The Insilico Molecular Docking of the Compounds of the Crude Extract of Dacryodes Edulis Leaves Against Alpha Enolase to Assess Its Activities on the Treatment of Bacterial Pneumonia

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Abstract- ***The leaves of Dacryodes edulis (Burseraceae) is an herb used locally for the treatment or management of earache, respiratory problems, and generalized pneumococcal diseases. The identification of the phytochemical components, and their inhibitory activities on alpha- enolase on the development of Streptococcus pneumonia has been studied. The leaves were screened for phytochemicals using gas chromatography-mass spectroscopy (GC-MS), and Fourier-transform infrared spectroscopy (FTIR). in silico molecular docking against alpha-enolase via reduction of the plasminogen binding of Streptococcus pneumoniae was used to access the inhibitory effect of the leaves sample on the development of Streptococcus pneumonia. Results revealed presence of alkaloids, flavonoids, tannins, phenols, saponins and terpenoids. Major functional groups were O-H, and N–H groups. Out of 12 compounds with medicinal properties, molecular docking revealed 7,9-Di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione (-4.3 kcal/mol), Viridiflorol (-4.1 kcal/mol), and 2,4-Di-tert-butylphenol (-4.1kcal/mol) as promising inhibitors that fabricates finest attractive charges and conventional hydrogen bonds with Streptococcus pneumoniae alpha-enolase compared with standard antibiotic Amoxicillin (-5.0Kcal/mol). These compounds could inhibit the role of alpha-enolase to restrain plasminogen binding, invasion and progression of Streptococcus pneumoniae.***

Indexed Terms- Bioactive compounds, phytochemical, Dacryodesedulis, chromatography, pneumococcal.

I. INTRODUCTION

The therapeutic capability of plants has long been recognized since the antediluvian age when man and animals chew raw herbs to treat infections and sickness. The therapeutic properties of plants are believed to be product of the plant's primary and secondary metabolites [1]. The primary metabolite of plants (proteins, carbohydrates, and amino acids) are produced during basic physiological processes that aid plant growth, while the secondary metabolite is a large number of specialized compounds produced from biosynthetic enzymatic reactions of the primary metabolites. Investigations done on some plants have shown that these compounds possess different culinary, medicinal, and nutraceutical properties [2,3]. With the emergence of new diseases and resistance of some pathogens to available antibiotics, medicinal plants have been a resort, and the search for plants with therapeutic effect has increased, consequently the misuse of traditional antibiotics has also increased. Pharmaceutical companies are determined to produce masses of new antibacterial from plant isolates.

Streptococcus pneumoniae is a Gram-positive, alpha-hemolytic and facultative anaerobic bacterium which belongs to Streptococcus genus that has been considered as one of the human pathogens for severe infections causing life threatening diseases such as pneumonia, meningitis, otitis media, and sepsis among infants and young children globally. In 2008, 1.6 million deaths of children (less than the age of 5 years) were caused by invasive pneumococcal disease (IPD) [2]. The world health organization (WHO) ranked *S.*

pneumoniae at 12th place among critical pathogens because of its expanding ability of resistance against present antibiotics [4]. *S. pneumoniae* exhibits several factors that assist in disseminating and invading the host body system. However, binding of exposed cell surface proteins of *S. pneumoniae* with plasminogen (PLG) as a profibrinolytic component, initiate proteolytic activity, is a significant step in pathogenesis to invade human. Once PLG is converted into active plasmin, it degrades the extracellular matrix and assists pathogen to transmigrate across tissue barriers into deeper tissue sites [5]. A study supervised by Pancholi and Fischetti revealed that *alpha-enolase* is involved in the pathogenic effect of *S. pneumoniae* because of potent cell-surface PLG binding protein. Over and above, *alpha-enolase* possesses a significant PLG binding as compare to other cell-surface proteins [6, 7]. Hassan et al. (2021) reported the enzyme *alpha-enolase of S. pneumoniae* as a potential target in inhibiting *S. pneumoniae progression*. Surface-associated *alpha-enolase* is a multifunctional glycolytic enzyme persuaded in cellular stress, parasitic infections, autoantigen activates, and the reproduction, growth, and development of organisms [7]. Therefore, inhibiting *S. pneumoniae alpha-enolase* can be a route of development of protein-based vaccine for treatment of invasive pneumococcal disease (IPD).

Dacryodes edulis commonly known in English as African pear, bush butter, or native pear is widely claimed to have originated from the southeastern part of Nigeria where it is called “ube” [5]. The plant is a dioecious shade-loving species that grows to a height of 12–42 m, with feathery-like shiny leaves and it is found in the humid tropical zone of the non-flooded forest [2]. In many parts of West Africa including Nigeria, local medicine practitioners have made numerous claims regarding the diverse pharmacological uses of leaves of this plant. Chewed with kola nut, the leaves have been employed as an antiemetic. Sap from the leaves is used as an ear drop to treat ear infections, while decoctions are prepared as a remedy for fever and headache [7,8,9]. Some preparations from the leaves are employed to treat digestive tract disorders, toothache, and to cure skin diseases, such as rashes, scabies, ringworm, and wound. Local medicine practitioners in southwest Cameroun also claim that the leaves can be employed as plaster to treat snake bites [7]. The synergistic effect of decoction of the leaves with other plant leaves has been claimed to ameliorate high blood sugar, hypertension, and also ease labor pains [10]. With so many claims of therapeutic applications of this plant by local medicine practitioners, researchers have investigated the chemical properties of these leaves to correlate and validate these claims. Phytochemical screening of the leaves has revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, and glycoside [5,11]. Potential antimicrobial, anti-oxidant and antimalarial properties of *dacryodes edulis* leaf extract had been reported by Ezeabara *et al*. [11]; Ononamadu *et al*. [5]; Hassan-Olajokun *et al*. [12]. Some researchers have also reported the possible employment in the treatment and management of diabetes and hypoglycemia [5,13,14]. So far to the best of our knowledge, there is no report on the use of the leaves for the treatment of invasive pneumococcal disease. Computational methods such as homology modeling, molecular docking, and dynamic simulations, have been successfully used to study potential compounds against invasive pneumococcal disease. Computer-aided docking simulation has been proposed as one of the target-based methods for the discovery of inhibitors of alpha enolase.[17] Therefore, the aim of this study is to identify broad-spectrum molecular targets from the leaves of *dacryodes edulis* with high efficacy and no significant adverse implication on human systems. Identification of novel molecular targets and lead compounds for the treatment of invasive pneumococcal disease will be a means of overcoming the rising antibiotic resistance in bacteria.

II. MATERIAL AND METHODS

- Chemicals and reagents

The chemicals were of analytical grade and were sourced from a licensed analytic grade chemical distributor at Onitsha, Anambra State, Nigeria.

- Sample collection

The leaves of Dacryodes edulis were plucked from a pear tree at Umuehie, Umuezeala Nsu of Ehime Mbano local government in Imo State, Nigeria. The herbarium sample was collected and verified by a professional taxonomist at the Imo State University

Owerri Nigeria and given a voucher specimen number IMSUH- 472 and was deposited at the Imo state University herbarium. The fresh leaves were shade dried for 3 weeks at room temperature to obtain dry leaves, then coarsely powdered using a mortar. The ground sample (500 g) was macerated in 1000 mL of methanol (99%) for 72 h in airtight glass cylinders. The extract was filtered with Whatman No.1 filter paper and concentrated in vacuo at 55 °C using a rotary evaporator model Yamato RE301 digital rotary evaporator. This procedure is a replica of the method by Ikpa and Maduka [2].

- Qualitative Phytochemical Screening

Qualitative phytochemical screening of the plant extract was carried out according to Njoku [15] and Ikpa and Maduka [2], investigating the presence of the following phytochemicals: alkaloids, saponins, tannins, phenols, terpenoids, steroid, glycosides, and flavonoids.

- FTIR Analysis of the *Dacryodes edulis* Sample Extract

Fourier transform infrared spectrophotometer (SHIMADZU Model no 84008 at the National research institute, Zaria) was used to determine the functional groups present in the *D. edulis* leaves extract. The shifts of the FTIR peak numbers were closely scrutinized to accurately identify distinctive functional groups in the leave extract. The results were interpreted using sigma Aldrich/merch IR spectrum chart.

- Gas Chromatography-Mass Spectrometry Analysis of *Dacyrodes edulis* extract

The Gas chromatography-mass spectrometric investigation of the phytochemical profile of the extract was analyzed at the Shimadzu Training Center for the Analytical Instrument (STC) in Lagos, Nigeria. The analysis was done using the GC-MS-QP2010SE SHIMADZU instrument. The conditions for the analysis is described as follows: injection temperature was set at (200 °C), injection mode split ratio was at (1:1), carrier gas (Helium) flow rate was (1.56 mL/min), the system temperature was programmed from 60 °C (at 10 °C/min) to 160 °C (held for 2 min) then at (10 °C/min) to 250 °C and the injection volume was 0.5µL. Mass spectrophotometer condition: Ion source temperature 200 °C and Interface temperature 250 °C solvent cut time 4.5 min and the acquisition was in the scan mode. The results were interpreted using the NIST GC–MS library [17].

- Molecular docking

- Protein preparation

The X-ray diffraction-based crystal structure of *S. pneumoniae alpha-enolase* (PDB ID: 1W6T) in complex with MG and 2PE inhibitors, was retrieved from Protein Data Bank (PDB) with 2.0 A° resolution [18]. The complex bonds and water molecules were removed in the PyMol software. Auto dock tools (ADT) were utilized to add polar hydrogens and Kollman united charges to increase susceptibility towards electronegative atoms [19].

- Preparation of ligand library

The PubChem database was used to retrieve structures of the ligand. ADT was adopted to check Gasteiger charges for selected ligands, and a torsion count widget was chosen to classify rotatable and non-rotatable bonds of ligands.

- Docking and screening

In this study, the authors have also performed molecular docking simulation studies of small molecules present in the leaves of *D. edulis* G against *S. pneumoniae alpha-enolase* to understand the molecular interaction analysis of these compounds and its binding site. In this study, we used AutoDock vina in PYRX software for molecular docking and BIOVIA Discovery Studio to visualize results. AutoDock Vina implements sophisticated optimization and Pepto-Grid algorithm for rescoring the rate of ligand atom's appearance at a given grid box with specific characteristics [25, 26]. The results are evaluated using X-score implemented by AutoDock Vina, it is a weighted sum of stearic interactions, the interaction of hydrophobic atoms, and hydrogen bonding [25]. Swift, adequate scoring, and binding algorithm give rise to confident results that make it valuable in the spectrum range of docking tools.

III. RESULTS AND DISCUSSION

- Phytochemical screening result

The phytochemical analysis of the methanolic leaf extract of *D. edulis* revealed the presence of alkaloids and flavonoids in high concentration, tannins, phenols, and saponins were in moderate concentration while the phytochemicals with the least concentration in the study were Terpenoids, glycosides, and steroids. The result presented in (Table1) is similar to the report made by Olasunkanmi and Adeniyi [17] and Hassan-Olajokun *et al.* [12]. The phytochemical compounds detected have been reported to be biologically active and thus contribute to the therapeutic application of *D. edulis.* Alkaloids have been used as an analgesic, anticancer and bactericidal agents, they account for the antimicrobial activity of the plant leaves [18]. Flavonoids and phenols show effective antibacterial activity due to complex formation with extracellular and soluble proteins, in the same vein complexes with the cell walls of bacteria leading to the ultimate death of the bacteria. Flavonoids also possess anti-inflammatory, anti-allergic, antiviral, anti-thrombotic, vasodilatory, and antioxidant properties [19, 20]. The antitrypanosomal and antileishmanial activities of flavonoids have also been reported [21]. Studies suggest that the presence of flavonoids in the human diet may reduce the risk of coronary heart disease, lowering the risk of various cancers [22], and also prevent menopause symptoms [23]. Tannins have been reported to exhibit numerous therapeutic properties as they have been used for the treatment of burns and other wounds due to their hemorrhagic and antiseptic potentials [24]. They have also been used as anthelmintics, antioxidants, and antiviral, anticancer, and also to chelate dietary iron. The mode of action of tannins has been said to be related to their ability to form strong complexes with proteins and other macromolecules thereby inactivating microbial adhesins, and some enzymes [25, 26]. Studies have shown that Saponins exhibit cytotoxic effects and growth inhibition against a variety of cells which accounts for their anti-inflammatory, anti-cancer properties and antitumor activity in animals [6]. Terpenoids show antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* and are used in antischistosomal therapy [27]. Glycosides and Steroids have also been reported in some literature to contain various therapeutic properties [13, 28]. The presence of these detected phytochemicals in *D.edulis* leaf extract justifies the local medicinal uses of the leaves.

Table 1 The result for the qualitative and quantitative phytochemical screening are presented

s/n	Phytochemical	Remark	Composition (%)
1	Alkaloids	+	4.66 ±0.14
2	Saponins	+	1.94 ±1.42
3	Tannins	+	2.67 ±0.01
4	Phenols	+	2.74 ±1.17
5	Terpenoids	+	O.54 ±1.15
6	Steroids	+	NA
7	Glycosides	+	0.34 ±0.20
8	Flavonoids	+	3.80 ± 0.04

+: Present

Value = Mean SD of triplicate values

NA not analyzed for quantitative analysis

- Functional Groups Present in *Dacryodes edulis* leaves Extract

The absorption spectrum of the methanol leaf extract of *Dacryodes edulis* was presented and summarized in table 2. The peaks from the spectrum in fig 1 showed shifts in absorption bands which were used to determine the functional groups present in the extract. Major functional groups detected were hydroxyl groups O-H and strong stretching carboxylic acid O–H. Primary and secondary amines and amides N–H stretch. O–H of Phenols and tertiary alcohols, C = O of Saturated Carbonyl compounds and carboxylic acids, as well as the absorption bands of Nitriles, S-H stretching of thiols. The functional group detected correlate with results from the phytochemical compounds detected, the functional groups detected is summarized in table 2 while Fig. 1 shows the spectrum. This result correlates with the investigation on the seed made by Ikpa and Maduka [2].

Fig1 FTIR spectra of the *Dacryodes edulis* seed extract

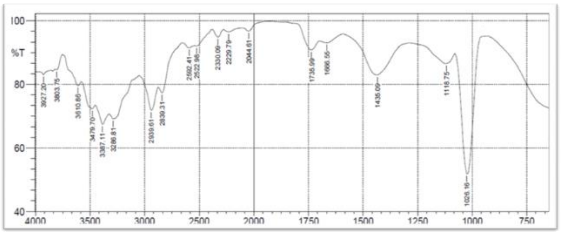


Table 2 Tabulated data showing the interpretation of the FTIR spectra

SN	λ (reference)	λ (test sample)	Functional grp	Comment
1	> 3500	3927.2	O-H non-bond	Oxygenated organic compounds
2	> 3500	3803.75	O-H stretch	Oxygenated organic compounds
3	> 3500	3610.86	O-H stretch	Acids and alcohols
4	3650-3200	3479.7	N-H, O-H	carboxylic acids; Amines,
5	3570-3200	3387.7	N-H stretching	Aliphatic primary amine
6	3570-3200	3286.81	O-H stretching	Alcohol or carboxylic acid
7	3000-2840	2939.61	C-H stretching	alkane
8	3000-2800	2839.31	O-H stretching N-H stretching	Alcohol or carboxylic acid or Aliphatic primary amine
9	2600-2550	2592.41	S-H stretching	Thiols
10		2522.98		
11	2376-2248	2330.09	C ≡N	Stretching Nitriles
12	2300–1990	2229.79	C–C	Multiple bonding beside C–C triple bond
13	2100–1800	2044.61	C=O, C=C=C, N=C=S	Allene, isothiocyanate, carbonyl
14	1750–1700	1735.99	C=O stretching	Carbonyl, esters, δ-lactone
15	1600-1400	1666.55	C=C stretching	Substituted alkenes
16	1465, 1450	1435.09	C-H stretching S-O stretching	Methylene and methyl groups, sulfate groups
17	1124-1087	1118.75	C-O stretching	Secondary alcohol
18	1150-1085	1026.16	=C-O-C	Ethers symmetric stretch

Where λ = wavelength

- GC-MS Result of the *Dacryodes edulis* leaves Extract

GC-MS is one of the best techniques used for the identification of bioactive constituents of volatile organic matter, long-chain branched-chain hydrocarbons, acids, alcohols, esters, etc. the GC-MS analysis in this study (Fig. 2) shows 52 retentions in the spectrum of the crude sample. However, 12 major peaks were selected due to compound composition which was determined with GC–MS normalization result. The selected and identified peaks have been presented in Table 3, The medicinal properties of some of the selected compounds have been discussed.

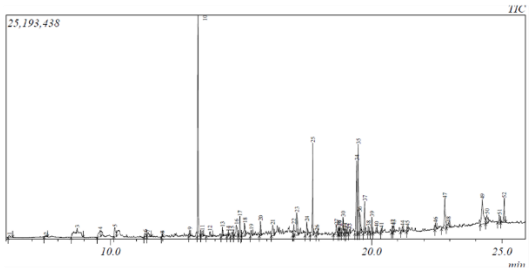
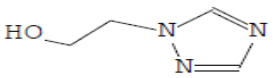
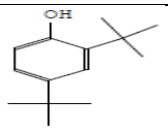
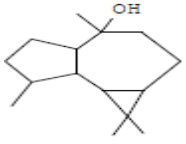
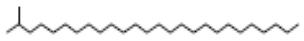
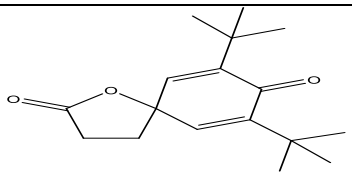
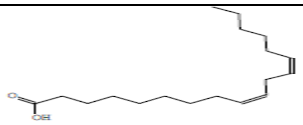
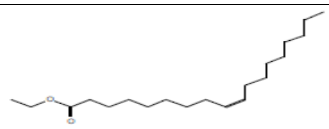
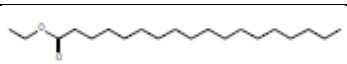
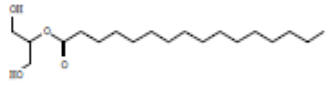
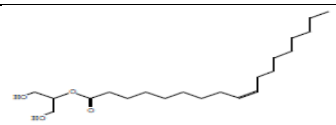
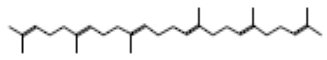


Fig. 2 The total ion chromatogram of the *Dacryodes edulis* leaf extract.

1-(2-Hydroxyethyl)-1,2,4-triazole is present as a core structural component in an array of drug classifications such as antimicrobial, anti-inflammatory, antitubercular, analgesic, antianxiety antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anesthetic, antidepressant, antiobesity, antioxidant, anti-Parkinson′s, antidiabetic, antihistaminic, and immune modulatory agents [31]. 2,4-Di-tert-butylphenolderivative is known for its antibacterial and anti-inflammatory activities [32, 33]. Octadecanoic acid has also been reported to possess Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary, antipsychotic, and insectifuge activities [34]. The potential medicinal use of n-Hexadecanoic acid as an antioxidant, hypocholesterolemic, nematicide, pesticide, and lubricant, antiandrogenic, as well as a flavoring agent, hemolytic, and a 5-alpha reductase inhibitor has been reported [35]. Squalene a triterpenoid and precursor for steroids has a role in topical skin lubrication and protection [36]. Although there are speculations that ethyl oleate is a toxic mediator of ethanol in the body (pancreas, liver, heart, and brain) [37]. Ethyl oleate has been used as a solvent for pharmaceutical drug preparations involving lipophilic substances, it is also used as a lubricant and a plasticizer for plastic productions [38]. 7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione possess noticeable pharmacological activities including antineoplastic, antimicrobial and antiviral activities [39]. The use of (E)-9-Octadecanoic acid ethyl ester for perfumery purposes has also been reported.

Table 3: Compounds identified from the GC-MS analysis of the *dacryodes edulis* leaves extract

s/n	IUPAC name	Chemical name	MF	Pubchem id	% conc	MW	Structure
1	1-(2-Hydroxyethyl)-1,2,4-triazole	-	$C_4H_7N_3O$	550925	5.25	113	
2	2,4-Di-tert-butylphenol	-	$C_{14}H_{22}O$	7311	15.00	206	
3	(1aR,4S,4aS,7R,7aS,7bS)-1,1,4,7-tetramethyl-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulen-4-ol	Viridiflorol	$C_{15}H_{26}O$	11996452	2.06	222	

4	2-methylhexacosane	-	$C_{27}H_{56}$	150931	2.02	380	
5	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	-	$C_{17}H_{24}O_3$	545303	3.56	276	
6	cis-9,cis-12-Octadecadienoic acid	Linoleic acid	$C_{18}H_{32}O_2$	5280450	6.80	280	
7	ethyl (Z)-octadec-9-enoate	Ethyl oleate	$C_{20}H_{38}O_2$	5363269	8.36	310	
8	(E)-9-Octadecenoic acid ethyl ester	Ethyl elaidate	$C_{20}H_{38}O_2$	5364430	2.94	310	
9	Octadecanoic acid, ethyl ester	Ethyl stearate	$C_{20}H_{40}O_2$	8122	3.02	312	
10	1,3-dihydroxypropan-2-yl hexadecanoate	2-Palmitoylglycerol	$C_{19}H_{38}O_4$	123409	3.80	330	
11	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2-Monoolein	$C_{21}H_{40}O_4$	5319879	5.68	356	
12	(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	Squalene	$C_{30}H_{50}$	638072	2.19	410	

- Molecular docking Result of the *Dacryodes edulis* leaves Extract

The compounds were docked to the active site of the pneumococcal alpha-enolase comprising of residues $_{248}$FYDKERKVY$_{256}$ (fig 3b) (Bergmann et al., 2003).

This site is exposed on the surface of the alpha-enolase and contributes 570 $A^{\circ 2}$ of the total surface area supporting the notion that this is the prime plasmin(ogen)-binding site. The active site is part of L3, one of three catalytic loops that closes the active site upon substrate binding (Ku¨hnel et al., 2001). When displayed on the surface of pneumococcal cells, the glycolytic activity of alpha-enolase is no longer relevant, freeing this loop for other functions. Systematic permutation of the pneumococcal peptide $_{248}$FYDKERKVY$_{256}$ indicates that D250, E252, K251 and K254 are the active residues critical for plasmin(ogen)-binding (Ehinger et al., 2004, Bergmann et al., 2003). The results demonstrated that the adopted inhibitors were inside the pocket of the active site of alpha-enolase and inhibited successfully with possible interactions, but some of them showed a negligible binding affinity with active site residues. The results ranked based on binding affinity, an inhibitor with a lower value of binding affinity is supposed to established strong interaction on a specific active site (Table 4).

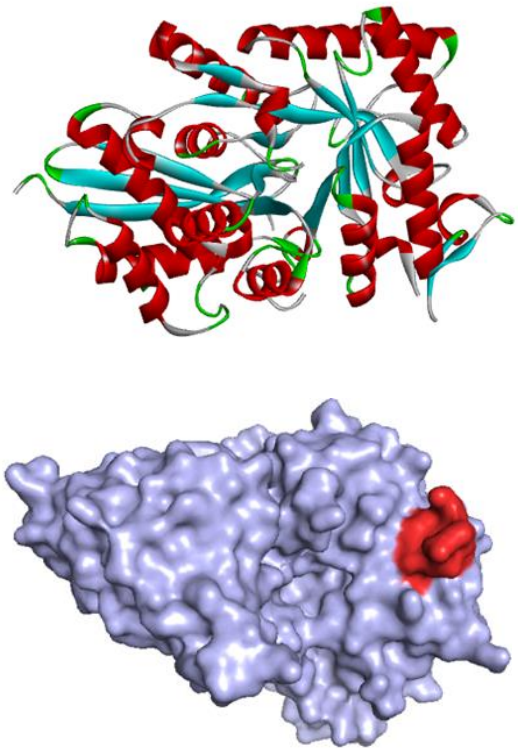


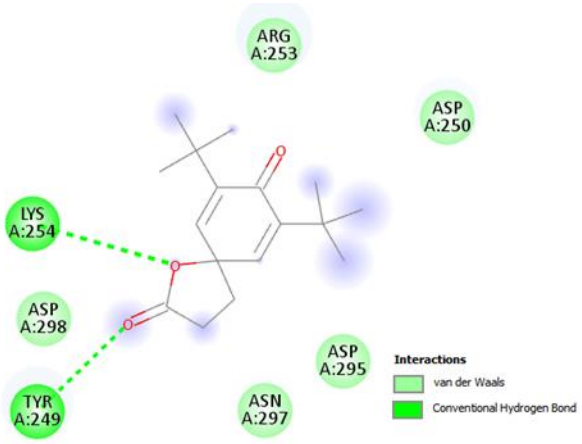
Fig 3: Visualization of alpha-enolase (1W6T) chain a (a) cartoon model (b) surface model with the active site colored in red.

Table 4: Inhibitors ranked based on their binding affinity

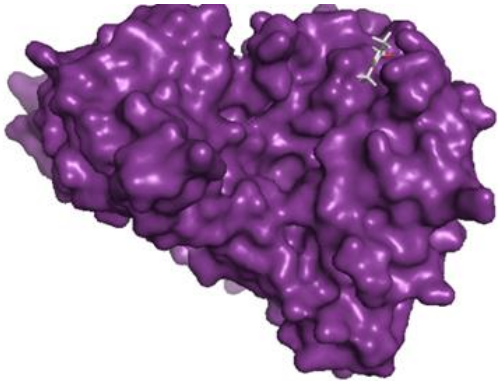
s/n	Compound	Pubchem id	PYRX (kcal/mol)	VINA (kcal/mol)
1	Amoxicillin	33613	-5.0	-5.0
2	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	545303	-4.3	-4.3
3	Viridiflorol	11996452	-4.1	-4.1
4	2,4-Di-tert-butylphenol	7311	-4.1	-4.1
5	Squalene	638072	-3.7	-3.8
6	2-Palmitoylglycerol	123409	-3.3	-3.3
7	Linoleic Acid	5280450	-3.2	-4.1
8	9-Octadecenoic acid, ethyl ester	5364430	-3.2	-2.8
9	2-Monoolein	5319879	-3.1	-3.4
10	1H-1,2,4-Triazole-1-ethanol	550925	-3.1	-3.2
11	Ethyl oleate	5363269	-2.9	-3.1
12	Ethyl stearate	8122	-2.9	-2.9
13	2-Methylhexacosane	150931	-2.7	-3.1

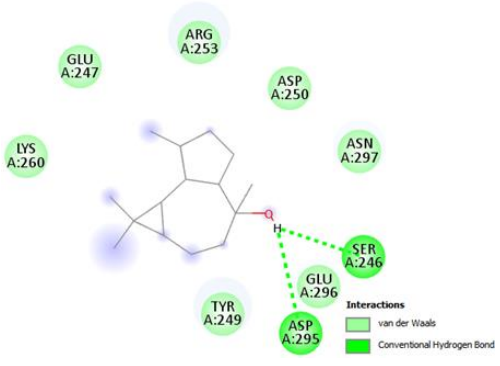
Both docking tools (PYRX and VINA) are showing the same ranking of the receptor-ligand complex based on energy scores. This ranking homogeneity cross-validated our results that some of the identified compounds showed significant potential to block active sites of enolase protein of *S. pneumoniae* with predicted binding affinity. Out of the 12 identified compounds in the extract, 3 compounds showed binding affinity close to the that of the reference drug(amoxicillin). Amoxicillin is a drug used in

treating *S. pneumonia*, and it was used as the reference drug for control in this study. Amoxicillin showed the minimum value of the binding affinity of -5.0kcal/mol. Therefore, it displayed the strongest interaction with alpha-enolase among all docked ligands discussed in this study. It constructed conventional hydrogen bonds with binding residues of SER246, ASP295, TYR249, and LYS 260. It also constructed hydrophobic Van der Waal's interactions with LYS 254, ASP250, ARG 253, GLU296, and also had a Pi-Pi T-shaped interaction with TYR249. Although Amoxicillin had the highest binding affinity score. It had only weak hydrophobic interactions with two of the major active residues LYS 254, and ASP250. The high binding affinity score due to the strong binding with other residues at the active site. The closest ligand to the control was 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione. It showed the minimum value of binding affinity of -4.3 kcal/mol. It constructed conventional hydrogen bonds with binding residues of LYS 254, and TYR 249. Moreover, residues of ASP254, ASP295, ASN297, ASP250 and ARG253 fabricated van der Waal's interactions that assist to bind the ligand more strongly inside the pocket of alpha-enolase to inhibit its activity. The compound has a strong conventional hydrogen bond with on of the major residues (LYS254) and also had hydrophobic interaction with ASP250. Viridiflorol and 2,4-Di-tert-butylphenol were also selected as prospective inhibitor due to their rank with the smilar binding energy of -4.1 Kcal/mol. Viridiflorol fabricated hydrogen bonds with protein residues of SER246, and ASP295, while most of its interactions were hydrophobic van der Waal's interactions with the residues LYS260, GLU247, ARG253, ASP250, ASN297, GLU296, and TYR249. However, it did not construct strong bond with the major active residues, instead it had a weak Van der Waal's interaction with ASP250, this arises doubts on the potential of ligand to inhibit the activity of alpha-enolase. The 2,4-Di-tert-butylphenol constructed hydrogen bond with the residue of ASP250, other interactions include van der waal's interactions with LYS254, ASN297, SER246, and GLU296. It also stabilized it nucleus with pi-anion interactions with ASP295 intercalating the binding site of the receptor by transferring of charge and it also had a p-pi T-shaped interaction with TYR249. Moreover, ASP250 residue is a major binding residue that was constructed by 2,4-Di-tert-butylphenol using the carbon-hydrogen (C-H) bond, these interactions may have escalated the stability of ligand on the binding site. while the rest of the ligands evinced ineffectual binding affinity score and bond formation. Therefore, the top three ligands the tendency to bind to the active site and prevent plasmin(ogen) binding. They can serve as leads in treating the disease.

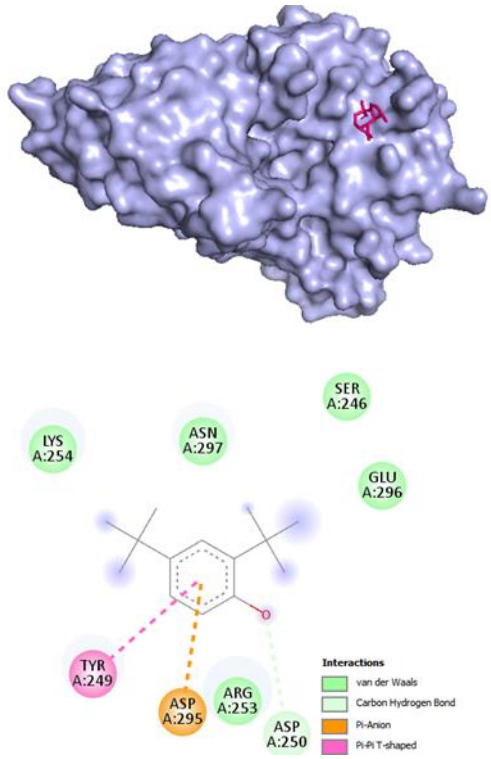


7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione)

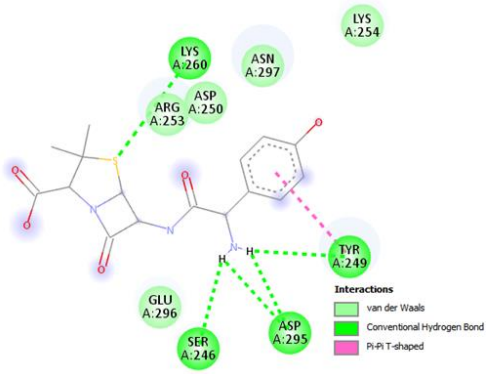




Viridiflorol



2,4-Di-tert-butylphenol



Amoxicillin

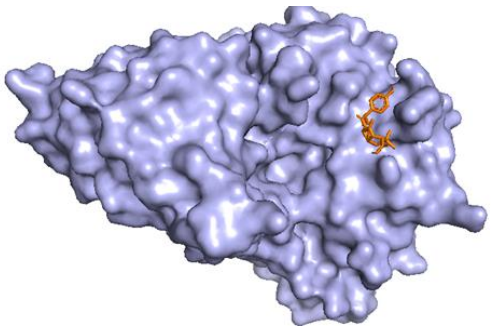


Fig. 4 Interactions of ligands with alpha-enolase residues of active„ pocket sites

CONCLUSION

The methanol extract of *D. edulis* leaves has been examined qualitatively and quantitatively for Phytocompounds using both classical and spectroscopic methods, inhibition of the *S.pneumonia alpha-enolase* enzyme by the identified compounds was also assayed using molecular docking. The results from the present study revealed that some of the identified compounds can be available for medicinal purposes. Enormous information has been garnered from this study that justifies claims made by local medicine practitioners on the therapeutic activity of the leaves. However, the researchers further suggest investigations be done by isolation of the bioactive compounds and rendering them available to pharma-companies as drug leads.

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