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Molecular Docking and Virtual Screening of *Boswellia dalzielii* Phytochemicals as Potential Inhibitors of *Rabies lyssavirus.*

Emmanuel Chuks Oranu [1], Chigozie Celestina Ezeagha [1,*], Afomachukwu Jessica Madueke [1], Blessing Ejiro Otarigho [2], joseph Orjiakor [1] and Ifechukwu Kingsley Etuka [1]

[1] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University Igbariam, Anambra State, Nigeria.*
[2] *Department of Pharmacology and Therapeutics, Faculty of Basic Medical science, Delta State University, Delta State, Nigeria.*

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Abstract

Rabies is a fatal viral encephalitic disease caused by the *Rabies lyssavirus* that has the highest case fatality rate of any infectious disease. Infection with rabies virus takes either the furious form or paralytic form defined by neurological complications or widespread paralysis respectively. This study was aimed to employ computational methods and tools to predict, analyse, and understand the potential antiviral effects of phytochemical compounds from *Boswellia dalzielii* (hutch) on this virus. To achieve this, the target protein of *Rabies lyssavirus* was selected from protein data bank. The receptor (8B8V) was prepared using Chimera-1.10.1, PyMOL-2.3.0, and Autodock tools-1.5.6, and the same was done for the reference ligand (PEG), while the phytochemicals were selected from Drugbank and PubChem database. The phytochemicals which were screened out were then extracted and prepared using Autodock tools-1.5.6. Validation of docking protocol was done after which molecular docking was carried out using Linux operating system (Ubuntu)-20.04. The docking results were analysed and visualised using PyMOL-2.3.0 and the front runners were screened on Datawarrior based on the Lipinski's Rule of Five and toxicity. Following the completion of the aforementioned procedures, the reference ligand was observed to have a mean binding energy of -3.3 Kcal/mol. While the 149 phytoconstituents including the 10 front-runners had lower binding energies ranging from -8.6 to -3.8 Kcal/mol indicating better binding affinity than the reference ligand. Comparing the phytochemicals with the reference ligand of the selected protein, it can be concluded that these phytochemicals exhibit better binding affinity, with Gallocatechin (-8.6 Kcal/mol) having the highest binding affinity, thus, a better anti-rabies activity, however, it failed the Lipinski rule but Luteolin (-7.7 Kcal/mol), Epicatechin (-7.6 Kcal/mol) and Cianidanol (-7.3 Kcal/mol) passed the Lipinski rule and toxicity studies and are also part of the front runners and can be predicted to have better anti-rabies activity.

Keywords: *Rabies lyssavirus*; Phytochemicals; Molecular docking; *Boswellia dalzielii*; Binding affinity; Reference ligand; Gallocatechin; Anti-viral activity**.**

1. Introduction

Lyssa viruses cause rabies, which is widely regarded as the deadliest known encephalitic disease known. The prototype, known as *Rabies lyssavirus* (RABV), is thought to infect all terrestrial mammals. Transmission is by virus-laden saliva, typically through the bite of an infected animal, but sometimes through other ways such as scratches and, in rare cases, organ transplants [1, 2].

* Corresponding author: Ezeagha Chigozie Celestina

This single-stranded, negative-sense RNA virus is typically transmitted via the bite of an infected animal, with domestic dogs serving as the primary reservoir and vector for human transmission in many regions of the world [3].

Rabies, or 'rabere' in Latin, means 'to be mad.' The sickness has been known since the beginning of civilization. Rabies was first officially documented in the pre-mosaic Eshmuna code of Babylon in the twenty-third century BC. However, in the 1880s, Louis Pasteur identified a virus as the source of the sickness. Though rabies is a preventable viral zoonosis by vaccines [4], it remains an important public health issue in developing countries, as evidenced by the fact that globally, this devastating disease is responsible for more than 60,000 human deaths, while approximately 15 million people receive rabies post-exposure prophylaxis (PEP) annually [5].

Plants have been employed as medicinal agents for many years, initially in the form of traditional concoctions and later as isolated active drug moieties, with the transfer of knowledge and expertise from one generation to the next [6]. The utilisation of indigenous plants for the management of human ailments dates back to the ancient civilisations of China and India. Currently, there is a growing emphasis and interest in the production of medicinal substances, with a particular focus on the field of Phytochemistry [7].

Phytochemicals, commonly referred to as phytonutrients, are substances that exist naturally in plants and contribute to their flavour, colour, and ability to resist diseases. Phytochemicals have sparked widespread interest in scientific research due to their potential health-promoting and disease-preventive characteristics [8]. Epidemiological studies have established links between high intake of phytochemical-rich foods and decreased risk of chronic diseases such as cardiovascular disease, cancer, diabetes, and neurological disorders [9].

Boswellia dalzielii, commonly known as “African frankincense”, is a tree in the Burseraceae family, belonging to the Boswellia genus and the B. dalzielii species. The wooden savanna stands about 13 metres tall and has a pale, papery bark that peels and is ragged [10]. The bark produces whitish gum resin that dries quickly and is friable. The plant products (such as gum resin) and various plant parts are commonly used in traditional medicine [11].

It is abundantly found in West Africa in different countries such as Ghana, Niger, Ivory Coast, Upper Volta and Northern Nigeria, where the Hausa-speaking inhabitants of Nigeria call it ‘Hano’ or ‘Ararrabi’ [11]. Moreover, in Burkina Faso, it is called ‘Kumdagneogo’, ‘Tree Man’ or ‘Volta’, and in Ghana, it is called ‘Piangwogu’, while in Ethiopia, it is known as ‘Etan’ [7].

B. dalzielii is the frankincense-producing plant found in West Africa.It is used to treat, among others, rheumatism, septic sores, venereal diseases and gastrointestinal disorders. Despite its widespread use in traditional medicine in the sub region, the West African species is understudied compared to its more well-known counterparts. Phytochemical examination of the plant confirmed the absence of alkaloids while saponins, tannins, flavonoids, cardiac glycosides, steroids, and terpenes were found to be present [11].

The term '*in silico*' is a modern word that refers to computer-based experimentation and is connected to the more widely known biological terms *in vivo* and *in vitro. In silico* pharmacology (also known as computational therapeutics and computational pharmacology) is a rapidly expanding field that encompasses the development of strategies for employing software to acquire, evaluate, and integrate biological and medical data from a variety of sources. It explicitly outlines the use of this data in the development of computational models or simulations that can be used to generate predictions, propose hypotheses, and, eventually, give discoveries or breakthroughs in medicine and therapies [12, 13].

2. Materials and methods

Several software programmes are required to investigate the activities of the constituents of *Boswellia dalzielii* against *Rabies lyssavirus* through molecular docking and post-docking analysis. These software programmes have demonstrated high reliability and effectiveness in *in silico* research, making them valuable resources in drug discovery and design. They include PyMOL (v 2.3.0), Chimera (v 1.10.1), Autodock tools (v 1.5.6), Datawarrior 550, MGL Tools (v 1.5.6), and OpenBabel (v 2.4.1). Biological [Protein Data Bank (PDB)] and Chemical (PUBCHEM and Drug bank) databases are used for bioinformatics and cheminformatics mining respectively [14].

Molecular docking and dynamics simulation documents include;

- Conf.Text: This Ubuntu text document inputs binding site scores based on grid box size and position for accurate binding site representation in molecular docking simulations.

- Bash Binbash.sh: This ODT document contains codes for molecular docking procedures. Finally, a window 10 pro laptop.

2.1. Bioinformatics Mining- Identification of Target Sites

A search on protein data bank (PDB) for possible target proteins of *Rabies lyssavirus* with the following factors; a resolution of below (because the lower the Armstrong the better the resolution), an organic compound as a ligand and its relation to *Boswellia dalzielii* pathogenesis was determined.

2.2. Cheminformatics mining

Constituents from *Boswellia dalzielii* with potential antiviral activities against *Rabies lyssavirus* were identified and about 150 phytochemicals downloaded from Drugbank and Pubchem which were screened on Datawarrior using the Lipinski's Rule of Five, thus phytochemicals with molecular weight higher than 500 Da, hydrogen bond donors higher than 5, hydrogen bond acceptors higher than 10 and partition coefficient higher than 5 were screened out. Based on toxicity they were also screened for tumorigenicity, mutagenicity, effect on reproduction, irritant and undesirable functions.

2.3. Selection and Preparation of Target Protein

After a thorough search on Protein databank, Rabies virus RNA free nucleoprotein- phosphoprotein complex with the code 8B8V was identified and selected for the study. Its resolution is 2.30 Å. The ligand present in the protein is Di(Hydroxyethyl)ether with the chemical formula; $C_4H_{10}O_3$.

The protein of choice (8B8V) was downloaded from protein databank in pdb format. The protein preparation was conducted in both Chimera and Autodock tools.

In Chimera, the similar chains were deleted leaving only two chains (A and B). This was saved as "Deleted 8B8V" as an SDF file.

All ligands except the ligands of choice [Di(Hydroxyethyl)ether] were deleted. Again, this was saved as "Deleted 8B8V+Ligand"

The ligand of choice was then deleted, leaving only the protein behind and the file was saved as "Filtered 8B8V"

The target protein saved as the file "Filtered 8B8V" was then prepared on Autodock Tools. This was done by opening the protein on Autodock tools to edit it by adding polar only hydrogen and Kollman charges. It was then saved as a pdbqt file.

2.4. Selection and Preparation of Target Ligands

The reference ligand was effectively determined with the following factors; first it was going to be an organic molecule, the second requirement is that it must bind to a binding site, this was observed using Pymol and Chimera, after observation Di(Hydroxyethyl)ether was selected.

The reference ligand was found and downloaded from Protein Data Bank in ideal sdf format.

The Preparation of ligands and phytochemicals were done in both Open Babel in Ubuntu and Autodock tools in Windows 10pro using the following process;

The ligands were converted from sdf to mol2 format in Open Babel in Ubuntu using the command line: Is + enter + obabel (ligand) -O ligand.mo12. After which the mol2 files were copied and transferred to Windows where they were prepared in Autodock tools in windows by computing Gesteiger charges and making all bonds non rotatable. The file was then saved as pdpqt format and ready for docking.

2.5. Validation of Docking Protocol

Here, the proteins are ready to be docked and now the binding site was determined. The program used is the Autodock tools in Windows 10pro and the reference ligand was isolated from the protein in Chimera and introduced alongside the prepared protein in Autodock tools. A grid box was introduced and made to cover the ligand in the binding site and the centre and dimension recorded in table 1.

Table 1 Centre and Dimension of Grid Box for prepared 8B8V+ligand

Grid box	Center	Dimension
X	36.068	16
Y	38.908	16
Z	16.676	16

2.6. Molecular Docking

Docking simulations are conducted in the Autodock-vina in Ubuntu. The pdpqt files which included the protein 8B8V, the ligands (phytochemicals) and reference ligand [Di(Hydroxyethyl)ether] with the code e introduced into a folder in Ubuntu, then three copies of this folder was created and opened in terminal using the command line; 'bash binbash.sh' which was used to dock the four folders.

2.7. Post Docking Analysis

After the docking was successfully conducted (in four places), all results were compiled on excel and the mean alongside the standard deviation for each of the phytochemicals was calculated. The mean binding affinity of each of the ligands was then compared to the reference ligand and the frontliners were ascertained. These ligands within the binding pocket were visualised on pymol, to differentiate the ligands within the binding pocket, they were each coloured separately. The forces of interaction between the amino acids and the ligands were also visualised. Snapshots were made for easy visualisation of the affinity within the binding pocket and saved as PNG.

3. Results

There were about 150 phytochemicals downloaded from Drugbank and PubChem, then molecular docking was conducted with these ligands against the target protein and their binding energies were determined on Ubuntu.

Only the first 10 compounds with better binding affinities (the frontrunners) were screened on Datawarrior using the Lipinski Rule of Five and their toxicity profiles considered.

The tables below contain the 150 phytochemicals with their binding energies and the first 10 compounds with better binding affinity as compared with the reference ligand (PEG) which was determined after docking with the selected protein 8B8V. Also shows the results obtained after the first screening of the ligands based on the Lipinski rule/toxicity screening done and results which were collated after comparison of the phytochemicals with the reference ligand during the post docking analysis.

Table 2 Binding energies, mean binding energies and standard deviation of all the docked phytochemicals against the reference ligand PEG.

S/N	LIGANDS	E 1	E 2	E 3	E 4	MEAN	STANDARD DEVIATION
1	Gallocatechin	-8.6	-8.6	-8.6	-8.6	-8.6	0.0
2	Robinetin	-8.3	-8.2	-8.2	-8.2	-8.2	0.1
3	Isorhamnetin	-8.1	-8.1	-8.1	-8.1	-8.1	0.0
4	Epicatechin-3-gallate	-8.3	-8.2	-8.3	-7.3	-8.0	0.5
5	Apigenin	-7.8	-7.8	-7.8	-7.8	-7.8	0.0
6	Luteolin	-7.7	-7.7	-7.7	-7.7	-7.7	0.0
7	Epicatechin	-7.6	-7.6	-7.5	-7.5	-7.6	0.1
8	Epigallocatechin-3-gallate	-7.5	-7.5	-7.5	-7.5	-7.5	0.0
9	Kaempherol	-7.4	-7.4	-7.4	-7.4	-7.4	0.0

10	Cianidanol	-7.3	-7.3	-7.3	-7.3	-7.3	0.0
11	Quercetin	-7.3	-7.3	-7.3	-7.3	-7.3	0.0
12	Butein	-7.2	-7.2	-7.2	-7.2	-7.2	0.0
13	Myricetin	-7.1	-7.1	-7.1	-7.1	-7.1	0.0
14	Epigallocatechin	-7.3	-7.3	-6.6	-6.5	-6.9	0.4
15	Daidzein	-6.9	-6.9	-6.9	-6.9	-6.9	0.0
16	Baicalin	-6.4	-7	-7	-7	-6.9	0.3
17	Beta-pinacene	-6.8	-6.8	-6.8	-6.8	-6.8	0.0
18	Caryophyllene alcohol acetate	-7.3	-6.3	-7.1	-6.3	-6.8	0.5
19	Genistein	-6.7	-6.7	-6.8	-6.7	-6.7	0.0
20	Biochanin A	-6.7	-6.7	-6.7	-6.7	-6.7	0.0
21	Incensole	-6.7	-6.7	-6.7	-6.7	-6.7	0.0
22	Eupalestin	-6.5	-6.5	-6.5	-6.5	-6.5	0.0
23	Cembrenol	-6.3	-6.4	-6.5	-6.5	-6.4	0.1
24	Resveratrol	-6.3	-6.3	-6.3	-6.3	-6.3	0.0
25	Serratol	-5.9	-6.1	-6.1	-6.1	-6.1	0.1
26	Alpha-Longipinene	-6	-6	-6	-6	-6.0	0.0
27	Incensole acetate	-6	-6	-6	-6	-6.0	0.0
28	m-Camphorene	-5.9	-5.7	-5.9	-6	-5.9	0.1
29	Sinensetin	-5.9	-5.8	-5.9	-5.9	-5.9	0.1
30	Alpha-Patchoulene	-5.8	-5.8	-5.8	-5.8	-5.8	0.0
31	Alpha-Selinene	-5.8	-5.8	-5.8	-5.8	-5.8	0.0
32	Caryophyllene oxide	-5.8	-5.8	-5.8	-5.8	-5.8	0.0
33	Alpha-amyrin	-6.2	-5.6	-6.3	-4.8	-5.7	0.7
34	Gamma-Eudesmol	-5.5	-5.8	-5.8	-5.8	-5.7	0.2
35	Trans-Geranylgeraniol	-5.9	-5.6	-5.7	-5.7	-5.7	0.1
36	Widdrol	-5.7	-5.7	-5.7	-5.7	-5.7	0.0
37	Alpha-copaene	-5.6	-5.6	-5.6	-5.6	-5.6	0.0
38	Alpha-cubebene	-5.6	-5.6	-5.6	-5.6	-5.6	0.0
39	Bergamotene	-5.6	-5.6	-5.6	-5.6	-5.6	0.0
40	Beta-caryophyllene	-5.6	-5.6	-5.6	-5.6	-5.6	0.0
41	9-epicaryophyllene	-5.6	-5.6	-5.6	-5.6	-5.6	0.0
42	(-)-beta-Chamigrene	-5.6	-5.5	-5.6	-5.6	-5.6	0.0
43	Lilial	-5.6	-5.6	-5.5	-5.6	-5.6	0.0
44	Zerumbone	-5.6	-5.5	-5.5	-5.5	-5.5	0.0
45	Alpha-cadinol	-5.5	-5.5	-5.5	-5.5	-5.5	0.0
46	Beta-selinene	-5.5	-5.5	-5.5	-5.5	-5.5	0.0
47	Cyperene	-5.5	-5.5	-5.5	-5.5	-5.5	0.0

48	Italicene	-5.5	-5.5	-5.5	-5.5	-5.5	0.0
49	Tau-Muurolol	-5.5	-5.5	-5.5	-5.5	-5.5	0.0
50	Viridiflorol	-5.5	-5.5	-5.5	-5.5	-5.5	0.0
51	(-)-gamma-Cadinene	-5.5	-5.3	-5.5	-5.5	-5.5	0.1
52	Alpha-humulene	-5.5	-5.4	-5.4	-5.5	-5.5	0.1
53	Gallic acid	-5.4	-5.5	-5.4	-5.5	-5.5	0.1
54	Aromadendrene	-5.4	-5.4	-5.4	-5.4	-5.4	0.0
55	Alpha-bourbonene	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
56	Beta-longipinene	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
57	Beta-patchoulene	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
58	Beta-santalene	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
59	D-allose	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
60	Germacrene A	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
61	Isolongifolene	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
62	Protocatechuic acid	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
63	Viridiflorene	-5.3	-5.3	-5.3	-5.3	-5.3	0.0
64	p-Camphorene	-5.3	-5.2	-5.1	-5.3	-5.2	0.1
65	Beta-guaiene	-5.2	-5.2	-5.2	-5.2	-5.2	0.0
66	Alpha-Pinene oxide	-5.1	-5.1	-5.1	-5.1	-5.1	0.0
67	Beta-himachalene	-5.1	-5.1	-5.1	-5.1	-5.1	0.0
68	Bornyl acetate	-5.1	-5.1	-5.1	-5.1	-5.1	0.0
69	Thymol	-5.1	-5.1	-5.1	-5.1	-5.1	0.0
70	Tribulin	-5.1	-5.1	-5.1	-5.1	-5.1	0.0
71	6-epi-Shyobunol	-5.1	-5.1	-5.1	-5.1	-5.1	0.0
72	Alpha-Terpinyl acetate	-5	-5	-5	-5	-5.0	0.0
73	Beta-D-Xylopyranose	-5	-5	-5	-5	-5.0	0.0
74	Trans-Pinocarveol	-5	-5	-5	-5	-5.0	0.0
75	Trans-Verbenol	-5	-5	-5	-5	-5.0	0.0
76	Linalyl acetate	-5	-4.9	-4.9	-5	-5.0	0.1
77	Verbenone	-4.9	-5	-4.9	-5	-5.0	0.1
78	Beta-farnesene	-4.8	-4.8	-5.2	-4.8	-4.9	0.2
79	Chrysanthenone	-4.9	-4.9	-4.9	-4.9	-4.9	0.0
80	Cis-Verbenol	-4.9	-4.9	-4.9	-4.9	-4.9	0.0
81	6-Camphenone	-4.9	-4.9	-4.9	-4.9	-4.9	0.0
82	Methyl salicylate	-4.8	-4.9	-4.8	-4.9	-4.9	0.1
83	Alpha-terpineol	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
84	Beta-fenchol	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
85	Carvacrol	-4.8	-4.8	-4.8	-4.8	-4.8	0.0

86	Carvone	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
87	Cis-Pinocamphone	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
88	Exo-Fenchol	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
89	Myrtenal	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
90	Trans-Sabinol	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
91	1-8-Cineole	-4.8	-4.8	-4.8	-4.8	-4.8	0.0
92	Beta-bisabolene	-4.5	-4.5	-4.5	-5.6	-4.8	0.6
93	o-Creosol	-4.8	-4.7	-4.8	-4.7	-4.8	0.1
94	Perillene	-4.9	-4.6	-4.6	-4.7	-4.7	0.1
95	Umbellulone	-4.7	-4.7	-4.7	-4.7	-4.7	0.0
96	p-Cymen-8-ol	-4.7	-4.7	-4.7	-4.6	-4.7	0.1
97	Pinocarvone	-4.7	-4.7	-4.7	-4.6	-4.7	0.1
98	Terpinen-4-ol	-4.7	-4.6	-4.7	-4.7	-4.7	0.1
99	Carvenone	-4.7	-4.6	-4.6	-4.7	-4.7	0.1
100	Trans-Pinocamphone	-4.6	-4.6	-4.7	-4.6	-4.6	0.1
101	(+)-trans-Limonene oxide	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
102	Alpha-campholenal	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
103	Borneol	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
104	Camphor	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
105	Isopinocamphone	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
106	p-Cymene	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
107	Pyrogallic acid	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
108	Tricyclene	-4.6	-4.6	-4.6	-4.6	-4.6	0.0
109	Rosefuran	-4.7	-4.5	-4.5	-4.5	-4.6	0.1
110	Alpha-terpinene	-4.6	-4.5	-4.5	-4.5	-4.5	0.0
111	Linalool	-4.5	-4.6	-4.3	-4.7	-4.5	0.2
112	(-)-cis-Limonene oxide	-4.5	-4.5	-4.5	-4.5	-4.5	0.0
113	(+)-cis-Limonene oxide	-4.5	-4.5	-4.5	-4.5	-4.5	0.0
114	(Z)-Chrysanthemol	-4.5	-4.5	-4.5	-4.5	-4.5	0.0
115	Alpha-phellandren-8-ol	-4.6	-4.6	-4.4	-4.4	-4.5	0.1
116	Beta-cyclocitral	-4.5	-4.5	-4.5	-4.5	-4.5	0.0
117	Delta-3-carene	-4.5	-4.5	-4.5	-4.5	-4.5	0.0
118	3-p-menthene	-4.5	-4.5	-4.5	-4.5	-4.5	0.0
119	Undecan-2-one	-4.3	-4.4	-4.6	-4.6	-4.5	0.2
120	Myrcene	-4.6	-4.5	-4.6	-4.1	-4.5	0.2
121	Cis-sabinene hydrate	-4.5	-4.4	-4.4	-4.4	-4.4	0.0
122	(-)-trans-Limonene oxide	-4.4	-4.4	-4.4	-4.4	-4.4	0.0
123	Alpha-thujene	-4.4	-4.4	-4.4	-4.4	-4.4	0.0

124	Beta-terpinene	-4.4	-4.4	-4.4	-4.4	-4.4	0.0
125	Cis-p-menth-8-ene	-4.4	-4.4	-4.4	-4.4	-4.4	0.0
126	Cuminal	-4.4	-4.4	-4.4	-4.4	-4.4	0.0
127	Gamma-terpinene	-4.4	-4.4	-4.4	-4.4	-4.4	0.0
128	Trans-thujone	-4.4	-4.4	-4.4	-4.4	-4.4	0.0
129	D-limonene	-4.4	-4.4	-4.3	-4.4	-4.4	0.1
130	Terpinolene	-4.3	-4.4	-4.3	-4.4	-4.4	0.1
131	Alpha-phellandrene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
132	Alpha-pinene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
133	Beta-fenchene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
135	Beta-pinene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
136	Camphene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
137	Carvotanacetone	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
138	Isoterpinolene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
139	m-Cymene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
140	Myrcenol	-4.1	-4.4	-4.4	-4.3	-4.3	0.1
141	o-Cymene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
142	Thuja-2-4(10)-diene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
143	Toluene	-4.3	-4.3	-4.3	-4.3	-4.3	0.0
144	Alpha-fenchene	-4.2	-4.2	-4.2	-4.2	-4.2	0.0
145	Beta-ocimene	-4.3	-4.3	-3.8	-4.3	-4.2	0.3
146	(Z)-Salvene	-4.2	-4	-4.2	-4.2	-4.2	0.1
147	Lavandulol	-4.1	-4.1	-4.1	-4.1	-4.1	0.0
148	Beta-Citronellene	-4.5	-3.7	-3.7	-4.1	-4.0	0.4
149	Santolina triene	-3.8	-3.8	-3.8	-3.8	-3.8	0.0
150	PEG reference ligand	-3.4	-3.2	-3.2	-3.3	-3.3	0.1

Table 3 Binding energies, mean binding energies and standard deviation of the docked front-runners.

S/N	LIGANDS	E 1	E 2	E 3	E 4	MEAN	STANDARD DEVIATION
1	Gallocatechin	-8.6	-8.6	-8.6	-8.6	-8.6	0.0
2	Robinetin	-8.3	-8.2	-8.2	-8.2	-8.2	0.1
3	Isorhamnetin	-8.1	-8.1	-8.1	-8.1	-8.1	0.0
4	Epicatechin-3-gallate	-8.3	-8.2	-8.3	-7.3	-8.0	0.5
5	Apigenin	-7.8	-7.8	-7.8	-7.8	-7.8	0.0
6	Luteolin	-7.7	-7.7	-7.7	-7.7	-7.7	0.0
7	Epicatechin	-7.6	-7.6	-7.5	-7.5	-7.6	0.1

8	Epigallocatechin-3-gallate	-7.5	-7.5	-7.5	-7.5	-7.5	0.0
9	Kaempherol	-7.4	-7.4	-7.4	-7.4	-7.4	0.0
10	Cianidanol	-7.3	-7.3	-7.3	-7.3	-7.3	0.0
150	PEG reference ligand	-3.4	-3.2	-3.2	-3.3	-3.3	0.1

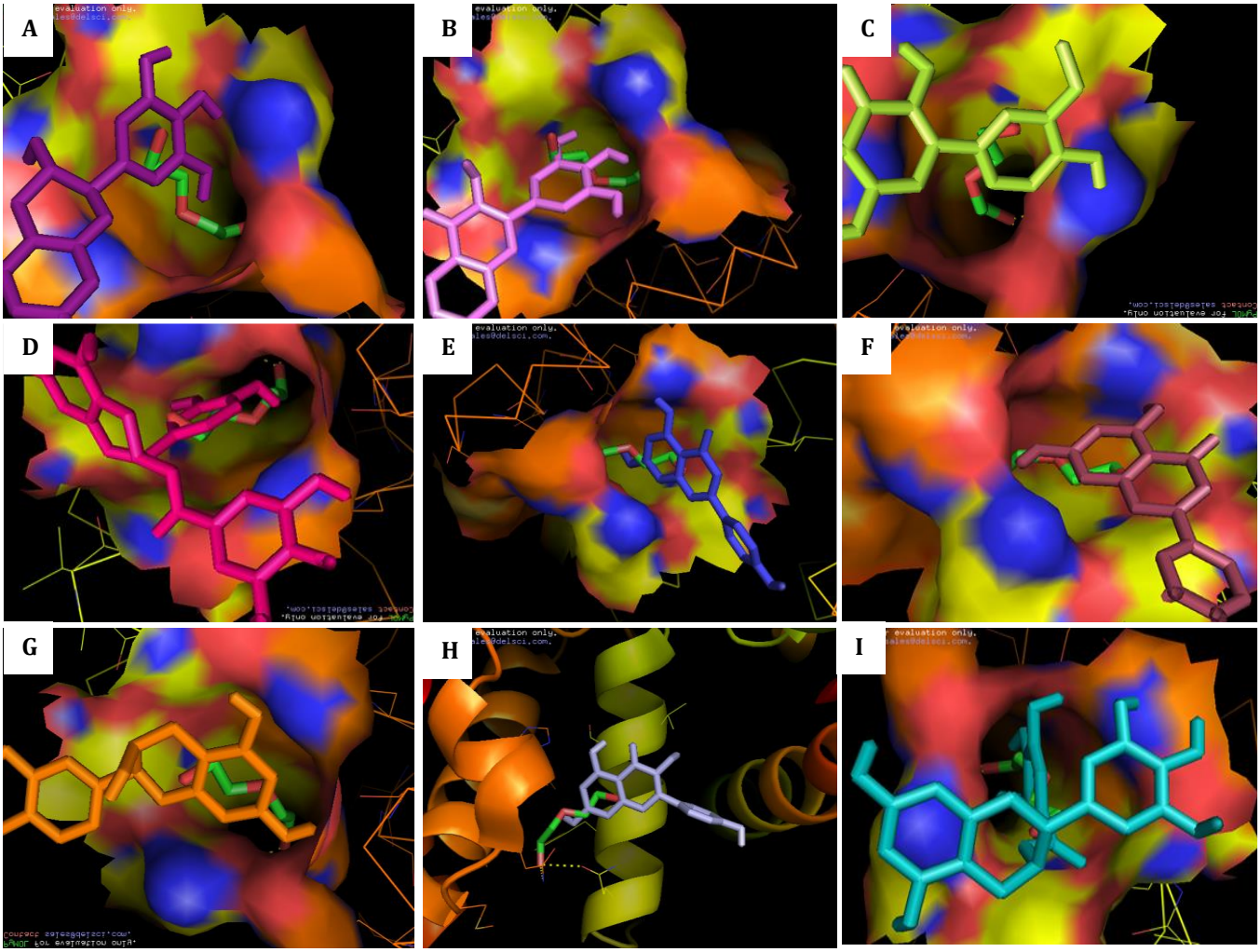

Figure 1 The first nine front runner ligands on the table and the reference ligand PEG within the binding pocket of the selected protein 4IVV

Table 4 Molecular descriptors of the front-runners

S/N	LIGANDS	CHEMICAL FORMULA	MOL. WEIGHT	ClogP	HYDROGEN BOND ACCEPTORS	HYDROGEN BOND DONORS
1	Gallocatechin	$C_{15}H_{14}O_7$	306.3	1.2	7	6
2	Robinetin	$C_{15}H_{10}O_7$	302.2	1.5	7	5
3	Isorhamnetin	$C_{16}H_{12}O_7$	316.3	1.8	7	4
4	Epicatechin_3_gallate	$C_{22}H_{18}O_{10}$	442.4	2.4	10	7
5	Apigenin	$C_{15}H_{10}O_5$	270.2	2.3	5	3
6	Luteolin	$C_{15}H_{10}O_6$	286.2	2.0	6	4
7	Epicatechin	$C_{15}H_{14}O_6$	290.3	1.5	6	5
8	Epigallocatechin_3_ Gallate	$C_{22}H_{18}O_{10}$	458.4	2.1	11	8
9	Kaempherol	$C_{15}H_{10}O_6$	286.2	1.8	6	4
10	Cianidanol	$C_{15}H_{14}O_6$	290.3	1.5	6	5

Table 5 Toxicity profile of the front-runners

S/N	Ligands	Mutageni-City	Tumorigeni-City	Effect On Reproduction	Irrritant Effect
1	Gallocatechin	None	None	None	None
2	Robinetin	High	None	None	None
3	Isorhamnetin	High	None	None	None
4	Epicatechin-3-gallate	None	None	None	None
5	Apigenin	High	None	None	None
6	Luteolin	None	None	None	None
7	Epicatechin	None	None	None	None
8	Epigallocatechin-3-gallate	None	None	None	None
9	Kaempherol	High	None	None	None
10	Cianidanol	None	None	None	None

4. Discussion

The strength of a protein-ligand complex is determined by the intermolecular interactions between these binding parameters, and molecular docking uses scoring functions to provide a quick and basic estimate of the binding affinity. Scoring functions aim to estimate binding affinity, which is directly related to the Gibbs energy of binding [15, 16, 17].

Based on this principle, a more negative Gibbs binding energy indicates a stronger and more spontaneous binding interaction. The more negative the value, the stronger the binding affinity between the molecules and the more positive the value, the weaker the binding affinity. Thus, a drug with a highly negative Gibbs binding energy will bind tightly to its target, which is desirable in drug design.

Table 2: The binding energies, mean binding energies and standard deviation of the 150 phytoconstituents including the reference ligand following molecular docking against the target protein on Ubuntu were calculated. These

phytoconstituents were arranged in order of decreasing affinity to the target protein, with Gallocatechin having the highest affinity (-8.6 Kcal/mol) and PEG reference ligand having the least affinity (-3.3 Kcal/mol), thus, indicating that all the phytochemicals have better binding affinity than the reference ligand.

Table 3: The binding energies, mean binding energies and standard deviations of the first 10 phytoconstituents with better binding affinities (front-runners) to the target protein also arranged in order of decreasing affinity to the target protein with Gallocatechin having the highest affinity (-8.6 Kcal/mol) and Cianidanol having the least affinity (-7.3 Kcal/mol).

Figures A-h: The binding of the front-runners (ligands) in the binding pocket of the target protein-reference ligand complex were visualised on PyMOL and their interaction with the reference ligand in the binding pocket were observed**.**

Table 4: Only the first 10 compounds with better binding affinities (front-runners) were screened on Datawarrior using the Lipinski's Rule of Five (Ro5), which states that:

- No more than five hydrogen bond donors
- No more than ten hydrogen bond acceptors
- Molecular mass less than 500 Da
- Partition coefficient not greater than five.

In drug discovery, the Rule of 5 states that poor absorption and permeation are more likely when:

- The molecular weight is greater than 500 Da
- The cLogP is greater than 5
- There are more than 5 hydrogen-bond donors (sum of O-Hs and N-Hs)
- There are more than 10 hydrogen-bond acceptors (sum of Ns and Os).

There is a correlation between increasing molecular weight and reduced permeability in the gut and into the central nervous system [18]. This means that drugs with higher molecular weight are less likely to be effective when taken orally compared to those with lower molecular weight.

Lipophilicity is a physicochemical property that is frequently expressed as the logarithm of the ratio of drug that partitions into the organic phase to that in the aqueous phase (cLogP). It is generally considered to be extremely important to the rate of absorption, therefore, compounds that displayed a cLogP less than 5 were more likely to be orally bioavailable.

A compound's ability to permeate a membrane bilayer can be hindered by a compound's abundance of hydrogen-bond donor and acceptor groups. Instead of partitioning into the lipophilic environment found in a cellular membrane, the molecule will rather partition into a solvent with a strong hydrogen bond, such as water [19, 20].

Consequently, the front-runners were initially screened to verify that these phytochemicals which would be employed for further analysis would be orally active or bioavailable.

Majority of the front-runners satisfied Lipinski's rule of 5 except Gallocatechin (6 hydrogen bond donors), Epicatechin-3-gallate (7 hydrogen bond donors) and Epigallocatechin-3-gallate (11 hydrogen bond acceptors and 8 hydrogen bond donors)**.** Based on this principle, these three exemptions are not druggable for oral administration while the other seven are druggable.

Table 5: Toxicological assessment of novel drug entities is critical in drug discovery and development. Any adverse effects upon use of such medications on humans, animals, plants, or the environment pose significant risks. Predicting drug toxicity using conventional animal models has long been standard practice in this industry. However, the requirement for such sustainable alternatives emerged as a result of the financial burden and essential demand of ethical permits for in vivo animal experimentation.

In this context, *in silico* toxicology models provide an alternative to computationally analyse, simulate, and predict the toxicity of new drugs/compounds. With the promising ability to reduce (or replace) the use of animals to evaluate toxicological outcomes, computational models are competent enough to offer various advantages, including cost-efficiency, rapid data availability, and advanced capacity to standardise processes [21].

The phytochemicals were screened based on Mutagenicity, Tumorigenicity, Effect on reproduction and Irritant effects, all of which to ensure that they are non-toxic and safe for use.

The results show that most of the front-runners are void of toxicity except Robinetin, Isorhamnetin, Apigenin and Kaempherol which have high mutagenicity. Mutagenicity refers to their potential to induce permanent transmissible changes in the amount or structure of the genetic material of cells or organisms [22]. Therefore, based on toxicity screening, these four are not druggable unlike the other six phytochemicals which are druggable.

5. Conclusion

In conclusion, the *in silico* study conducted on the phytochemical constituents of *Boswellia dalzielii* against *Rabies lyssavirus* has provided significant insights into the potential of natural compounds as anti-rabies agents. This study clearly illustrates that Luteolin (-7.7 Kcal/mol), Epicatechin (-7.6 Kcal/mol) and Cianidanol (-7.3 Kcal/mol) passed the Lipinski rule and toxicity studies and are also part of the front runners and can be predicted to have better anti-rabies activity. Application of Lipinski's Rule of Five and Toxicity assessment ensured favorable drug-like properties and safety profiles of druggable candidates. These findings suggest several candidates are viable anti-rabies agents, with others requiring possible optimization.Thus, the study underscores the value of computational methods in identifying and refining natural compounds for anti-rabies drug development whilst saving cost, time and resources.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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