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In silico evaluation of isovitexin and other antihelminthic compounds activities found in *Anthocleista djalonensis* leaf.

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Abstract

Objective: Soil-transmitted helminth infections, which include whipworms, roundworms such as Ascaris, are highly prevalent worldwide, particularly affecting impoverished and marginalized communities. These infections occur when one or more parasitic intestinal roundworms infest the body. The identification of the bioactive compounds responsible for a particular medicinal property of an herbal extract and the elucidation of their mechanism of action are essential stages in drug discovery and development. The aim of this study is evaluate by *in silico* studies isovitexin and other antihelminthic compounds found in *Anthocleista djalonensis* leaf.

Methods: The 3D structures of the proteins were obtained from Protein Data Bank with PDB codes of 3VR9, 5HGQ, 5TIW, 7NWY. The individual receptors were prepared for molecular docking simulations with AutoDockTools and saved as pdbqt. Phytochemicals were obtained from PubChem in SDF-3D format. The reference ligands were identified and obtained from the literature. Using Autodock tools, the ligands were prepared and saved as pdbqt for molecular docking simulation. Validation of docking protocol was done. The molecular docking was done using Autodock Vina-4.2.6 in the Linux operating system (ubuntu). The docking process was repeated 4 times for each of the protein and each of the ligand for the calculation of the average and standard deviation. The docking results were analysed and visualized using PyMol-v1.3r1-ed win32.

Results: Isovitexin, β-sitosterol, 3-QCA, 5-QCA and quercetin were the frontrunner compounds that had higher binding affinities in the active site of at least one of the four screened drug targets when compared to the reference inhibitors of these receptors.

Conclusion: In conclusion, *Anthocleista djalonensis* leaf can be said to have antihelminthic activities.

Keywords: Antihelminthics; Molecular Docking; *Anthocleista Djalonensis*; *In* Silico Studies**.**

1. Introduction

Parasitic worms affect more than one-quarter of the world's population, with soil-transmitted helminthiases (STH) accounting for about 1.5 billion infections[2]. STH is one of the neglected tropical diseases (NTDs) that affects mainly people living in regions of high poverty, without adequate sanitation, and in close contact with infectious vectors,

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domestic animals, and livestock [19]. Parasite eggs present in human faeces contaminate the soil where they embryonate and are taken back into the intestinal tract through poorly treated drinking water and foods [11]. Children constitute the most vulnerable group to worm infestation; pregnant women also suffer impaired immunity and a lower quality of life [11]. Based on the location of the adult parasite in the body, helminthiases may clinically present as intestinal (whipworms, intestinal roundworms, and hookworms), or tissue (trematodes, hydatid tapeworms, and tissue roundworms) parasites [11]. Mild symptoms include abdominal pain, nausea, diarrhoea, and loss of appetite, and in children, severe cases may lead to anaemia, eosinophilia, stunted growth, malnutrition, pneumonia, and poor physical and cognitive development [19]. High-intensity infections could result in intestinal obstruction requiring surgery and death in cases of *Strongyloides stercoralis* [18]. Control measures mainly include periodic deworming, health education, and improvements in environmental sanitation. Seasonal chemotherapy with synthetic anthelmintics remains the primary measure to eliminate or reduce infecting helminthes.

In Africa, about 80% of the population largely depend on traditional remedies for their primary healthcare needs [17]. Plants form the larger part of these traditional remedies and have historically been used in treating internal parasites and other diseases in humans and livestock [12]. There is increasing research into natural products, especially medicinal plants, as sources of new antiparasitic agents. *Anthocleista djalonensis* is a member of the family *Gentianaceae* and formerly *Loganiaceae,* it has a common name called Cabbage tree; Anthocleista are trees and shrub like plants in the major group of Angiosperms [4]. It is called Sapo in Yoruba, Kwarii in Hausa, Okpokolo in Igbo and Osuo in Bayelsa state in southern Nigeria and it is wide spread in West Africa. It grows as a small tree and reaches stature heights of up to 15m its slender trunk has a diameter of 40 cm. The opposite and regularly set leaves are divided into petiole and leaf blade; the petiole is 1 to 9 cm long while the leaf blade is simple, elongated, elliptical to obovate-elliptic having a length of 9 to 35 cm in young plants of 115 cm and 5 to 17 cm width, the young plants width is about 50 cm with a heart-shaped, plump or wedge-shaped base, wedge-shaped upper end and a smooth edge [14]. Abba (2024) identified Isovitexin as one of the major chemical constituents of *Anthocleista djalonensis* leaves found in the Anambra region of Nigeria. The study used HPLC to analyse and detect the abundance of isovitexin and its derivatives in Ethyl Acetate and n-butanol fractions of locally sourced *A. djalonensis* [1].

For the purpose of this study, four essential proteins were identified to be used in the *in-silico* Anti-Heltminthic evaluation. They are Mitochondrial rhodoquinol-fumarate reductase, Sulfotransferase, Lysyl-trna synthetase and Carbonic anhydrase. Mitochondrial rhodoquinol-fumarate reductase is a critical enzyme in the anaerobic energy metabolism of parasites, making it a promising target for drug development in the treatment of helminthiasis and other related conditions. The antischistosomal prodrug oxamniquine is activated by a sulfotransferase enzyme within *Schistosoma mansoni* parasites, making it a species-specific treatment effective against S. mansoni adult worms [5]. Lysyl-tRNA synthetase (LysRS) is a key enzyme in protein biosynthesis, responsible for attaching lysine to its corresponding tRNA molecule. In the context of helminthiasis, LysRS has been identified as a potential target for drug development due to its selective inhibitory properties. Carbonic anhydrase inhibitors have shown promise as potential anthelminthic agents due to their ability to target essential metabolic processes in parasites. These inhibitors have been extensively studied for their therapeutic applications in various diseases, including brain ischemia, glaucoma, and epilepsy. [3]

2. Materials and Methods

2.1. Materials

The research work requires certain materials, tools, software and essentially useful web sites:

A personal computer with internet access and Linux operating system (Ubuntu- 12.04), windows operating system.

2.1.1. Tools and Softwares

- An autodock tool vs-1.5.6 downloaded from http://mgltools.edu. It is used for proteins and ligands preparation and conversion of file format.
- Chimera – 1.9. it is used in the preparation of proteins (removal of residues, water, and other unwanted components of the protein).
- African Natural Compounds Database PyMol- v1.3rl-edu downloaded from http://delsci.com. It is used for viewing 3D structures of proteins and ligands.
- Molinspiration downloaded from www.molinspiration.com. This tool is used to sketch compounds from their SMILES format, predict biological activities and determine the physio-chemical properties of the compounds.

- Protein databank (PDB) gotten from www.rcsb.org. it is used for molecular characterization of proteins, download of 3D structures and fasta file format of biological molecules.
- ZINC database gotten from zinc.docking.org. it is used for physio-chemical properties prediction, download the mol2 file format and SMILE of ligands

2.2. Method

2.2.1. Identification of all the Phytochemicals Possibly Present in Anthocleista djalonensis

A literature search was conducted to identify phytochemicals that have been isolated and identified in extract of *Anthocleista djalonensis*. Thirty phytochemicals were found in the published literature, identified in the leaf extracts. The phytochemicals obtained are listed as follows: Sweroside, Stigmasterol, β-sitosterol, amplexine, djalonenol, djalonensone, loganic acid, swertiamarin, p-coumaric acid, Quercitrin, Quercetin, Isogentisin, Shikimic acid, Gallic acid, 3-Caffeoylquinic acid, (+)-Catechin, Delphinidin-3,5-diglucoside, 5-Caffeoylquinic acid, Vanillic acid, Caffeic acid, (-)-Epicatechin, Syringic acid, Ferulic acid, 3,5-Dicaffeoylquinic acid, Naringin, Rutin, Hyperoside, Resveratrol, Amarogentin, Kaempferol-3-O-glucoside [15]

2.2.2. Studying the drug-likeness of the identified phytochemicals

The drug-likeness of the identified phytochemicals of *Anthocleista djalonensis* was evaluated *in silico* using the Datawarrior chemoinformatics application. The *In-silico* was done based on Lipinski's rule of five. Lipinski's rule of five is a concept frequently used in drug discovery. This rule helps to predict if a biologically active molecule is likely to have the chemical and physical properties to be orally bioavailable. The Lipinski rule bases pharmacokinetic drug properties on specific physicochemical properties such as hydrogen bond donors, hydrogen bond acceptors, Molecular mass, and Partition coefficient [10].

2.2.3. Preparation of the Receptors for Molecular Docking Simulation

The 3-D structures of the receptors/proteins were obtained from Protein Data Bank (http://rscb.org) with the respective Protein Data Bank (PDB) code: 3VR9 for Mitochondrial Rhodoquinol-Fumarate Reductase, 5HGQ for sulfotransferase, 5TIW for lysyl-tRNA synthetase and 7NWY for carbonic anhydrase. PYMOL tool was employed to gain insight into the ligands binding to the receptors and to edit the protein bound to the ligand. The individual receptors were prepared for molecular docking simulations with AutoDockTools (ADT). In the preparation, polar hydrogen was added to the receptors and saved in protein data bank, partial charge and atom type (PDBQT) file format. The pdbqt file format is the structural format needed for the protein and ligand to be in before carrying out molecular docking simulation. The electrostatic Grid boxes and 3-D affinity of different sizes and centres, as indicated in the table below, were created around the active sites of the proteins and saved as a conf file.

2.2.4. Preparation of Ligands (compounds)

The phytochemicals with no Lipinski violation and the reference ligands were noted for molecular docking simulation. Phytochemicals were obtained from PubChem in SDF-3D format. The reference ligands were identified and obtained from the literature. These reference ligands also included Compounds Co-crystallized with the receptors on Protein Data Bank and approved drugs. Using Autodock tools, the ligands were prepared for molecular docking simulation. All rotatable bonds, Torsions, and Geistegers charges were assigned and saved as pdbqt files.

2.2.5. Validation of docking protocol

To validate the molecular docking protocol, the simulation of the reference ligands (Ligands co-crystalized with the protein) with the prepared receptor was simulated using Autodock Vina® on a Linux platform using a configuration file and script. Docked conformations were visualized in PyMol-1.4.1 and compared with the experimental crystal structures of the co-crystallized compounds.

2.2.6. Molecular Docking Simulation

Molecular docking simulations were carried out in four replicates on a Linux platform using AutoDockVina® and associated tools after validation of the docking protocols. The Estimated Binding free energy values (kcal/mol ± S.E.M.) were ranked to enable the identification of the frontrunner phytochemicals.

3. Results

Table 1 The PDB repository codes and names of the proteins used in the receptor preparation

	PDB Protein code	Full name of Protein
1	3VR9	Mitochondrial-rhodoquinol-fumerate reductase from the parasitic *Ascaris suum* with the specific inhibitor Flutolanil
2	5HGQ	Loa Loa Lysyl-tRNA synthetase in complex with cladosporin
3	5TIW	Schistosoma Heamatobium (Blood Fluke) Sulfotransferase/Racemic Oxamniquine Complex
4	7NWY	Crystal Structure of alpha carbonic anhydrase from Schistosoma mansoni with 4-(3-(4-fluorophenyl)ureido)benzenesulfonamide

Table 2 Grid box parameters used in the molecular simulations. This set boundary where docking occurred.

	3VR9		5HGQ		5TIW		7NWY	
	Centers	Sizes	Centers	Sizes	Centers	Sizes	Centers	Sizes
X	-91.811	18	6.43	13	-19.462	12	-6.923	20
Y	-22.138	22	-25.135	29	18.822	21	5.821	40
Z	64.043	32	-18.422	17	25.569	32	12.685	32

Table 3 A Table of the Drug-likeness evaluation parameters of the chemical compounds detected in *Anthocleista dialonensis* extract.

S/N	Molecule Name	Total Mol. Weight	Clogp	H-Acceptors	H-Donors	Polar Surface Area
1	(-)-Epicatechin	290	1.51	6	5	110.38
2	(+)-Catechin	290	1.51	6	5	110.38
3	3,5-Dicaffeoylquinic acid	516	0.80	12	7	211.28
4	3-Caffeoylquinic acid	354	-0.77	9	6	164.75
5	5-Caffeoylquinic acid	354	-0.77	9	6	164.75
6	Amarogentin	587	0.89	13	6	201.67
7	Amplexin	200	-0.05	4	2	66.76
8	Caffeic acid	180	0.78	4	3	77.76
9	Delphinidin-3,5-diglucoside	627	-3.15	17	11	285.75
10	Djalonenol	200	-0.05	4	2	66.76
11	Djalonensone	272	2.57	5	2	75.99
12	Ferulic acid	194	1.06	4	2	66.76
13	Gallic acid	170	0.11	5	4	97.99
14	Hyperoside	464	-0.35	12	8	206.60
15	Isogentisin	258	2.61	5	2	75.99

16	Kaempferol-3-O-glucoside	448	0.00	11	7	186.37
17	loganic acid	538	-4.24	15	9	245.29
18	Naringin	581	-0.74	14	8	225.06
19	p-coumaric acid	164	1.13	3	2	57.53
20	Quercetin	302	1.49	7	5	127.45
21	Quercitrin	448	0.58	11	7	186.37
22	Resveratrol	228	2.83	3	3	60.69
23	Rutin	611	-1.26	16	10	265.52
24	Shikimic acid	174	-1.62	5	4	97.99
25	Stigmasterol	413	7.60	1	1	20.23
26	Sweroside	358	-1.66	9	4	134.91
27	Swertiamarin	374	-2.48	10	5	155.14
28	Syringic acid	198	0.66	5	2	75.99
29	Vanillic acid	168	0.73	4	2	66.76
30	β-sitosterol	415	7.86	1	1	20.23

Table 4 The Result of Molecular Docking Simulation of Mitochondrial Rhodoquinol-Fumarate Reductase with the Phytochemicals of *Anthocleista djalonensis* using flutolanil and nafuredin as reference compounds.

S/N	Compounds	Mean Estimated Binding Free Energy (Kcal/mol) ± Standard Deviation
1	β-sitosterol	-12.40 ± 0.00
2	Flutolanil	-9.60 ± 0.00
3	Nafuredin	-9.40 ± 0.12
4	Sweroside	-8.93 ± 0.03
5	Resveratrol	-8.83 ± 0.03
6	Stigmasterol	-8.78 ± 0.19
7	5-Caffeoylquinic_acid	-8.70 ± 0.00
8	Quercetin	-8.70 ± 0.00
9	Djalonensone	-8.48 ± 0.03
10	3-Caffeoylquinic_acid	-8.45 ± 0.10
11	Swertiamarin	-8.40 ± 0.23
12	(+)-Catechin	-8.30 ± 0.00
13	(-)-Epicatechin	-8.18 ± 0.03
14	Isogentisin	-7.80 ± 0.00
15	Isovitexin	-7.40 ± 0.12
16	P-coumaric_acid	-7.00 ± 0.00
17	Ferulic_acid	-6.93 ± 0.03
18	Caffeic_acid	-6.88 ± 0.03

19	Gallic_acid	-6.20 ± 0.00
20	Syringic_acid	-6.20 ± 0.00

Table 5 The Result of Molecular Docking Simulation of lysyl-trna Synthetase with the Phytochemicals of *Anthocleista djalonensis* using cladosporin as a reference compound.

S/N	Compounds	Mean Estimated Binding Free Energy (Kcal/Mol) ± SEM
1	β-sitosterol	-12.50 ± 0.00
2	Cladosporin	-9.90 ± 0.00
3	3-Caffeoylquinic_acid	-9.83 ± 0.03
4	5-Caffeoylquinic_acid	-9.60 ± 0.00
5	(+)-Catechin	-9.20 ± 0.00
6	Isogentisin	-9.20 ± 0.00
7	Quercetin	-9.08 ± 0.19
8	(-)-Epicatechin	-8.25 ± 0.10
9	djalonensone	-8.18 ± 1.33
10	Sweroside	-8.00 ± 0.00
11	Resveratrol	-7.95 ± 0.03
12	swertiamarin	-7.83 ± 0.05
13	Caffeic_acid	-7.10 ± 0.00
14	Isovitexin	-7.10 ± 0.00
15	Stigmasterol	-7.03 ± 0.30
16	Ferulic_acid	-6.85 ± 0.03
17	Syringic_acid	-6.83 ± 0.03
18	Gallic_acid	-6.80 ± 0.00
19	p-coumaric_acid	-6.78 ± 0.03
20	Vanillic_acid	-6.45 ± 0.05

Table 6 The Result of Molecular Docking Simulation of Sulfotransferase with the Phytochemicals of *Anthocleista djalonensis* using oxamniquine as a reference compound

S/N	Compounds	Mean Estimated Binding Free Energy (Kcal/Mol) ± SEM
1	β-sitosterol	-11.50 ± 0.00
2	Quercetin	-9.40 ± 0.00
3	(+)-Catechin	-9.28 ± 0.05
4	(-)-Epicatechin	-9.10 ± 0.00
5	Stigmasterol	-8.98 ± 0.03
6	djalonensone	-8.80 ± 0.00
7	5-Caffeoylquinic_acid	-8.48 ± 0.03

8	Sweroside	-8.40 ± 0.00
9	3-Caffeoylquinic_acid	-8.20 ± 0.00
10	Isogentisin	-8.00 ± 0.00
11	Isovitexin	-8.00 ± 0.00
12	swertiamarin	-7.93 ± 0.03
13	Oxamniquine	-7.90 ± 0.00
14	Resveratrol	-7.90 ± 0.00
15	Ferulic_acid	-6.83 ± 0.03
16	Caffeic_acid	-6.78 ± 0.03
17	p-coumaric_acid	-6.73 ± 0.03
18	Syringic_acid	-6.70 ± 0.00
19	Vanillic_acid	-6.50 ± 0.00

Table 7 The Result of Molecular Docking Simulation of carbonic anhydrase with the Phytochemicals of *Anthocleista djalonensis* using 4-{[(4 fluorophenyl)carbamoyl] amino}Benzene- sulphonamide as a reference compound.

S/N	**Compounds**	**Estimated Binding Free Energy (Kcal/Mol) ± SEM**
1	β-sitosterol	-9.85 ± 0.03
2	3-Caffeoylquinic_acid	-9.70 ± 0.00
3	5-Caffeoylquinic_acid	-8.75 ± 0.07
4	Quercetin	-8.10 ± 0.00
5	(+)-Catechin	-7.80 ± 0.00
6	(-)-Epicatechin	-7.63 ± 0.05
7	4-{[(4 fluorophenyl)carbamoyl] amino}Benzene- sulphonamide	-7.58 ± 0.03
8	Isogentisin	-7.30 ± 0.00
9	swertiamarin	-7.23 ± 0.05
10	Isovitexin	-7.20 ± 0.00
11	Sweroside	-7.13 ± 0.03
12	Caffeic_acid	-7.10 ± 0.00
13	djalonensone	-6.90 ± 0.00
14	Resveratrol	-6.73 ± 0.07
15	Ferulic_acid	-6.60 ± 0.00
16	Gallic_acid	-6.60 ± 0.00
17	Vanillic_acid	-6.60 ± 0.00
18	Stigmasterol	-6.43 ± 0.11
19	p-coumaric_acid	-6.40 ± 0.00
20	amplexine	-5.80 ± 0.10

4. Discussion

We identified four essential proteins in worms, which served as potential drug targets in this study. We simulated the binding of the chemical compounds reportedly present in *Anthocleista djalonensis* using molecular docking to predict the binding affinity of these compounds on the active sites of these enzymes and to predict whether the binding and inhibition of these enzymes is the mechanism of anthelminthic action of the bioactive compounds in the plant. Because of the structural similarity of the enzymes in a wide range of worms [6] the result obtained in this *in silico* study could explain the hypothetical mechanisms of actions of the compound screened in this study against a wide range of worms.

The parameter use to predict Drug-likeness is Lipinski's rule of five, which state that any compound that fail more than one rule athough of the five rules will likely not to be bioavailable. Therefore, these hypothetical finding was able to predict drug likeness found in the *Anthocleista djalonensis* against standard compound with protein or ligand. Hence, Lipinski's rule of five is a concept molecular docking simulation used in drug discovery. This rule helps to predict if a biologically active molecule is likely to have the chemical and physical properties to be orally bioavailable.

The results obtained in the Autodock vina simulation are estimated ligand binding free energy [13]. The estimated binding free energy is inversely propositional to the predicted binding affinity. Thus, our result was presented in order of increasing estimated binding (Decreasing binding affinity).

Isovitexin, also known as homovitexin or saponaretin, is a flavonoid found in several medicinal plants [20]. Isovitexin and its structurally related compound, vitexin, have been reported to possess a wide range of pharmacological activity, which includes anti-cancer, anti-inflammatory, anti-hyperlgesic and neuroprotective effects [8]. Isovitexin is a major constituent of *A. djalonensis* extract [1]. So, it is an essential phytoconstituent of consideration in this study. Based on the *in silico* result, Isovitexin had a higher binding affinity (-8.00 ±0.00) at the active site of the crystal structure of Schistosoma heamatobium sulfotransferase than the reference inhibitor, Oxamniquine (-7.90 ± 0.00). The higher binding affinity exhibited by Isovitexin indicates that Isovitexin might inhibit sulfotransferases found in worms. So, based on the *in silico* studies, Isovitexin might be one of the anthelminthic bioactive compounds found in the leaf extracts of *A. djalonensis*. And one of the possible mechanisms of the anthelminthic action of isovitexin, as predicted by this *in silico* study, is by inhibiting sulfotransferase, a critical enzyme necessary for the survival of parasitic worms. Thus, isovitexin is one of the frontrunner compounds identified in the *in silico* study performed in this study and will serve as a promising drug candidate in discovering novel anthelminthic compounds from natural products.

β-sitosterol is another promising frontrunner compound identified in this study. It has a higher binding affinity (-12.40 ± 0.00)in the active site of the crystal structure of parasitic *Ascaris suum* mitochondrial rhodoquinol-fumarate reductase than the reference inhibitor, Flutolanil (-9.60± 0.00); higher binding affinity (-12.50 ± 0.00) at the active site of the crystal structure of *Loa loa* lysyl-tRNA Synthetase than the reference inhibitor, Cladosporin (-9.90 ± 0.00); higher binding affinity (-9.85 ± 0.03)at the active site of the crystal structure of *Schistosoma mansoni* carbonic anhydrase than the reference inhibitor (-7.58 ±0.03); and higher binding affinity (-11.50 ±0.00)at the active site of the crystal structure of *Schistosoma heamatobium* sulfotransferase than the reference inhibitor, Oxamniquine (-7.90 ± 0.00). The high binding affinity of β-sitosterol on mitochondrial rhodoquinol-fumarate reductase obtained in this *in silico* study suggests that it could bind to and inhibit the enzyme, mitochondrial rhodoquinol-fumarate reductase. The enzyme plays an essential role in energy metabolism in worms. By inhibiting this enzyme, β-sitosterol would decrease ATP production, resulting in the collapse of the mitochondrial membrane potential and paralysis of the worm [16].

Another promising compound identified is 3-Caffeoylquinic_acid (3-CQA). It has a higher binding affinity (-9.70±0.00) in the active site of the crystal structure of Schistosoma mansonicarbonic anhydrase than the reference inhibitor, 4-{[(4 fluorophenyl)carbamoyl]amino}Benzene-sulfonamide (-7.58 ± 0.03); higher binding affinity (-8.20 ± 0.00)at the active site of the crystal structure of Schistosoma heamatobium sulfotransferase than the reference inhibitor, Oxamniquine (-7.90 ± 0.00). It also had an appreciable binding affinity (-9.83 ± 0.03) at the active site of the crystal structure of *Loa loa* lysyl-trna Synthetase when compared to the reference inhibitor, Cladosporin (-9.90 ± 0.00).

5-Caffeoylquinic acid (5-CQA) is another promising compound identified .It has a similar high binding affinity to its isomer 3-CQA in this study against lysyl-tRNA Synthetase, Sulfotransferase and carbonic anhydrase. 3-CQA and 5-CQA are chlorogenic acids [9].

Quercetin is another promising compound identified in this study. It had higher binding affinity (-9.40 ± 0.00) at the active site of the crystal structure of *Schistosoma heamatobium* sulfotransferase than the reference inhibitor, Oxamniquine (-7.90 ± 0.00) and higher binding affinity (-8.10 ± 0.00) at the active site of the crystal structure of *Schistosoma mansoni* carbonic anhydrase than the reference inhibitor (-7.58 ±0.03). Quercetin is a flavonoid that has

elicited anthelminthic activities in *in vitro* studies, and its mechanism of actions has been shown to involve causing oxidative stress in the nervous system of worms [7].

5. Conclusion

This research successfully studied the *in silico* binding affinities of the reported *Anthocleista djalonensis* extract's chemical constituents on essential proteins in parasitic worms. β-sitosterol activities on the four enzymes screened in this study will be adequate to fully understand how β-sitosterol elicits all the reported actions and effects. Hence, we recommend Isovitexin, β-sitosterol, 3-QCA, 5-QCA and quercetin as hit compounds for further studies in discovering novel anthelminthic agents from plant sources.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

References

[1] Abba, C. C., Egwuatu, M. C., Johnson, O., Eze, P. M., Eze, N. K., and Obidiegwu, O. C. (2024). Isovitexin, the main phytoconstituent of Anthocleista djalonensis, may be responsible for the plant's antimalarial properties. *Magna Scientia Advanced Biology and Pharmacy, 11(2), 026-032.*

[2] Bharti B., Bharti S., Khurana S. (2018). Worm infestation: diagnosis, treatment and prevention. *Indian Journal of Pediatrics, 85(11):1017–1024*

[3] Bulli, I., Dettori, I., Coppi, E., Cherchi, F., Venturini, M., Mannelli, L., … and Pedata, F. (2021). Role of carbonic anhydrase in cerebral ischemia and carbonic anhydrase inhibitors as putative protective agents. *International Journal of Molecular Sciences, 22(9), 5029.*

[4] De Wilde, J. J. F. E. (2011). Novitates Gabonenses 73. A new species of Anthocleista (Gentianaceae) from Gabon. Blumea-Biodiversity, *Evolution and Biogeography of Plants, 56(1), 1-3.*

[5] Ezebuo, F. and Uzochukwu, I. (2020). Schistosomal sulfotransferase interaction with oxamniquine involves hybrid mechanism of induced-fit and conformational selection. *Current Computer - Aided Drug Design, 16(4), 451-459.*

[6] Giunti, S., Andersen, N., Rayes, D., and De Rosa, M. J. (2021). Drug discovery: Insights from the invertebrate Caenorhabditis elegans. *Pharmacology Research and Perspectives, 9(2), e00721.*

[7] Goel, V., Sharma, S., Chakroborty, N. K., Singla, L. D., and Choudhury, D. (2023). Targeting the nervous system of the parasitic worm, Haemonchus contortus with quercetin. Heliyon, 9(2).

[8] He, M., Min, J. W., Kong, W. L., He, X. H., Li, J. X., & Peng, B. W. (2016). A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia, 115, 74-85.

[9] Liang, N., and Kitts, D. D. (2015). Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. Nutrients, 8(1), 16.

[10] Lipinski, C. A., Lombardo, F., Dominy B. W and Feeney, P.J (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev, 46(1-3):3-26.

[11] Liu M., Panda S. K., Luyten W. (2020). Plant-based natural products for the discovery and development of novel anthelmintics against nematodes .Biomolecules,10(3): 426.

[12] Ndjonka D., Abladam E. D., Djafsia B., Ajonina-Ekoti I., Achukwi M. D. and Liebau E. (2013). Anthelmintic activity of phenolic acids from the axlewood tree Anogeissus leiocarpus on the filarial nematode Onchocerca ochengi and

drug-resistant strains of the free-living nematode Caenorhabditis elegans. Journal of Helminthology, 88(4): 481–488.

[13] Nguyen, N. T., Nguyen, T. H., Pham, T. N. H., Huy, N. T., Van Bay, M., Pham, M. Q., ... and Ngo, S. T. (2020). Autodock Vina Adopts More Accurate Binding Poses but Autodock4 Forms Better Binding Affinity. J. Chem. Inf. Model, 60, 204-211.

[14] Okenwa, C. J., Chikelu, O. R., and Nwogbo, C. E. (2015). Phytochemical, proximate and total antioxidant analysis of Ogbu (Ficus exasperata) and Uvuru (Anthocleista djalonensis) healthy green leaves. International Journal of Pharmacy and Medical Sciences, 5(2), 48-53.

[15] Onocha, P. A., Okorie, D. A., Connolly, J. D., and Roycroft, D. S. (1995). Monoterpene diol, iridoid glucoside and dibenzo-α-pyrone from Anthocleista djalonensis. Phytochemistry, 40(4), 1183-1189.

[16] Otero, L., Martínez-Rosales, C., Barrera, E., Pantano, S., and Salinas, G. (2019). Complex I and II subunit gene duplications provide increased fitness to worms. Frontiers in Genetics, 10, 1043.

[17] WHO. Schistosomiasis and soil-transmitted helminthiases: numbers of people treated in 2018. Wkly Epidemiol Rec 2019; 94: 601–12.

[18] WHO. WHO Factsheet: Soil-Transmitted Helminthiases . Geneva, Switzerland: World Health Organization; 2020.

[19] WHO. Regional Office for Africa. Helminthiasis, 2020. Available from: https://www.afro.who.int/healthtopics/helminthiasis#:~:text=It%20is%20an%20infestation%20with (Assessed on July 4th, 2023).

[20] Xiao, L., Chen, X. J., Feng, J. K., Li, W. N., Yuan, S., & Hu, Y. (2023). Natural products as the calcium channel blockers for the treatment of arrhythmia: Advance and prospect. Fitoterapia, 105600.