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Original Research Article

Molecular Docking of Phytochemicals from *Vernonia amygdalina* and *Nauclea latifolia* as Potential Inhibitors of Drug Targets in *Ascaris lumbricoides*

Janet F. Akinseye[1], Uba D. Somtochukwu[1], Sunday A. Buru[1,2]*, Bernard O. Oluboyo[1], Adebimpe H. Egbebi[1], Agu C. Chidera[1], Thomas H. Zagi[2]

[1]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria.*
[2]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Allied Health and Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria*

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ABSTRACT

Ascariasis is a parasitic infection caused by the intestinal roundworm *Ascaris lumbricoides.* It is one of the soil-transmitted helminths (STHs), also known as geohelminths. Human and animal ascariasis constitutes one of the most important global public health challenges with worldwide distribution, with the majority of cases found in the tropics, such as Nigeria, India, and China. The study was performed in silico on secondary metabolites isolated from medicinal plants *Vernonia amygdalina* and *Nauclea latifolia* based on an ethnobotanical survey made in Nigeria. Maestro 9.3 docking suite was used in protein preparation, ligand preparation, receptor grid formation, and ligand docking against different *A. lumbricoides* specific proteins (3VR9, 7N09, Beta-tubulin) that have been documented as potential and conventional drug targets. One hundred and eighty-two phyto-compounds *of N. latifolia* and Bitter leaf were obtained from examination of already published works of literature Journals and used in this study for the generation of library of compounds respectively. The result showed the docking score of the two plants to each of the specific proteins and also the ligand interactions of these phytochemicals to the specific protein. The investigations of this current project through molecular docking studies have shown that phytochemicals from the medicinal plants; *V. amygdalina* and *N. latifolia* can serve as potential inhibitors and/or lead compounds for the development of novel inhibitors of these targets for the treatment of ascariasis. It also provides the molecular basis of their anti-helminthic activities, possibly through the inhibition of metabolic processes in the mitochondria.

Keywords: Molecular Docking, Ascariasis, Medicinal Plant, Druggable targets

Introduction

Ascaris lumbricoides is an intestinal parasitic roundworm of the genus *Ascaris*, known to cause Ascariasis in humans.[1] *A. lumbricoides* is one of the soil-transmitted helminths (STHs), also known as geohelminths.[2, 3, 4] Ascariasis is a perfect example of an environmental disease.[5] Human and animal Ascariasis constitutes one of the most important global public health challenges especially with the current drive toward "One Health" with worldwide distribution, the majority of the cases found in the tropics and subtropical countries.[6] Globally, an estimated 807 million - 1.2 billion people are infected with *A. lumbricoides.*[7] Nigeria has a prevalence of 25.4%. Children between 2 to 10 years old are the most affected due to the heavy infections they harbor and because of their vulnerability to nutritional deficiencies.[6] Transmission is enhanced since individuals can be asymptomatically infected and can continue to shed eggs for years.[8]

*Corresponding author. E mail: sunday.buru@kasu.edu.ng
Tel: +2349097082712

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New anti-helminthic drugs are urgently needed as drug resistance to climate change and environmental concerns arise through protein binding sites, enzymes, etc.[9] Indigenous medicinal plants for the control of intestinal parasites has been practiced for centuries, especially in developing countries, however scientific validation of these traditional practices has been lacking.[10, 11] With this, tremendous expansion in the use of traditional medicine, in particular, herbal medicines has led to a plethora of ethnobotanical investigations conducted. These, later, have proven to be one of the most reliable approaches for the discovery of new drugs.[12] In addition, secondary metabolites (phytochemicals) such as flavonoids, tannin, phenols, terpenoids, etc. Medicinal plants have shown greater and rapid action than generic drugs in the treatment of many microbial and viral infections. This study aimed to (1). Promote the rational use of traditional medicine for the treatment of Ascaris infection. The secondary metabolites from the identified plant species were used to generate ligands, which were used for Virtual screening, Molecular docking, and computational design towards various proteins, receptors, chokepoints, and enzymes of *A. lumbricoides*. (2) Investigate the phytochemicals from some selected medicinal plants as potential inhibitors of identified drug targets for the treatment of Ascariasis, and to make appropriate recommendations on medicinal plants capable of treating Ascariasis since most children infected might not be able to afford anti-parasitic agent. Therefore, anti-helminthics resistance in parasites is spreading and the inefficacy of chemical anti-parasitic compounds is threatening human and animal health.[13] New anti-helmintic drugs are urgently needed with low cost and easy accessibility for treatment. *V. amygdalina,* commonly referred to as Bitter leaf, belongs to the daisy family Asteraceae. Indigenous to tropical Africa and is found wild or cultivated all over Sub-Saharan Africa.[14, 15] *V. amygdalina,* as commonly called, is a highly appreciated vegetable in West and Central Africa and can be consumed in various dishes.[16] One

of the most common medicinal uses of *V. amygdalina* is for the treatment of intestinal worms including nematode.[14] This is as a result of unaffordable antihelminthics, especially in a resource-poor setting in most developing countries, as the majority of the population lives in the rural areas where these medicinal plants are readily available.[17] These factors paved the way for herbal remedies as alternative anthelmintics.[18] Although some work has been done on the anthelmintic activity of *V. amygdalina* most of them were *in vivo*.[19, 20] Therefore, this study intends to verify further the anthelmintic activity of these plants and instill more confidence in their use as an alternative to anthelmintic drugs. It is therefore imperative that research studies to find phytochemicals with strong inhibitory potential against Ascariasis should be encouraged.

Materials and Methods

A. lumbricoides protein (Database of Apicomplexa Transcriptomes) was sourced from molecular experimentation and was used for this study to ascertain the antihelminthics activity of plant-derived secondary metabolites against *A. lumbricoides* that affects humans.

Molecular Docking
Maestro 9.3[21] docking suite was used in protein preparation, ligand preparation, receptor grid formation, and ligand docking against different *A. lumbricoides*-specific proteins that have been documented as potential and conventional drug targets. The target proteins were selected by searching for host proteins that directly or indirectly affect the progression of the infection or aid the life cycle of *A. lumbricodes*. The following proteins were used for this work 3vr9, 7N09 and Beta-Tubulin, because they play an important role in the physiology, metabolic process, and survival of the parasite, and the crystal structures were downloaded from the RCSB (Protein Data Bank). The crystal structures of the plant's secondary metabolites were obtained from databases of PubChem. The selected secondary metabolites of *N. latifolia* and *V. amygdalina* respectively were docked against active sites of the various proteins. The procedure protein and ligand files was opened on Maestro and the proteins and ligands preparation were done using the standard operating procedure.[21]

Ligand Selection and Preparation
One hundred and eighty-two phytochemicals of *N. latifolia* and *V. amygdalina* were obtained from screening and used for this study respectively. They were obtained from already published literature and used in the generation of a library of compounds. The phytochemicals for each plant were copied and prepared using the LigPrep interface in Schrodinger with an OPL3 force field, at neutral pH, Desalt, and tautomers was automatically selected and only 1 stereoisomer of the ligands was generated each. It was necessary to convert the ligands prepared from the 2D structures of the ligands to 3D structures.

Protein Selection and Preparation
X-ray crystallized three-dimensional structure of the target proteins was retrieved from RCSB (Protein Data Bank). On maestro, a selected protein was copied into the project table. It was then highlighted for preparation. The 3D structure was viewed and assessed with the Maestro interface and prepared using a protein preparation wizard. The protein was first preprocessed. Then the addition of hydrogen led to the generation of the state. The protein was optimized, water molecules were removed and lastly, the protein was minimized so that it conformed to the Maestro suite.

Glide Grid Preparation
After protein and ligand preparation, the grid was developed. This was carried out with the receptor grid generation tool in maestro which defined the area around the active sites in terms of co-ordinates x, y, and z. as indicated in the results.

Extraction of Control Ligand
The co-crystalized ligand was extracted to make the active site accessible for new ligands. It was also reduced to validate which of the docking score was used to compare the test ligands.

Ligand Docking
The docking analysis was accomplished using the ligand docking tool on Maestro. The prepared library of ligands (phytochemicals 107 and 75 respectively for *V. amygdalina* and *N. latifolia*) was docked into the active site of the target proteins. The ligands were treated as flexible while the protein receptors were treated as rigid. The ligand docking was set at extra precision (XP). Docking analysis was performed on the test and control co-crystallized ligands to search for the best-docked conformation between ligand and protein. The conformation with the most favorable (least) free binding affinity (docking score) was selected for analyzing the interactions between the target receptor and ligands.[22, 23]

Statistical Analysis
Analysis was carried out using Graph Pad Prism 8.0 (Graph Pad Software Inc., San Diego, CA), CompuSyn Software by ComboSyn Inc., for phytochemical combination analysis.

Results and Discussion

Molecular Docking of V. amygdalina and N. latifolia
Molecular docking was performed to estimate the binding affinity of one hundred and seven and seventy-five phytochemicals of *V. amygdalina* and *N. latifolia* respectively across three different receptors. With less binding energy, a ligand has a higher possibility of being medicinal.

Heat Map of V. amygdalina and N. latifolia
Heat Map of V. amygdalina
Error! Reference source not found. shows the heat map of the one hundred and seven compounds for *V. amygdalina*. The heat map below shows the activities of all the phytochemicals screened for *V. amygdalina* on all the receptors. The green colours indicated the smallest values with the highest binding energy, the middle values were indicated by black colours, while the highest values showed the lowest binding energy which was indicated by red colours. The white space on any receptor indicated that no phytochemical was docked on that receptor. The range for the highest value was -5. Some of the phytochemicals that had the highest values include 2-Pentanol, 2-Aminopyrazine. For the middle values, there were some phytochemicals had black colours present on some of the receptors and they include 2-(methylio) chlorogenic acid, Diosmetin. The range for the middle value was -10. For the lowest values with the highest binding energy, the phytochemicals involved were Cynarine, Cymaroside and Luteolin -7- glucuronide. The range for the lowest values was -15.

Heat Map of N. latifolia
Figure 2 shows the heat map of seventy-five *N. latifolia* compounds. The heat map below shows the activities of all the phytochemicals screened for *N. latifolia* on all the receptors. The green colour indicated the smallest values with the highest binding energy, while the highest values showed the lowest binding energy and which was indicated by red colours. The white space on any receptor indicated that no phytochemical was docked on that receptor. Some of the phytochemicals that had the highest values include Allyl alcohol, Allyl methyl sulfide, Allyl methyl disulfide, and 1, 3- Dithiole. For the middle values, some phytochemicals had black colours present on some of the receptors and they include Angustine, Allixin, methyl 9H- pyridoxal (3,4-b) indole -1 – carboxylate. The lowest values which indicate phytochemicals with the highest binding energy involve Gamma-glutamylphenylalanine, 5-(methylthio) chlorogenic acid.

Docking Scores of Selected Compounds
The values of all the docked *V. amygdalina* ligands had a range of -3.2 to -11.912 kcal/mol while the values of *N. latifolia* docked to mitochondrial iodoquinol-fumarate reductase ranged from -1.336 – 10.715 kcal/mol. The docking score of the selected *V. amygdalina* and *N. latifolia* ligands for mitochondrial iodoquinol-fumarate were shown in Tables 1 and 2 respectively.

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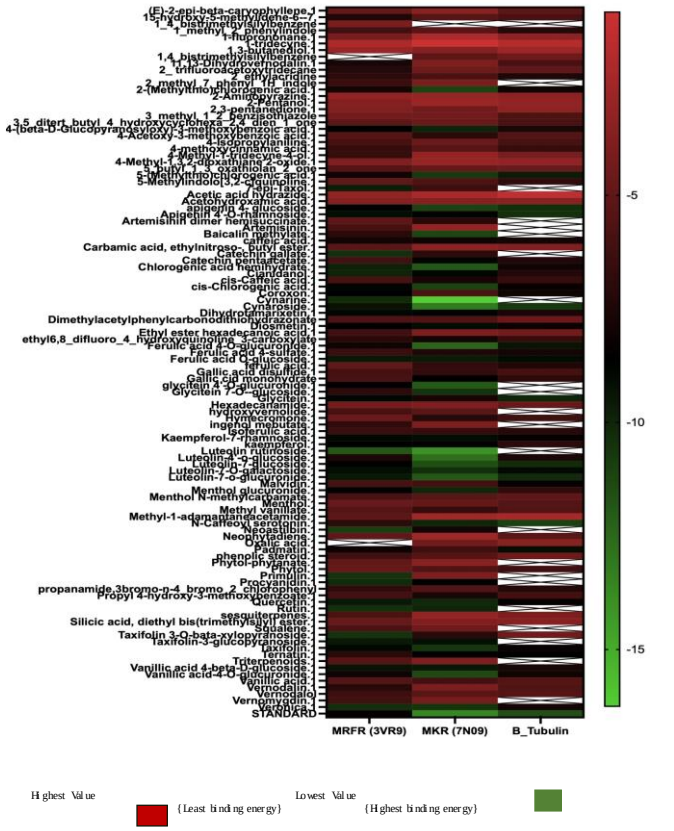

Figure 1: Heat map of the compounds of *V. amygdalina*

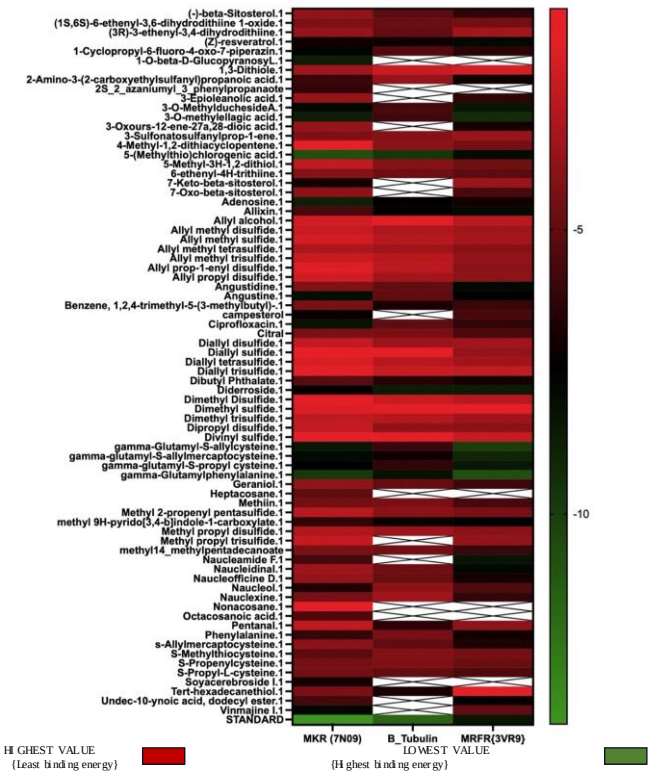

Figure 2: Heat map of the compounds of *Nauclea Latifolia*

Table 1: Docking and MM-GBSA scores of the top five lead compounds of the Mitochondrial Rhodoquinol -Fumarate Reductase receptor for *V. amygdalina*

S/N	PubChem ID	Compound	Glidescore (kcal/mol)	MMGBSA
1	44258082	Luteolin rutinoside	-11.912	-51.57
2	442437	Neoastilbin	-10.936	-45.17
3	94409	Primulin	-10.516	-55.66
4	493319	Taxifolin 3-O-betaxylopyranoside	-10.51	-48.19
5	5280805	Rutin	-10.459	-35.86
6	CCL	FTN (Standard)	-8.39	-48.77

Table 2: Docking and MM-GBSA scores of the top five lead compounds of the Mitochondrial Rhodoquinol -Fumarate Reductase receptor for *Nauclea latifolia*

S/N	PubChem ID	Compound	Glidescore (kcal/mol)	MMGBSA
1	111299	Gamma-glutamylphenylalanine	-10.715	-47.42
2	11346811	Gamma-glutamyl-S-allylcysteine.	-10.04	-49.99
		3-O-methylellagic acid.		
3	13915428		-9.317	-51.83
4	101630431	gamma-glutamyl-S-allylmercaptocysteine	-9.01	-48.1
		Diderroside		
5	23760099	FTN (Standard)	-8.622	-36.37
6	CCL		-8.39	-48.77

The docking score of the reference compound i.e., FTN (1-methylthoxy) phenyl)-2-(trifluoromethyl) benzamide gave a binding energy of -8.39 kcal/mol. The values of all the docked *V. amygdalina* ligands ranged from -1.145 to -11.097 kcal/mole while the value of all *N. latifolia* docked to beta-tubulin ranged from -1.57 to -9.652 kcal/mol. The docking score of the selected *V. amygdalina* and *N. latifolia* ligands for beta-tubulin are shown in Tables 3 and 4 respectively. The docking score of the reference compound DLW (2-methoxyphenyl)-methyl-carbonimidoyl)-3-oxidanyl-5-phenyl-cyclohex-2-ene-1 gave a binding energy of -11.621kcal/mol. The values of all the *V. amygdalina* ligands docked from -0.965 to -16.249kcal/mol while the value of all *N. latifolia* docked to Ascaris Mitochondrial Ketoreductase ranged from -1.168kcal/mol to -10.772kcal/mol. The docking scores of the selected *V. amygdalina* and *N. latifolia* ligands for Ascaris Mitochondrial Ketoreductase were shown in Tables 5 and 6 respectively. The docking score of the reference compound NAD (Nicotinamide-adenine-dinucleotide) gave a binding energy of -13.694kcal/mol. Furthermore, MM-GBSA (Molecular Mechanics/Generalized Born Surface Area) is a popular computational method used to predict the binding free energy of a ligand to a protein. MM-GBSA can be used to study the binding of phytochemical compounds to their protein targets, providing insights into their mechanism of action and aiding the design of more potent compounds. Several studies have used MM-GBSA to investigate the binding of phytochemical compounds to proteins. Tables 1, 2, 3, 4, 5 and 6 show the docking scores and the MM-GBSA of the five lead compounds for both *V. amygdalina* and *N. latifolia.*

Table 3: Docking and MM-GBSA scores of the top five lead compounds of the Beta-tubulin receptor for *V. amygdalina*

S/N	Pub Chem ID	Compound	Glidescore (kcal/mol)	MMGBSA
1	16659826	N-Caffeoyl serotonin	-11.097	-33.3
2	5280637	Cynaroside	-10.751	-36.3
3	74338461	Apigenin4'-O-rhamnoside	-10.461	-36.53
4	5491384	Apigenin4- glucoside	-10.389	-27.55
5	13093775	Luteolin-7-glucoside	-10.318	-30.23
6	CCL	DLW (Standard)	-11.621	-17.73

Table 4: Docking and MM-GBSA scores of the top five lead compounds of the Beta-tubulin receptor for *Nauclea latifolia*

S/N	PubChem ID	Compound	Glidescore (kcal/mol)	MMGBSA
1	102094725	5-methyl(thio)chlorogenic acid.	-9.652	-28.52
2	23760099	Diderroside	-8.617	-14.23
3	111299	Gamma, glutamylphenylalanine	-8.194	-28.94
4	60961	Adenosine	-7.345	-32.83
5	86374	Allixin	-7.334	-40.97
6	CCL	DLW (Standard)	-11.621	-17.73

Selected Ligand Interaction

The figures presented below demonstrate how the best five ligands for *V. amygdalina* and *N. latifolia* respectively, interact with protein receptors in comparison to the reference molecule. The compounds depicted in these pictures were found to interact with their respective target proteins through varieties of molecular interactions, including hydrogen bonds, pi-pi stacking, and hydrophobic interactions, and these interactions were thought to be the basis for the compound's activity.

Ligand Interaction of V. amygdalina and N. latifolia with Mitochondrial Iodoquinol-Fumarate Reductase

Error! Reference source not found. and **Error! Reference source not found.** depict a collage of several images including; Figures 3a-3b, Figures 3c-3f, and Figures 4a-4e. In Fig 3a, the reference ligand, the reference ligand (FTN) showed a hydrogen bond with amino acid residues (TRP – 197, TYR – 107) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with (ILE – 242, TYR – 107, TRP – 197, TRP – 196, PRO – 193, TYR – 63, LEU – 60, TRP – 69). In Fig 3b, Luteolin rutinoside from Bitter leaf showed hydrogen bond with amino acid residues (TRP – 197, TYR – 107, LEU – 60) and the arrow indicates that these residues are donating to form the hydrogen bonds. Pi-pi stacking was seen with the amino acid residue (TRP – 69). Hydrophobic bonds were formed with PRO – 193, ILE – 242, TRP – 196, TRP – 197, TRP – 69, TRY – 107, LEU – 60, TYR – 63, VAL – 77. In Fig 3c, Neoastilbin from *V. amygdalina* showed hydrogen bond with amino acid residues (TRP – 197, TYR – 107, LEU – 60) and the arrow indicates that these residues are donating to form the hydrogen bonds. Pi-pi stacking was seen with the amino acid (TRP – 69). Hydrophobic bonds were formed with ILE – 242, MET – 70, VAL – 73, TRP – 197, TRP – 196, TYR – 1007, PRO – 163, LEU – 60. In Fig 3d, Primulin from Bitter leaf showed hydrogen bond with amino acid residues (TRP – 197, TYR – 107) and the arrow indicates that these residues are donating to form the hydrogen bonds. Pi-pi stacking was seen with the amino acid residues (TYR – 107, TRP – 197). Hydrophobic bonds were formed with TYR – 63, LEU – 60, TRP – 69, TRP – 197, TRP – 196, ILE – 242, PRO – 193.In Fig 3e, Taxifolin 3-o beta-xylopyranoside from *V. amygdalina* showed hydrogen bond with amino acid residues (TYR – 107, LEU – 60) with the arrow indicating that these residues are donating to form the

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hydrogen bonds. Pi-pi stacking was seen with the amino acid residue (TRP – 69). Hydrophobic bonds were formed with PRO – 193, TRP – 196, TRP – 197, TRP – 69, VAL – 77, TYR – 107. In Fig 3f, Rutin from *V. amygdalina* showed hydrogen bond with amino acid residues (ARG – 109, TRP – 197) and the arrow indicates that these residues are donating to form the hydrogen bonds. Pi-pi stacking was seen with amino acid residue (TRP – 69). Hydrophobic bonds were formed with TYR – 63, LEU – 60, TRP – 69, ILE – 242, PRO – 193, TRP – 197, TRP – 196, VAL – 77, VAL – 112. In Fig 4a, Gamma-glutamyl from *N. latifolia* showed hydrogen bond with amino

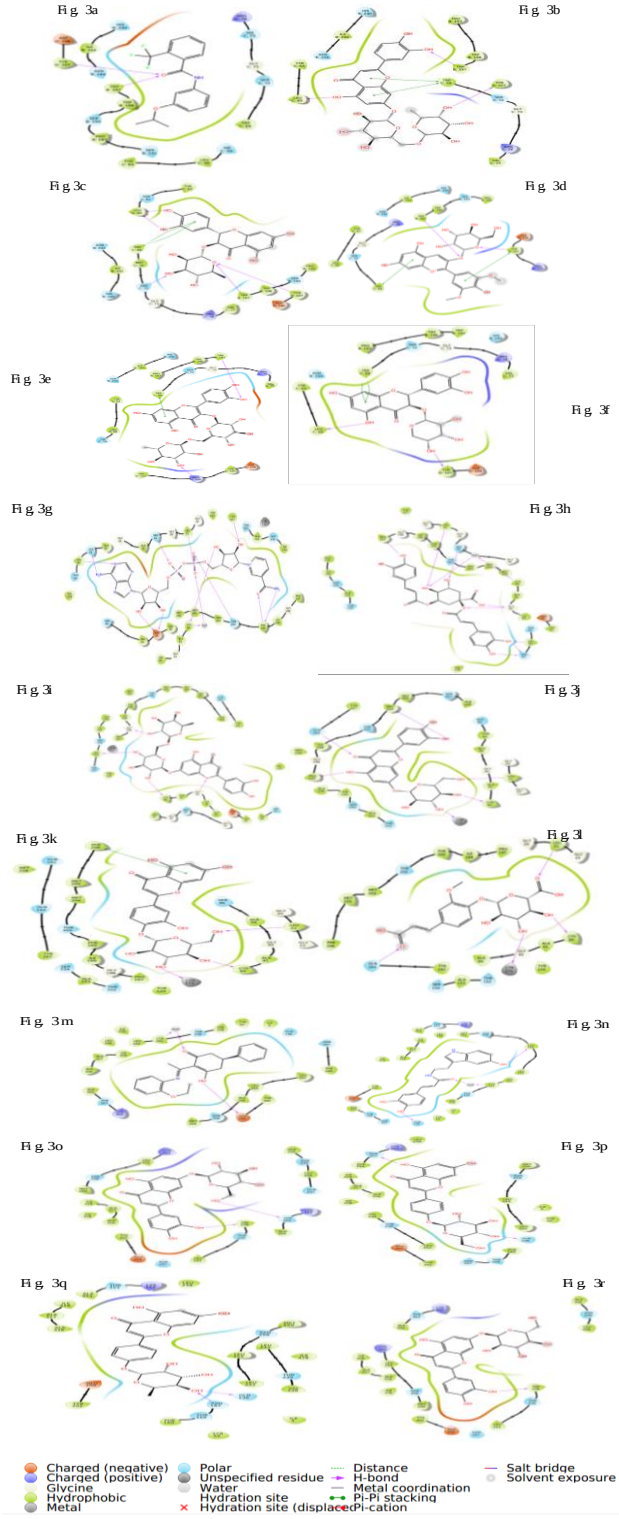

Figure 3: 3a-3f, 3g-3l; 3m-3r 2D Interaction of the amino acid residues with *V. amygdalina*

Key: Fig.3a: FTN STANDARD (1methylethoxyphenyl-2- trifluoromethyl benzamide) Fig.3b -Luteolin rutinoside, Fig.3c - Neoastilbin, Fig.3d - Primulin, Fig.3e -Taxifolin 3-o beta-xylopyranoside, Fig.3f -Rutin. Fig.3g– NAD STANDARD (Nicotinamide adenine dinucleotide),Fig.3h –Cynarine, Fig.3i – Luteolin rutinoside, Fig.3j- Cynaroside, Fig.3k – Luteolin-4-o-glucoside, Fig.3l – Ferulic acid – 4 -o -glucuronide. Fig.3m – DLW STANDARD (2-ethoxyphenyl)-methylcarbonimidoyl) -3- oxidanyl -5-phenyl-cyclohex-2en-1-one**,** Fig.3n – N-caffeoyl serotonin, Fig.3o – Cynaroside, Fig.3p – Apigenin 4-O- rhamnoside, Fig.3q – Apigenin 4-glucoside, Fig.3r – Luteolin -7-glucoside.

acid residues (TYR – 107, SER – 72, ARG – 76, HIS – 240 and the arrow indicates that these residues are donating to form hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with PRO – 193, LEU – 60, TYR – 107, TRP – 196, PHE – 138, LEU – 60,In Fig 4b, Gamma-glutamyl -S- allylcysteine from *N. latifolia* showed hydrogen bond with amino acid residues (TYR – 107, SER – 72, ARG – 76, HIS – 240) and the arrow indicates that these residues are donating to form a hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were seen with ILE – 242, PHE – 138, TYR – 107, TRP – 196, TRP – 197, PRO – 193, LEU -60, TRP – 69. In Fig 4c, 3-o methylellagic acid from *N. latifolia* showed hydrogen bonds with amino acid residues (TYR – 107, LEU – 60, ASN – 244) and the arrow indicates that these residues are donating to form a hydrogen bonds. Pi-pi stacking was seen with amino acid residue (TRP – 69). Hydrophobic bonds were formed with ILE – 242, TRP – 196, TRP – 197, PRO – 193, TRP – 63, LEU – 60, TYR – 63, TYR – 107. In Fig 4d, Gamma-glutamyl-S- allymercaptocysteine from *N. latifolia* showed hydrogen bonds with amino acid residues (SER – 72, TRP – 69, HIS – 240, HEM – 201, ARG – 76) and the arrow indicates that these residues are donating to form a hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed (TYR – 63, LEU – 60, TRP – 69, MET – 70, PRO – 193, TRP – 196, TRP – 197, TYR – 107, PHE -138. In Fig 4e, Diderroside from *N. latifolia* showed hydrogen bonds with amino acid residues (TRP – 197, ASP – 106) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with VAL – 77, PHE – 138, ILE – 242, TYR – 107, PRO -103, TRP – 196, TRP – 197, LEU – 60, TYR – 63, TRP – 69. The amino acid residues with negative charges are shown in red, positive charges in velvet, polar in cyan. Hydrophobic bonds were seen in green, undefined residues in grey. Hydrogen bond interaction is shown as pink-arrow, pipi stacking as green line and pi-cation as red line.

Ligand Interaction of V. amygdalina and N. latifolia with Ascaris Mitochondrial Ketoreductase

In Fig 3g, the reference ligand (NAD) showed hydrogen bonds with amino acid residues (ASP – 40, CYS – 90, LEU – 21, THR – 202, TYR – 167, PHE – 200, VAL – 64) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with VAL – 64, ALA – 62, ALA – 18, LEU – 21, TYR – 124, TYR – 167, ALA – 153, PHE – 200, ILE – 199, PRO – 197, LEU – 41, LEU – 39, MET – 205, MET – 204. In Fig 3h, Cynarine from *V. amygdalina* showed hydrogen bonds with amino acid residues (GLY – 92, VAL – 64, SER – 63, LEU – 21, PRO – 197) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with ALA- 156, ILE – 155, PRO – 197, ILE – 199, PHE – 200, TYR – 167, LEU – 21, PRO – 203, MET – 204, MET – 205, CYS – 90, ALA – 91, VAL – 64. In Fig 3i, Luteolin rutinoside from *V. amygdalina* showed hydrogen bonds with amino acid residues (GLY – 92, CYS – 90, ALA – 153, GLY – 198) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed MET – 258, MET – 204, MET – 205, PHE – 208, TYR – 167, ILE – 199, ALA – 156, PRO – 197, ALA – 153, TYR – 124. In Fig 3j, Cynaroside from *V. amygdalina* showed hydrogen bonds with amino acid residues (LEU – 21, CYS – 90, GLN – 164, PRO – 197, MET -204) and the arrow indicates that these residues a donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with TYR – 167, MET – 204, MET -205, PHE – 208, TYR -95, LEU – 21, ALA – 91, CYS -90, ILE – 155, PRO -197, ILE – 199, MET -258. In Fig 3k, Luteolin -4-o-

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glucoside from *V. amygdalina* showed hydrogen bonds with amino acid residues (LEU – 21, CYS – 90, LYN -171) and the arrow indicates that these residues are donating to form the hydrogen bonds. Pi-pi stacking was seen with the amino acid residue (PHE 208). Hydrophobic bonds were seen with PHE – 208, MET – 205, MET – 208, PHE - 200, ILE – 199, PRO -197, TYR – 124, CYS – 90, ALA -91, ALA – 94, ALA – 153, TYR – 167. In Fig 3l, Ferulic acid -4-o-glucuronide from *V. amygdalina* showed hydrogen bonds with amino acid residues (LYN – 171, CYS – 90, LEU – 21, GLN – 164) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with PHE – 200, ILE – 199, PRO – 197, LEU – 21, CYS – 90, ALA – 91, ALA – 153, TYR – 167, TYR – 124, ALA – 94. In Fig 4f, 5-methyl(thio)chlorogenic acid from *N. latifolia* showed hydrogen bonds with amino acid residues (CYS – 90, LEU – 21, SER – 91, GLN – 164) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with MET – 258, TYR – 167, PRO – 197, ILE – 199, PHE – 200, PRO – 203, MET – 204, MET – 205, ALA -153, ILE – 155. In Fig 4g, Gamma-glutamylphenylalanine from *N. latifolia* showed hydrogen bonds with amino acid residues (ALA -153, TYR – 167, THR – 202, GLY – 198, PHE – 200, ALA -153) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with MET – 258, MET -204, MET – 205, ILE – 199, CYS – 90, ALA – 91, ALA – 94, ILE – 155. In Fig 4h, Adenosine from *N. latifolia* showed hydrogen bonds with amino acid residues (CYS – 90, TYR – 167) and the arrow indicates that these residues are donating to form the hydrogen bond. No pi-pi stacking was seen. Hydrophobic bonds were seen with PHE – 200, ILE – 199, PRO – 197, ALA – 153, MET – 204, MET – 205, TYR – 167, TYR – 124, CYS – 90. In Fig 4i, 3-o methylellagic acid from *N. latifolia* showed hydrogen bond with amino acid residues (ALA – 153, GLY – 198, THR – 202) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with ILE – 199, PHE – 200, PRO – 203, MET – 204, MET – 205, LEU -21, PRO – 197, ILE – 155, TYR – 124, TYR – 167, CYS -90, ALA – 91. In Fig 4j, 1-O -beta – D – Glucopyranosyl – N –{(2R)-2-hydroxypalmitoyl} sphingosine from *N. latifolia* showed hydrogen bond with amino acid residues (GLN – 164, GLY – 198, PRO – 197, PHE – 200) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with TYR -167, PHE – 97, TYR – 95, PRO – 197, ILE. – 199, PHE – 200, LEU – 21, MET -204, MET -205, MET -258, PHE – 208, ALA- 156, LEU – 216, VAL – 212, PHE – 215.The amino acid residues with negative charges are shown in red, positive charges in velvet, polar in cyan. Hydrophobic bonds were seen in green, undefined residues in grey. Hydrogen bond interaction is shown as pink-arrow, pipi stacking as green line and pi-cation as red line.

Ligand Interaction of V. amygdalina and N. latifolia with Beta-Tubulin
Error! Reference source not found.3 and **Error! Reference source not found.**4 contains images depicted by the captions Figures 3m-3r. In Fig 3m, the reference ligand (DLW) showed hydrogen bonds with amino acid residues (GLU – 200, CYS – 241) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with ALA – 354, ALA – 316, LEU – 248, ILE – 318, ILE – 378, LEU – 242, ALA – 256, LEU – 255, TYR – 202, PHE – 169, TYR – 52, VAL – 238, MET – 259. In Fig 3n, N- caffeoyl serotonin from *V. amygdalina* showed hydrogen bonds with amino acid residues (CYS – 241, GLN – 136) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with ILE – 318, ALA – 317, ALA – 316, ILE – 378, ALA – 354, LEU – 255, LEU – 252, LEU – 242, VAL – 38, ILE – 4, TYR – 52, PHE – 169. In Fig 3o, Cynaroside from *V. amygdalina* showed hydrogen bonds with amino acid residues (VAL – 238, THR – 353) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with MET – 259, ILE – 318, ILE – 378, ALA – 316, ALA – 317, TYR – 202, LEU – 242, VAL – 238, LEU – 248, ALA – 250. In Fig 3p, Apigenin 4-O-rhamnoside from *V. amygdalina* showed hydrogen bonds with amino acid residues (ASN – 167, GLN – 136) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with PHE – 169, TYR – 52, ILE – 4, LEU – 252, VAL – 238, LEU – 255, LEU – 242, MET – 259, LEU – 248, ALA – 354, ILE – 318, ALA – 317, ALA – 316, TYR – 102. In Fig 3q, Apigenin 4-glucoside from *V. amygdalina* showed hydrogen bonds with amino acid residues (THR – 353, GLN – 136) and the

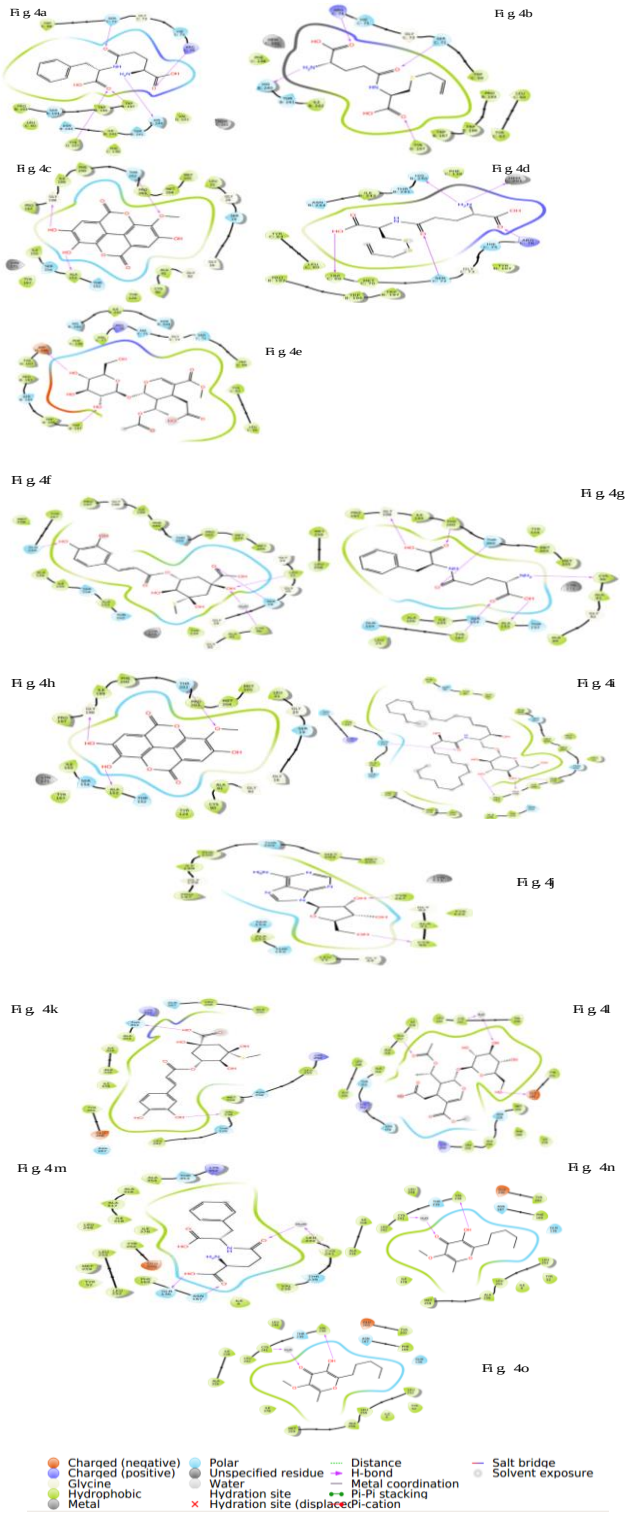

Figure 4: 4a-4e, 4f-4j, 4k-4o; 2D Interactions of the amino acid residues with Nauclea latifolia

Key: Fig.4a– Gamma-phenylalanine, Fig.4b– Gamma-glutamyl-S-allylcysteine, Fig.4c– 3-O methyellagic acid, Fig.4d – Gamma-glutamyl -s-allylmercaptocysteine, Fig.4e- Diderroside Fig.4f: 5-methyl(thio)chlorogenic acid, Fig4g: Gamma-glutamyl phenylalanine, Fig 4h– Adenosine, Fig4i: 3-O methyl ellagic acid, Fig4j: 1-O -beta-D-glucopyranosyl -N- [(2R) – 2- hydroxy palmitoyl] sphingosine., Fig.4k-5-methylethyl(thio)chlorogenic acid, Fig.4l- Diderroside, Fig.4m – Gamma-glutamyl phenylalanine, Fig .4n - Adenosine, Fig.4o -Allixin.

arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with ALA. – 354, ILE – 318, ALA – 317, ALA – 316, VAL – 238, ILE – 378, LEU – 242, TYR – 202, LEU – 255, ILE – 4, TYR – 52, MET – 259, PHE – 169. In Fig 3r, Luteolin -7-O-glucoside from *V. amygdalina* showed a hydrogen bond with amino acid residue (VAL – 238) and the arrow indicates that this residue is donating to form a hydrogen bond. No pi-pi stacking was seen.

Hydrophobic bonds were formed with TYR 202, MET – 259, LEU – 242, VAL – 238, ALA -250, ALA – 354, ALA – 317, ALA – 316, LEU – 255, MET – 259, ILE – 318.In Fig 4k, 5-methyl(thio)chlorogenic acid from *N. latifolia* showed hydrogen bonds with amino acid residues (THR – 353, VAL – 238) and the arrow indicates that these residues are donating to form hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with VAL – 238, MET – 259, LEU – 255, LEU – 242, TYR – 202, ALA – 316, ILE – 378, ALA – 354, ALA – 250. In Fig 4l, Diderroside from *N. latifolia* showed hydrogen bonds with amino acid residues (ALA – 317, GLU – 200, CYS – 241) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed ALA – 317 ILE – 318, LEU – 242, CYS – 241, MET – 259, ALA – 256, LEU – 255, ILE – 378, PHE – 268, ALA – 354, ALA – 316. In Fig 4m, Gamma-glutamyl phenylalanine from *N. latifolia* showed a hydrogen bonds with amino acid residues (ASN – 167, GLN – 136, CYS – 241) and the arrow indicates that these residues are donating to form the hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with LEU – 252, TYR – 52, MET – 259, TYR – 202, ILE – 378, LEU – 248, ALA – 317, ALA – 316, ALA – 354, CYS – 241, ILE – 4. In Fig 4n, Adenosine from *N. latifolia* showed a hydrogen bond with amino acid residue (VAL – 238) and the arrow indicates that this residue is donating to form a hydrogen bond. No pi-pi stacking. Hydrophobic bonds were formed with LEU – 252, LEU – 255, MET – 259, ILE – 378, TYR – 202, LEU – 242, CYS – 241, ALA – 316, ALA – 354 and LEU – 248. In Fig 4o, Allixin from *N. latifolia* showed hydrogen bonds with amino acid residues (CYS – 241, VAL – 238) and the arrow indicates that these residues are donating to form hydrogen bonds. No pi-pi stacking was seen. Hydrophobic bonds were formed with CYS – 241, LEU – 242, LEU – 248, VAL – 238, LEU – 252, TYR – 52, ILE – 4, LEU – 255, ALA – 256, MET – 259, ILE – 378, ALA – 316, ILE – 318, PHE – 169.The amino acid residues with negative charges are shown in red, positive charges in velvet and polar in cyan. Hydrophobic bonds were seen in green, with undefined residues in grey. Hydrogen bond interaction is shown as pink-arrow, pipi stacking as green line, and pi-cation as red line.

Table 5: Docking and MM-GBSA scores of the top five lead compounds of *V. amygdalina* on Ascaris Mitochondrial Ketoreductase

S/N	PubChem ID	Compound	Glidescore (kcal/mol)	MMGBSA
1	5281769	Cynarine	-16.249	-83.94
2	44258082	Luteolin rutinoside	-14.118	-61.42
3	5280637	Cynaroside.	-13.165	-58.53
4	5319116	Luteolin-4'-o-glucoside	-12.784	-63.59
5	6443140	Ferulic acid 4-O-glucuronide	-12.373	-53.51
6	CCL	NAD (Standard)	-13.694	-120.88

Table 6: Docking and MM-GBSA scores of the top five lead compounds of *Nauclea latifolia* on Ascaris Mitochondrial Ketoreductase

S/N	PubChem ID	Compound	Glidescore (kcal/mol)	MMGBSA
1	102094725	5, Methyl(thio) chlorogenic acid	-10.772	-44.2
2	111299	Gamma, glutamyl phenylalanine	-9.744	-39.33
3	60961	Adenosine	-8.805	-40.05
4	13915428	3-O-methylellagic acid	-8.749	-43.44
5	101948670	1-O-beta-D-Glucopyranosyl	-8.715	-65.36
6	CCL	NAD (Standard)	-13.694	-120.88

Mitochondrial Rhodoquinol-Fumarate Reductase aids in energy metabolism and the electron transport chain in the mitochondria.[23] The findings of the docking investigation demonstrated that Luteolin rutinoside from *V. amygdalina* and Gamma-glutamyl phenylalanine from *N. latifolia* . had the best docking scores of -11.912 and – 10.715 kcal/mol respectively. When compared with the reference standard (FTN) with docking score and MMGBSA of -8.39 kcal/mol and -48.77 respectively, the ligands formed a more stable receptor-ligand complex (as shown in Tables 1 and 2 respectively). This implies that Luteolin rutinoside and Gamma-glutamyl phenylalanine have a higher binding affinity to this target and can actively inhibit Mitochondrial Rhodoquinol-Fumarate Reductase.[24] This will lead to the disruption of the energy metabolism and electron transport in the ascaris mitochondria, thereby leading to the death of the parasite. The Ascaris Mitochondrial Ketoreductase helps in the metabolic pathway of the organism.[25] The findings of the docking investigation demonstrated that Cynarine from *V. amygdalina* and 5-methyl(thio)chlorogenic acid from *N. latifolia* had the best docking scores of -16.249 and -10.772 kcal/mol respectively. When compared to the reference standard (NAD) with the docking score of -13.694 kcal/mol, this implies that the two ligands can inhibit Ascaris Mitochondrial Ketoreductase, [26] thereby interfering with the energy metabolism and disrupting the normal

functioning of the worm in the host system. Beta-tubulin is essential for Ascaris cellular processes like cell division, intracellular transport, and survival.[27] The findings of the docking investigation demonstrated that N-caffeoyl serotonin from *V. amygdalina* and 5-methyl (thio) chlorogenic acid from *N. latifolia* had the top docking scores of -11.097 and -9.652 kcal/mol respectively. When compared to the reference standard (DLW) with the docking score of -11.621 kcal/mol, it means that N-caffeoyl serotonin and 5-methyl (thio) chlorogenic acid can actively inhibit Beta-tubulin. This study supported the claims that *V. amygdalina* and *N. latifolia* have good potential in the treatment of ascariasis and helminths infections and revealed the phytochemicals in each plant responsible for the reported activities and the possible targets/receptor they act on for their activity. From the experiment, it could be hypothesised, that the secondary metabolites from *V. amygdalina* and *N. latifolia* may work synergistically to achieve their anti-helminthic activity, and possibly through multi-target inhibition of key ascaris proteins.[28]The compounds with good binding scores and affinity from this study should be taken for MD simulation to gain insight on the nature of their interaction at the target receptor relative to that of the standard drugs (e.g. Albendazole on B-tubulin). In addition, these will inform a decision to carry out a bioassay-guided isolation/fractionation of these active compounds for *in-vitro* or *in-vivo* study to elucidate their anti-helminthic activities, or for rational drug design and optimization for chemical synthesis.[29] More ascaris targets (e.g Ascaris MSP, GST, CYP) should be screened against these phytochemicals from *N. latifolia* and *V. amygdalina* to know their effects on these key ascaris targets. It is important to note that the efficacy and safety of *V. amygdalina* and *N. latifolia* for the treatment of ascariasis has not been thoroughly investigated in clinical trials, therefore, further research is needed to determine the potential of the two plants as a viable treatment for ascariasis.

Conclusion

The molecular docking studies have shown that secondary metabolites from the medicinal plants; *V. amygdalina* and *N. latifolia* can serve as potential inhibitors and/or lead compounds for the development of novel inhibitors of these targets for the treatment of ascariasis. It also provides the molecular basis of their anti-helminthic activities, possibly through the inhibition of metabolic processes in the mitochondria.

Conflict of interest

The authors declare no conflict of interest.

Authors Declaration

The authors hereby declare that the work presented in this article is, original and that any liability for claims relating to the content of this article will be borne by them.

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