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Gas Chromatography – Mass Spectrometry Analysis and in Silico Antimalarial Activity Studies of Compounds from Leaves Extracts of *Mitragyna inermis* (Willd.) Kuntze

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAO conceptualized the idea, designed and reviewed the manuscript. Author ATA developed the methodology, analysis and writing. Author HTA assisted to develop some aspects of the methodology, software and writing. Author NNSNMK participated in writing and editing of the draft. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Malaria remains the deadliest infectious diseases in many tropical and subtropical regions, including Nigeria and other West African countries where its transmission occurs all year round. In many inhabitants, medicinal plants are traditionally used as remedies against the symptoms of acute malaria because of their efficacious properties demonstrated by their phytoconstituents. *Mitragyna inermis* is one of the medicinal plants used by traditional healers in Nigeria for the treatment of various human diseases including malaria.
Methods: We identified the phytochemical constituents of the methanol leaves extract of *M. Inermis* using gas chromatography-mass spectrometry (GC-MS) technique. Furthermore, the *in silico* antimalarial study was conducted by investigating the binding interactions of the identified compounds with plasmepsin II, a key enzyme implicated in malaria pathogenesis using EH58 reference ligand by employing molecular docking techniques.
Results: A total number of 40 compounds were identified from the extract of *M.inermis*, and cis-13,16-docasadienoic acid (12. 33 %) was identified as the major phytochemical. Other phytochemicals like Pyrrolo[1,2-a] pyazine-1,4-dione, hexahydro-3-(methylpropyl), 3-benzyl-6-methyl-2,5-piperazinedione, 2,5 dibenzyloxynitrobenzene, carbonic acid, 2-dimethylaminoethyl neopentyl ester were found but in trace amounts. The results of molecular docking studies predicted interactions of compounds from *M. inermis* with plasmepsin II enzyme. Five top-scoring bioactive compounds were selected based on their binding energies (docking scores) upon docking with target protein, with compound 2, (2,5-dibenzyloxynitrobenzene) exhibiting the best binding affinity. ADME properties indicated favorable drug-like characteristics for these compounds, while toxicity predictions showed hepatotoxicity and immunotoxicity. Pharmacokinetic assessments revealed high gastrointestinal absorption, blood-brain barrier permeability for compound 2, and inhibition potential against CYP enzymes for certain compounds, offering insights into their therapeutic potential against malaria.
Conclusion: The molecular docking analysis revealed the potential of phytochemicals from *M. inermis* to interact effectively with plasmepsin II enzyme, showing promising antimalarial potentials. The identified compounds exhibited favorable drug-like properties and minimal toxicity concerns, highlighting their potential as candidates for further exploration in the development of antimalarial agents.

Keywords: Malaria; phytochemicals; Mitragyna inermis; plasmepsin II; molecular docking.

1. INTRODUCTION

"Malaria, an infectious disease caused by *Plasmodium* f*alciparum*, a protozoan parasite remains the deadliest infectious diseases in many tropical and subtropical regions, including Nigeria and other West African countries where its transmission occurs all year round. In 2022, the global incidence of malaria was estimated at 249 million cases, resulting in 608,000 deaths. In Africa, about 94% of these cases (233 million) were reported, with Nigeria exhibiting a notably high incidence compared to other African nations. Specifically, the Democratic Republic of Congo accounted for 12%, Uganda for 5%, and Mozambique for 4 %. Remarkably, over 50 % of all malaria-related deaths occurred in just four countries, with Nigeria leading at 31 %, followed by the Democratic Republic of the Congo at 12 %, Niger at 6 %, and Tanzania at 4 %. This emphasizes the concentrated impact of malaria in these regions" [1]. The use of synthetic drugs currently dominates malaria treatments and management, but the widespread emergence of resistant malaria parasites to hitherto effective drugs such as chloroquine, pyrimethamine, and proguanil constitutes global concerns [2]. "Thus, there is an intensified need for exploring medicinal plants which may serve as a springboard for new phytotherapies that could affordably treat malaria, especially among less privileged native people living in rural areas. Among the medicinal plants with historical antimalarial property is *M. inermis"* [3]. It is called

Ewe Okobo in Yoruba and Giyayya in Hausa. *M. inermis* (Willd.) Kuntze belongs to the family Rubiaceae [4] is a shrub grown in West African region on a low alluvial plain and swampy savanna [5]. Various parts of this plant are used to treat many ailments. The bark is used to treat fever, high blood pressure, dysentery, syphilis, wounds and epilepsy [6]. The root, bark and leaves have been reported to treat anorexia, constipation and leprosy [6]. Moreover, the leaves are widely used as an antimalarial [3-4] and anthelmintic [7]. It also serves as a stimulant and a diaphoretic agent [8]. *Mitragya inermis* has been reported as a pain killer and for treating arthritis, epilepsy, nasopharyngeal afflictions, stomach troubles, and venereal diseases [9]. In the present study, the leaves extract of *Mitragya inermis* was investigated and its phytochemical constituents were identified by means of GC-MS technique. In this study, we investigated the leaf extract of *Mitragya inermis* and identified its phytochemical constituents using the GC-MS technique. Additionally, an *in silico* antimalarial study was conducted to explore the binding interactions of the identified compounds with plasmepsin II, a key enzyme implicated in malaria pathogenesis using EH58 reference ligand as a benchmark by employing molecular docking techniques.

2. MATERIALS AND METHODS

Sample: "The leaves of *M. inermis* were collected from Alapo Vilage, Ilorin, Kwara State, Nigeria and taxonomically identified and authenticated by Mr Odewo A. Samuel (Department of Forest Conversation and Protection, Forestry Research Institute of Nigeria (FRIN)), Ibadan Oyo State, Nigeria, where its voucher specimen (FHI112952) was deposited" [3].

Preparation of extract: "The leaves of *M. inermis* were washed under running tap water, cut into smaller pieces and dehydrated by air at room temperature for seven days to ensure a crispy texture. The dehydrated leaves were pulverized, weighed, and stored in a polyethylene bag for further analysis" [10]. 150 g of the pulverized leaves material was macerated in 95% methanol at ambient temperature for 72 hours. The extract was filtered and evaporated under reduced pressure at 40 ^{o}C to afford 12.5 g and percentage yield 8.3%.

Gas Chromatography- Mass Spectrometric Analysis: The crude extract was analyzed using SHIMADZU GC-MS QP2010 Ultra coupled with MS-5973-634071 Series, at column oven temperature of 60.0 °C (increasing to 270 ºC in 7 min at flow rate of 10 ml/min). Injection temperature of 250.0 °C with split flow injection and linear velocity flow control modes. The velocity pressure was maintained at 100.0 kPa with total flow rate of 102.6 ml/min, column flow rate of 2.16 ml/min and linear velocity of 37.9 cm/sec. A purge flow rate of 3.0 ml/min and a split ratio of 45.1 were used. The ion source temperature was 230.0 °C, interface temperature of 250.0 °C, solvent cut time of 4.50 min. The MS start time was 6.0 min; end time was 26.0 min, scan event time of 0.30 sec, scan speed of 1666. The start m/z of 35.00 and end m/z of 450.00.

Molecular docking: Twenty nine (29) compounds from GC-MS analysis of methanol extract of the leaves of *M.inermis* were selected for molecular docking studies. The target protein Plasmepsin II (PDB ID: 1LF3), N-(3-[(2-benzo [1,3 dioxol-5-yl-ethyl) [3-(1-methyl-3-oxo-1,3-dihydro-isoindol-2-yl)-propionyl]-amino]-1-benzyl-2-(hydroxypropyl)-4-benzyloxy-3,5 dimethoxy-benzamide (EH58) was docked with the selected compounds using BIOVIA, Discovery Studio (version 2021) and PyRx version 8.0 software. The binding energies were calculated accordingly. The ligands and the target protein were prepared by following the approved standard procedures for protein and ligand preparation, and the files were submitted to PyRx. The acquired binding energy,binding contacts of each ligand, and the docked data were analyzed using Discovery Studio Visualizer.

Ligand molecule preparation: The structures of selected compounds 1–29 and Plasmepsin II (PDB ID: 1LF3) were retrieved through the PubChem compound database at NCBI (http://pubchem. ncbi. nlm. nih.gov/). The 3D crystal structure of the protein (Plasmepsin II (PDB ID: 1LF3)) was retrieved from the Protein Data Bank (PDB).

Preparation of Target protein: The Discovery Studio software was used to process and prepare the protein and convert raw PDB structure into prepared protein models. The crystal structure of the protein was prepared by removing the water molecules present in the structure. Then, Discovery Studio software was used to analyze protein structure, hydrogen bond interactions and non-bond interactions of ligands with the active site residues and generations of high-quality images.

Docking: The prepared ligand conformers were docked against the prepared target protein Plasmepsin II (PDB ID: 1LF3) structure to evaluate their binding and interactive potentials at the active pockets of the protein in comparison with a co-crystallized standard, EH58 using PyRx software to perform the docking. The various conformations for ligand in the docking procedure were generated and the final energy refinement of the ligand pose was performed. The docking score of the best pose into the target proteins for all the tested bioactive compounds was calculated.

ADMET drug-likeness and toxicity analysis: The drug likeness of the compounds was predicted using the Swiss ADMET server which is based on Lipinski's rule ((http://www.swissadme.ch/) [11]. In addition, "the potential of the compounds to exhibit hepatotoxicity, carcinogenicity, immunotoxicity, cytotoxicity, and mutagenicity was determined using webserver (admetSAR webserver (http://lmmd.ecust.edu.cn/admetsar2/)" [12].

3. RESULTS

Result of GCMS Analysis: The GC-MS analysis demonstrated the existence of various categories of phytochemicals in the methanol leaves extract of *M.inermis*. The total ion chromatogram (TIC) of the extract presented in Table 1 showed the retention time and signals corresponding to the phytochemicals present in the extract. Total forty (40) phytochemicals were suggested, and their molecular formula, molecular weight and percentage area are presented in Table 1.

Result of molecular docking studies: The predicted potential interaction of selected compounds with plasmepsin II enzyme by molecular calculations showing the binding energy and amino acid residues of plasmepsin II enzyme interacted with each compound and the hydrogen bonds are given in Table 2. The binding energy with a higher negative value corresponds to a more stable interaction between the compounds, ligand and target enzyme. Twenty-nine (29) phytochemicals were selected and docked with target protein (plasmepsin II) to predict their binding affinities and five top-scoring compounds were selected based on their binding energies (docking scores) upon docking with Plasmepsin II (PDB ID: 1LF3) (Tables 3 and 4). To predict the binding modes of active compounds with plasmepsin II and identify the interacting amino acid residues, the 2D interactions of the top five active compounds (2–6) alongside with the 3D interaction of plasmepsin II were created, as shown in Figs. 1a-e.

Results of ADME property: The results of the ADME properties which revealed the predicted lipophilicity, water solubility, drug-likeness, and bioavailability scores of five selected compounds from *M.inermis* are presented in Tables 5-7.

4. DISCUSSION

A total number of 40 phytochemicals was identified from the leaves of *M.inermis* Cis-13,16-docasadienoic acid (12. 33 %) was indicated as the major phytochemical followed by Methyl 5-oxopryrrolidine-2-carboxylate (7.71 %), Benzyl hydrazinecarboxylate (7.67 %), Nicotinamide, (7.45 %), Palmitic acid (6.05 %), 3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (5.65 %), 2,4-imidazolidinedione, 5-(2-methylpropyl)-,(S)- (4.42 %), 1-acetylpiperidin-4-one (3.60 %), 1-phenethyl-pyrrolidin-2,4-dione, (3.50 %). Other phytochemicals such as Pyrrolo[1,2-a] pyazine-1,4-dione, hexahydro-3-(methylpropyl), 3-benzyl-6-methyl-2,5-piperazinedione, 2,5-dibenzyloxynitrobenzene, Carbonic acid, 2-dimethylaminoethyl neopentyl ester etc. were also found but in trace amounts.

Among the 29 compounds, 2,5-dibenzyloxynitrobenzene (2), exhibited the best binding affinity to plasmepsin II in terms of a low binding energy of – 8.2 kcal/mol; however, its binding energy was lower than that of reference ligand, EH58, the potent antimalarial drug with binding energy of − 9.5 kcal/mol interacted with GLY 216, SER 218, SER 218, VAL 78 residues of the Plasmepsin II (PDB ID: 1LF3) active site (Fig. 1a) and formed hydrophobic interactions with ILE 290, ASN 288, ILE14, ALA219, THR221, THR 217, MET15, ASP34, ASP214, ILE 300, SER 37, GLY 36, TYR 192, ILE 123, SER 79, PHE 111, ILE 32, PHE 120, THR 114, TYR192, TYR 77, PHE 294, LEU 292 (Table 4). Compound 2 (2,5-dibenzyloxynitrobenzene) was predicted to strongly interacted with four hydrogen bonds with SER 79, GLY 216, and π-sigma with TYR 77 and π-anion with ASP 214 (Fig. 1b). Additionally, it was stabilized through hydrophobic interactions with residues ILE300, ILE212, TYR192, GLY36, ASP214, PHE 294, VAL 78, ASP34, ILE123, PHE 111, MET 115, ILE 32, PHE 120, THR 114, TYR 77 (Table 4).

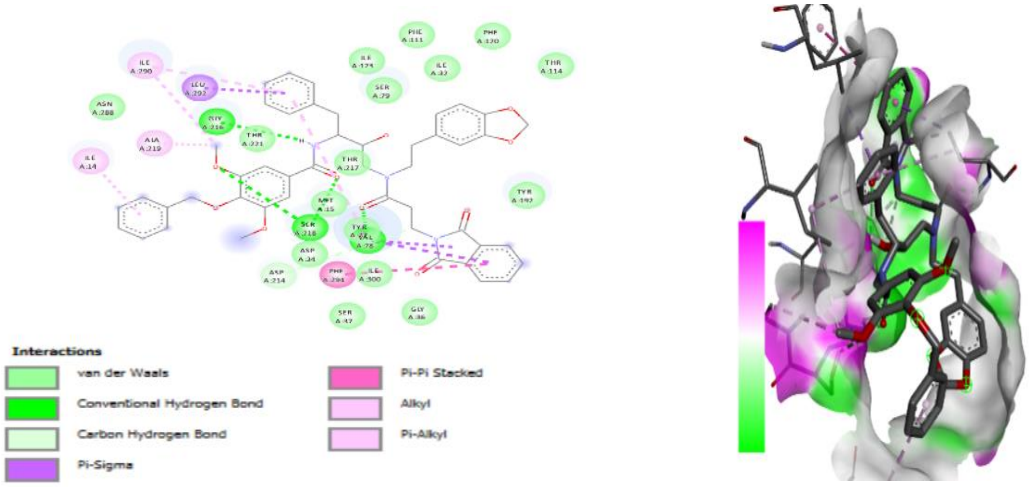


Fig. 1a. Molecular interaction of amino acid residue of plasmepsin with EH58, 2D left 3D right.

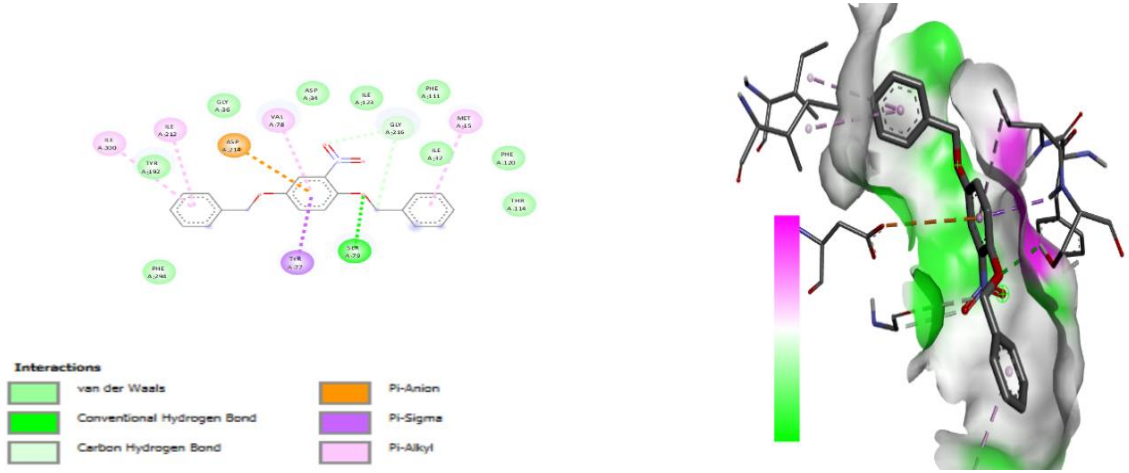


Fig. 1b. Molecular interaction of amino acid residue of plasmepsin with 2,5 dibenzyloxynitrobenzene, 2D left 3D right.

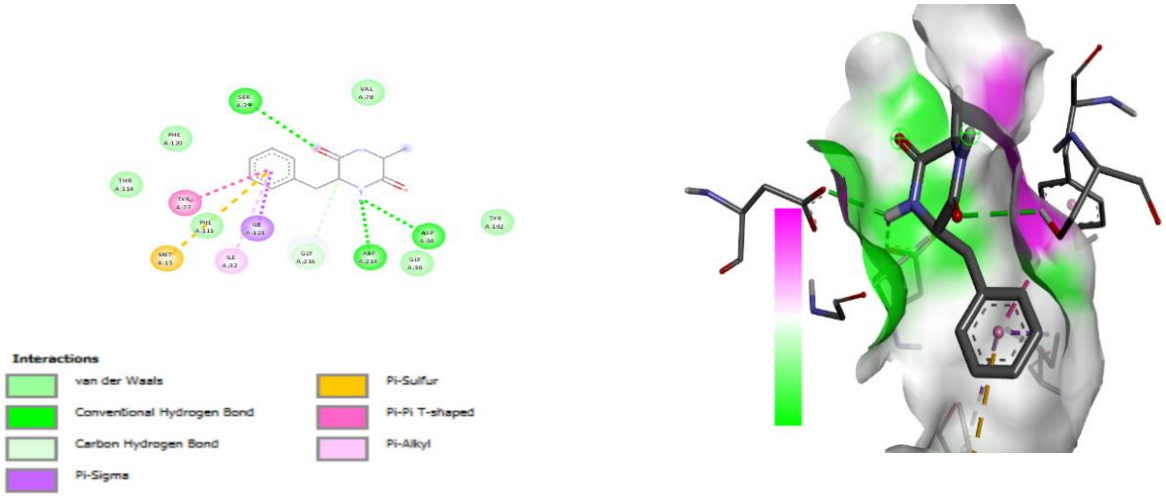


Fig. 1c. Molecular interaction of amino acid residue of plasmepsin with 3-benzyl-6-methylpiperazine-2,5-dione, 2D left 3D right.

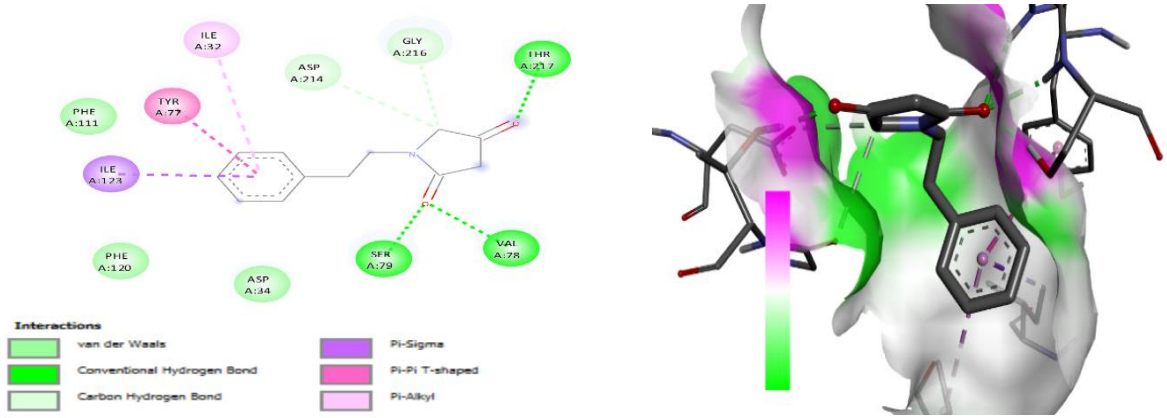


Fig. 1d. Molecular interaction of amino acid residue of plasmepsin with 1-phenethyl-pyrrolidin-2,4-dione, 2D left 3D right

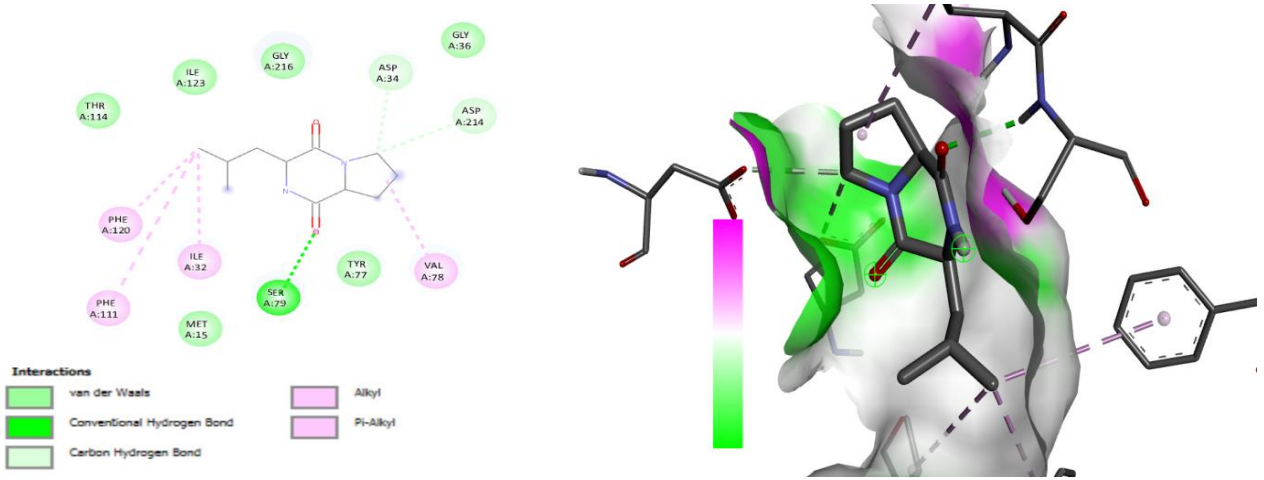


Fig. 1e. Molecular interaction of amino acid residue of plasmepsin with pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, 2D left 3D right.

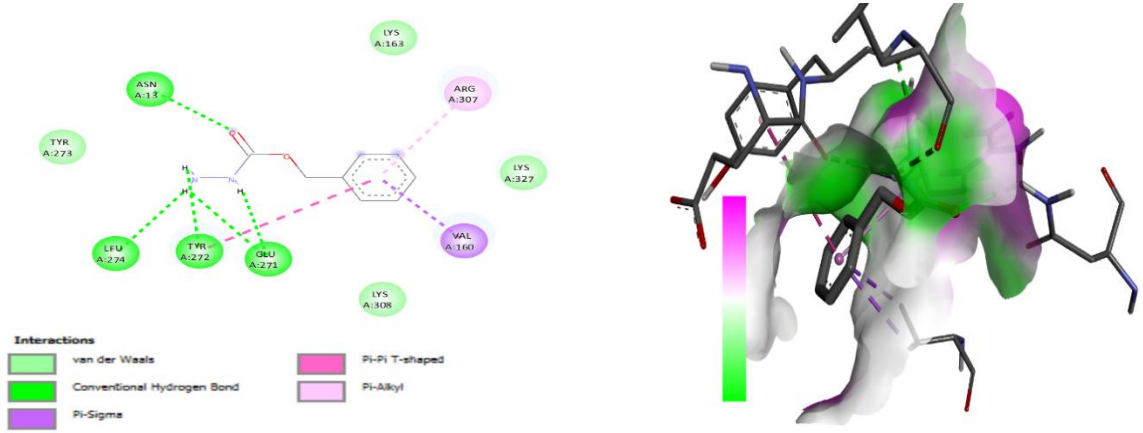


Fig. 1f. Molecular interaction of amino acid residue of plasmepsin with Benzylhydrazine carboxylat, 2D left 3D right

Table 1. GC-MS profile of phytochemicals in *M.inermis* leaves extract

S/N	COMPOUND	Molecular Formula	Molecular weight	% AREA
1.	Furan-2(5H)-one	$C_4H_4O_2$	85	0.3
2	3-hydroxy-4,4-dimethyldihydrofuran-	$C_6H_{10}O_3$	131	0.58
3.	Pyrrolidin-2-one	C_4H_7NO	86	1.71
4	*(E)*-3-methyldec-3-ene	$C_{11}H_{22}$	154	0.45
5	2-(piperazin-1-yl)ethanamine	$C_6H_{15}N_3$	130	0.78
6.	4-vinyl-1*H*-imidazole	$C_5H_6N_2$	95	1.65
7.	2,3-dihydrobenzofuran	C_8H_8O	121	1.37
8.	Dianhydromannitol	$C_6H_{10}O_4$	147	1.43
9.	Methyl palmitate	$C_{17}H_{34}O_2$	270	1.00
10.	1-acetylpiperidin-4-one	$C_7H_{11}NO_2$	142	3.60
11.	Methyl 5-oxopryrrolidine-2-carboxylate	$C_6H_9NO_3$	143	7.71
12.	Palmitic acid	$C_{16}H_{32}O_2$	257	6.05
13.	Nicotinamide	$C_6H_6N_2O$	123	7.45
14.	3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	211	5.65
15.	Benzyl hydrazinecarboxylate	$C_8H_{10}N_2O_2$	167	7.67
16.	2,4-imidazolidinedione, 5-(2-methylpropyl)-,(S)-	$C_7H_{12}N_2O_2$	157	4.42
17.	1H-Imidazole,2-ethyl-4,5-dihydro-4-methyl-	$C_6H_{12}N_2$	113	0.65
18.	Propanamide, 3-(1-piperazinyl)-	$C_7H_{15}N_3O$	157	0.95
19.	9,12-octadecadienoic acid (Z,Z)- methyl ester	$C_{19}H_{34}O_2$	294	2.94
20	9-octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	297	1.22
21.	3-pyrrolidin-2-yl-propionic acid	$C_7H_{13}NO_2$	143	1.84
22	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466	2.93
23.	1-phenethyl-pyrrolidin-2,4-dione	$C_{712}H_{13}NO_2$	203	3.50
24.	Cis-13,16-docasadienoic acid	$C_{22}H_{40}O_2$	336	12.33
25.	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	1.30
26.	2,2,4,4-tetramethyl-6-oxabicyclo[3.1.0]hexan-3-one	$C_9H_{14}O_2$	154	3.32
27	Propanoic acid,2,2-dimethyl-,2-phenylethyl ester	$C_{13}H_{18}O_2$	206	0.81
28.	Pyrrolo[1,2-a]pyazine-1,4-dione,hexahydro-3-(methylpropyl)	$C_{11}H_{18}N_2O_2$	210	0.67
29.	Hexadecanamide	$C_{16}H_{33}NO$	255	0.95
30.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.59
31	3-benzyl-6-methyl-2,5-piperazinedione	$C_{12}H_{14}N_2O_2$	218	0.65
32.	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.62

S/N	COMPOUND	Molecular Formula	Molecular weight	% AREA
33.	L-proline, N-valeryl-, heptadecyl ester	$C_{27}H_{51}NO_3$	437	1.55
34.	2,5-dibenzyloxynitrobenzene	$C_{20}H_{17}NO_4$	335	0.62
35.	Carbonic acid, 2-dimethylaminoethyl neopentyl ester	$C_{10}H_{21}NO_3$	203	0.24
36.	N,N'-Dibutylidene-hydrazine	$C_8H_{16}N_2$	140	0.74
37	8,11-octadecadienoic acid, methyl ester	$C_{19}H_{34}O$	294	1.55
38.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	0.18
39.	Oxacycloheptadec-8-en-2-one, (8Z)	$C_{16}H_{28}O_2$	252	3.98
40.	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.33

Table 2. The molecular docking score (Kcal/mol) of ligands against targeted proteins

S/N	Compound	PubMed CID	Docking score with plasmepsin II (PDB ID: 1LF3)
1	EH58	446918	-9.5
2	Furan-2 (5H)-one	10341	-3.4
3	3-hydroxy-4,4-dimethyldihydrofuran-2(3H)-one	989	-4.9
4	Pyrrolidin-2-one	12025	-3.6
5	2-(piperazin-1-yl)ethanamine	8795	-4.0
6	4-Vinyl-1H-imidazole	271079	-3.9
7	2,3-dihydrobenzofuran	10329	-5.0
8	Dianhydromannitol	23619611	-4.1
9	1-acetylpiperidin-4-one	122563	-4.5
10	methyl5-oxopyrrolidine-2-carboxylate	500249	-4.6
11	Nicotinamide	936	-4.9
12	3-isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	102892	-5.5
13	Benzylhydrazine carboxylate	79242	-6.5
14	2,4-Imidazolidinedione, 5-(2-methylpropyl)-, (S)-	100892	-5.5
15	1H-Imidazole, 2-ethyl-4,5-dihydro-4-methyl-	13604	-4.1
16	propanamide,3-(1-piperazinyl)	544697	-4.7
17	3-Pyrrolidin-2-yl-propionic acid	550965	-5.1
18	1-phenethyl-pyrrolidin-2,4-dione	568711	-6.4
19	cis-13,16-docasadienoic acid	5312554	-5.5
20	linoleic acid ethyl ester	5282184	-5.3
21	2,2,4,4-tetramethyl-6-oxabicyclo [3.1.0]hexan-3-one	550924	-5.2
22	Hexadecamide	69421	-5.2

S/N	Compound	PubMed CID	Docking score with plasmepsin II (PDB ID: 1LF3)
23	Propanoic acid, 2,2-dimethyl-2-phenylethylester	105516	-5.7
24	3-benzyl-6-methylpiperazine-2,5-dione	139767	-7.0
25	2,5-dibenzyloxynitrobenzene	350342	-8.2
26	(8Z)-1-oxacycloheptadec-8-en-2-one	5365703	-7.4
27	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	7074739	-6.0
28	N,N'-Dibutylidene-hydrazine	9578454	-5.0
29	Carbonic acid, 2-dimethylaminoethylneopentyl ester	58096382	-5.0
30	heptadecyl 1-pentanoylpyrrolidine-2-carboxylate	91695474	-5.7

EH58 (Standard inhibitor of 1LF3)

Table 3. Names and docking scores of the top-five compounds of *Mitragyna inermis* against targeted proteins

S/N	Compound	PubMed CID	Docking score with plasmepsin II (PDB ID: 1LF3)
1	EH58	446918	-9.5
2	2,5-dibenzyloxynitrobenzene	350342	-8.2
3	3-benzyl-6-methylpiperazine-2,5-dione	139767	-7.0
4	Benzylhydrazine carboxylate	79242	-6.5
5	1-phenethyl-pyrrolidin-2,4-dione	568711	-6.4
6	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	7074739	-6.0

EH58: N-(3-[(2-benzo[1,3] dioxol-5-yl-ethyl)[3-(1-methyl-3-oxo-1,3-dihydro-isoindol-2-yl)-propionyl]-amino]-1-benzyl-2-(hydroxypropyl)-4-benzyloxy-3,5-dimethoxy-benzamide.

Table 4. Interaction of the top five (5) compounds against the target Plasmepsin II (PDB ID: 1LF3)

Pubchem CID	Compound	Hydrogen bond	Hydrophobic and other interactions
446918	EH58	GLY 216, SER 218, SER 218, VAL 78	ILE 290, ASN 288, ILE14, ALA219, THR221, THR 217, MET15, ASP34, ASP214, ILE 300, SER 37, GLY 36, TYR 192, ILE 123, SER 79, PHE 111, ILE 32, PHE 120, THR 114, TYR192, TYR 77, PHE 294, LEU 292
350342	2,5-dibenzyloxynitrobenzene	SER 79, GLY 216, GLY 216	ILE300, ILE212, TYR192, GLY36, ASP214, PHE 294, VAL 78, ASP34, ILE123, PHE 111, MET 115, ILE 32, PHE 120, THR 114, TYR 77
139767	3-benzyl-6-methylpiperazine-2,5-dione	SER 79, ASP 34, ASP 214, GLY 216	ILE 123, THR 114, PHE 120, TYR 77, PHE 111, MET 15, ILE 32, GLY 36, TYR 192, VAL 78
79242	Benzylhydrazine carboxylate	LEU 274, TYR 272, GLU 271, ASN 13	VAL 160, TYR 273, LYS 308, LYS 327, ARG 307
568711	1-phenethyl-pyrrolidin-2,4-dione	SER 79, VAL 78, THR 217, ASP 214, GLY 216	ILE 123, PHE 111, TYR 77, ILE 32, ASP 34, PHE 120
7074739	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	SER 79, ASP 34, ASP 214	THR 114, ILE 123. GLY 216, GLY 36, PHE 120, PHE 111, ILE 32, MET 15, TYR 77, VAL 78

Table 5. Predicted lipophilicity, water solubility, druglikeness and bioavailability scores of test compounds

Cpd	Mol.Weight (g/mol)	Consensus LogP	Silicos-IT LogSw	ESOL Class	Lipinski Violations	Veber Violations	Muegge violations	Bioavail-ability
C1	799.86	5.03	7.07	Poorly soluble	2	2	4	0.17
C2	335.35	3.59	2.70	Moderately soluble	0	0	0	0.55
C3	218.25	0.63	1.53	Very soluble	0	0	0	0.55
C4	203.24	1.48	2.34	Very soluble	0	0	0	0.55
C5	210.27	0.96	1.01	Very soluble	0	0	0	0.55
C6	166.18	0.86	0.06	Very soluble	0	0	1	0.55

C1: EH58, C2. 2,5-dibenzyloxynitrobenzene, C3. 3-benzyl-6-methylpiperazine-2,5-dione, C4. 1-phenethyl-pyrrolidin-2,4-dione, C5. Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, C6. Benzylhydrazine carboxylate.

Table 6. Toxicity profile of compounds from *Mitragyna inermis*

Target	C1	C2	C3	C4	C5	C6
Hepatotoxicity	+	+	+	+	+	+
Carcinogenicity	-	-	-	-	-	-
Immunotoxicity	+	+	+	+	+	+
Mutagenicity	-	-	-	-	-	-
Cytotoxicity	-	-	-	-	-	-

(+) = active, (-) = inactive. C1: EH58, C2. 2,5-dibenzyloxynitrobenzene, C3. 3-benzyl-6-methylpiperazine-2,5-dione, C4. 1-phenethyl-pyrrolidin-2,4-dione, C5. Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, C7. Benzylhydrazine carboxylate

Table 7. Pharmacokinetics prediction output of some compounds from *Mitragyna inermis*

Cpd	GI absorption	BBB permanent	PGP substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 Inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Logkp (cm/s)
C1	Low	No	Yes	No	Yes	Yes	No	Yes	-7.00
C2	High	Yes	No	Yes	Yes	Yes	Yes	No	-5.02
C3	High	No	No	No	No	No	No	No	-7.63
C4	High	No	No	No	No	No	No	No	-6.52
C5	High	No	No	No	No	No	No	No	-6.79
C6	High	No	No	No	No	No	No	No	-6.65

C1: EH58, C2. 2,5-dibenzyloxynitrobenzene, C3. 3-benzyl-6-methylpiperazine-2,5-dione, C4. 1-phenethyl-pyrrolidin-2,4-dione, C5. Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, C6. Benzylhydrazine carboxylate

Compound 3 (3-benzyl-6-methylpiperazine-2,5-dione) possessed a low binding energy of -7.0 kcal/mol and interacted with four hydrogen bonds with SER 79, ASP 34, ASP 214, GLY 216 as well as hydrophobic interactions with ILE 123, THR 114, PHE 120, TYR 77, PHE 111, MET 15, ILE 32, GLY 36, TYR 192, VAL 78. Compound 4 (Benzylhydrazine carboxylate) strongly interacted with residues in the active site region of plasmepsin II target with a binding energy of -6.5 kcal/mol. It formed four hydrogen bonds with LEU 274, TYR 272, GLU 271, ASN 13. Compound 5 (1-phenethyl-pyrrolidin-2,4-dione) interacted with different amino acid residues with a binding energy of -6.4 kcal/mol similar to compound 4. It formed five hydrogen bonds with SER 79, VAL 78, THR 217, ASP 214, GLY 216. Compound 5 formed additional hydrophobic interactions with ILE 123, PHE 111, TYR 77, ILE 32, ASP 34, PHE 120. Compound 6 (Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-) interacted with residues SER 79, ASP 34, ASP 214 through three hydrogen bonds. It possessed the lowest binding energy of -6.0 kcal/mol. It formed additional hydrophobic interactions with THR 114, ILE 123. GLY 216, GLY 36, PHE 120, PHE 111, ILE 32, MET 15, TYR 77, VAL 78. The main energy contributors to the interactions between the compounds and 1LF3 were π-stacking, π-sigma, π-anion, hydrogen bonding, van der Waals and hydrophobic bonds.

After the molecular docking studies of 29 phytochemicals with plasmepsin II protein target, the absorption, distribution, metabolism, excretion and toxicity (ADMET) of the five (5) best dock scored phytochemicals were screened using the online tool “admetSAR webserver (http://lmmd.ecust.edu.cn/admetsar2/)” [12] to predict their pharmacokinetic properties.

ADMET properties include absorption: water solubility, human intestinal absorption, P-glycoprotein substrate, skin permeability; distribution: blood-brain barrier (BBB) permeability; metabolism: cytochrome (CYP) inhibitors, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 substrate; excretion: drug total clearance; toxicity, hepatotoxicity, immunotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. The molecular weights of the compounds were between 335.35-166.18. Log S values of the compound ranged from 7.07 of reference compound, (poorly soluble), 2.70, moderately soluble for 2,5-dibenzyloxynitrobenzene and 1.53, 2.34, 1.01 and 0.06 values for C3, C4, C5 and C6 respectively which indicate very soluble. The Log P ranged from 0.63 to 3.59. The study revealed that no compound violated Lipinski rule except the reference ligand. All identified compounds from *M.inermis* were predicted to pass the Veber’s and Muegge’s rule. For the bioavailability predictions, all compounds scored 0.55 except the referenced standard ligand which scored 0.17. The Toxicity profile (Table 7) depicts that all compounds include reference ligand (EH58) were actively hepatotoxicity and immunotoxicity based on the prediction but none of the compounds were predicted to be carcinogenic, mutagenic, and cytotoxic. Table 7 showed the pharmacokinetic prediction output of the selected compounds. All the five compounds showed high GI absorption. Compound 2 (2,5-dibenzyloxynitrobenzene) displayed the ability to cross the blood-brain-barrier only. None of the compounds from the plant were glycoprotein substrate permeability except reference ligand. EH58 and 2,5- dibenzyloxynitrobenzene were predicted to be inhibitor of CYP2C19, CYP2C9, CYP2D6. EH58 was predicted to be an inhibitor of CYP3A4 as shown in the Table 7; all compounds are skin permeants with logkp ranging from -7.63 to -5.02 cm/s. ADMET screening revealed favorable lipophilicity, water solubility, and bioavailability scores for the selected compounds. None of the compounds violated Lipinski's rule, and all adhered to Veber's and Muegge's rules for bioavailability. Toxicity predictions indicated potential hepatotoxicity and immunotoxicity, while none of the compounds were found to be carcinogenic, mutagenic, or cytotoxic. Pharmacokinetic predictions demonstrated high gastrointestinal absorption for all compounds, with Compound 2 exhibiting blood-brain barrier permeability. EH58 and Compound 2 were predicted to inhibit various cytochrome enzymes, while all compounds displayed skin permeability.

5. CONCLUSION

Out of the 29 phytochemicals that were selected for screening, 2,5-dibenzyloxynitrobenzene displayed the highest binding affinity against plasmepsin II. Following this were 3-benzyl-6-methylpiperazine-2,5-dione, benzylhydrazine carboxylate, 1-phenethyl-pyrrolidin-2,4-dione, and pyrrolol [1,2-a] pyrazine-1,4-dione hexahydro-3-(2-methylpropyl)-. These five compounds along with EH58 (the standard

ligand) exhibited hydrogen bond interactions with active site amino acid residues such as GLY216 and VAL78 at different positions. The primary contributors to the energy in these interactions between plasmepsin II and the ligands were hydrogen bonds as well as hydrophobic and hydrophilic bonds. Unlike the standard compound EH58 which did not exhibit adequate druglike properties or good ADMET profiles; these five compounds demonstrated satisfactory druglike properties and favorable ADMET profiles. Therefore, further studies are necessary to develop them into effective antimalarial drugs. Structural models of their interactions at plasmepsin II active sites will be useful for designing future antimalarial agents.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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