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Molecular Docking of the Inhibitory Activities of Selected Phytochemicals in Artemisia Afra Against NADH-Ubiquinone Oxidoreductase of Plasmodium Falciparum (PfNDH2)

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Abstract: Nicotinamide Adenine Dinucleotide Hydrogen (NADH)-ubiquinone oxidoreductase in Plasmodium falciparum (PfNDH2) constitute a feasible target for anti-malarial drug discovery. This work aims at investigating the inhibitory activities of selected phytochemicals in Artemisia Afra against NADH-Ubiquinone Oxidoreductase of Plasmodium Falciparum. 50 phytochemicals were selected based on structural stability. Quantum mechanical Density Functional Theory (DFT) studies with B3LYP at 6-311G* level was done on pfNDH2 as the apoprotein control. Pharmacokinetic ADMET profiling, bioactivity assessment, physicochemical studies, molecular docking was used to study the PfNDH2 inhibiting activities of the 50 compounds from Artemisia afra. Out of these 50 phytochemicals, 2,4,6-Triphenyl-1,3 dioxane (2,4,6 TPD), chamazulene, aromadendrene, 1-epi-bBicyclosesquiphellandrene (1-EBSP) and cis-muurola-3,5-diene (CM3,5D) passed the physicochemical properties of the Lipinski rule of 5, binding mode, molecular interaction and ADMET calculations. These five compounds also showed high binding affinity of -8.9 kJ/mol, -7.7kJ/mol, -7.3kJ/mol, -7.1kJ/mol and -7.1kJ/mol at the binding pores of PfNDH2 respectively. The reactivity of these compounds was also investigated by the electron donating and accepting activities of the compounds using Density Functional Theory calculated Higher Occupied Molecular Orbital, Lower Unoccupied Molecular Orbital energy and HOMO/LUMO energy gap revealed the stability of the compounds due to the low energy gap values obtained. The values obtained showed that aromadendrene and chamazulene were potential inhibitors of PfNDH2 and were the most potent and therefore, recommended for therapeutic efficacy investigation.

Keywords: Molecular Docking, Artemisia Afra, Plasmodium Falciparum, Phytochemicals

1. Introduction

Malaria is one of the most dangerous infectious diseases and the development of drug resistant parasites only exacerbate the situation. The advent of drug-resistant malarial strains has increased continuously in recent times and has led to a great challenge for health globally. Malaria, a mosquito borne disease that is life threatening has been receiving innovative attention for effective anti-malaria drugs. The latter unfolding of multi drug resistant strains of malaria pathogen Plasmodium falciparum persist to cause deaths in the torrid and semiarid region, but malaria vaccine are not yet available for efficient and effective treatment. As

a result, it dictate for new and effective anti-malaria drugs are increasing rapidly. So, new anti-malaria drugs with innovative approaches to targets are absolutely required to address drug-resistant malaria.

One of the most commonly used plants in traditional medicine in South and West Africa is Artemisia afra. it is an aromatic shrub used to treat various disorders including coughs, colds, influenza, and malaria. The treatment of a wide ranging and unrelated list of health conditions and symptoms indicates that A. afra can, or should be considered a panacea. Infusion or decoction often made into syrup for bronchial treatment is the usual preparation. A. afra is used to treat coughs and colds, chills, dyspepsia, loss of appetite, stomach-ache and other gastric derangements, colic, croup, whooping-cough, gout and as purgative, flu, headaches, inflammation, gout, sore throat, malaria, diabetes, bladder and kidney disorders, asthma, constipation as well as numerous other health problems [1] It is also used by insertion of fresh leaves into the nostrils to clear clogged nose [2].

Molecular Docking Studies of Lonchocarpus cyanescens Triterpenoids as Inhibitors for Malaria showed that Structure-based methods remain one of the most logical approaches in drug discovery. [3]

Docking analysis of Proguanil and its analog with Human Dihydrofolate Reductase receptor carried out using HEX and Argus lab docking software showed that some of the modified drugs are better than the commercial drugs available in the market [4].

The computational method showed the advantage in saving time, cost and resources. It is feasible to block the interaction of NAD–dependent protein deacylases protein from selected compounds using virtual screening based on pharmacophore and molecular docking studies to serve as a preliminary study of designing an inhibitor against Plasmodium falciparum [5].

The predicted structure of Pftk in molecular-docking study of malaria drug target enzyme transketolase in Plasmodium falciparum was reported to serve first hand in the future development of effective Pftk inhibitors with potential anti-malarial activity [6].

The high binding energy obtained in molecular docking, synthesis and in vitro antimalarial evaluation of certain novel curcumin analogues showed that the designed compounds have good affinities for the PfATP-6 protein and that this could be responsible for its antimalarial activity against the Plasmodium falciparum species [7].

Synthesis, molecular docking and antiplasmodial activities of Tetrahydro-β-Carbolines was reported as a promising compound endowed with the highest antiplasmodial activity, highest selectivity, and lack of cytotoxicity. In silico simulations carried out for (1*S*,3*R*)-**7** provided useful insights into its possible interactions with enzymes essential for parasite metabolism [8].

An in-silico approach to Molecular docking analysis of apigenin and quercetin from ethylacetate fraction of Adansonia digitata with malaria-associated calcium transport protein showed that it could lead to further development of potent calcium transporter inhibitors for the prevention and treatment of malaria and related conditions [9].

Docking analysis obtained from quantitative Structure-activity relationship and molecular docking of Some Pyrrolones as antimalarial agents against Plasmodium Falciparum revealed that three of the studied compounds with binding affinity values of -10.7 kcal/mol, -10.9 kcal/mol and -11.1 kcal/mol possess higher potency than standard antimalarial drugs with binding affinity values of -8.8 kcal/mol, -9.5 kcal/mol and -9.0 kcal/mol. This showed that the information obtained by the QSAR and molecular docking results will offer important structural insights for the design of novel and highly potent antimalarial from the pyrrolones [10].

Molecular docking and QSAR studies for modeling of antimalarial activity of hybrids 4-anilinoquinoline-triazines derivatives with the wild-type and mutant receptor *pf*-DHFR showed that the quantitative structure-activity relationship (QSAR) and docking studies performed for previously reported 4-anilinoquinoline and 1,3,5-triazines based molecular hybrids. The generated model also showed good correlation coefficients (R^2 = 0.70) and test set prediction coefficient (R^2 = 0.74). These outcomes showed the good predictive ability of the conventional QSAR model [11].

Series of methods have been used by the researchers in finding efficient and effective drugs for curing various diseases which involves long time laboratory works, too much capital and energy but this present work focused on the use of one of the tools of Computer aided drug design (CADD) called molecular docking which has also been used by various researchers to discover the potent among the selected phytochemicals isolated from Artemisia afra.

Molecular Docking is a computational modeling method of Drug Discovery mostly used to assist in understanding protein-ligand interaction [12-16] and forecasting the protein-ligand binding mode. Relative to the traditional method, it is efficient, effective, fast and minimizes cost compared to the traditional method. Therefore, this current work targeted to check the inhibitory activities of selected phytochemicals in Artemisia afra against NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2) for anti-Malarial drug discovery in order to give scientific evidence for their conventional uses.

NADH-ubiquinone oxidoreductase of Plasmodium falciparum (PfNDH2) appeared to be a viable target for anti-malarial drug development.

2. Methodology

2.1. Ligands Preparation

There were 50 selected Compounds from Artemisia afra used against the target receptor (NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2)). Those compounds were downloaded from a drug database

called PubChem, an open chemistry database, and a drug bank consisting of substance, compound, and bioassay (https://pubchem.ncbi.nlm.nih.gov/). [17] The following compounds were downloaded: 2-Ethyl-(E)-2-butenal-CID_5362897, Cyclohexene, 3-methyl-6-(1-methylethylidene)-CID_30248, Tricyclo [2.2.1.0(2,6)] heptane, 1,3,3-trimethyl-CID_79022,2Hydrazinopyridine-CID_78645, p-Cymene-CID_7463, Eucalyptol-CID_2758, 2-Methylbutanoic anhydride-CID_102642, 2-Carene-CID_79044, Cyclooctene, 3-(1-methylethenyl)-CID_5367373, cis-Hexenyloctyne carbonate- CID_5365699, Bicyclo (3.1.0) hexane, 6-isopropylidene-1-methyl-CID_57822716, Benzene, tert-butyl- CID_108164, Bornyl acetate- CID_16028, Undecanal-CID_1234, 1-Decen-3-yne-CID_520646, D-Limonene- CID_440917, Bicyclo [2.2.1]hept-2-ene, 1,7,7-trimethyl- CID_71357940, Cyclohexanone, 2-(1-mercapto-1-methylethyl)-5-methyl-CID_6951713, Benzoic acid, 3-methoxy-, methyl ester-CID_4962354, 2,4,6-Triphenyl-1,3-dioxane- CID_568919, Caryophyllene- CID_5281515, β-copaene- CID_19725, Benzaldehyde, O-(diethylboryl)oxim- CID_10656207, (+)-epi-Bicyclosesquiphellandrene- CID_521496, Aromandendrene- CID_91354, Bicyclogermacrene-CID_13894537, cis-muurola-3,5-diene- CID_51351708, Phosphine, dimenthoxy-menthyl- CID_609493, Caryophyllene oxide- CID_10681562, Benzoic acid, nonadecyl ester- CID_3078322, p-Menth-8-ene, 3-methylene- CID_564761, Quinoline, 4-propyl- CID_588471, Benzene, 1,1'-(diazomethylene)bis- CID_10883673, 5,9-Hexacosadienoic acid, methyl- CID_5312573, 2-Benzothiazolamine, 4-methyl- CID_15132, Chamazulene-CID_10719, Hexane, 1,6-dibromo- CID_12368, Undecane, 4-cyclohexyl- CID_8186, 2,15-Hexadecanedione-CID_654321, Benzene, 1-ethyl-2,3-dimethyl- CID_13621, Benzene, 1-methyl-3-(1-methylethyl)-CID_139845, Phytol-CID_5280435, Endo-Borneo- CID_120151. These compounds served as the ligand molecules used in determining their potency against NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2). The downloaded compounds were converted to 3-dimensional structures saved in .pdb format for the effective virtual examination exercise employing SMILES and Online Translator) and Discovery studio then later minimized to acquire lowest energy and most stable conformer before docking.

2.2. Target Receptor Preparation

Crystal structure of Nicotinamide Adenine Dinucleotide Hydrogen (NADH)-ubiquinone oxidoreductase of Plasmodium falciparum (PfNDH2) describe a possible target for anti-malarial drug discovery (Protein ID: 5jwa) was downloaded [18] and It undergoes further treatment using BIOVIA Discovery Studio Software (version 19.1) [19] to remove the unwanted molecules and prevent molecular interaction during virtual screening.

2.3. Active Sites Determination

Amino acids residues of the active site of target receptor NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2) were determined with (Uniprot) (www.uniprot.org) and Discovery Studio. Result obtained were contrast, justified and validated with the experimental data from literature for NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2) [20]. The amino acids residues reported are: GLY47, SER48, GLY49, TRP50, GLY51, ILE69, SER70, PRO71, ARG72, THR76, THR78, PRO79, ASN92, GLU116, CYS117, ALA147, VAL148, GLY149, ALA150, ILE157, LYS162, VAL167, LYS168, VAL206, THR211, ASP221, LYS225, LEU268, ASN269, TYR277, VAL278, SER309, ILE352, GLY353, ASP354, CYS355, PRO434, THR435, ALA436, GLN437, ALA439, PHE460, HIS479, TRP511, ARG514, PHE518, PHE521, LYS525, TYR527, ARG529.

2.4. Molecular Docking Simulations

All atoms and complexes including water molecules attached with our target receptor (PDB ID: 5jwa) were detached using [19] in order to have a clean and accurate virtual screening. Pyrx-virtual screening tools (Autodock Vina and Open Babel,) were used for the docking process while Autodock tool-1.5.6 program [21] was used to determine the grids, the dimension and binding centre of 5JWA (-5.825,-58.985,22.747) and (103.170, 100.056, 129.571 Å) for (x, y, z) respectively with 1.000 Å spacing, then the docking scores and other calculations were carried out using AutoDock Vina, MGL tools- 1.5.6, PyMOL Console Edu and BIOVIA Discovery studio 4.5.

2.5. Pharmacokinetics (ADMET) and Drug-Likeness Properties Evaluation

ADMET properties related to Chemical absorption, distribution, metabolism, excretion, and toxicity were evaluated [22, 23]. The drug-likeness of the selected compounds was assessed using Mol inspiration Online Tool (https://molinspiration.com). This is important in drug discovery and development and a high-quality drug candidate should have sufficient efficacy against the therapeutic target and also display suitable ADMET properties at a therapeutic dose level.

2.6. Density Functional Theory (Quantum Mechanics)

Density Functional Theory of Quantum mechanical calculations were done for the five key compounds (Hits) obtained from the virtual screening. Structural optimization was done using Spartan'14 software with DFT/B3LYP at 6-311G* levels. This was done to obtain the frontier orbital energies, the highest occupied molecular orbital (HOMO), the lowest occupied molecular orbital (LUMO) and energy gap. Information on the stability and reactivity of the compounds are also provided.

3. Results and Discussion

Molecular docking is a computer-based method used in drug discovery to discover of new compounds of medicinal nature by predicting ligand-target interactions and the binding affinity of the ligand to the protein on a molecular interaction. Crystal structure of NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2) with PDB ID 5JWA (Figure 1) was used as the target protein in this research. Docking was performed on Fifty (50) compounds to PfNDH2 target protein with code 5JWA and the binding affinities of the selected compounds are as shown in Table 1. 2,4,6- Triphenyl-1,3-dioxane (2,4,6-TPD) had -8.9 kJ/mol, Chamazulene (Cha) had -7.7kJ/mol, Aromandendrene (Aro) was -7.3kJ/mol while the binding energy values for both (+)-epi-Bicyclosesquiphellandrene (1-EBSP) and Cis-muurola-3,5diene (CMD) is -7.1JK/mol. This implies that the selected compounds have higher binding affinity comparing to others. For 5jwa-2,4,6-TPD, the amino acid residues participating in the hydrogen bonding formation with their respective H-Bond distance include Pro79 (4.14 Å, 4.74 Å, 5.30 Å), Thr78 (4.16 Å), Leu406 (4.64 Å), Ala436 (4.30 Å) while that of 5jwa-CHA includes Trp50 (4.32 Å), Pro79 (4.30 Å), Ala436 (4.79 Å), Leu473 (3.77 Å), Lys470 (3.89 Å), Leu507 (5.21 Å), Val503 (4.58 Å), Tyr504 (4.22 Å), 5jwa-ARO include Phe155 (3.88Å, 4.08 Å, 4.27 Å), Ile157 (5.26Å), Val206 (4.80 Å), Tyr277 (3.81 Å), Val278 (4.76 Å), Trp307 (5.28Å), Ser217(2.56 Å) while that 5jwa-1-EBSP include Phe155 (5.08 Å), Ile157 (5.30 Å), Tyr277 (3.81 Å), Val278 (4.18 Å), Trp307(4.61 Å), Ser217(2.56 Å) of and that of 5jwa-CMD are Phe155 (5.24 Å), Ile157 (5.31 Å), Val206 (4.26 Å), Tyr277(3.86,4.69,5.42 Å), Val278 (5.15 Å), Trp307 (4.68 Å). Hydrophobic and electrostatic interactions are also reported and for 5jwa-2,4,6-TPD, the hydrophobic interactions include Trp30, Phe77, Pro210, Thr211, Thr435, Gln437, Gly471, Leu473, 5jwa-CHA include Ser309 while for 5jwa-ARO we have Tyr444, Asn448, Ile466, Ser464 and for5jwa-1-EBSP, the hydrophobic and electrostatic interactions include Gly245 as well, that of 5jwa-CMD include Ser309. The molecular interaction is shown in Figure 2.

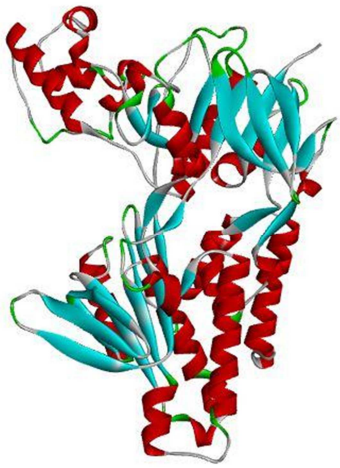
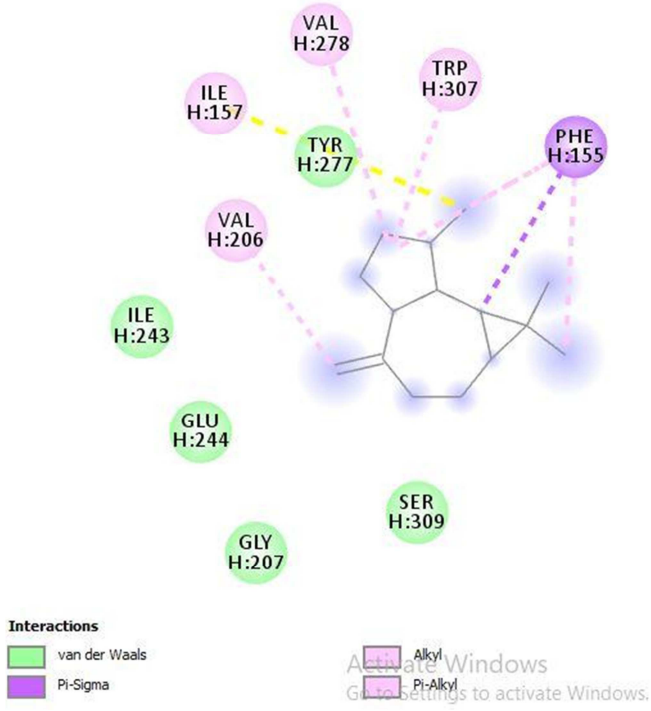
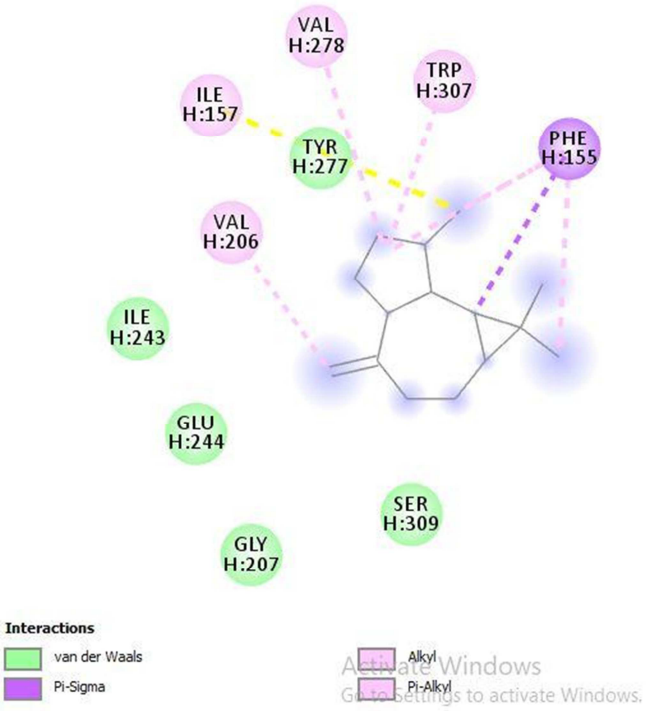


Figure 1. *Crystal structure of NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2) (PDB ID: 5jwa).*



A-1



A-2

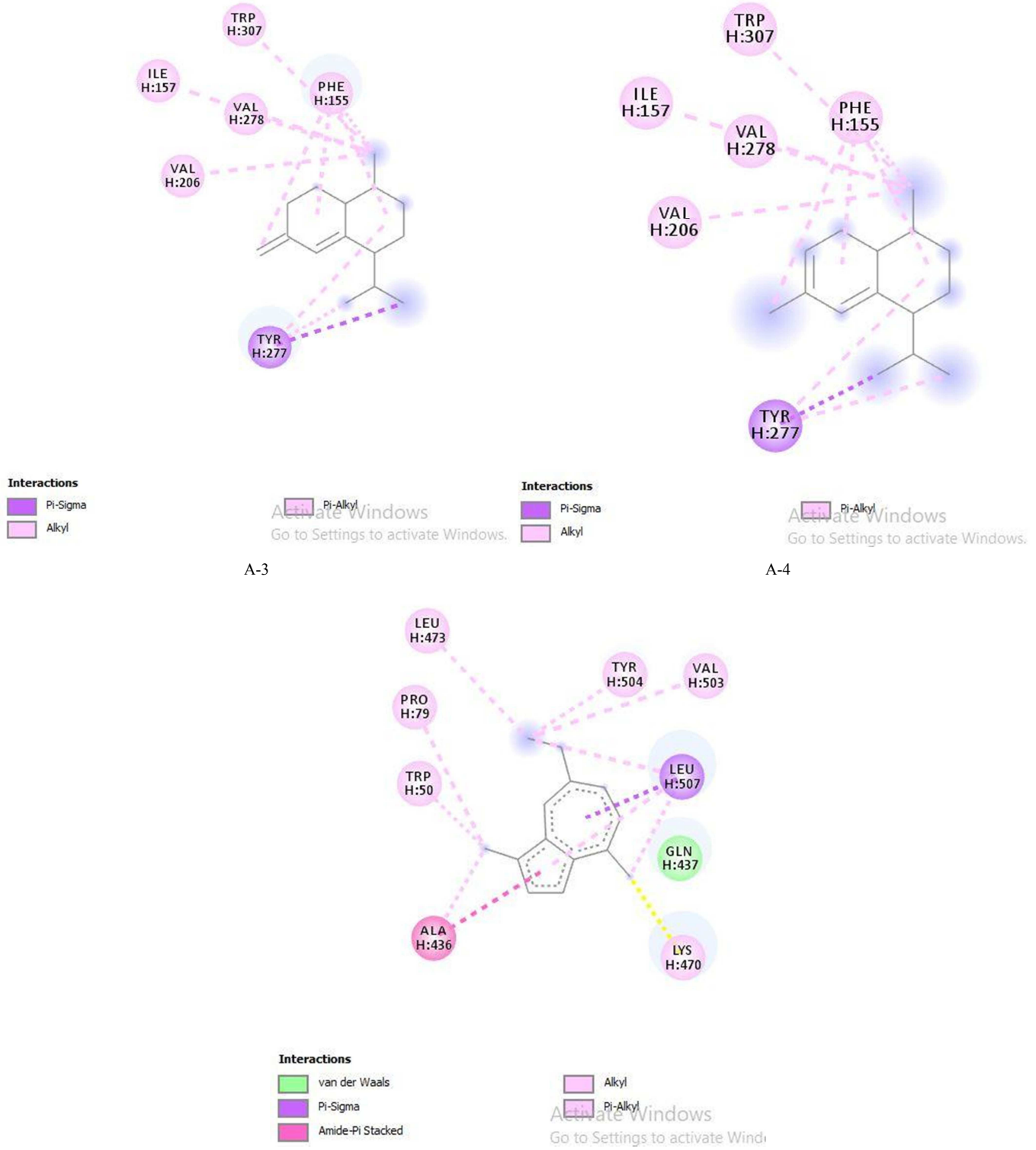


Figure 2. *Amino acid interactions of 2,4,6-Triphenyl-1,3-dioxane (A-1); (+)-epi-Bicyclosesquiphellandrene (A-2); Aromandendrene (A-3); Cis-muurola-3,5diene (A-4); Chamazulene (A-5) with the active site (binding pocket) NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2) (PDB ID: 5jwa).*

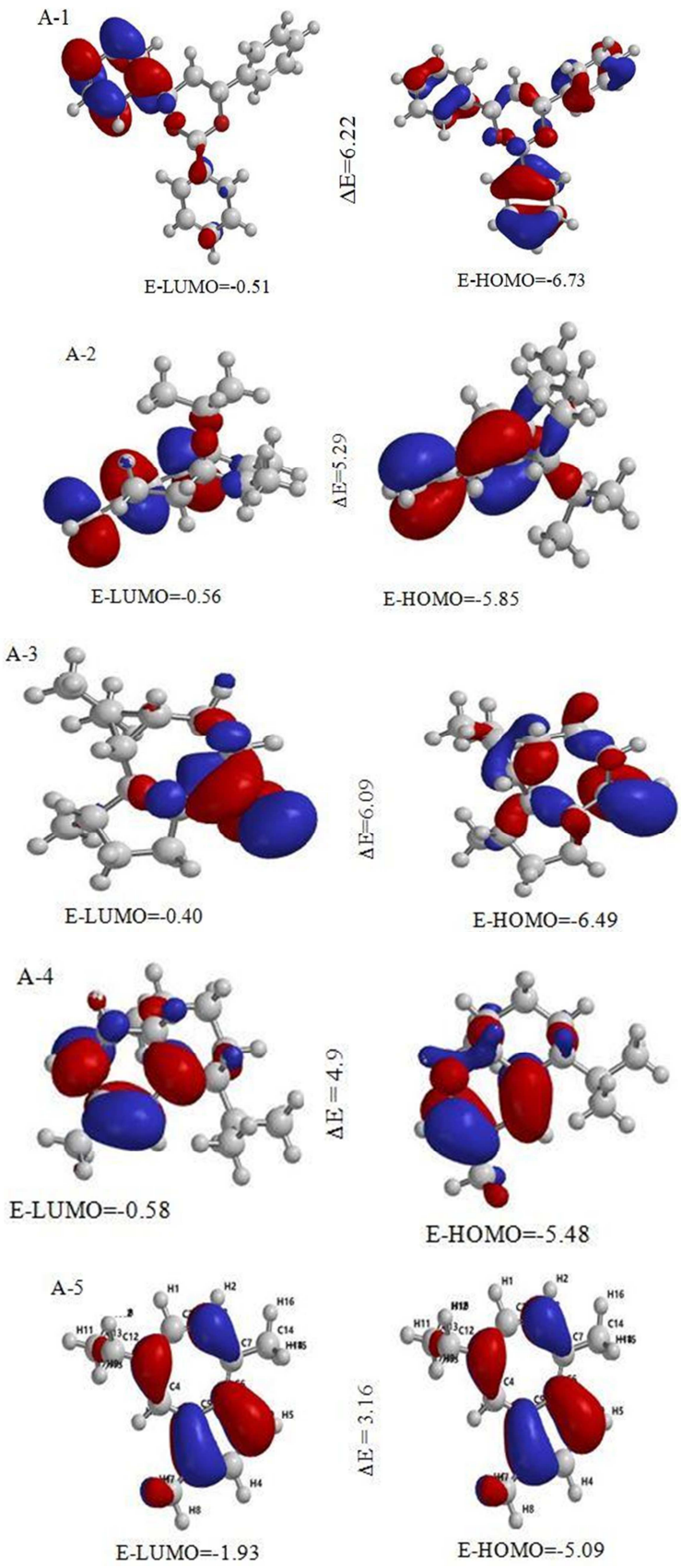


Figure 3. *Shows the highest occupied molecular orbital (HOMO), the lowest occupied molecular orbital (LUMO) respectively for each of the compounds.*

A-1=2,4,6-Tripheny l-1,3-dioxane (2,4,6TPD); A-2 = (+)-epi-Bicyclosesquiphellandrene (1EBSP); A-3 = Aromandendrene (ARO); A-4 = Cis-muurola-3,5diene (CMD); A-5= Chamazulene (CHA)

3.1. Drug Likeness of the Selected Hit Compounds

Table 1. *Binding affinity, H-bond interaction, Electrostatic/hydrophobic interactions and inhibition constant of the selected Hit Compounds.*

Ligands	Binding Affinity (ΔG), kcal/mol	5jwa Receptor amino acids forming H-bond with ligands (H-Bond Distance, Å)	Electrostatic/ Hydrophobic Interactions	Inhibition Constant (Ki), µM
2,4,6- Triphenyl-1,3-dioxane	-8.9	Pro79 (4.14 Å, 4.74 Å, 5.30 Å), Thr78 (4.16 Å), Leu406 (4.64 Å), Ala436 (4.30 Å)	Trp30, Phe77, Pro210, Thr211, Thr435, Gln437, Gly471, Leu473	0.30
(+)-epi-Bicyclosesquiphellandrene	-7.1	Phe155 (5.08 Å), Ile157 (5.30 Å), Tyr277 (3.81 Å), Val278 (4.18 Å), Trp307 (4.61 Å), Ser217 (2.56 Å)	Gly245	6.28
Aromandendrene	-7.3	Phe155 (3.88Å, 4.08 Å, 4.27 Å), Ile157 (5.26Å), Val206 (4.80 Å), Tyr277 (3.81 Å), Val278 (4.76 Å), Trp307 (5.28 Å), Ser217 (2.56 Å)	Tyr444, Asn448, Ile466, Ser464	47.53
Cis-muurola-3,5diene	-7.1	Phe155 (5.24 Å), Ile157 (5.31 Å), Val206 (4.26 Å), Tyr277 (3.86,4.69,5.42 Å), Val278 (5.15 Å), Trp307 (4.68 Å)	Ser309	6.28
Chamazulene	-7.7	Trp50 (4.32 Å), Pro79(4.30 Å), Ala436 (4.79 Å), Leu473 (3.77 Å), Lys470 (3.89 Å), Leu507 (5.21 Å), Val503 (4.58 Å), Tyr504 (4.22 Å)	Gln437	2.28

Table 2. *Druglikeness of the of the selected Hit compounds.*

Compounds	Heavy atoms (HA)	Molecular Weight (MW)	RO5 violations	Hydrogen bond donor (HBD)	Hydrogen bond acceptor (HBA)	miLog P
A-1	24	316.40	1	0	2	5.15
A-2	15	204.36	1	0	0	5.52
A-3	15	204.36	0	0	0	4.85
A-4	15	204.36	0	0	0	5.74
A-5	15	184.28	0	0	0	4.84

A-1 = 2,4,6- Triphenyl-1,3-dioxane; A-2 = (+)-epi-Bicyclosesquiphellandrene; A-3 = Aromandendrene; A-4 = Cis-muurola-3,5diene; A-5= Chamazulene

Table 3. *ADMET analysis of the selected Hit compounds.*

Absorption & Distribution	A-1	A-2	A-3	A-4	A-5
BBB (+/-)	0.9825 (BBB+)	0.9439 (BBB+)	0.9725 (BBB+)	0.9460 (BBB+)	0.9850 (BBB+)
HIA+	0.9953 (99.53%)	0.9975 (99.75%)	0.9925 (99.25%)	1.0000 (100%)	0.9970 (99.70%)
Aqeous Solubility (Log S)	-2.9844	-5.2875	-5.1273	-5.2483	-4.4978
Metabolism					
CYP450 2C19 Inhibitor	0.6455 Inhibitor	0.5970 Non-Inhibitor	0.6360 Non-Inhibitor	0.6441 Non-Inhibitor	0.8015 Non-Inhibitor
CYP450 1A2 Inhibitor	0.5070 Non-Inhibitor	0.6768 Non-Inhibitor	0.6302 Non-Inhibitor	0.7018 Non-Inhibitor	0.5318 Non-Inhibitor
CYP450 3A4 Inhibitor	0.9033 Non-Inhibitor	0.9109 Non-Inhibitor	0.9119 Non-Inhibitor	0.9302 Non-Inhibitor	0.9223 Non-Inhibitor
CYP450 2C9 Inhibitor	0.6849 Non-Inhibitor	0.6622 Non-Inhibitor	0.6547 Non-Inhibitor	0.7071 Non-Inhibitor	0.8765 Non-Inhibitor
CYP450 2D6 Inhibitor	0.9383 Non-Inhibitor	0.9174 Non-Inhibitor	0.9224 Non-Inhibitor	0.9182 Non-Inhibitor	0.8743 Non-Inhibitor
Excretion					
Biodegradation	0.5249 Not biodegradable	0.8164 Not biodegradable	0.8901 Not biodegradable	0.7352 Not biodegradable	0.8374 Not biodegradable
Toxicity					
AMES Mutagenesis	0.6100 Non Ames Toxic	0.6386 Ames Toxic	0.7852 Non-Ames Toxic	0.6044 Non-Ames Toxic	0.8133 Non-Ames Toxic
Acute Oral Toxicity	0.5549 III	0.7969 III	0.5833 III	0.8388 III	0.8388 III
Eye Irritation (YES/NO)	NO	NO	NO	NO	NO
Eye Corrosion (YES/NO)	NO	NO	NO	NO	NO
hERG Inhibition	0.9337 Weak inhibitor	0.8089 YES	0.9643 Weak inhibitor	0.9643 Weak inhibitor	0.9701 Weak inhibitor
Carcinogenicity	0.5180 Non-Carcinogenic	0.4547 Non-Carcinogenic	0.4839 Non-Carcinogenic	0.4839 Non-Carcinogenic	0.4314 Non-Carcinogenic

A-1 = 2,4,6- Triphenyl-1,3-dioxane; A-2 = (+)-epi-Bicyclosesquiphellandrene; A-3 = Aromandendrene; A-4 = Cis-muurola-3,5diene; A-5= Chamazulene

Assessment of the physicochemical parameters and drug-likeness of compounds is one of the important step in drug discovery and design, but the potential active, according to Lipinski, an efficient oral therapeutic drug must obey the 'rule of five' with only one (1) violation and according to the rule, an orally bioavailable drug must possess molecular weight (MW) ≤ 500Da, hydrogen bond donor (HBDs) ≤ 5, hydrogen bond acceptor (HBAs) ≤ 10 and logP (octanol-water partition coefficient) ≤ 5 [24]. Descriptors of oral bioavailability are essential as they predict the permeability and absorption of drug across biological membrane such as epithelium cell. Partition coefficient value (LogP) is especially important in predicting intestinal absorption of drugs. Considering all the ligands) binding affinity (Table 1), inhibition constant, (Table 2) efficacy and safety profile using the Molinspiration online (http://www.molinspiration.com/), and ADMET SAR-2 web-server (Table 3) [21], Aromandendrene; Cis-muurola-3,5diene; Chamazulene were qualified as hit compounds for further analysis and are coded A-3, A-4 and A-5 respectively. Drug-likeness of all the ligands were evaluated with Molinspiration online (http://www.molinspiration.com/) as shown in Table 2, it is apparent from the table that none of them had more than one violation of the 'rule of five' which is a good indication for satisfactory oral bioavailability and permeability.

3.2. ADMET Analysis of the Selected Compounds

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profile of a molecule is an essential assay in the early stage of drug discovery. ADMET have been and still are significant reason for attrition in drug discovery and the data obtained compliment the selection and identification of molecules with optimal safety therapeutic profile dose along the discovery process rather than at the final stage, as it helps in avoiding waste of time and precious resource on drug molecules that may eventually be discarded [25]. ADMET profile of selected but potent phytochemicals from A. afra as computed using ADMET SAR-2 web-server [5] were as shown in Table 3, as part of the drug ADMET profile, a drug molecule should have good human intestinal absorption (HIA), solubility (log S) range between -1 to -5, and should not be toxic. [25] All the selected hit, A-1, A-2, A-3, A-4 and A-5 are well absorbed in the human intestine and were found to cross the blood-brain barrier, although an oral drug does not necessarily need to cross the blood-brain barrier but for only central nervous system target drugs [26]. Prediction shows that the five (5) selected hit compounds were non-inhibitor of the cytochrome P450 which is the microsomal enzyme, an indication of a good metabolic activity of the drug in the hepatocytes [27] The Ames toxicity value express the potential of a drug molecule to cause mutation in DNA and could be a major reason for excluding a drug molecule along the discovery process [28]. Similarly, the hits compound possesses type III acute oral toxicity values (slightly toxic) which could easily be converted to type IV (nontoxic) during hit-lead optimization.

3.3. Bioactivity of Selected Compounds

Table 4 shows the Bioactivity parameters of the five (5) selected hit compounds. The higher the binding energy the lower the inhibition constant as shown in the .inverse relationship between binding Energy and inhibition constant (Equation 1), indicating that the inhibition constant of a potential hit compound is expected to have values ranging between 0.1-1.0μM and not exceed 10nM for a potent drug [26]. Inhibition constant value of the hit compounds range from 0.30 to 6.28 μM. This shows that the five (5) selected compounds are qualified drug candidates with 2,4,6- Triphenyl-1,3-dioxane (0.30 μM) being the most potent of all in terms of their binding affinities. It was also observed form the result obtained for Ligand Efficiency (LE), Fit Quality (FQ), and Ligand-efficiency-dependent lipophilicity (LELP) according to equation 2-5 that all the hit compounds had values within the recommended fit quality of ≥0.8 [29].

$$Ki = e^{\left[\frac{-\Delta G}{RT}\right]} \quad (1)$$

Where R = Gas constant (1.987 × 10–3 kcal/K-mol); T = 298.15 (Absolute Temperature); ki = Inhibition constant

$$LigandEfficiency(LE) = -B.E \div Heavyatoms(H.A)\_ -. \quad (2)$$

$$LE_{scale} = 0.873e^{-0.026 \times H.A} - 0.064 \quad (3)$$

$$FQ = LE \div LE_{scale} \quad (4)$$

$$LELP = LogP \div LE \quad (5)$$

HOMO and LUMO energy was calculated for the five top hit compounds (2,4,6-TPD, 1-EBSP, Aro, CMD and Cha) using the quantum mechanical Density Functional Theory (DFT), the result is as shown in Figure 3. Chamazulene (Cha) has the lowest energy gap of 3.16eV with -5.09eV and -1.93eV HOMO/LUMO energy value respectively. In comparison, the values of HOMO and LUMO energies of the other compounds are 2,4,6-TPD (-6.73eV and -0.51eV), 1EBSP (-5.85eV and -0.56eV), Aro (-6.49eV and -0.40eV) and CM3,5D (-5.48eV and 0.58eV) with an energy gap of 6.22eV, 5.29eV, 6.09eV and 4.9eV respectively (Table 4). The chemical species reactivity is described by the frontiers orbitals, the highest occupied molecular orbital (HOMO), and the lowest occupied molecular orbital (LUMO). The HOMO and LUMO energy values describe the ability of the compounds to donate and accept electrons. Energy gap value gives the difference between the LUMO and HOMO energy and this represents the intramolecular charge transfer and kinetic stability. Low chemical reactivity and high kinetic stability are associated with a large energy gap and this is used to predict the strength and stability of the compounds. In contrast, compounds with small energy gaps are more reactive with less kinetic stability [30].

Table 4. *Showing the Bioactivity of the selected Compounds.*

BIOACTIVITY	A-1	A- 2	A- 3	A- 4	A- 5
AutoDock Vina docking score (kcal/mol)	-8.9	-7.1	-7.3	-7.5	-7.7
Ki (μM)	0.30	6.28	4.18	6.28	2.28
miLog P	5.15	5.52	4.85	5.74	4.84
Ligand Efficiency (LE) /kcal/mol/heavy atom)	0.37	0.47	0.61	0.55	0.51
LE- Scale	0.54	0.68	0.73	0.71	0.68
Fit Quality (FQ)	0.92	0.90	1.06	0.98	0.97
Ligand-efficiency-dependent lipophilicity (LELP)	13.89	11.66	7.97	10.51	9.43

A-1 = 2,4,6- Triphenyl-1,3-dioxane; A-2 = (+)-epi-Bicyclosesquiphellandrene; A-3 = Aromandendrene; A-4 = Cis-muurola-3,5diene; A-5= Chamazulene

Table 5. *Shows the highest occupied molecular orbital (HOMO), the lowest occupied molecular orbital (LUMO) and the energy gap.*

COMPOUNDS	HOMO (eV)	LUMO (eV)	Energy Gap (ΔE) (eV)
2,4,6 TPD	-6.73	-0.51	6.22
1EBSP	-5.85	-0.56	5.29
Aro	-6.49	-0.40	6.09
CMD	-5.48	-0.58	4.9
Cha	-5.09	-1.93	3.16

2,4,6-Triphenyl-1,3-dioxane (2,4,6TPD); (+)-epi-Bicyclosesquiphellandrene (1EBSP); Aromandendrene (Aro); Cis-muurola-3,5diene (CMD); Chamazulene (Cha).

Table 6. *Shows the downloaded 50 Ligands with their respective CID and Binding energy value.*

Compounds	CID	Binding energy value
2-Ethyl-(E)-2-butenal	CID_5362897	-4.7
Cyclohexene, 3-methyl-6-(1-methylethylidene)	CID_30248	-6.3
Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl	CID_79022	-5.5
2-Hydrazinopyridine	CID_78645	-5.8
p-Cymene	CID_7463	-6.3
Eucalyptol	CID_2758	-5.5
2-Methylbutanoic anhydride	CID_102642	-5.2
2-Carene	CID_79044	-5.6
Cyclohexanol, 1-methyl-4-(1-methylethenyl) – cis	CID_8748	-5.7
Thujone	CID_261491	-5.8
Artemiseole	CID_521927	-5.9
Camphor	CID_2537	-5.3
Terpinen-4-ol	CID_11230	-6.0
3,5-Dimethylcyclopentene	CID_522549	-5.5
(-)-Myrtenol	CID_88301	-5.4
Cyclooctene, 3-(1-methylethenyl)	CID_5367373	-6.6
cis-Hexenyloctyne carbonate	CID_5365699	-5.1
Bicyclo (3.1.0) hexane, 6-isopropylidene-1-methyl	CID_57822716	-4.6
Benzene, tert-butyl	CID_108164	-5.1
Bornyl acetate	CID_16028	-4.8
Undecanal		-4.8
1-Decen-3-yne	CID_520646	-4.3
D-Limonene	CID_440917	-6.4
Bicyclo [2.2.1] hept-2-ene, 1,7,7-trimethyl	CID_71357940	-5.1
Cyclohexanone, 2-(1-mercapto-1-methylethyl)-5-methyl	CID_6951713	-5.0
Benzoic acid, 3-methoxy-, methyl ester	CID_4962354	-5.6
2,4,6-Triphenyl-1,3-dioxane	CID_568919	-8.9
Caryophyllene	CID_5281515	-6.5
β-copaene	CID_19725	-6.7
Benzaldehyde, O-(diethylboryl)oxim	CID_10656207	-6.3
(+)-epi-Bicyclosesquiphellandrene	CID_521496	-7.1
Aromandendrene	CID_91354	-7.3
Bicyclogermacrene	CID_13894537	-5.8
cis-muurola-3,5-diene	CID_51351708	-7.1
Phosphine, dimenthoxy-menthyl	CID_609493	-7.5
Caryophyllene oxide	CID_10681562	-5.9
Benzoic acid, nonadecyl ester	CID_3078322	-4.9
p-Menth-8-ene, 3-methylene	CID_564761	-6.3
Quinoline, 4-propyl	CID_588471	-6.5
Benzene, 1,1'-(diazomethylene)bis	CID_10883673	-6.1

Compounds	CID	Binding energy value
5,9-Hexacosadienoic acid, methyl	CID_5312573	-5.6
2-Benzothiazolamine, 4-methyl	CID_15132	-5.8
Chamazulene	CID_10719	-7.7
Hexane, 1,6-dibromo	CID_12368	-3.6
Undecane, 4-cyclohexyl	CID_8186	-5.4
2,15-Hexadecanedione		-4.9
Benzene, 1-ethyl-2,3-dimethyl	CID_13621	-5.9
Benzene, 1-methyl-3-(1-methylethyl)	CID_139845	-5.7
Phytol	CID_5280435	-5.5
Endo-Borneo	CID_120151	-5.0

4. Conclusion

The results obtained from this research analyzing the inhibitory activities of selected phytochemicals in Artemisia afra against PfNDH2 for anti-malaria drug discovery shows that five (5) of the compounds, 2,4,6-Triphenyl-1,3-dioxane (2,4,6TPD); (+)-epi-Bicyclosesquiphellandrene (1EBSP); Aromandendrene (Aro); Cis-muurola-3,5diene (CMD); Chamazulene (Cha) escaped through most of the analysis but two of them aromadendrene and chamazulene were still more potent considering the quantum mechanistic study and they were the potential compounds for anti-malaria drug development and therefore, recommended for therapeutic efficacy investigation and adoption to join the existing drugs for malaria.

References

[1] Anneke du Toit and Frank van der Kooy (2019) Artemisia afra, a controversial herbal remedy or a treasure trove of new drugs? Journal of Ethnopharmacol. doi: 10.1016/j.jep.2019.112127 Vol244 15 112 -127

[2] Ian E. Cock and Sandy F. Van Vuuren (2020) The traditional use of southern African medicinal plants in the treatment of viral respiratory diseases: A review of the ethnobotany and scientific evaluations, journal of Ethnopharmacol. 262: 113194. doi: 10.1016/j.jep.2020.113194

[3] Adejoro I. A., Waheed S. O., Adeboye O. O. (2016) Molecular Docking Studies of Lonchocarpus cyanescens Triterpenoids as Inhibitors for Malaria. J Phys Chem Biophys 6: 213. doi: 10.4172/2161-0398.1000213

[4] Paul D Lyne (2002) Structural base virtual screening: An overview Drug Discovery Today Volume 7, Issue 20, Pages 1047-1055.

[5] Cheng F, Li W, Zhou Y, et al (2012) admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties. J Chem Inf Model 52 (11): 3099-3105. https://doi.org/10.1021/ci300367a

[6] Md. Anayet Hasan, Md. Habibul Hasan Mazumder, Afrin Sultana Chowdhury, Amit Datta and Md. Arif Khan (2015) Molecular-docking study of malaria drug target enzyme transketolase in Plasmodium falciparum 3D7 portends the novel approach to its treatment Source Code for Biology and Medicine 10: 7 2-14 DOI 10.1186/s13029-015-0037-3

[7] Chandrajit Dohutia1, Dipak Chetia, Kabita Gogoi, Dibya Ranjan Bhattacharyya, Kishore Sarma (2017) Molecular docking, synthesis and in vitro antimalarial evaluation of certain novel curcumin analogues. Brazilian Journal of Pharmaceutical Sciences http://dx.doi.org/10.1590/s2175-97902017000400084

[8] Anna Jaromin, Beata Gryzio, Marek Jamrozik, Silvia Parapini, Nicoletta Basilico, Marek Cegia, Donatella Taramelli and Agnieszka Zagorska (2021) Synthesis, Molecular Docking and Antiplasmodial Activities of New Tetrahydro-β-Carbolines Int. J. Mol. Sci. 22 (24), 13569; https://doi.org/10.3390/ijms222413569

[9] Akinwunmi O. Adeoye a, c,*, John O. Olanlokun b, Habib Tijani c, Segun O. Lawal b, Cecilia O. Babarinde b, Mobolaji T. Akinwole b, Clement O. Bewaji c (2019). Molecular docking analysis of apigenin and quercetin from ethylacetate fraction of Adansonia digitata with malaria-associated calcium transport protein: An in silico approach. volume 5, Issue 9, E02248 https://doi.org/10.1016/j.heliyon..e02248

[10] Zaharaddeen Shehu, A. Uzairu an Balarabe S. Sagagi (2018) Quantitative Structure Activity Relationship (QSAR) and Molecular Docking Study of Some Pyrrolones Antimalarial Agents against Plasmodium Falciparum. 1-17DOI: 10.18596/jotcsa.346661

[11] Hanine Hadni and Menana Elhallaoui (2019) Molecular docking and QSAR studies for modeling the antimalarial activity of hybrids 4-anilinoquinoline-triazines derivatives with the wild-type and mutant receptor pf-DHFR. Heliyon Vol. Issue 8 https://doi.org/10.1016/j.heliyon.e02357.

[12] Shehu Z, Uzairu A, Sagagi B. (2018) Quantitative Structure-Activity Relationship and Molecular Docking Study of Some Pyrrolones Antimalarial Agents against Plasmodium Falciparum. JOTCSA.; 5 (2): 569–84. DOI: http://dx.doi.org/10.18596/jotcsa.346661.

[13] Isaiah A. Adejoro, Sodiq O. Waheed Omolara O. Adeboye, Frederick U. Akhigbe (2017). Molecular Docking of the Inhibitory Activities of Triterpenoids of Lonchocharpus cyanescens against Ulcer. Journal of Biophysical Chemistry: 8,1-11. https://doi.org/10.4236/jbpc.2017.81001.

[14] Olawale F. Akinyele, Emmanuel G. Fakola, Oluwatoba E. Oyeneyin. Omolara O. Adeboye, Ayowole O. Ayeni, Justinah S. Amoko and Temitope A. Ajayeoba (2020) Molecular Docking Study of Primaquine-Favipiravir Based Compounds as Potential Inhibitors of COVID-19 Main Protease. European Reviews of Chemical Research, 2020, 7 (1) 3-15DOI: 10.13187/ercr.2020.1.3

[15] O. O. Adeboye, F. O. Oyeleke, S. A. Agboluaje, Molecular Docking of Inhibitory Activities of Syzygium Cordatum against Mycobacterium Tuberculosis, Adv. J. Chem. A, 2022, 5 (2), 147-163. DOI: 10.22034/AJCA.2022.310927.1286 URL: http://www.ajchem-a.com/article_146908.htm

[16] Shyamalambica P, Tapas Ranjan Samal, Mavuduri Jagannath (2019) Molecular-Docking Studies of Malaria Drug Target Enzyme Nad – Dependant Protein Deacylases Sir2a in Plasmodium Falciparum – A Novel Approach to its treatment. Research Journal Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences 5 (1) 243 – 261. DOI: 10.26479/2019.0501.2

[17] Kim S. Han L. Yu B. Hähnke V. D. Bolton E. E. Bryant S. H. (2016) PubChem structure-activity relationship (SAR) clusters J Cheminform 7 33 Pubchem (https://pubchem.ncbi.nlm.nih.gov/

[18] protein data bank RCSB (http://www.rcsb.org/pdb)

[19] BIOVIA, Discovery Studio Software Dassault Systèmes, [19.1], San Diego: Dassault Systèmes, [2019].

[20] Yang Y, Yu Y, Li X, Li J, Wu Y, Yu J, Ge J, Huang Z, Jiang L, Rao Y, Yang M (2017). Target Elucidation by Cocrystal Structures of NADH-Ubiquinone Oxidoreductase of Plasmodium falciparum (PfNDH2) with Small Molecule To Eliminate Drug-Resistant Malaria. J Med Chem. 9; 60 (5): 1994-2005. doi: 10.1021/acs.jmedchem.6b01733. Epub. PMID: 28195463.

[21] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009) AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexiblity. Journal of Computational Chemistry, 30, 2785-2791. http://dx.doi.org/10.1002/jcc.21256

[22] H. Yang, C. Lou, L. Sun, J. Li, Y. Cai, Z. Wang, W. Li, G. Liu, Y. Tang (2019), (https://lmmd.ecust.edu.cn/admetsar2/).

[23] (https://molinspiration.com)

[24] Lipinski CA. Lead profiling Lead- and drug-like compounds : the rule-of-five revolution. Drug Discovery Today. 2004; 1 (4): 337-341. doi: 10.1016/j.ddtec.2004.11.007

[25] Tsaioun K, Kates SA (2010) ADMET for Medicinal Chemists: A Practical Guide. John Wiley and Sons, Singapore 145-200. https://doi.org/ 10.1002/9780470915110

[26] Hughes J. P., Rees S, Kalindjian SB, Philpott KL (2011) Principles of early drug. Br J Pharmacol 162: 1239-1249. https://doi.org/10.1111/j.1476-5381.2010.01127.x

[27] Stevens E (2014) Lead Discovery. In: Jaworski A (ed) Medicinal Chemistry: Modern Drug Discovery Process. Pearson, pp 247–272.

[28] Mccarren P, Springer C, Whitehead L (2011) An investigation into pharmaceutically relevant mutagenicity data and the influence on Ames predictive potential. J Cheminform 3 (51): 1-20. https://doi.org/10.1186/1758-2946-3-51

[29] Hopkins AL, Keserü GM, Leeson PD, et al (2014) The role of ligand efficiency metrics in drug discovery. Nat Rev Drug Discov 13 (2): 105-121. https://doi.org/10.1038/nrd4163

[30] Heßelmann A. DFT-SAPT Intermolecular Interaction Energies Employing Exact-Exchange Kohn-Sham Response Methods. J Chem Theory Comput. 2018 Apr 10; 14 (4): 1943-1959. doi: 10.1021/acs.jctc.7b01233. Epub 2018 Apr 2. PMID: 29566325.