aromatase by natural compounds—a pharmacophore guided molecular modelling simulations. *South Afr. J. Bot.* 2019;120, 230. 10.1016/j.sajb.2018.06.019
[31] Jackson LK, Goldsmith EJ, Phillips MA. X-ray Structure Determination of Trypanosoma brucei Ornithine Decarboxylase Bound to d-Ornithine and to G418: insights into substrate binding and odc conformational flexibility. Journal of Biological Chemistry, 2003; 278 (24): 22037-22043. https://doi.org/10.1074/jbc.M300188200.
[32] Joosten RP, Long F, Murshudov GN and Perrakis A. The PDB_REDO Server for Macromolecular Structure Model Optimization. *IUCrJ* **2014**, *1*, 213–220.
[33] Wade RC, Goodford PJ. The role of hydrogen-bonds in drug binding. Prog Clini Biol Res. 1989;289: 433-444.
[34] Kaitin KI. Obstacles and opportunities in new drug development. Clin Pharm Therap 2008; 83: 210-212.
[35] Matias M, Campos G, Silvestre S, Falcao S, Alves G. Early preclinical evaluation of dihydropyrimidin(tho)ones as potential anticonvulsant drug candidates. Eur J Pharm. 2017; 102:264-274.
[36] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev. 1997; 23: 3-25.
[37] Palm K, Luthman K, Ungell AL, Strandlund G, Artursson P. *J Pharm Sci.* 1996;85:32–39. doi: 10.1021/js950285r.