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In Silico Studies of Involvement of Ornithine Decarboxylase and HSP-90 Gene in the Anti-Trypanosomal Activities of *Alchornea laxiflora* Benth

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Abstract: African trypanosomiasis is a zoonotic disease of sub-Saharan Africa caused primarily by extracellular protozoa belonging to the *Trypanosoma* genus, including *Trypanosoma brucei brucei*. *Alchornea laxiflora* Benth (Pepe in Yoruba) is a prevalent species of tropical plant known for its numerous medicinal properties, including the ability to treat protozoan infections. Ornithine decarboxylase and HSP-90 validated potential targets for anti-trypanosomal drugs, and they were used to investigate the mechanism underlying the anti-trypanosomal effects of *A. laxiflora*. Using Autodock Vina, the interaction of previously identified compounds from *A. laxiflora* with the Ornithine decarboxylase and HSP-90 genes of *Trypanosoma brucei brucei* was investigated. Out of 34 compounds screened from *Alchornea laxiflora*, 7 compounds (Quercetin, Rutin, Quercetin-3-O-beta-D-glucopyranoside, Quercetin-3-O-L-rhamnopyranoside, Quercetin-3-O-alpha-D-arabinopyranoside, Quercetin-3-O-rhamnoside, Quercetin-3-O-beta-D-galactopyranoside) were found to be potent against *Trypanosoma brucei brucei* based on their binding affinity and binding free energy. The hit molecules were further screened for ADME profiles. Quercetin and Quercetin-3-O-beta-D-glucopyranoside were considered the ideal drug candidates because they showed moderation for ADME properties and obeyed Lipinski's rule of five. This study confirmed the use of *Alchornea laxiflora* in the treatment of trypanosomosis, and the compounds most likely responsible for its anti-trypanosomal effect are Quercetin and Quercetin-3-O-beta-D-glucopyranoside, which inhibit the ornithine decarboxylase and HSP-90 genes of *Trypanosoma brucei brucei.*

Keywords: *Alchornea laxiflora*; molecular docking; ornithine decarboxylase; HSP-90 genes; trypanosomosis; *Trypanosoma brucei brucei.*

1. Introduction

African trypanosomiasis, also known as sleeping sickness in humans and Nagana in animals, continues to be a significant health and economic issue in sub-Saharan Africa, affecting around 50 million people and 48 million cattle, leading to substantial productivity losses and fatalities [1]. The disease is caused by extracellular protozoa of the genus *Trypanosoma*, with species such as *Trypanosoma brucei brucei* primarily responsible [2].

Nagana affects livestock, causing high mortality rates and presenting symptoms such as intermittent fever, anemia, anorexia, poor coat condition, emaciation, lethargy, swollen lymph nodes, abortion, infertility, reduced milk yield, submandibular edema, ascites, and ocular discharge [1]. African Animal Trypanosomiasis (AAT) remains a major obstacle to development in sub-Saharan Africa, severely hindering sustainable livestock production and food security [1]. The economic impact is profound, with high morbidity and mortality in susceptible animals, expensive treatment costs, and reduced meat and milk production exacerbating the situation [2]. Sustained control efforts have reduced human African trypanosomiasis cases, yet the disease continues to threaten millions in 36 countries in sub-Saharan Africa, particularly affecting rural populations dependent on agriculture and livestock [1]

Chemotherapy, chemoprophylaxis, and vector control programs have been the mainstay of trypanosomiasis control in Africa without an effective vaccine against the disease. Several chemical compounds and biological molecules have been tried to overcome *T. brucei* infection, including melarsoprol, pentamidine, and glycoprotein antigens [3].

Current first-line treatment depends on eflornithine (d, l-α-difluoromethylornithine) therapy, particularly in combination with nifurtimox [3]. Eflornithine is a mechanism-based inhibitor of ornithine decarboxylase, which catalyzes a key step in the biosynthesis of polyamines that facilitate the growth and proliferation of the parasite [4]. Other enzymes of polyamine biosynthesis could also serve as drug targets for treating parasitic infections [5,6].

Ornithine Decarboxylase (ODC), an essential enzyme, catalyzes the initial phase in the polyamine biosynthetic pathway of *T. brucei* [7]. ODC is dependent on 5'-pyridoxyl phosphate (PLP) and is an obligate homodimer. L-ornithine is converted into 1,4-diaminobutane, also known as putrescine, which serves as a precursor for the biosynthesis of the polyamines spermine and spermidine. Polyamines are essential components of living cells, regulating functions of polynucleotides (RNA and DNA) by interacting with them, contributing to the stability of biological membranes, and enhancing the catalytic activity of certain enzymes. Polyamines are crucial for protozoan cell proliferation and are regarded as essential nutrients for protozoan parasites. Inhibiting biosynthetic enzymes or knocking down genes leads to polyamine depletion, decreasing trypanothione levels and cell mortality [8,9]. Therefore, ODC is a principal drug target enzyme that inhibits the proliferation of *Trypanosoma brucei* within the living system.

The 90-kDa heat shock protein (HSP90) family consists of essential, highly conserved, and abundant molecular chaperones [10,11] that aid in the proper folding and maturation of a specific set of substrates known as client proteins [12,13]. Many client proteins of human HSP90 are involved in protein folding and degradation, signaling pathways, cellular trafficking, cell cycle regulation, and differentiation [14-16].

In *Trypanosoma* and *Leishmania*, the HSP90 machinery is crucial for environmental sensing and life cycle regulation [17]. In silico analyses of the HSP90/HSPC family in intracellular kinetoplastid parasites have been published [18]. For survival in both the insect vector and mammalian host, trypanosomes rely on heat-shock proteins. Heat shock protein 90 (HSP90) plays a vital role in the cellular stress response. Inhibiting its interactions with chaperones and co-chaperones is being explored as a potential therapeutic strategy for various diseases [19].

Exploring traditionally asserted medicinal plants for their biological activity led to the development of a number of antiprotozoal medications.

According to "The Plant List" (http://www.theplantlist.org), there are 55 species in the *Alchornea* genus. *Alchornea laxiflora* (Benth.) Pax & K. Hoffm is commonly found in tropical rainforests across America, Africa, and Asia. This species is a deciduous understory tree or shrub that can grow up to 6 meters and is typically found in lowland tropical forests, wetlands, riverine vegetation, mixed deciduous woodlands, sub-montane forests, and semi-deciduous tropical rainforests [20, 21]. *A. laxiflora* has been documented for its use as an anti-venom for snake bites [22]. Its extracts are used to treat malaria, piles, dysentery, eczema, congestion, and high fever [23]. Okokon *et al*. [24] investigated the in vitro antiplasmodial activity of crude ethanol, petroleum ether, chloroform, ethyl acetate, and butanol extracts of *A. laxiflora* leaves against two strains of *Plasmodium falciparum*. Osho et al. [25] also reported on the in vitro and in vivo anti-trypanosomal activities of crude extracts of *A. laxiflora* leaves against *Trypanosoma brucei brucei*.

2. Materials and Methods

2.1. Preparation of natural compounds.

Compounds previously characterized by *Annona muricata* were retrieved from different literature. The compounds were prepared using a ligprep panel on Maestro 11.5 with an OPLS3 force field at pH 7.0 +/- 2.0. In the ligprep tab, desalt and generate tautomers options were selected. The stereoisomer computation was left to generate 32 per ligand at most. The output format was left as Maestro.

2.2. Protein preparation.

The crystal structures of ornithine decarboxylase with (PDB ID-1NJJ) and HSP90 of *T. brucei* (PDI ID-3OPD) were retrieved from the protein data bank (PDB) and uploaded on the workspace of Maestro (version 11.8.) The obtained three-dimensional crystal structure was prepared via a protein preparation wizard.

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 a flexible ligand sampling set followed by extra precision (XP) with ligand sampling restricted to none (refine only). The best-docked structure for each ligand was chosen using the model energy score (e-model) that combines the glide score, the non-bonded interaction energy, and the excess internal energy of the generated ligand conformation.

2.4. ADME predictions.

The lead compounds' absorption, distribution, metabolism, excretion (ADME), and molecular properties were predicted using Qikprop. This evaluates the acceptability of hit compounds based on Lipinski’s rule of five, provides ranges for comparing a particular molecule’s properties with those of 95 % of known drugs, and retrieves the most similar drugs available.

3. Results and Discussion

3.1. Docking analysis.

Docking studies are carried out to obtain accurate ligand conformation and orientation predictions within a targeted binding site. In the present study, 87 compounds from *Alchornea laxiflora* were screened, and compounds with high docking scores were picked. About five compounds have shown high binding affinity with ornithine decarboxylase (ODC) and HSP-90 proteins of *Trypanosoma brucei brucei*.

Table 1 presents the molecular docking results of the respective ligand-protein complex with ODC. The docking score of the hit compounds ranged from -4.356 to − 10.171 kcal/mol. Quercetin attained the highest binding affinity with a -10.171 kcal/mol score. The next ranked compounds are rutin, Quercetin-3-O-beta-D-glucopyranoside, and Quercetin-3-O-beta-D-galactopyranoside with docking scores of -9.368 kcal/mol, -8.87 kcal/mol, and -8.448 kcal/mol respectively.

Table 1. The molecular docking results of the respective ligand-protein complex with ODC.

S/N	Title	Entry name	Docking score
1	Quercetin	Quercetin.1	-10.171
2	Rutin	Rutin.1	-9.368
3	Quercetin-3-O-beta-D-glucopyranoside	Quercetin-3-O-beta-D-glucopyranoside.1	-8.87
4	Quercetin-3-O-beta-D-galactopyranoside	Quercetin-3-O-beta-D-galactopyranoside.1	-8.448
5	Ellagic acid	entry.1	-7.505
6	quercetin-3-O-alpha-D-arabinopyranoside	quercetin-3-O-alpha-D-arabinopyranoside.1	-7.005
7	quercetin-7-O-glucopyranoside	quercetin-7-O-glucopyranoside.1	-6.924
8	quercetin-3-O-L-rhamnopyranoside	quercetin-3-O-L-rhamnopyranoside.1	-6.842
9	3-O methyl ellagic acid	3-O methyl ellagic acid.1	-6.741
10	4735	Pentamidine.1	-5.966
11	Quercetin-3,4'-disulphate	Quercetin-3,4'-disulphate.1	-5.354
12	Quercetin-3,7,3',4-tetrasulphate	Quercetin-3,7,3',4-tetrasulphate.1	-5.354
13	Quercetin-3-O-rhamnoside	Quercetin-3-O-rhamnoside.1	-5.331
14	Quercetin-7,4-disulphate	Quercetin-7,4-disulphate.1	-5.256
15	Quercetin-3,4'-diacetate	Quercetin-3,4'-diacetate.1	-4.356

The molecular docking results of the respective ligand-protein complex with HSP90 are presented in Table 2, with docking scores of hit compounds ranging from 3.889 kcal/mol to -13.112 kcal/mol. Rutin attained the highest binding affinity with a score of -13.112 kcal/mol followed by Quercetin-3-O-beta-D-glucopyranoside (-12.295 kcal/mol), quercetin-3-O-alpha-D-arabinopyranoside (-11.975 kcal/mol), Quercetin-3-O-rhamnoside (-11.883kcal/mol) and Quercetin-3-O-beta-D-galactopyranoside (-11.38 kcal/mol) in that order.

Table 2. The molecular docking results of the respective ligand-protein complex with HSP90.

S/N	Title	Entry name	Docking score
1	Rutin	Rutin.1	-13.112
2	Quercetin-3-O-beta-D-glucopyranoside	Quercetin-3-O-beta-D-glucopyranoside.1	-12.295
3	quercetin-3-O-alpha-D-arabinopyranoside	quercetin-3-O-alpha-D-arabinopyranoside.1	-11.975
4	Quercetin-3-O-rhamnoside	Quercetin-3-O-rhamnoside.1	-11.883
5	Quercetin-3-O-beta-D-galactopyranoside	Quercetin-3-O-beta-D-galactopyranoside.1	-11.38

S/N	Title	Entry name	Docking score
6	quercetin-7-O-glucopyranoside	quercetin-7-O-glucopyranoside.1	-10.928
7	quercetin-3-O-L-rhamnopyranoside	quercetin-3-O-L-rhamnopyranoside.1	-10.832
8	Quercetin	Quercetin.1	-10.806
9	Quercitrin	Quercitrin.1	-9.872
10	Ellagic acid	entry.1	-8.973
11	3-O methyl ellagic acid	3-O methyl ellagic acid.1	-8.781
12	Quercetin-3,7,3',4-tetrasulphate	Quercetin-3,7,3',4-tetrasulphate.1	-7.46
13	Quercetin-7,4-disulphate	Quercetin-7,4-disulphate.1	-7.417
14	Quercetin-3,4'-disulphate	Quercetin-3,4'-disulphate.1	-7.072
15	Quercetin-3,4'-diacetate	Quercetin-3,4'-diacetate.1	-6.441
16	4735	Pentamidine.1	-4.463
17	3-O-beta-D-glucopyranosyl-beta-sitosterol	3-O-beta-D-glucopyranosyl-beta-sitosterol.1	-3.825
18	3-O-acetyl oleanolic acid	3-O-acety oleanolic acid.1	3.383
19	6475119	3-O-acetyl-ursolic acid.1	3.889

Rampogu *et al.* [26] reported that the docking score reflects the inhibitory activities of the ligand in the protein-ligand complex. Therefore, this result suggests that the hit compounds derived from *Alchornea laxiflora* are promising agents as inhibitors of ODC and HSP90 of *Trypanosoma brucei brucei.*

3.2. Hydrogen bonding interaction.

The binding site of ornithine decarboxylase (ODC) includes several crucial residues such as Asp-364, Arg-22, Leu-25, Leu-339, Pro-340, Gln-341, Glu-343, Leu-382, Glu-384, and Asp-385. Among these, Pro-340, Glu-384, Asp-385, and Asp-243 are particularly significant as they form hydrogen bonds with the inhibitor [27, 28].

The interacting residues of the ODC protein with lead compounds are listed in Table 3. Our results revealed that some of the inhibitors were involved in hydrogen bond interaction with GLU 384, ASP385, ASP44, and ASP47. Quercetin, rutin, and Quercetin-3-O-beta-D-glucopyranoside formed hydrogen interaction with GLU384. A similar interaction with potential inhibitors was also reported by Jamabo et al. [27].

Table 3. Docking score of lead compounds of *Alchornea laxiflora* with interacting residues of ODC.

S/N	Compound name	Docking score	Interacting residues	Number of H- bonds	Salt bridge	Pi cation
1	Quercetin	-10.171	ASP385, GLU384, PRO340, ASP243	4	3	-
2.	Rutin	-9.368	ASP47, ASP44, THR21, ASP385, GLU384	5	-	-
3.	Quercetin-3-O-beta-D-glucopyranoside	-8.87	GLU343, GLU384	4	-	-
4.	quercetin-3-O-L-rhamnopyranoside	-6.842	ARG242, ASP44, ASP47, ARG50	7		

Furthermore, water-mediated interactions have been shown to occur between the ligand and the residues Asn91, Gly122, Phe123, Asp78, and Asn36 at the HSP90 binding site [27]. Interestingly, all lead compounds as proposed inhibitors of HSP90 from this study (Rutin, Quercetin-3-O-beta-D-glucopyranoside, quercetin-3-O-alpha-D-arabinopyranoside, Quercetin-3-O-rhamnoside and Quercetin-3-O-beta-D-galactopyranoside) have hydrogen bond interaction with ASP78, ASN91, and PHE123. Table 4 gives the interacting residues of the HSP90 with lead compounds.

Table 4. Docking score of lead compounds of *Alchornea laxiflora* with interacting residues of HSP-90.

S/N	Compound name	Docking score	Interacting residues	Number of H- bonds	Salt bridge	Pi-Pi stacking
1	Rutin	-13.112	ASP87, LYS43, ASP78, PHE123	5	-	1
2.	Quercetin-3-O-beta-D-glucopyranoside	-12.295	ASN91, LYS43, GLY120, ASP78	4	-	-
3.	quercetin-3-O-alpha-D-arabinopyranoside	-11.975	ASN91, PHE 123, ASN36	3	-	1
4.	Quercetin-3-O-rhamnoside	-11.883	ASN91, ASP78, PHE123, GLY 120	3	-	1
5.	Quercetin-3-O-beta-D-galactopyranoside	-11.38	ASP78, GLY82, PHE123	3	-	1

Hydrogen bonds' significance in evaluating ligand binding specificity has been well-documented [29,30].

The hit molecules were further accessed to interact profiles with the protein targets. Figures 1-9 show the 2D diagram of the best-hit compounds, illustrating their intermolecular interaction at the active sites of ornithine decarboxylase and HSP-90.

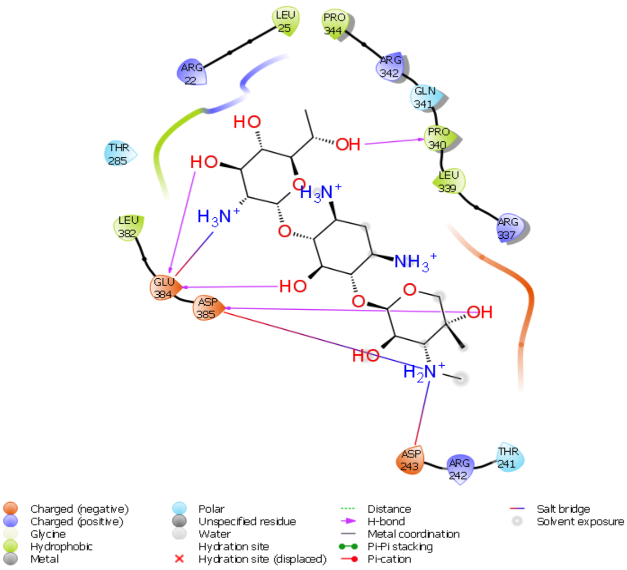

Figure 1. The binding pose of Quercetin from Alchornea laxiflora with *T. brucei* ornithine decarboxylase shows critical amino acid interaction within the protein's active site in 2D.

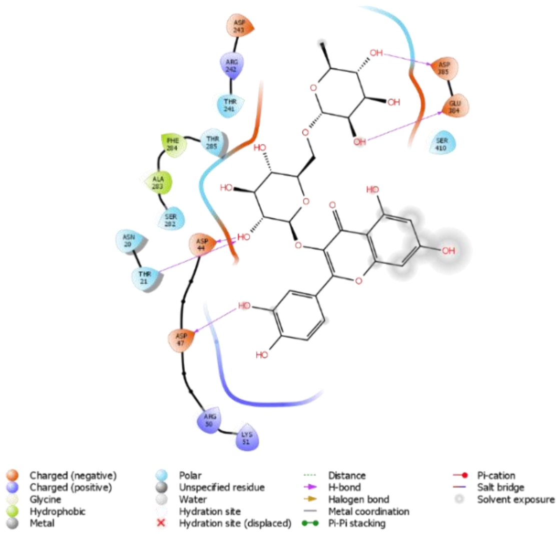

Figure 2. The binding pose of rutin from Alchornea laxiflora with *T. brucei* ornithine decarboxylase shows critical amino acid interaction within the protein's active site in 2D.

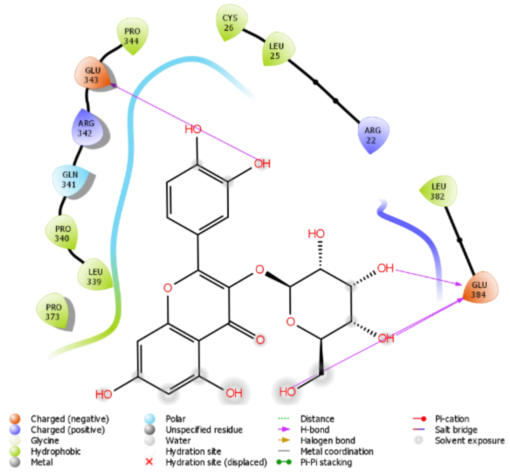

Figure 3. Binding pose of quercetin-3-O-beta-D-glucopyranoside from *Alchornea laxiflora* with *T. brucei* ornithine decarboxylase showing critical amino acid interaction within the protein's active site in 2D.

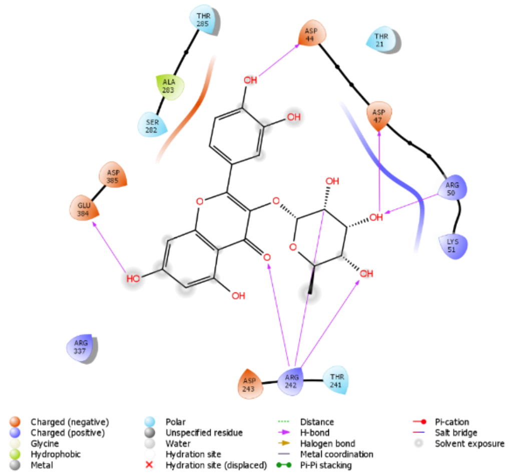

Figure 4. Binding pose of quercetin-3-O-L-rhamnopyranoside from *Alchornea laxiflora* with *T. brucei* ornithine decarboxylase showing critical amino acid interaction within the protein's active site in 2D.

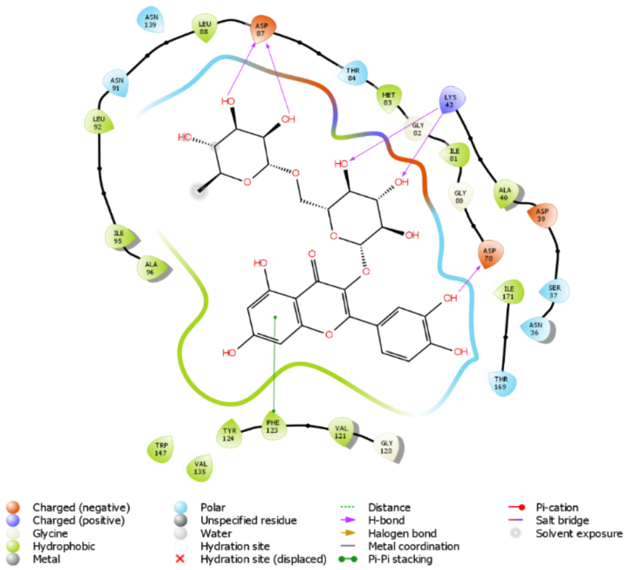

Figure 5. The binding pose of rutin from Alchornea laxiflora with *T. brucei* HSP90 shows critical amino acid interaction within the protein's active site in 2D.

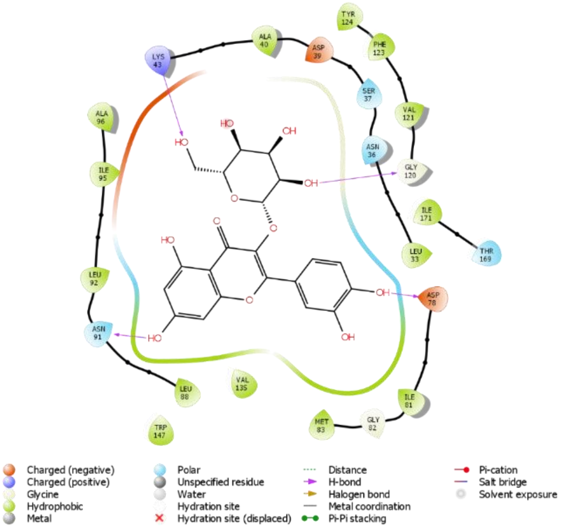

Figure 6. The binding pose of Quercetin-3-O-beta-D-glucopyranoside from Alchornea laxiflora with *T. brucei* HSP90 shows critical amino acid interaction within the protein's active site in 2D.

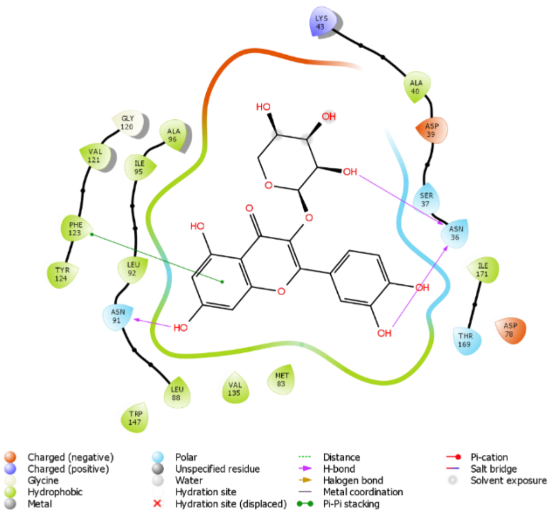

Figure 7. Binding pose of quercetin-3-O-alpha-D-arabinopyranoside from *Alchornea laxiflora* with *T. brucei* HSP90 showing critical amino acid interaction within the protein's active site in 2D.

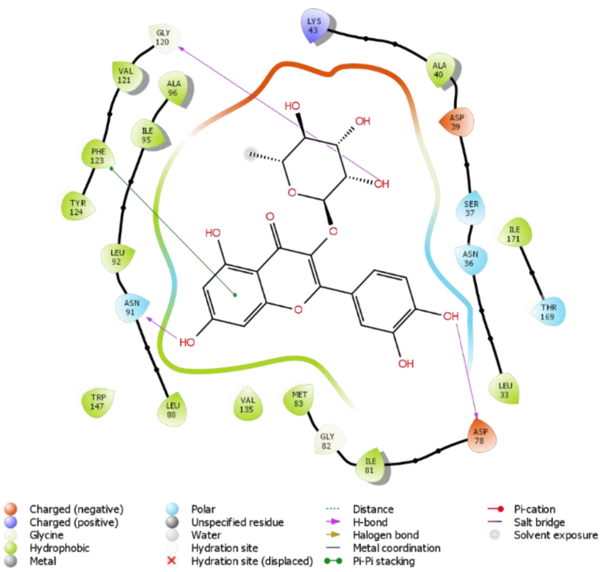

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Figure 8. The binding pose of Quercetin-3-O-rhamnoside from Alchornea laxiflora with *T. brucei* HSP90 shows critical amino acid interaction within the protein's active site in 2D.

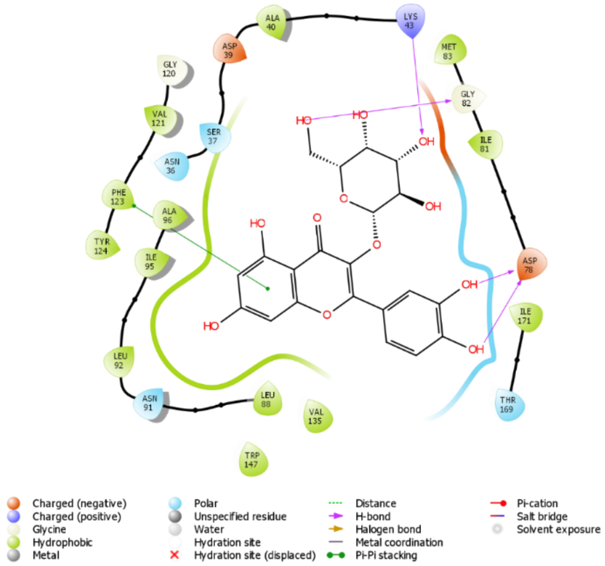

Figure 9. Binding pose of Quercetin-3-O-beta-D-galactopyranoside from *Alchornea laxiflora* with *T. brucei* HSP90 showing critical amino acid interaction within the protein's active site in 2D.

3.3. ADME studies.

The predicted ADME properties (Table 5) include a number of rotatable bonds, the molecular weight of the molecule, the number of hydrogen bond acceptors, the prediction of binding to human serum albumin, the number of hydrogen bond donors, predicted octanol-water partition coefficient and a number of violations of Lipinski's rule of five (RO5).

Table 5. The physicochemical properties of the seven hit compounds from *Alchornea laxiflora*.

S/N	Entry name	Molecular Weight	H-B Donor	H-B acceptor	QPlogPo/w	QPlogBB	PSA	Rule Of Five
1	Quercetin	302.24	4	5.25	0.358	-2.407	143.351	0
2	Rutin	610.524	9	20.55	-2.534	-5.193	271.721	3
3	Quercetin-3-O-beta-D-glucopyranoside	464.38	4	15	0.84	-1.926	199.459	1
4	quercetin-3-O-L-rhamnopyranoside	448.382	6	12.05	-0.432	-2.839	190.435	2
5	quercetin-3-O-alpha-D-arabinopyranoside	434.356	6	12.05	-0.755	-3.091	198.236	2
6	Quercetin-3-O-rhamnoside	448.382	6	12.05	-0.714	-3.169	198.426	2
7	Quercetin-3-O-beta-D-galactopyranoside	448.382	6	11	2.12	0.327	39.204	2

[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.

Evaluating the efficacy and toxicity of new drug candidates is one of the most essential aspects of drug discovery. The implementation 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. A number of methods and instruments exist to evaluate the physicochemical properties of a molecule that can influence its pharmacokinetic and pharmacodynamic properties.

Before a compound is designated a drug candidate, the Lipinski rule of five (Ro5) is one of the essential criteria [31]. Prospective drug candidates that adhere to the Ro5 generally have lower attrition rates during clinical trials, thereby increasing their chances of becoming and remaining marketable [32, 33].

From our study, the pharmacokinetic properties of Quercetin and Quercetin-3-O-beta-D-glucopyranoside from *Alchornea laxiflora* obeyed the rule of five, indicating their potential as drug-like candidates and potential inhibitors of ODC and HSP90 of *Trypanosoma brucei brucei.*

4. Conclusions

In the current study, Quercetin and Quercetin-3-O-beta-D-glucopyranoside from *Alchornea laxiflora* have shown superior potential as inhibitors of ornithine decarboxylase (ODC) and heat shock protein 90 (HSP-90) in *Trypanosoma brucei brucei*. As promising drug candidates, these compounds exhibit excellent ADME (absorption, distribution, metabolism, and excretion) characteristics and favorable docking scores. Consequently, further in vitro and in vivo biological studies are necessary to confirm their therapeutic efficacy in treating trypanosomiasis.

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Conflicts of Interest

The authors declare no conflict of interest.

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