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# COMPUTATIONAL INSIGHTS INTO LEAF COMPOUNDS OF *Pterocarpus santalinoides* AGAINST FabH PROTEIN RECEPTOR

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## ABSTRACT

Diarrhea remains one of the most prevalent diseases in Nigeria, especially among children aged between 1 and 5. The death rate by diarrheal disease is about 1.5 million per year, which include 620 thousand children below the age of 5, and 320 thousand adults above the age of 70, Diarrheal disease has been ranked as the second leading cause of mortality of children. Ethno medical and scientific reports abound on using the extract from the leaf of *Pterocarpus santalinoides* for treatment of diarrhea of any kind. GCMS analysis was carried out to determine the bioactive compound present in the leaf extract responsible for the efficacy and result revealed the presence of 13 bioactive compounds with Oxiraneundecanoic acid, 3-pentyl-cis-methyl ester (37.89 %), Oleic Acid (35.49%) and 9-Octadecenoic acid (Z)-, methylester (12.35%) as the most abundant. The result indicated that the efficacy of the plant against diarrhea diseases could be linked to 9-Octadecenoic acid (Z)-, methylester found in the crude extract. The bioactive compounds from the extract were docked on the diarrhea protein crystal structure of beta-ketoacyl-acp synthase iii + malonyl-coa FabH (1HNJ) to know their binding affinities and compared with that of co-crystallize ligand and standard drug. The docking result showed that the cocrystallize ligand has the best binding affinity of -7.6 kcal/mol, followed by the phytocompound 9-Octadecenoic acid (Z)-, methylester (6.7 kcal/mol). 9,12-Octadecadienoic acid, methyl ester has affinity -6.3 kcal/mol, Aspidospermidin-17-ol and oleic acid have affinity ( -6.1 kcal/mol) while Hexadecanoic acid, 14-methyl-, methyl ester and phytol both have (-6.0kcal/mol) respectively while Undec-10-ynoic acid, undecyl ester has affinity (-5.9kcal/mol). The result revealed that the co-crystallize ligand has the best affinity followed by all the phytocompounds from the extract while the commercial drug has less affinity but with a very good interaction. All the compounds from the extract have good binding affinities within the range of -6.7 – 4.9kcal/mol. The interaction results of the extracts with the disease protein proved the anti-diarrhea efficacy of the plant as acclaimed ethnomedically therefore validating its traditional use against diarrhea diseases.

Keyword: *Pterocarpus santalinoides,* diarrhea, co-crystallize ligand, FabH protein.

## INTRODUCTION

Diarrhea is defined as an increase in the frequency, fluidity or volume of bowel movement and associated with increased frequency of bowel sound, wet stools and abdominal pain [1]. Diarrhea is a common gastrointestinal problem characterized by loose watery stool and mild to severe dehydration [2]. Diarrhea, a common disease in tropical countries and can be interpreted as an incidence of daily stool exceeding 200 g comprised of 60 to 95% of water [3]. Infants and children suffer from diarrhea most, and mortality from diarrhea is high compared with other diseases [4]. The condition is as result of harmful bacteria, viruses, and other pathogens and is characterized by frequent watery bowel movements, higher solute levels in



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the intestines, excessive loss of electrolytes, decreased ability of the intestine to absorb necessary electrolytes, and increased movement of the intestines [5]. Globally, 4 – 5 million death cases of human occur annually as a result of diarrhea [6]. Infants and children suffer from diarrhea most, and mortality from diarrhea is high compared with other diseases [1].

Diarrhea remains the number one killer disease among Nigerian children aged 1 – 5 years, killing about 194,000 children in Nigeria below five years annually [7,8]. It is the leading cause of malnutrition among under-five children [1]. Over 10% of death in children, about 800, 000 die each year as a result of diarrhea [9]. Every child in African environment before the age of 5 years has had atleast one case of diarrhea with some having up to three per year [10]. Major diarrhea-causing bacteria include *Shigella flexneri*, *Salmonella typhi*, and *E. coli*. The fungus *Candida albicans* has also been identified as a major causal agent [4]. There are three clinical types of diarrhea, namely, acute watery diarrhea, which may last several hours or days and includes cholera, acute bloody diarrhea, also called dysentery and persistent diarrhea, lasting 14 days or longer [11]. Diarrheal disease is a leading cause of child mortality and morbidity in the world, and mostly results from contaminated food and water sources. Worldwide, 780 million individuals lack access to improved drinking-water and 2.5 billion lack improved sanitation. Diarrhea due to infection is widespread throughout developing countries [11].

Diarrhea occurs when the secretion and absorption capacity of the small intestine become upset by bacterial or other pathogenic infections. In severe conditions, especially in infants and older adults, diarrhea can be lethal because of excessive water loss and electrolytes from the body. The most severe threat posed by diarrhea is dehydration. During a diarrheal episode, water and electrolytes (sodium, chloride, potassium and bicarbonate) are lost through liquid stools, vomit, sweat, urine and breathing. Dehydration occurs when these losses are not replaced [12]. The frequency of diarrheal infection and mortality is high in underdeveloped countries with low hygiene, and approximately 525,000 deaths occur because of diarrheal infection worldwide [13,14].

In developing countries, it is more common, where young kids have diarrhea approximately three times/year. Although various synthetic drugs are being prescribed as standard therapy for diarrhea, they have side effects. It is possible to prescribe the herbal medicine for diarrhea, which is safe and effective [2].

Herbal medicines are believed to be effective in curing diarrhea, and for many years, plants and plant extracts have been used to treat various gastrointestinal ailments, including diarrhea [2,13]. However, herbal medicines used in the treatment of diarrhea in African rural communities are unlikely to be replaced soon by modern medicines [15]. Nowadays, the integration of herbal medicine into modern medical practices is highly advocated [16].



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The use of medicinal plants for diarrhea results in improvement of the symptom. Different plant species have been found useful in the treatment of diarrhoea which include *Garcinia cola, Azardirchta indica, Bryophyllum pinnatum, Physcalis bransilensis, Mangifera indica, Ocimum gratisimum L, Ocimum basilium, Pterocarpus santalinoides L* [8]. In this research the efficacy of *Pterocarpus santalinoides* in diarrhea treatment against FabH protein will be studied.

*Pterocarpus santalinoides* is an evergreen, small to medium sized tree species in *Fabaceae* family with a remarkable bi-continental distribution, native to tropical western Africa. The tree grows to 9–12 m tall, with a trunk up to 1m in diameter and flaky bark [17]. The leaves are pinnate, 10–20 cm long, with 5–9 leaflets. The flowers are orange-yellow, produced in panicles. The fruit is a pod 3.5–6 cm long, with a wing extending three-quarters around the margin [18].

*Pterocarpus santalinoides* DC, also known as "Red sandal wood" in English, is a native plant in Nigeria. It is called gbengbe in Yoruba, gyadar kumi in Hausa, and nturukpa in Igbo language [19]. The tender leaves of the plant are used as soup vegetable in Eastern Nigeria, and extracts of its leaves, stem bark, and roots are used in ethno-medicine for the treatment of various ailments including inflammatory and cardiovascular diseases (heart attack and stroke) [20]. Research reports in available literature showed that *P santalinoides* leaf extract possesses hepatoprotective and antioxidant activities, with glucose and lipid lowering properties [21]. Aged people traditionally use *P. santalinoides* leaves for soup and as medicine because it is believed to help them cope with old age-related cardiovascular diseases such as weak/failing heart and stroke [22]. No form of toxicity has been reported with the folkloric use of the leaves of *P. santalinoides* as vegetable and ethno-medicine in humans, and as fodder or ethno-medicine in livestock [20]. In this research the efficacy of the plant is studied as an antidiarrheal agent against Fab enzyme using molecular docking to compare the binding affinity of its phytochemical with that of co crystalline ligand and commercial drug.

## MATERIALS AND METHODS

### *Plant Collection and Identification*

Fresh leaf of *Pterocarpus santalinoides* was harvested from uncultivated farm land located in Prefab Aladinma in Owerri Municipal Local Government Area of Imo State Nigeria and was identified by Professor F.N Mbagwu of the Department of Botany Imo State University, Owerri as *Pterocarpus santalinoides* from *Fabaceae* family.

### *Preparation of the Sample for Analysis*

The harvested *Pterocarpus santalinoides* leaf was washed with water to remove sand and dirt,



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and then dried at room temperature for two weeks. The plant material after complete dryness was pulverized with new corona mechanical grinder 2013 model, weighed and stored in amber coloured Winchester bottle for analysis.

***Extraction of Phytochemicals***

The pulverized *Pterocarpus santalinoides* leaf 200g was measured and percolated in a stoppered container containing 500ml of redistilled ethanol (98%) and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter had dissolved. The mixture was then clarified by filtration and later concentrated with rotary evaporator at $55^0$C to get the crude sample for GCMS analysis [23].

***GC-MS Analysis***

The GC-MS analysis was done at Zaria, kaduna state Nigeria. The compounds in the sample were identified using agilent GC-MS (Agilent 19091-433HP, USA) coupled to a mass spectrophotometer. The initial column temperature was 35°C with a hold time of 3 minutes. The temperature was programmed to rise by 8°C/min with a final temperature of 280°C. In the process, 1µl of the sample was injected into the port and immediately vaporized and moved down the column with helium as the carrier gas with flow rate of 1 ml/min. The MS Spectrum was taken at 70 eV. The identification of the compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in NIST14 structural library [24].

The compounds identified from GCMS and their structures are shown in table 1

***Identification and Preparation of Ligands***

The 3D structure-data files (SDF) of the compounds in the crude extract sample and anti-diarrhea drug were identified and downloaded from the PubChem database. They were minimized in PyRx virtual screening tool, using Universal Force Field at 200 steps and converted to AutoDock ligands (pdbqt) and then used for the docking analysis. Identification and preparation of molecular targets crystal structure of beta-ketoacyl-acp synthase iii + malonyl-coa (FabH) with PDB ID :1HNJ was identified and downloaded from the Protein Data Bank (PDB). The interfering crystallographic water molecules and co-crystallized ligand were removed, and minimization of the energy of the protein was then done using Biovia Discovery studies [25].

***Docking Procedure and Analysis of Results***

The screening of the phytochemical compounds from the seed extract was performed by docking them on selected binding pockets of proteins beta-Ketoacyl-acyl carrier protein synthase III (FabH) and ranked based on their binding affinities. The site multiple docking of the ligands and proteins were done with Autodock Vina in PyRx software. A rigid-flexible docking was performed after setting a grid box surrounding the binding sites of the receptors at exhaustiveness = 8, center x = 27.48, center y = 11.99, center z = 27.63, size x = 29.80, size y = 31.82, size z =



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23.26. The molecular docking results were organized on an Excel spreadsheet, and the Heat Map of the data and the interactions were viewed using the Biovia discovery studio.

***The Protein***

Beta-Ketoacyl-acyl carrier protein synthase III (FabH) is a condensing enzyme that plays central roles in fatty acid biosynthesis. Three-dimensional structures of *E. coli* FabH in the presence and absence of ligands have been refined to 1.46A resolution. The structures of improved accuracy revealed detailed interactions involved in ligand binding (From PDB).

B-Ketoacyl-acyl carrier protein (ACP) synthase III (the FabH gene product) condenses acetyl-CoA with malonyl-ACP to initiate fatty acid biosynthesis in the dissociated, type II fatty acid synthase systems typified by *Escherichia coli*. The accumulation of malonyl-acyl carrier protein (ACP) following the inhibition of a reconstituted fatty acid synthase system by acyl-ACP implicated synthase III (FabH) as a target for acyl-ACP regulation [26]. Therefore, the FabH protein was purified and its biochemical and regulatory properties examined. FabH exhibited a Km of 40 mM for acetyl-CoA and 5 mM for malonyl-ACP. FabH also accepted other acyl CoAs as primers with the rank order of activity being acetyl-CoA ' propionyl-CoA >> butyryl-CoA. It utilized neither hexanoyl-CoA nor octanoyl-CoA. AcylACPs suppressed FabH activity, and their potency increased with increasing acyl chain length between 12 and 20 carbon atoms. Nonesterified ACP was not an inhibitor. Acyl-ACP inhibition kinetics were mixed with respect to acetyl-CoA, but were competitive with malonyl-ACP, indicating that acyl-ACPs decrease FabH activity by binding to either the free enzyme or the acyl enzyme intermediate. These data support the concept that the inhibition of chain initiated at the b-ketoacylACP synthase III step contributes to the attenuation of fatty acid biosynthesis by acyl-ACP.

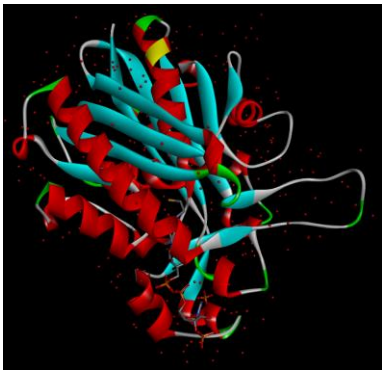


Plate 1 :Unprepared protein

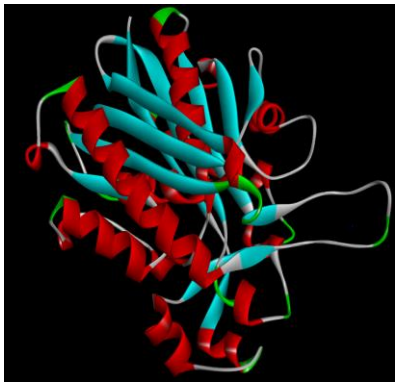


Plate 2: Prepared protein

Plate 1 is the original picture of diarrhea protein as retrieved from protein databank (http://www.rcsb.org) with the co-crystallized ligand, which were used to validate the docking



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protocols for the binding sites while plate 2 is the prepared proteins as used for the docking, it was

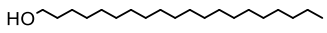
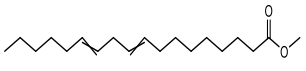
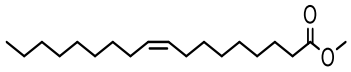
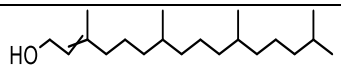
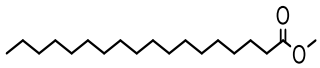
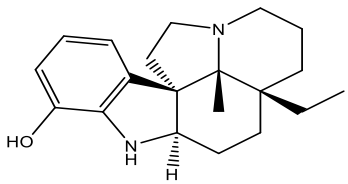
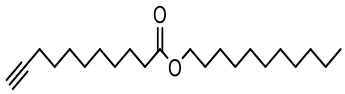
prepared by removing water of crystallization and unwanted protein chains in Discovery studio.

## RESULTS AND DISCUSSION

### *Chemical Composition of the Leaf Extract*

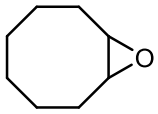
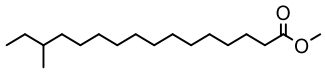
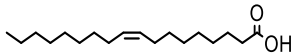
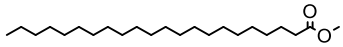
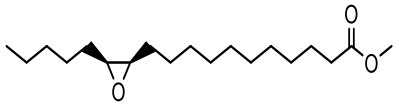
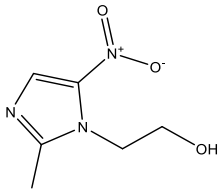
The GC–MS analysis of *Pterocarpus santalinoides* leaf extract gave 13 peaks for the bioactive compounds, their percentage composition, retention time, structures and formulas were recorded in the Table 1.

The Table 1: GC-MS Result of the phytochemicals from *Pterocarpus santalinoides* leaf extract

| s/n | RT | Compound name | Percentage | Pubchem ID | Chemical formula | Structure |
|---|---|---|---|---|---|---|
| 1 | 20.304 | Hexadecanoic acid, methyl ester | 3.96 | 8181 | $C_{17}H_{34}O_2$ |  |
| 2 | 21.812 | 1-Eicosanol | 0.40 | 12404 | $C_{20}H_{42}O$ |  |
| 3 | 23.375 | 9,12-Octadecadienoic acid, methyl ester | 0.69 | 5284421 | $C_{19}H_{34}O_2$ |  |
| 4 | 23.573 | 9-Octadecenoic acid (Z)-, methylester | 12.35 | 5354176 | $C_{21}H_{38}O_4$ |  |
| 5 | 23.956 | Phytol | 0.72 | 5280435 | $C_{20}H_{40}O$ |  |
| 6 | 24.153 | Methyl stearate | 2.49 | 8201 | $C_{19}H_{38}O_2$ |  |
| 7 | 24.878 | Aspidospermidin-17-ol | 0.91 | 12308697 | $C_{22}H_{30}N_2O_3$ |  |
| 8 | 25.144 | Undec-10-ynoic acid, undecyl ester | 0.21 | 91692431 | $C_{22}H_{40}O_2$ |  |



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| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | 27.130 | 9-Oxabicyclo[6.1.0]nonane | 0.54 | 67513 | $C_8H_{14}O$ |  |
| 10 | 27.694 | Hexadecanoic acid, 14-methyl-, methyl ester | 0.68 | 520159 | $C_{18}H_{36}O_2$ |  |
| 11 | 29.891 | Oleic Acid | 35.49 | 445639 | $C_{18}H_{34}O_2$ |  |
| 12 | 30.966 | Docosanoic acid, methyl ester | 3.68 | 13584 | $C_{23}H_{46}O_2$ |  |
| 13 | 33.497 | Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis- | 37.89 | 91692407 | $C_{19}H_{36}O_3$ |  |
| 14 | | Flagyl | | 4173 | $C_6H_9N_3O_3$ |  |

The phytochemical components were presented in the order from the highest to the least percentage as follows: Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis- (37.89) > Oleic Acid (35.49) > 9-Octadecenoic acid (Z)-, methylester (12.35) > Hexadecanoic acid, methyl ester (3.96) > Docosanoic acid, methyl ester(3.68) > Methyl stearate (2.49) > Aspidospermidin-17-ol(0.91) > Phytol (0.72) >9,12-Octadecadienoic acid, methyl ester (0.69) > Hexadecanoic acid, 14-methyl-, methyl ester (0.68) > 9-Oxabicyclo[6.1.0]nonane (0.54) > 1-Eicosanol (0.40) > Undec-10-ynoic acid, undecyl ester (0.21) Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis-, Oleic Acid and 9-Octadecenoic acid (Z)-, methylester have over 85% of the percentage composition of the plant

***Oleic Acid***

Oleic acid is an octadec-9-enoic acid, in which the double bond at C-9 has Z (cis) stereochemistry, it is an omega-9 fatty acid. Oleic acid can be made by the body and can also be found in foods. Highest levels are found in olive oil and other edible oils. Oleic acid is most commonly used for preventing heart disease, reducing cholesterol and inflammation. It is used to prevent cancer and other conditions [26]. Oils with oleic acid are used to replace saturated fats in diet, Oleic acid may be responsible for the hypotensive (blood



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pressure reducing) effects of olive oil that is considered a health benefit [27]. A 2017 review found that diets enriched in oleic acid are beneficial for regulating body weight [28].

***Methyl stearate***

Methyl stearate is a fatty acid methyl ester and an octadecanoate ester. It has a role as a metabolite. It is a natural product found in *Humulus lupulus*, *Ostrinia nubilalis*, and other organisms with data available.it can serve as emulsifier and stabilizer [29].

***9-octadecanoic acid methyl ester***

It has antioxidant, anti-inflammatory and antimicrobial activity [13], a drug used for treatment or prevention of cardiac arrhythmias. Anti-arrhythmia drugs may affect the polarisation-repolarisation phase of the action potential, its excitability or refractoriness, or impulse conduction or membrane responsiveness within cardiac fibres. 9-octadecanoic acid methyl ester Has other uses as Antifungal, antiarthitic, antitumour, anticancer and anticoronary, [30, 31].

***Hexadecanoic acid methyl ester***

This can be Antioxidant, Nematicide, Pesticide, Antibacterial, Antifungal, Antiarthritic, Antitumor; Anticancer, Anticoronary, Anti-inflammatory, Hypocholesterolemic and Hepatoprotective [32]. Hexadecanoic acid is also anticytotoxicity, apoptotic and anticancer [33].

***Aspidospermidin-17- ol***

Aspidospermidine is an alkaloid isolated from plants in the genus *Aspidosperma*. It has been a popular target for total synthesis, due to the fact that it provides a good showcase for synthetic strategies also because its structure is similar to many other important bioactive molecules [34,35].

Alkaloids such as aspidospermine and haplocine from *Aspidosperma* sp. inhibited CQ-resistant and sensitive strains of *P. falciparum* [34]. It is active against chloroquine resistant strains of *Plasmodium falciparum*, several species of *Aspidosperma* plants are referred to as remedies for the malaria treatment, especially *Aspidosperma nitidum*. As a medicinal plant, it is used also as a natural anti-inflammatory. Its fractionated extracts were assayed in vitro for activity against malaria parasites and for cytotoxicity [36].

Oxiraneundecanoic acid, 3-pentyl-, cis-methyl ester and phytol are used as therapeutic agents, they were known for their antioxidant and anticancer activity [37, 38].

The 13 compounds from the leaf extracts, the control drug flagy and the co-crystallize ligand were docked on FabH protein to know their binding affinities and compared with that of a known anti-diarrhea drugs to determine the one with better binding affinity against the binding site of the disease protein.

The docking result showed that the co-crystallize ligand has the best binding affinity of -7.8kcal/mol followed by the phytocompound 9-Octadecenoic acid (Z)-, methylester (6.7 kcal/mol). 9,12-Octadecadienoic acid, methyl ester has affinity -



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6.3 kcal/mol, Aspidospermidin-17-ol and oleic acid have affinity -6.1 kcal/mol while Hexadecanoic acid, 14-methyl-, methyl ester and phytol both has -6.0kcal/mol respectively. Undec-10-ynoic acid, undecyl ester has affinity (-5.9kcal/mol). Hexadecanoic acid, methyl ester and 1-Eicosanol have same affinity (-5.6kcal/mol, while Docosanoic acid, methyl ester and Methyl stearate (-5.5kcal/mol). The leaf bioactive compound 9-Oxabicyclo [6.1.0] nonane has high affinity (-4.9 kcal/mol) close to that of the commercial drug flagy (-4.6 kcal/mol). The result revealed that the the cocrystallize ligand has the best affinity followed by all the phytocompounds from the extract. The result also showed that the bioactive compounds from the plant extract have better affinity than the commercial drug thereby supporting the efficacy of the plant in treatment of diarrhea. The docking result was recorded in table 2.

Table 2: Binding Affinity result of the phytocompounds, co-crystallize ligand and the standard drug with the disease protein.

| Phytocompound | Binding affinity |
|---|---|
| Aspidospermidin-17-ol | -6.1 |
| 1-Eicosanol | -5.6 |
| Docosanoic acid, methyl ester | -5.5 |
| Oleic Acid | -6.1 |
| Hexadecanoic acid, 14-methyl-, methyl ester | -6.0 |
| Phytol | -6.0 |
| 9,12-Octadecadienoic acid, methyl ester | -6.3 |
| 9-Octadecenoic acid (Z)-, methylester | -6.7 |
| 9-Oxabicyclo[6.1.0]nonane | -4.9 |
| Methyl stearate | -5.5 |
| Oxiraneundecanoic acid, 3-pentyl-,methyl ester, cis- | -6.0 |
| Undec-10-ynoic acid, undecyl ester | -5.9 |
| Standard drug FLAGYL | -4.6 |
| Hexadecanoic acid, methyl ester | -5.6 |
| Cocrystallize ligand | -7.8 |

The interaction of the compounds with better binding affinities and the commercial drug were carried out on the enzyme to confirm whether the docking was on the active site and pocket. The results were discussed below.

***Interaction Result of Phytocompound with Diarrhea Protein***

Figs. 1-6 were the interaction results of the extracts, cocrystallize ligand and commercial drug to determine the types of bond they formed with the disease protein, these were done using pyRx software and the results were recorded in Table 3.



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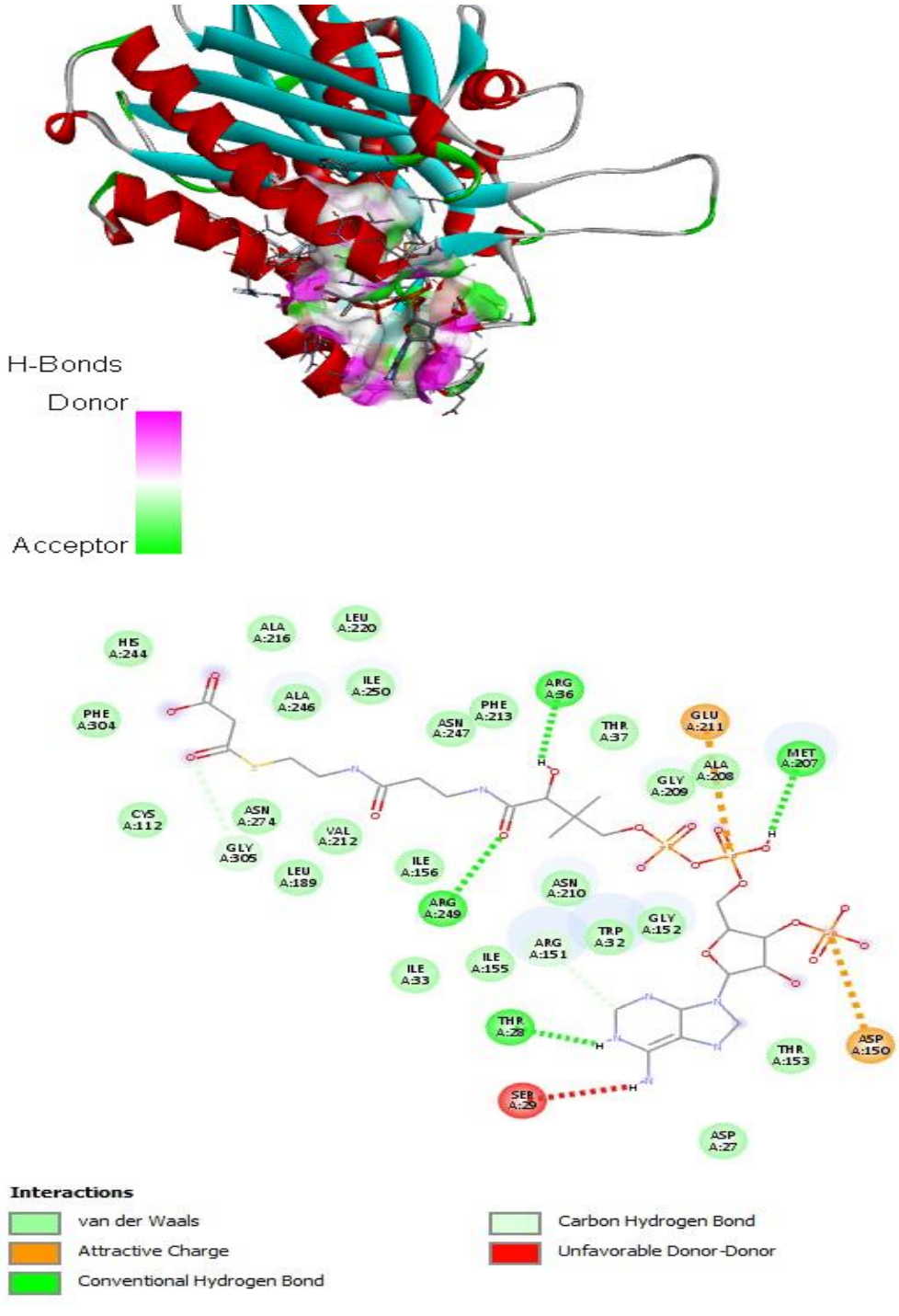



Fig 1: Interaction of protein with co crystallize ligand



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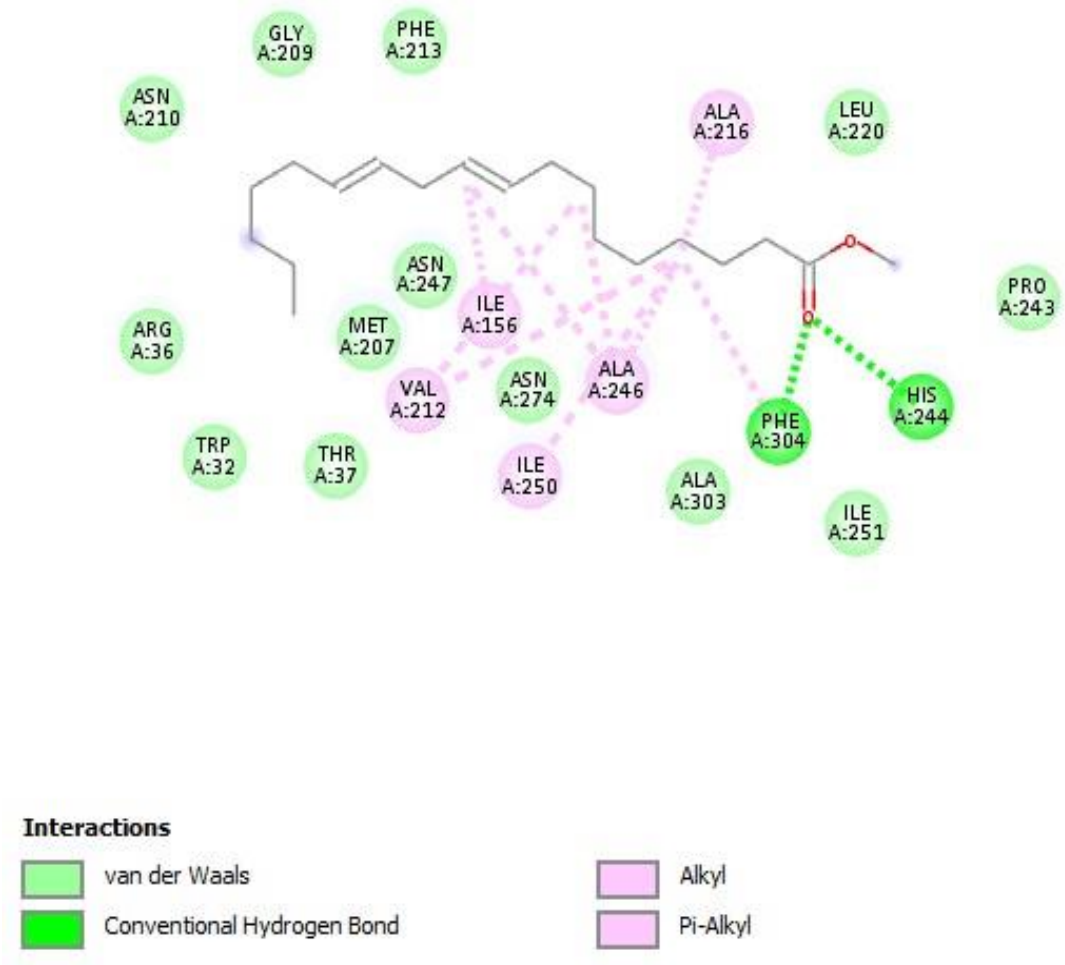



Fig 2: Interaction of protein with 9,12-Octadecadienoic acid, methyl ester



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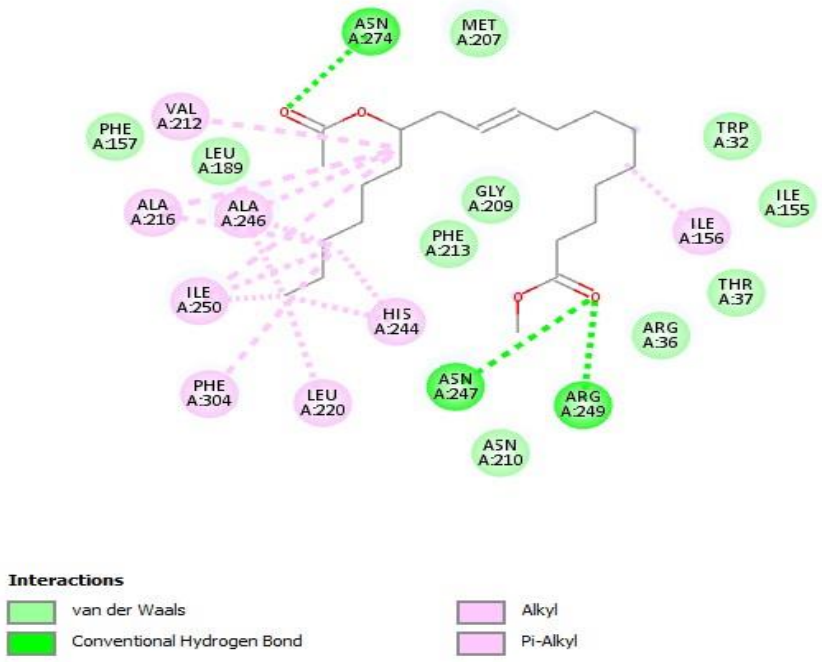



Fig 3: Interaction of protein with 9-Octadecenoic acid (Z)-, methylester

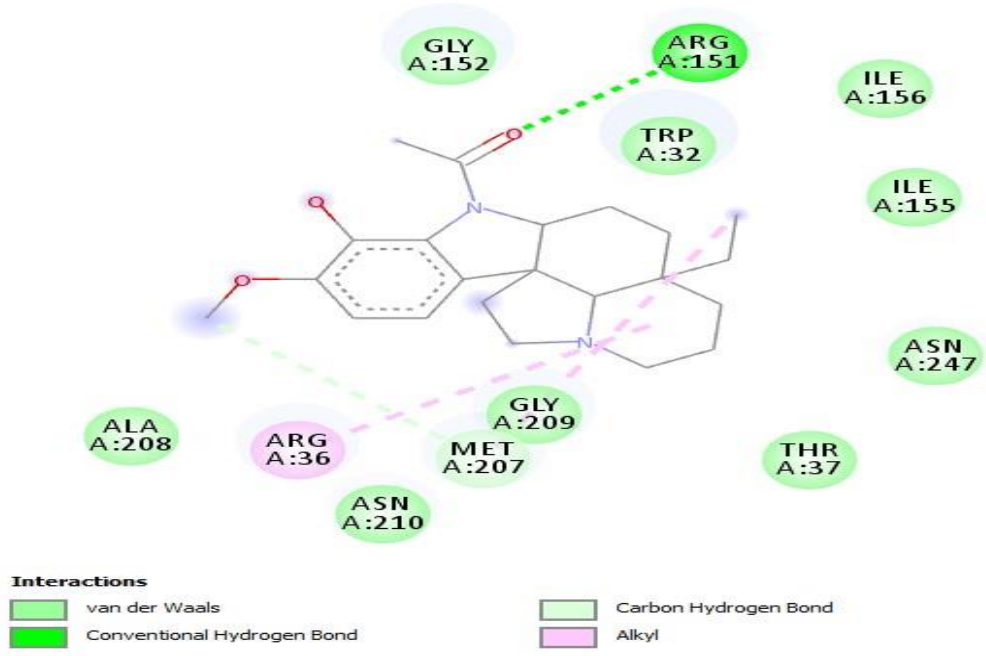



Fig 4: Interaction of the protein with Aspidospermidin-17-ol



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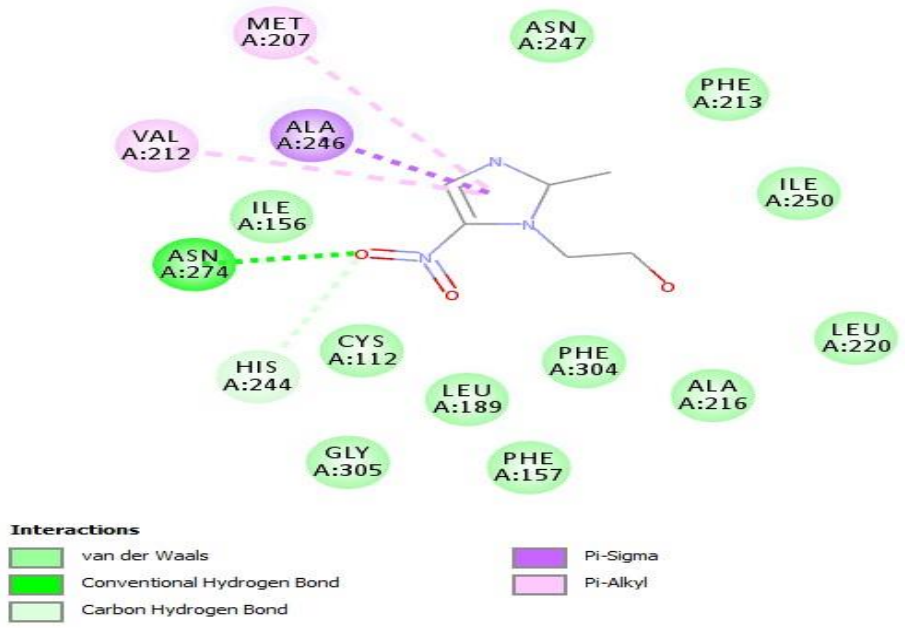



Fig 5 : Interaction of the protein with commercial drug Flagy

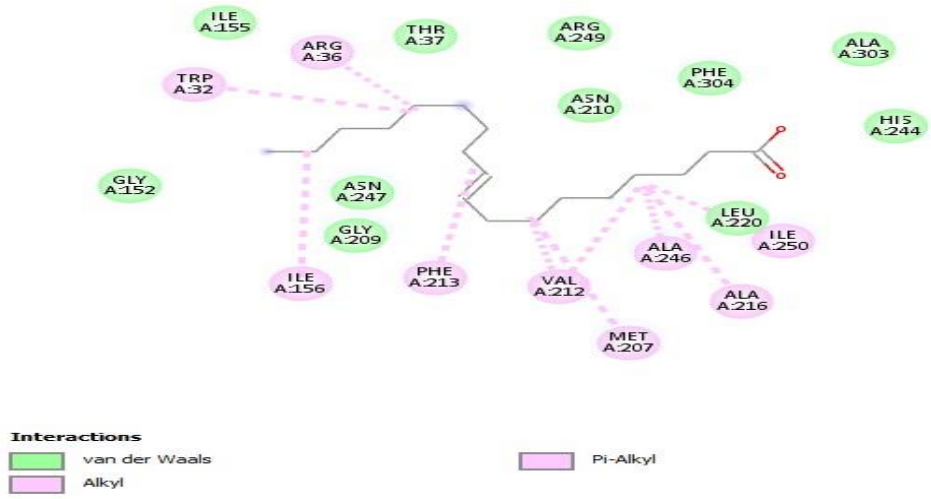



Fig 6: Interaction of the protein with Oleic acid.

The interaction results above showed that some of the identified compounds have significant potentials to block the active site of the enzyme.

Out of the 13 identified compounds, 4 compounds with binding affinities close to the co-crystallize ligand and the reference drug were used to study their interaction with the disease protein. Co-crystallize ligand has the minimum value of the binding affinity (-7.8kcal/ mol). it interacted with the FabH enzyme through four conventional hydrogen bond MET 207, ARG 36, ARG 249 and



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THR 28 and two carbon hydrogen bond GLY 305 and ARG 151. It has an attractive charge ASP 150 and an unfavourable donor-donor bond SER 29. Co-crystallize ligand also form hydrophilic vander waals bond with ASP 27, THR 153, ILE 33, ILE 55, TRP 32, GLY 152, ASN 210, GLY 209, ALA 208, THR 37, PHE 213, ASN 247, HIS 244, ALA 216, LEU 220,PHE 304, ALA 246, ILE 250, CYS 112, ASN 274, LEU 189, ILE 156, ASN 210,and PHE 213. Commercial drug flagyl although with the highest affinity has the highest interaction with conventional hydrogen bond ASN 274 and Carbon Hydrogen bond HIS 244. Pi sigma bond ALA 246 and Pi alkyl bond with MET 291 and VAL 212. It also has hydrophilic vander waal bond with ILE 156, ANS 247, PHE 213, ILE 250, LEU 220, ALA 216, PHE 157, LEU 189, CYS 42 AND GLY 305

Extract 9-octadecanoic acid (z) methylester with least affinity -6.7kcal/mol interact with the disease protein using three conventional hydrogen bond ASN 247, ARG 249 and ASN 274, Pi alkyl and Alkyl bond with VAL 212, ALA 216, ALA 246, ILE 250, PHE 304, LEU 220, HIS 244 and ILE 156. It also formed vander waal bond with MET 207, TRP 32, ILE 155, THR 37, ARG 36, ASN 210, GLY 209, PHE 213, LEU 189, and PHE 157. Another phytocompound Aspidospermidin-17-ol with affinity -6.1 constructed conventional hydrogen bond with ARG 151, carbon hydrogen bond with MET 207. It has an Alkyl bond with ARG 36 and vanderwaal bond with ALA 208, GLY 209, ASN 210, THR 37, ILE 155, ILE 156, TRP 32 and GLY 154. Bioactive compound 9.12-octadecanoic acid with affinity -6.3 formed two conventional hydrogen bond with amino acids PHE 304 and HIS 244. Pi alkyl and alkyl bonds with ILE 156, VAL 212, ALA 246. ILE 250, ALA 216. And hydrophobic vander waal bond with ASN 210, GLY 209, PHE 213ARG 36, TRP 32, THR 37, MET 207, ASN 247, AND 274, PRO 243 and LEU 220. Aspidospermidin-17-ol, 9,12-octadecanoic acid and 9-octadecanoic acid methyl ester have better interactions than Oleic acid. This an indication that the plant can be used in the treatment of diarrhea because most of the extracts were recorded to contain antimicrobial activity. The control flagyl although with the highest activity -4.7 kcal/ mol showed the best interaction indicating that binding affinity may not be the best way to determine efficacy of a compound.

Table 3. Docking results showing the type of interaction and amino acid involve

| Compound | Binding affinity | Type of interaction | Amino acid |
|---|---|---|---|
| 9,12-octadecanoic acid | -6.3 | Conventional hydrogen bond<br>Pi Alkyl and Alkyl<br><br><br><br>Vander waal | PHE 304.HIS 244<br><br>ILE 156, VAL212, ALA246, ILE 25ALA 216, |



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| | | | |
|---|---|---|---|
| | | | ASN 210,GLY 209, PHE 213,ARG36 TRP 32 THR37, MET 207, ASN 247, ASN274, ALA 303, ILE 251, PRO 243, LEU 220 |
| 9-octadecanoic acid (z) methyl ester | -6.7 | Conventional hydrogen bond<br><br>Pi Alkyl and alkyl<br><br><br><br><br>vander waal | ASN 247, ARG 249, ASN 274<br><br>VAL 212, ALA 216,ALA 246, ILE 250, LEU 220,ILE 156, HIS 244, PHE 304.<br>MET 207, TRP 32, ILE 155,THR 37, ARG 36, ASN 210, GLY209, PHE 213, LEU 189. PHE 157 |
| Aspidospermidin-17-ol | -6.1 | Conventional hydrogen bond<br><br>Carbon hydrogen bond<br><br>Alkyl<br><br>Vander waal | ARG 151<br><br>MET 207<br><br>ARG 36<br><br>ALA 208, GLY 209,ASN 210,THR 37, ILE 155, ASN 247, ILE 156, TRP 32, GLY 154 |
| Flagyl | -4.7 | Conventional hydrogen bond<br>Carbon hydrogen bond<br>Pi Sigma bond<br>Pi alkyl<br><br>Vander waal | ASN 274<br><br>HIS 244<br>ALA 246<br>MET 207, VAL 212<br><br>ILE 156, ASN 247, PH 213, ILE 250, LEU 220,LEU 189, GLY 305,CYS 42, ALA 216,PHE 157 |
| Oleic Acid | -6.1 | Alkyl | ILE 156, PHE 213, VAL 212, MET 207, ALA 246, ALA 216, |



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| | | | |
|---|---|---|---|
| | | | ILE 250, ARG 36, TRP 32 |
| | | Vander waal | ILE 155, THR 37, ARG 249, PHE 304, ALA 303, HIS 244, LEU 220, ASN 247, GLY 209, GLY 152 |
| Co crystallize ligand | -7.8 | Conventional hydrogen bond<br>Carbon hydrogen bond<br>Attractive charge<br>Unfavourable donor-donor<br>Vander waal | MET 207, ARG 36, ARG249, THR 28<br>GLY 305. ARG 151<br>ASP 150<br>SER 29<br>ASP 27, THR 153, ILE 33, ILE55, THR57, PHE 213, ASN247, TRP 32, GLY 209,GLY 152,ASN 210, ALA 208, HIS 244, ALA 216, LEU 220,PHE 304, ALA 246, ILE 250, CYS 110, ASN 274, LEU 189, ILE 156, ASN 210, PHE 213 |

## CONCLUSION

The result obtained from the GC-MS showed that *Pterocarpus santalinoides* leaf contain 13 bio-active compounds with known medicinal, biological and therapeutic properties with their percentage composition as follows: oxiraneundecanoic acid, 3-pentyl cis-methyl ester (37.89%), Oleic acid (35.49%), 9-octadecenoic acid (12.35%) Hexadecanoic acid methyl ester (3.96%) and Docosanoic acid, methyl ester (3.68%) were the most abundant compounds in the extract. Oleic acid and oxiraneundecanoic acid, cis -3-pentyl-methyl-ester, gave up to 73% of the total percentage of compound found in the extract. Hexadecanoic acid methyl ester and 9,12-octadecenoic acid have been reported to show



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anti-diarrhea activity thereby positively validating the claims made ethnomedically that *Pterocarpus santalinoides* can cure diarrhea diseases [31, 32]. Diarrheal disease has been ranked as the second leading cause of mortality of children. There are about 1.7 billion cases per year of diarrheal disease in children below the age of 5 across the continents. The death rate by diarrheal disease is about 1.5 million per year, which include 620 thousand children below the age of 5, and 320 thousand adults above the age of 70 [38]. The occurrence of childhood mortality in developing countries due to diarrheal diseases has been found to be between 9% and 34% [39, 40]. Diarrhea may be inflammatory, secretory, osmotic, or neurogenic as inflammation has been an underlying factor in most health condition like diabetes, arthritis, heart disease and cancer. *Pterocarpus santalinoides* leaf is reported to have been used medically as anti- inflammatory, anti-oxidant, anti-arthtis, anticancer etc, this can be attributed to the presence of phytochemicals like oleic acid, phytol, and Oxiraneundecanoic acid, cis -3-pentyl-, methyl ester, Aspidospermidine is an alkaloid and is reported to be responsible for the antimalarial and anti-diabetic activity of *Pterocarpus santalinoides.* This research validated the ethnomedical claims of this plant due to its potential phytochemicals.

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