

Molecular Docking Modeling Using In-silico Inhibitory Potentials of *Parkia biglobosa* Compounds against a Resistant *Trypanosoma brucei* strain

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Received on: April, 2024

Revised and Accepted on: May, 2024

Published on: July, 2024

ABSTRACT

In this study, the molecular docking model was used to investigate the antitrypanosomal inhibitory potential of *Parkia biglobosa*. Twenty-eight different compounds were identified as potential candidates; however, the returned binding energy (kcal/mol) suggested four suitable candidates (beta-sitosterol, epi-gallocatechin, epi-catechin-3-O-gallate, catechin and taraxerone). The antitrypanosomal activity of these compounds was investigated using a molecular docking assay against two proteins from *T. brucei brucei*, namely arginine kinase 3 (Accession No. EAN76668.1) and trans-sialidase (AlphaFold ID: AF-Q57YTZ-F1). Using the SwissAdme server (SwissADME), all herbal substances were screened in silico for drug-likeness according to the guidelines of Lipinsk, Egan and Veber. Molecular docking was performed to identify putative binding orientations and binding energies (BE) of drugs at the selected binding domains of trans-sialidase and arginine kinase 3 using Auto Dock Vina in the open-source Python Prescription 0.8 program. The returning binding energy (kcal/mol) of *P. biglobosa*'s druglike compounds indicates that they bind well to the active sites of both proteins. However, additional investigation is needed to test *P. biglobosa* in vitro or in vivo to see if it may be used as an effective and less expensive drug because it is a more accessible plant.

Keywords: *Parkia biglobosa*, In-silico, Animal trypanosomiasis, Resistant strain. phytochemical compounds, molecular docking.

1.0 INTRODUCTION

Trypanosomes are the causative agents of trypanosomiasis in vertebrates and humans. The trypanosome, an extracellular protozoan of the genus *Trypanosoma*, which is a hemoparasite, is widely distributed in tropical regions (Jimenez *et al.*, 2022; Kasozi *et al.*, 2022). *Trypanosoma brucei brucei* is a subspecies of the parasitic kinetoplastid belonging to the genus *Trypanosoma*. In sub-Saharan Africa, tsetse fly species spread this parasite, which causes vector-borne diseases in vertebrates (WHO, 2020; Shereni *et al.*, 2021). Increased malaise, anemia, emaciation and, if left untreated, death of the host are the hallmarks of the disease (Okello *et al.*, 2022). African trypanosomiasis, sometimes known as sleeping sickness in humans, causes animal trypanosomiasis in animals, also known as nagana in cattle and horses (Luzak, *et al.*, 2021). To date, three subspecies of *T. brucei* have been recognized: *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense* (Venturelli, 2022). Most medications used to treat AAT are unable to pass through the blood-brain barrier, infection in the bloodstream often returns following treatment. The shortcomings, toxicity, cost, and evolution of drug-resistant strains of the current medicines are compounded by these limitations (Mohd *et al.*, 2019; Álvarez-Bardón *et al.*, 2020; Ungogo *et al.*, 2020; Odeniran *et al.*, 2021;

Tempone *et al.*, 2021; Kasozi *et al.*, 2022; Pacheco and Santos, 2022; Francesconi *et al.*, 2024). The need for novel medication therapy is urgent because some present therapies have serious side effects that can be fatal to the patient (Wing-Fu Lai, 2019; Álvarez-Bardón *et al.*, 2020; Morais *et al.*, 2020; Peerzada *et al.*, 2021; Richards *et al.*, 2021; Ibrahim *et al.*, 2022; Hofer, 2023; Hofer, 2023). Medicinal plants have continuously drawn a lot of interest as a possible alternate source for ensuring the availability of chemotherapy for African trypanosomiasis. (Hzounda *et al.*, 2021; Tempone *et al.*, 2021; Dur̃ao, *et al.*, 2022). African trypanosomiasis has been treated using medicinal plants, according to numerous research (Luna *et al.*, 2019; Álvarez-Bardón *et al.*, 2020; Kourbeli *et al.*, 2021; Nekoei *et al.*, 2022; Pacheco and Santos, 2022; Kamdem and Boyom, 2023; Oroscó *et al.*, 2024). Compounds in *Parkia biglobosa* hold immense significance in drug discovery, elucidating its therapeutic potential against various ailments as the indigenous African plant boasts rich phytochemical diversity, with compounds like flavonoids and tannins exhibiting promising pharmacological activities (Oyebode *et al.*, 2018). Molecular docking provides the first line of proof for inhibition by determining the binding affinity between molecules and the binding pose of a ligand in the binding pocket of a macromolecule

(Gurung *et al.*, 2021). Docking studies enable the prediction of ligand-receptor interactions, aiding in the identification of potential lead compounds with high binding affinities and specificities (Ji *et al.*, 2020). By elucidating the molecular mechanisms underlying *Parkia biglobosa*'s bioactivity, docking facilitates rational drug design, paving the way for novel therapeutics against diseases. Ultimately, this approach accelerates drug development, emphasizing the plant's pharmacological exploitation in traditional medicine and modern pharmaceuticals. Thus, the purpose of this study was to assess the *In-silico* inhibitory prospects of compounds found in *Parkia biglobosa* against a Resistant Strain of *Trypanosoma brucei brucei* using two proteins; Trans-Sialidase (AlphaFold ID: AF-Q57Y TZ-F1) and Arginine kinase 3 (Accession No. EAN76668.1).

2.0 MATERIALS AND METHOD

2.1 Protein Sequence Selection and Retrieval

Two proteins were chosen from *T. brucei brucei*, specifically Trans-Sialidase (AlphaFold ID: AF-Q57Y TZ-F1) and Arginine kinase 3 (Accession No. EAN76668.1). We chose to use the protein sequences from the National Center for Biotechnology Information (NCBI) server (National Center for Biotechnology Information (nih.gov)) and AlphaFold's Deep Mind of the European Molecular Biology Laboratory (EMBL) server (AlphaFold Protein Structure Database (ebi.ac.uk)) because there were no structures for these proteins in protein databases. Homology Modelling and Preparation of Protein Structure for Molecular Docking.

In order to construct their 3D structures, the obtained protein sequences of Trans-Sialidase (AlphaFold ID: AF-Q57Y TZ-F1) and Arginine kinase 3 (Accession No. EAN76668.1) were uploaded into the workspace module of the SWISS-MODEL website (<https://swissmodel.expasy.org>). Next, using the appropriate tools in the UCSF-Chimera© (version 1.13) software (<http://www.cgl.ucsf.edu/chimera>) (Pettersen *et al.* 2004), the protein structures were prepared to be processed by docking and minimization.

2.2 Ligand Optimization, Retrieval, Drug-Likeness Screening, And Selection

Twenty-eight plant compounds were selected from *Parkia biglobosa* (Tringali 2000) through extensive literature mining. All plant compounds were *in silico*-wise analyzed for their druglikeness from the rules of Lipinski (Lipinski *et al.* 2001), Egan (Egan *et al.*, 2000), and Veber (Veber *et al.* 2002) through the SwissADME server (SwissADME) (Daina, Michielin, and Zoete 2017). Those compounds that scaled through the screening were obtained from a daughter webserver of NCBI called PubChem in the structure data file (SDF) (PubChem (nih.gov)). The 3D conformers of drug-like

compounds were uploaded in Python prescription suit (PyRx) and optimized via Open babel in PyRx (version 0.8) which converted the ligands energetically to the most stable structures using merck molecular force field 94 (MMFF94) (Halgren, 1996).

2.3 Simulating Molecular Docking

The molecular docking was carried out through AutoDock Vina in open-source Python Prescription 0.8 (Trott and Olson, 2010) to acquire possible binding orientations and binding energy (BE) of compounds at the selected binding domain of arginine kinase 3 and trans-sialidase. This region was covered with the aid of the grid box with dimensions (17.5878 x 21.5384 x 27.7438) Å and (26.7771 x 17.0536 x 21.5627) Å, and the centers was adjusted based on the site of binding in arginine kinase 3 and trans-sialidase respectively. This area in arginine kinase 3 was predicted using dog site scorer module (Volkamer *et al.*, 2012) of protein-plus server (<https://proteins.plus>) (Schöning-Stierand *et al.* 2020), and was predicted to comprise of Leu48, Asp74, Val74, Ile76, Gln77, Ser78, Gly79, Ser85, Gly86, Val87, Gly88, Leu89, Tyr90, Ala91, Pro92, Phe104, Val107, Ile108, Tyr111, His112, Pro160, Phe216, Ala219, Ala220, Phe292, Cys293, Pro294, Ser295 and Leu297. That of trans-sialidase binding site was reported to compose of Arg374, Arg298, Arg84, Asp146 and Arg102 (Lima *et al.* 2012). After the docking run, compounds with the least binding energy were subjected to molecular visualization in order to analyze their binding poses (3D) and molecular interaction fingerprints (2D) through PyMOL© Molecular Graphics (version 2.4, 2016, Schrödinger LLC) and LigPlot⁺ software (Laskowski and Swindells, 2011).

3.0 RESULTS AND DISCUSSION

3.1 Modeling homology in protein structure

The popular RCSB protein database does not currently contain the 3D protein structures of the proteins found in the parasite of interest that have crystallized. To create a protein structure that is appropriate for our investigation, we thus employ a homology modeling strategy. Following the construction of the two structures, the models were evaluated, and it was discovered that the arginine kinase 3 model from *T. b. brucei* (2J1Q) had 81.69% identity and 95.44% of its amino acid residues were located in the preferred region. This was also noted for trans-sialidase, which was modeled using a template from the deep mind server of Alpha Fold, including 92.63% of its residues in amino acids and 100% identity (Figure 1). These demonstrated the use of both forms for simulations of molecular docking and molecular dynamics.

3.2 Drug Likeness Screening

The outcome of this screening of compounds from the study plant evaluated in silico against the two enzymes of *T. brucei brucei* showed 16 compounds could be drug-

like after passing them through Lipinski's, Egan's and Veber's rules, oral bioavailability inclusive as presented in Table 1 below.

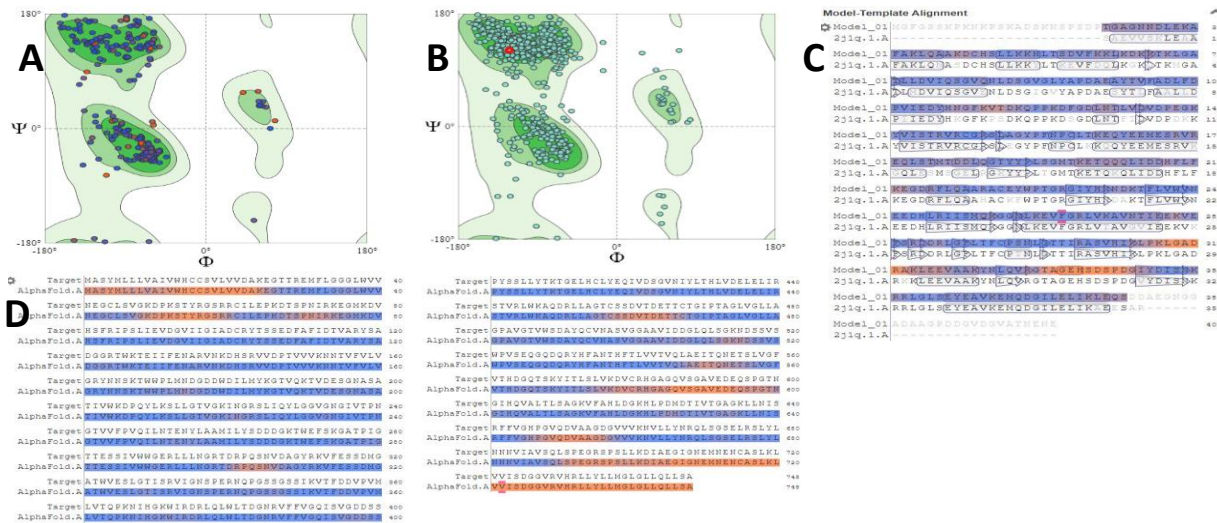


Figure 1: Homology modelled Protein structures' assessment. Ramachandran plots of a) AK 3 and b) TS. According to this plot, most of the amino acid residues were seen to occupy the favored regions. Multiple sequence alignments of c) AK 3 and d) TS against their templates from 2J1Q and AF-Q57YTZ-F1 with sequence identities of 81.69 % and 100% respectively.

Table 1: Drug-likeness Screening of Compounds in *Parkia biglobosa* Using SwissAdme Server

Molecule	PubChem ID	Lipinski #violations	Veber #violations	Egan #violations	Bioavailability Score
p-coumaric acid	637542	0	0	0	0.85
Trans-ferulic acid	445858	0	0	0	0.85
Caffeic acid	689043	0	0	0	0.56
Protocatechuic acid	72	0	0	0	0.56
Gallic acid	370	0	0	0	0.56
2-(2heptadecyloxy) tetrahydro	544115	1	1	1	0.55
2-Naphthalenol	8663	0	0	0	0.55
9-Octadecenamidine	1930	1	1	0	0.55
9-Octadecenoic acid	637517	1	1	1	0.85
beta Sitosterol	222284	1	0	1	0.55
beta-Amyrin	612816	1	0	1	0.55
Catechin	9064	0	0	0	0.55
4-O-methyl-epi-gallocatechin	176920	0	0	0	0.55
epi-gallocatechin	72277	1	0	0	0.55
epi-catechin 3-O-gallate	107905	1	1	1	0.55
epi-gallocatechin 3-O-gallate	65064	2	1	1	0.17
Hexadecanoic acid, methyl ester	8181	1	1	0	0.55
Lupeol	259846	0	0	1	0.55
Lupenone	92158	1	0	1	0.55

Lupeol acetate	92157	1	0	1	0.55
n-Hexadecanoic acid	985	1	1	0	0.85
Oleic Acid	445639	1	1	1	0.85
Taraxerone	92785	1	0	1	0.55
tButyltricyclo[4.2.1.1(2,5)]decane -9,10-diol	590981	0	0	0	0.55

3.3 Molecular Docking analysis

We performed molecular docking of the plant's predicted druglike compounds against two protein targets (AK 3 and TS), which are important to the pathology of animal trypanosome infection, in order to provide insight into *P. biglobosa's* potential against infections caused by *T. brucei brucei*. As evidenced by the returned binding energy (kcal/mol) in Table 2, this analysis shows that *P. biglobosa's* druglike compounds are effective binders to both proteins' active sites. The lower the binding energy, the likelihood is the affinity of compounds to the active site of the targeted proteins in *T. brucei brucei*. However, a more robust experiment is required to ascertain the binding potent of these compounds through in vivo, in vitro or molecular dynamics simulations.

Table 2: Binding energy (kcal/mol) of drug-like compounds from *Parkia biglobosa* when docked against *T. Brucei brucei's* arginine kinase 3 and Trans-Sialidase enzymes using auto dock vina

Molecule	PubChem ID	Binding Energy (kcal/mol)	
		Arginine Kinase 3	Trans-sialidase
p-coumaric acid	637542	-5.3	-6.2
Trans-ferulic acid	445858	-5.1	-6.5
Caffeic acid	689043	-5.4	-6.6
Protocatechuic acid	72	-5.0	-5.2
Gallic acid	370	-4.8	-6.0
2-(2- heptadecynyloxy) tetrahydro	544115	-5.5	-5.8
2-Naphthalenol	8663	-5.8	-5.6
9-Octadecenamide	1930	-4.3	-4.8
9-Octadecenoic acid	637517	-4.4	-5.4
beta Sitosterol	222284	-6.2	-7.4
Catechin	9064	-6.0	-7.7
4-O-methyl-epi-gallocatechin	176920	-5.3	-7.6
epi-gallocatechin	72277	-6.3	-7.8
epi-catechin 3-O-gallate	107905	-6.4	-8.3
Hexadecanoic acid, methyl ester	8181	-3.9	-4.9
Lupeol	259846	-6.3	-7.1
Lupenone	92158	-5.5	-5.8
n-Hexadecanoic acid	985	-4.5	-4.8
Oleic Acid	445639	-4.5	-5.9
Taraxerone	92785	-6.2	-8.2
tButyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	590981	-5.3	-6.3

3.4 Molecular Interaction Analysis

Aside from the returned binding energy, we look at the mechanism involved in the binding of these compounds with the targeted proteins. These entail that we look at the relationships that results from protein-compound complexes of those with good binding energy. From our present study, hydrogen bonds, and hydrophobic interactions were observed

to be present between the atoms of the plant compounds and side chains of the amino acid residues of AK 3 and TS (Figure 2 and 3).

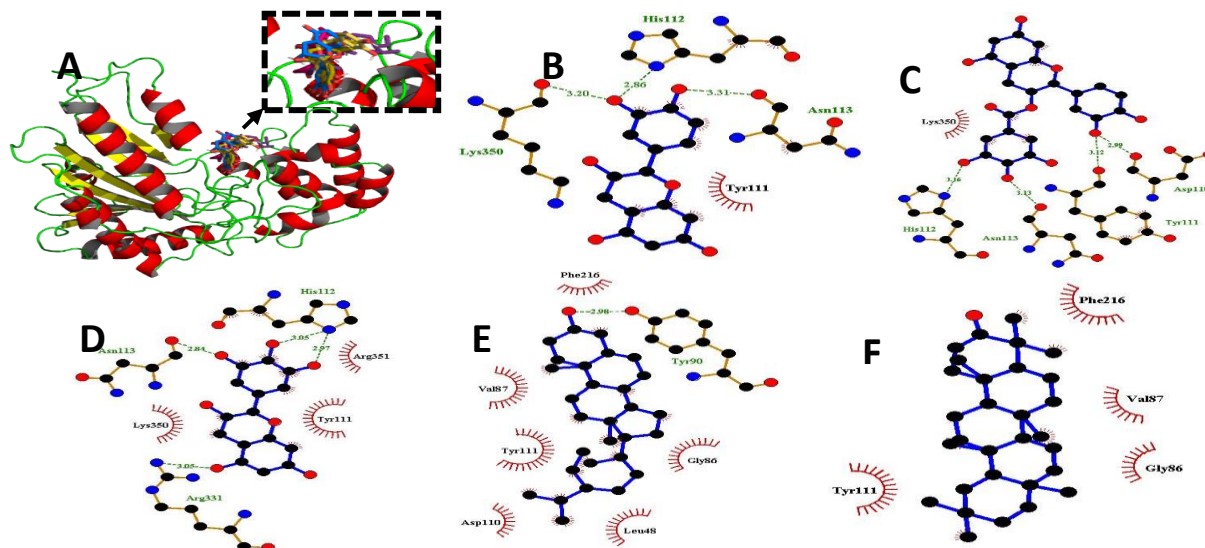


Figure 2: Molecular docking of selected compounds in *P. biglobosa* and arginine kinase 3 of *T. brucei brucei*. A) 3D binding poses of hit compounds in the binding pocket of AK 3. 2D molecular interaction analysis of b) catechin, c) epicatechin-3-O-gallate, d) epigallocatechin, e) β -sitosterol and e) Taraxerone with amino acid residues in the binding pocket of AK 3. Compounds are in blue stick, amino acid residues are in brown sticks, green dashes represent hydrogen bonds and red spiked-curve are hydrophobic interactions.

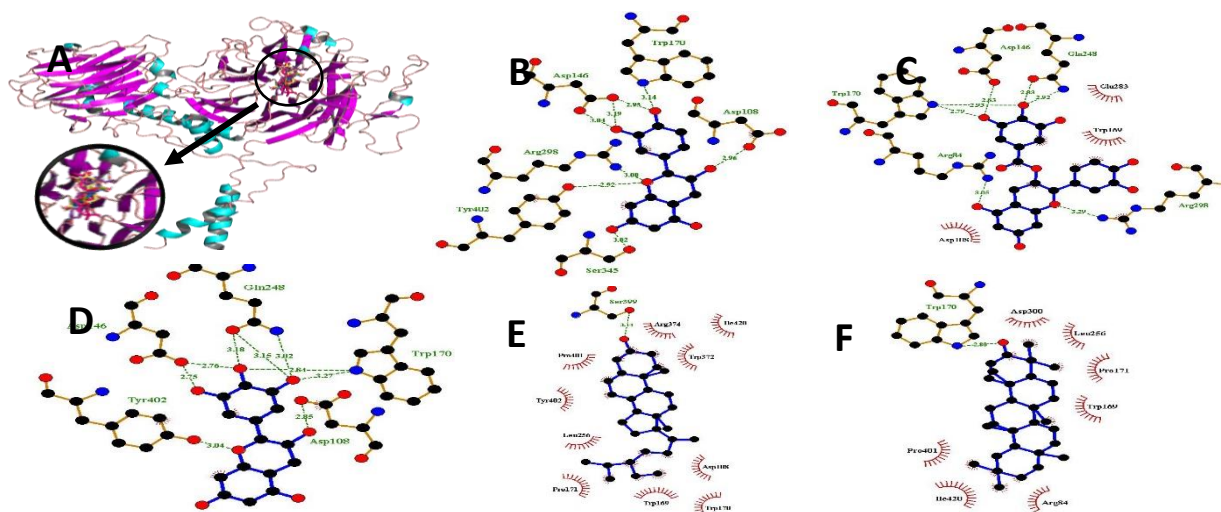


Figure 3: Molecular docking of selected compounds in *P. biglobosa* and trans-sialidase of *T. brucei brucei*. A) 3D binding poses of hit compounds in the binding pocket of TS. 2D molecular interaction analysis of b) catechin, c) epicatechin-3-O-gallate, d) epigallocatechin, e) β -sitosterol and e) Taraxerone with amino acid residues in the binding pocket of TS. Compounds are in blue stick, amino acid residues are in brown sticks, green dashes represent hydrogen bonds and red spiked-curve are hydrophobic interactions.

4.0 DISCUSSION

Trypanosoma brucei brucei, one of the causative agents of African Animal trypanosomiasis, poses a significant health threat, especially with the emergence of drug-

resistant strains (Okello *et al.*, 2022). *Parkia biglobosa*, a medicinal plant known for its diverse bioactive compounds, holds promise as a potential source of anti-trypanosomal agents. The molecular docking modeling

of compounds isolated from the plant against a resistant strain of *Trypanosoma brucei brucei*, focusing on their inhibitory effects on two crucial enzymes, arginine kinase 3 and trans-sialidase, implicated in the parasite's survival and virulence (Gurung *et al.*, 2021). Utilizing molecular docking techniques, we have explored the binding interactions between compounds from the plant and target proteins of the parasite. These in-silico studies offer valuable insights into the potential inhibitory effects of *Parkia biglobosa* compounds on key enzymes essential for the parasite's viability and pathogenesis.

Arginine kinase 3 plays a pivotal role in energy metabolism by catalyzing the transfer of phosphate groups, essential for ATP production in *Trypanosoma brucei brucei* and is one of the key enzymes, responsible for the parasites' metabolic plasticity, which maintains the cell energy homeostasis during environment changes (Pereira, 2014) Through molecular docking simulations, it has been observed that certain compounds from *Parkia biglobosa* exhibit favorable binding interactions with arginine kinase 3, by targeting this enzyme, the compounds may disrupt ATP synthesis, thereby impeding the parasite's energy production and ultimately compromising its survival. This observation was in line with previous finding some plant-derived polyphenolic pigments, such as anthocyanidins, were predicted to inhibit arginine kinase 3 (Valera-Vera *et al.*, 2021). Trans-sialidase is another critical enzyme in *Trypanosoma brucei brucei* involved in host-parasite interactions and immune evasion. Molecular docking studies have indicated promising binding interactions between compounds from *Parkia biglobosa* and trans-sialidase. Inhibition of trans-sialidase activity by these compounds could hinder the parasite's ability to modulate host immune responses, thus attenuating its virulence and aiding in host clearance of the infection. This finding supports the work of Vázquez-Jiménez *et al.*, (2021) who earlier detected 487 compounds derived from benzoic acid as potential trans-sialidase inhibitors with a more promising binding energy value (< -7.7 kcal/mol) than the known inhibitor 2,3-dehydro-2-deoxy-N-acetylneuraminic acid. It is noteworthy that neither of the chemicals (beta sitosterol, epi-gallocatechin, epicatechin 3-O-gallate, catechin, or taraxerone) violated any of the drug likeness guidelines (veber, egan, or Lipinski), which makes them great candidates for further drugs development. This observation has been supported by numerous studies on the anti-trypanosomal potential of different plant metabolites and molecular docking investigations (Boniface and Elizabeth, 2019; Álvarez-Bardón *et al.*, 2020; Morais *et al.*, 2020; Abdelfattah *et al.*, 2021; Tempone *et al.*, 2021; Fall *et al.*, 2022; Pone and Ferreira, 2022; Boniface *et al.*, 2023; El-Nashar *et al.* 2023; Orosco *et al.*, 2024).

5.0 CONCLUSION

The in-silico inhibitory potential of compounds from *Parkia biglobosa* against drug-resistant strains of *Trypanosoma brucei brucei*, targeting arginine kinase 3 and trans-sialidase, highlight the prospects of *Parkia biglobosa* against infections caused by *T. brucei brucei*. As evidenced by the returned binding energy (kcal/mol) in Table 2. The findings show *P. biglobosa's* druglike compounds are effective binders to both proteins' active sites. Molecular docking modeling provides a valuable initial screening tool for identifying promising lead compounds with anti-trypanosomal activity. Nonetheless, the findings underscore the importance of exploring natural sources, such as *Parkia biglobosa*, in the search for novel treatments against drug-resistant *Trypanosoma brucei brucei*.

6.0 RECOMMENDATION

The effectiveness and safety of these chemicals as possible medicines for drug-resistant *Trypanosoma b. brucei* need to be further experimentally validated through *in vivo* evaluation.

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