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Evaluation of the Antimicrobial Potentials of Selected Nut Extracts on Pathogenic Bacteria and Mycotoxigenic Fungi

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Abstract

The antimicrobial potential of nut extracts has garnered interest due to their nutritional benefits and bioactive compounds. This study evaluated the ethanoic extracts of walnut (*Tetracarpidium conophorum*), almond (*Prunus dulcis*), and "abere" (*Picralima nitida*) for inhibitory effects against various pathogenic bacteria, including *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, Shigella spp., and mycotoxigenic fungi such as Aspergillus flavus and Aspergillus fumigatus*. *In-silico* analyses provided further insights into the bioactive compounds identified through GC-MS, focusing on their drug-likeness and toxicity profiles. Two key compounds from *P. nitida* - Diethylphthalate (A1) and 3-oxo-2-pentyl-methylcyclopentaneacetic acid (A3) - demonstrated promising drug-like properties, with A1 being non-toxic and A3 showing mild carcinogenic potential. Molecular docking studies highlighted the extracts' potential as inhibitors against *Mycobacterium tuberculosis* and Dengue virus targets, with some extracts outperforming the reference drugs isoniazid and acetaminophen, although none surpassed ampicillin. This study underscores the importance of further research to isolate specific bioactive components, optimize extraction techniques, and explore the full antimicrobial potential of these nut extracts. Future studies should also consider the concentration, extraction methods, and target microorganisms to enhance the understanding of their antimicrobial efficacy.

Keywords: Antimicrobial potential; Pathogenic bacteria; *In-silico* analysis; Molecular docking studies.

INTRODUCTION

Walnut (*Tetracarpidium conophorum*), almond (*Prunus dulcis*), and "Abere" (*Picralima nitida*) are three notable nuts that are not only integral to many diets but also hold substantial nutritional and medicinal value. These nuts are celebrated for their unique health benefits and significant roles in both culinary and traditional medicine contexts. Walnuts, native to Nigeria and other parts of West Africa, are renowned for their rich content of omega-3 fatty acids and antioxidants. These compounds contribute to heart and brain health, as well as overall well-being. The nutritional profile of walnuts includes high levels of alpha-linoleic acid (ALA), vitamin E, B vitamins, magnesium, and phosphorus. ALA is particularly beneficial for cardiovascular health, cognitive function, and inflammation reduction (Gundesli *et al.*, 2021; Wu *et al.*, 2023). Beyond the nut itself, walnut by-products such as shells and green husks have shown promise in both medicinal and industrial applications, further extending the utility of this versatile nut (Liu *et al.*, 2021). However, challenges in walnut cultivation, including susceptibility to pests, diseases, and adverse climatic conditions, necessitate the adoption of sustainable practices to ensure long-term productivity (Hammed *et al.*, 2008; Malhotra *et al.*, 2008).

Almonds (*Prunus dulcis*), originally from the Middle East and now widely cultivated globally, particularly in California, are valued for their monounsaturated fats, fiber, and essential minerals such as magnesium, copper, and phosphorus. Almond consumption has been linked to improved heart health by lowering LDL cholesterol and oxidative stress. Additionally, their high fiber content supports weight management and digestive health (Kamil *et al.*, 2012; Ellis *et al.*, 2004). Almonds are rich in antioxidants, including vitamin E and polyphenols, which protect against oxidative damage (Milbury *et al.*, 2017). Despite their health benefits, almond cultivation raises environmental concerns due to high water usage and issues associated with monoculture practices (Ladizinsky *et al.*, 1999).

"Abere" (*Picralima nitida*) is a plant indigenous to West-Central Africa, including regions like Ivory Coast, Ghana, and Sierra Leone. Traditionally, "Abere" has been utilized in local medicine for treating various ailments, including malaria, fever, hypertension, jaundice, and gastrointestinal disorders (Nwaogu *et al.*, 2016; Orole *et al.*, 2017). The plant contains a range of phytochemicals, such as alkaloids, glycosides, and other

secondary metabolites, which contribute to its therapeutic effects. Sustainable harvesting practices are essential to ensure the continued availability of this valuable resource while minimizing environmental impact.

Antibiotic resistance has become a critical global health issue, largely driven by the overuse and misuse of antibiotics. This has sparked interest in alternative treatments, including phage therapy, antimicrobial peptides (AMPs), and probiotics, as promising solutions to combat resistant bacterial strains (Torres-Barceló *et al.*, 2018). Additionally, mycotoxins—harmful substances produced by fungi—pose significant risks by contaminating food and animal feed (da Rocha *et al.*, 2014). Research into the antibacterial, antigiardial, anticancer, and toxic properties of various nut extracts highlights their potential role in addressing these issues (Lebaratoux *et al.*, 2016). Bioactive compounds found in nuts, such as omega-3 fatty acids, antioxidants, proteins, and minerals, contribute to their antimicrobial properties and overall health benefits (Vincent *et al.*, 2017; Banel *et al.*, 2009; Chen *et al.*, 2019; Liu *et al.*, 2013). The seeds of *Picralima nitida* ("Abere") contain several key phytochemicals, including alkaloids (5.33±0.57 mg/g), tannins (9.60±0.05 mg/g), cyanogenic glycosides (3.39±0.03 mg/g), oxalates (4.36±0.002 mg/g), saponins (13.50±0.50 mg/g), flavonoids (5.50±0.50 mg/g), phenols (1.79±0.03 mg/g), and phytates (0.17±0.004 mg/g) (Nwaogu *et al.*, 2016). These compounds are integral to the plant's traditional medicinal uses and emphasize the need for sustainable harvesting practices to preserve this resource.

Walnuts and almonds offer significant economic and health benefits. Walnuts support global economies by providing income for farmers and contributing to the broader agricultural market. Medically, they are valued for their omega-3 fatty acids, antioxidants, and other nutrients that benefit heart health, reduce inflammation, and potentially lower the risk of chronic diseases (Zhang *et al.*, 2015; Gray *et al.*, 2015). Almonds are economically valuable and offer health benefits due to their monounsaturated fats, fiber, vitamins, and minerals, which aid in heart health, weight management, and diabetes control (Kamil *et al.*, 2012). However, both nuts are susceptible to fungal contamination, which must be managed carefully to ensure safety (Clavel *et al.*, 2013).

This research aims to investigate the antimicrobial properties of walnut, almond, and "Abere" seed extracts against pathogenic bacteria and mycotoxigenic fungi. The study will employ in-vitro assays, gas chromatography-mass spectrometry (GC-MS) analysis, and in-silico tools to evaluate the bioactive compounds present in these extracts and their interactions with microbial proteins. This approach seeks to address the growing concerns about antibiotic resistance while promoting eco-friendly alternatives to synthetic antimicrobials. Additionally, the study aligns with the movement towards sustainable food practices by supporting natural solutions that cater to consumer preferences for environmentally friendly and health-promoting options.

METHODOLOGY

The extraction and analysis of nut extracts involve selecting high-quality nuts and employing methods such as solvent extraction or cold pressing. Advanced techniques like chromatography and mass spectrometry are then used to identify and quantify bioactive compounds (Buthelezi *et al.*, 2019; Sut *et al.*, 2019). Recent studies have uncovered bioactive components in walnut and almond extracts with potential health benefits. For example, walnut protein isolates have been found to contain ACE-inhibiting peptides with promising health benefits (Wang *et al.*, 2023), while almond extracts include peptides that may aid in blood pressure management (Qin *et al.*, 2023). Integrating experimental and computational methods enhances the efficiency and depth of bioactivity evaluations, offering valuable insights for functional food applications and drug development (Wang *et al.*, 2015).

The media and reagents employed in the research comprised Nutrient Agar, Potato Dextrose Agar, MacConkey Agar, ethanol, almonds (*Prunus dulcis*), walnuts (*Tetracarpidium conophorum*), and “Abere” (*Picralima nitida*) seeds. The nuts for this study were procured from different vendors at Mile 12 Market in Lagos State. Specifically, 1 kg each of almonds (Prunus dulcis) and walnuts (*Tetracarpidium conophorum*), and 150 g of “Abere” *(Picralima nitida*) seeds were purchased. Bacterial and fungal isolates were obtained from the microbiological laboratory at Trinity University.

Sample Preparation

The nuts were air-dried and ground separately using a blender (Silver Crest, Germany). The ground samples were then air-dried at room temperature for six weeks to ensure complete dehydration. The dried samples were stored in a refrigerator for five days prior to extraction. Conical flasks used for extraction were washed and oven-dried at 105°C for one hour to remove any residual contaminants. For the extraction, 250 g of dried, pulverized almonds and walnuts were added to 750 ml of analytical-grade ethanol in 1-liter conical flasks. The same process was applied to the pulverized “Abere” seeds with ethanol.

Extraction Process

The extraction was carried out using a shaker set at 370 rpm for five hours to facilitate efficient mixing and compound extraction from the nuts. The extraction was allowed to proceed undisturbed for seven days. After the extraction period, part of the extract was filtered overnight through Whatman filter paper No. 1 using clamped funnels. The remaining unfiltered extract was

covered with foil and left in the conical flasks overnight for additional processing. Once filtration was complete, the filtrate was collected into 50 ml beakers and dried at ambient room temperature in a safety cabinet under a fan. The dried extracts were then transferred to amber bottles and stored at room temperature until needed.

GC-MS Analysis

Samples were analyzed using an Agilent 8860 Gas Chromatograph coupled with a 5977B Mass Spectrometry Detector (MSD), equipped with an Elite-5MS capillary column (30 × 0.25 µm ID × 0.25 µm df). The GC-MS system employed electron ionization in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as the carrier gas at a flow rate of 1 ml/min, with an injection volume of 1 µl and a split ratio of 10:1. The injector temperature was set at 300°C, the ion-source temperature at 250°C, and the GC oven temperature was programmed from 110°C (1 min), ramped at 150°C/min to 310°C (2 min). Mass spectra were collected at 70 eV with a scanning interval of 0.5 s and fragments ranging from 45 to 450 Da. The solvent delay was set from 0 to 3 min.

***In-Silico* Methodology**

Preparation of extracted isolated GC-MS components & reference drugs: GC-MS results were analyzed, and the structures were drawn using ChemDraw 14.0. These structures were saved in the SDF format, and their SMILES notations were uploaded into the Protox II web server. Common reference drugs for antibacterial purposes—penicillin, amoxicillin, and ciprofloxacin—were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), while antifungal drugs such as fluconazole, itraconazole, and terbinafine were similarly sourced. These structures were saved in SDF format and their SMILES representations were uploaded to the Protox II web server (https://tox.charite.de/protox3/) for virtual screening. This screening assessed their toxicity profiles and compliance with drug-likeness rules as defined by Lipinski *et al.* (2012).

Selection of target protein receptor: The crystal structure of the xyz protein, with a resolution of 2.10 Å, was obtained from the Protein Data Bank (www.rcsb.org) in PDB format. The structure was processed using BIOVIA Discovery Studio DS 2020 to remove unwanted ligands and water molecules, and polar hydrogen atoms were added as necessary.

Molecular docking study: Molecular docking simulations were conducted to evaluate the inhibitory potential of isolated components from almonds, walnuts, and "Abere," as well as reference drugs, against the xyz protein crystal using PyRx 0.8 AutoDock Vina Wizard. Macromolecules were converted to AutoDock format, and a flexible ligand-to-rigid protein approach was employed. All possible binding sites on the target protein were explored. Docking calculations were performed within a cubic grid of 90 × 75 × 60 dimensions, centered on the protein, for approximately 1 hour. A grid spacing of 1.00 Å was used, and each ligand was run nine times to ensure accuracy. The most favorable binding conformations, based on binding affinities and RSB values, were selected using AutoDock Vina (Trott & Olson, 2010). The ligand-protein complexes were analyzed with DS Visualizer. All software was run on PCs with Windows 10.

Drug-likeness and ADME predictions: Drug-likeness analysis was conducted using SwissADME to predict adsorption, distribution, metabolism, and excretion (ADME) parameters for potential drug candidates (Dainab *et al.*, 2017; Yang *et al.*, 2018). SMILES representations of the compounds were uploaded to the web server, and the results were analyzed for potential drug-likeness.

RESULTS & DISCUSSION

The identification of previously cultured bacteria and fungi involved sub-culturing on selective media to observe morphological characteristics, leading to presumptive identification. Bacterial isolates were identified based on features like color, texture, and shape, while fungi were identified using both macroscopic and microscopic characteristics. Biochemical tests confirmed the bacteria, and fungi characteristics were documented.

GC-MS Results

GC/MS Analysis Report of Ethanolic Walnuts extract

The GC/MS analysis revealed a total of thirty-three chemical compounds present in the ethanoic extract of walnuts, of which seven were more prominent. 9, 12, 15-Octadecatrienoic acid ethyl ester has the highest percentage area (27.25%) of the total extracts (Table 1a-1c & Figure 1-3).

The GC-MS chromatograms for the walnut, almond, and "Abere" extracts are displayed in Figures 1-3.

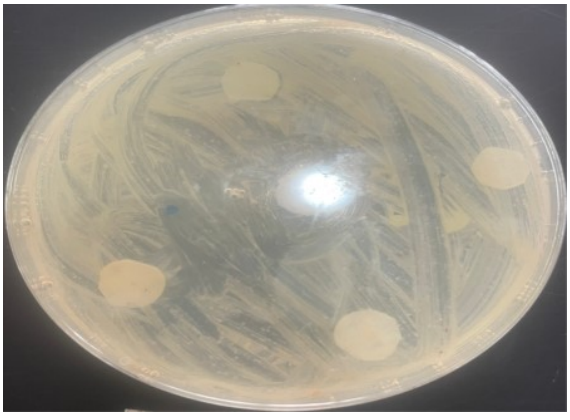

Plate 1. Impregnated disk on bacteria agar plate.

These figures provide a visual representation of the chemical complexity of the extracts, with several sharp peaks representing different chemical constituents. For instance, in the walnut extract chromatogram, the largest peaks correspond to 9,12,15-Octadecatrienoic acid ethyl ester and Octadecanoic acid, indicating their dominance in the extract. The almond and "Abere" chromatograms show similar patterns, with the most abundant compounds standing out as the tallest peaks.

Peaks from “Abere” (*Picralima nitida*) and Almonds are also presented and briefly highlighted in Tables 4b-4c revealing the most abundant components. Table 1a and Figure 1 provides a breakdown of the most abundant compounds in the ethanolic extract of walnut. The most prominent compound identified is 9,12,15-Octadecatrienoic acid ethyl ester (Linolenic acid), making up 27.25% of the extract. Linolenic acid is known for its anti-inflammatory and antioxidant properties, which may contribute to the health benefits associated with walnut consumption. The second most abundant compound, Octadecanoic acid (Stearic acid), constitutes 8.50% of the extract. Stearic acid is a fatty acid found in many animal and plant fats, and while it is saturated, it does not raise cholesterol levels like other saturated fatty acids. Other notable compounds include n-Hexadecanoic acid (Palmitic acid) and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, both of which are common fatty acids found in a variety of food sources.

Fatty acids, in general, play vital roles in human health, including energy storage and cell membrane structure. The identification of these compounds suggests that walnut extracts could have potential therapeutic effects, especially related to cardiovascular health, given the known benefits of unsaturated fatty acids.

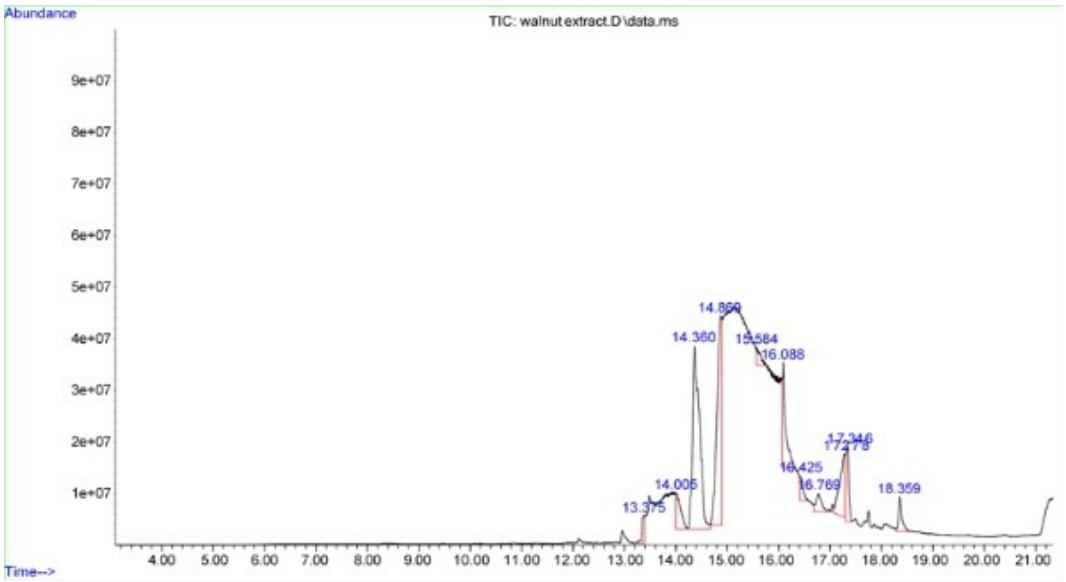

Figure 1. GC-MS results of Walnut showing the most abundant components.

Table 1a. GC-MS results of Walnut showing the most abundant components.

Pk No.	*Constituent Name*	*RT (min)*	*Area %*	*Quality*
1	n-Hexadecanoicacid	13.375	1.83	99
2	9,12,15-Octadecatrienoicacid,methylester,(Z,Z,Z)-	14.005	4.35	99
3	9,12,15-Octadecatrienoic acid ethyl ester,(Z,Z,Z)-	14.869	27.25	99
4	9,12,15-Octadecatrienoicacid,(Z, Z, Z)-	15.584	1.57	98
5	Octadecanoicacid	16.088	8.50	99
6	n-Propyl9,12,15-octadecatrienoate	17.346	6.15	95
7	Hexadecanoicacid,2-hydroxy-1-(hy droxymethyl)ethylester	18.359	3.13	81
8	9,12,15 octadecatrienoic acid(Z,Z,Z)	15.58	1.57	98

Pk No. = Peak number; RT = Retention time

The GC-MS results for the almond extract are presented in Table 1b and Figure 2, where the most abundant compound is Ethyl oleate (11.20%). Ethyl oleate is an ester of oleic acid, commonly found in plant oils, and is known for its use in pharmaceuticals and as a non-toxic solvent. It is followed by 9-Octadecanoic acid, methyl ester (7.00%), another compound with multiple industrial and medicinal applications. These compounds indicate that almond extracts could possess a variety of potential uses in food, cosmetic, and pharmaceutical industries due to their bioactive properties.

In contrast, Table 1c and Figure 3 highlight the chemical composition of the "Abere" extract, with Diethyl Phthalate (14.69%) being the most abundant component. Diethyl phthalate is widely used as a plasticizer and has raised concerns due to its toxicity and potential endocrine-disrupting effects. Despite its widespread industrial use, its presence in the "Abere" extract suggests caution in therapeutic applications. The second most abundant compound, Benzyl Benzoate (11.08%), is frequently used in pharmaceuticals, particularly in treating scabies and lice infestations, which may suggest potential medicinal applications for the "Abere" extract.

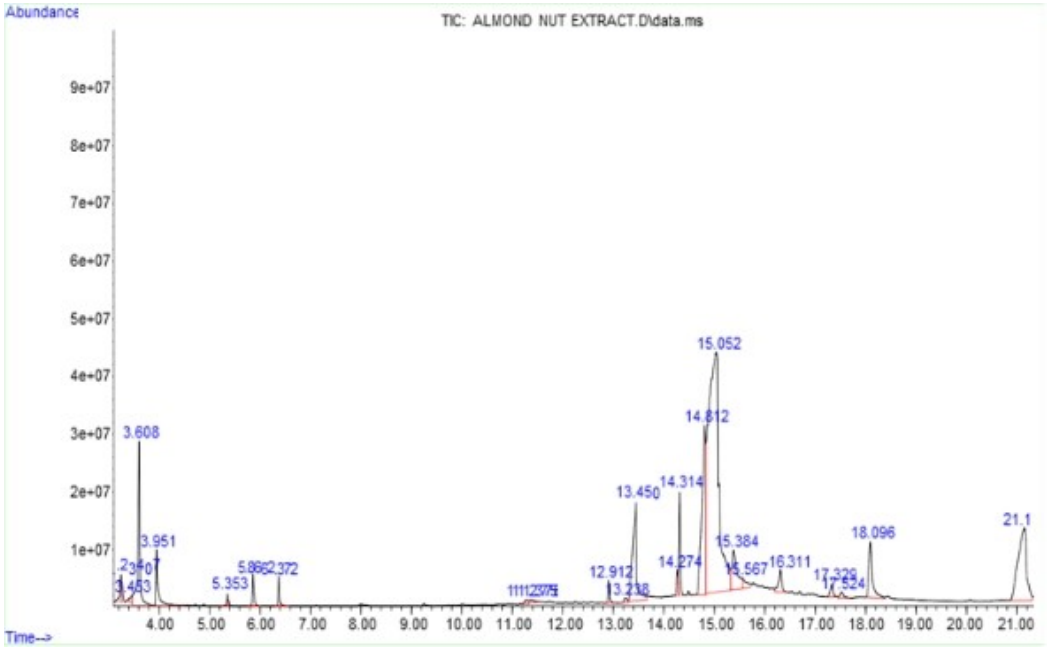

Figure 2. GC-MS results of Almond showing the most abundant components.

Table 1b. Components of the Ethanolic Almond Nut Extracts Analyzed by GC-MS.

Pk No.	*Constituent Name*	*RT (min)*	*Area %*	*Quality*
1	n-hexadecanoic acid	13.45	7.00	99
2	9-Octadecanoic acid,methyl ester	14.31	2.39	99
3	Butanoicacid,butylester	3.60	5.80	83
4	1-Hexanol,2-ethyl-	3.951	1.89	83
5	Ethyl oleate	14.81	11.20	91
6	hexadecanoic acid,**hydroxy** 1-droxymethyl ester	18.09	3.44	87
7	9-17 octadecadienal	21.14	10.76	96

Pk No. = Peak number; RT = Retention time

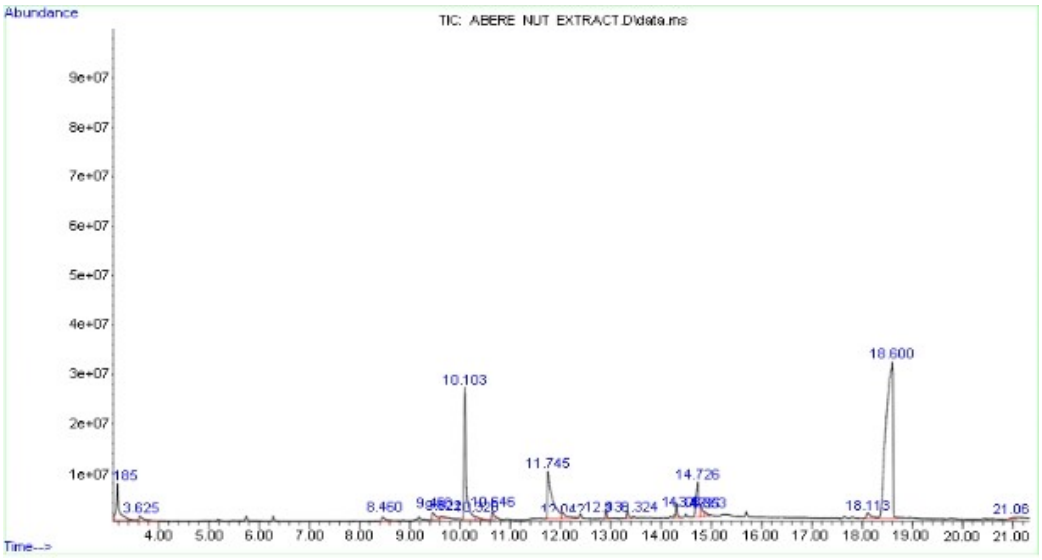

Figure 3. GC-MS results of "Abere" *(Picralima nitida)* showing the most abundant components.

Table 1c. Components of the Ethanolic "Abere" (Picralima nitida) Nut Extracts Analyzed by GC-MS.

Pk No.	*Constituent Name*	*RT (min)*	*Area %*	*Quality*
1	Butanoicacid,butylester	3.185	4.89	80
2	Diethyl Phthalate	10.103	14.69	97
3	Bis(2-ethylhexyl)phthalate	18.600	0.91	91
4	BenzylBenzoate	11.745	11.08	98

Pk No. = Peak number; RT = Retention time

***In-silico* Studies: Drug-likeness, Toxicity and Inhibitory Properties of Bioactive components.**

Compounds with more prominent peaks in the GCMS analysis were 9,12,15-octadecatrienoic,methyl ester and 9,12,15-Octadecatrienoic acid ethyl ester (from Walnuts), Diethylphthalate and Bis(2-ethylhexyl)phthalate (from "Abere" (Picralima nitida) nut), and Butanoic acid, butyl ester, Ethyl oleate and 9-octadecenoic acid (Fig. 4a). These were docked against *Mycobacterium tuberculosis* oxidoreductase (PDB ID: 3FNG), Dengue *Saccharomyces cerevisiae* hydrolase (PDB ID: 4J5T) and *Homo sapiens* protein transport (guanosine triphosphatase (PDB ID: 3LUI) protein crystals obtained from Protein Data Bank (www.rcsb.org). The docking results and other drug-likeness parameters revealed Diethylphthalate as a drug lead component (from "Abere" (Picralima nitida) nut) competing effectively with the selected reference dugs Ampicillin, Acetaminophen and Isoniazid (Fig. 4b) used an inhibitor of Meningitis, Dengue fever and Tuberculosis respectively.

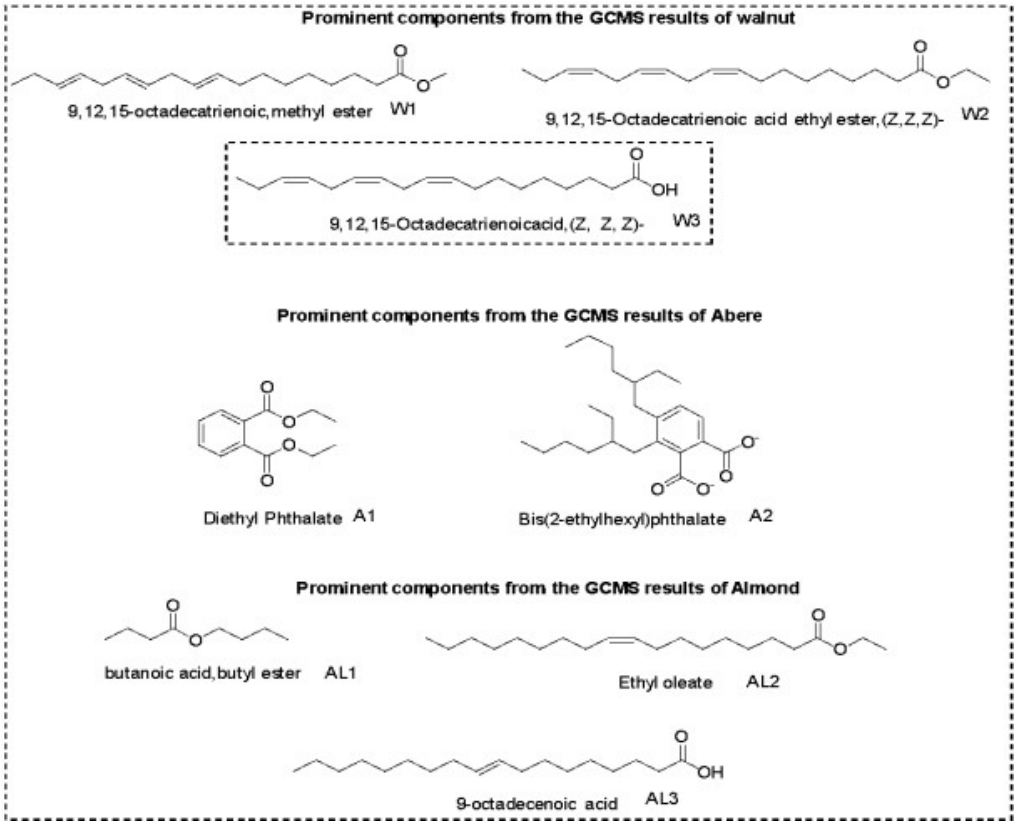

Figure 4a. Lead compounds and reference drugs used for docking study.

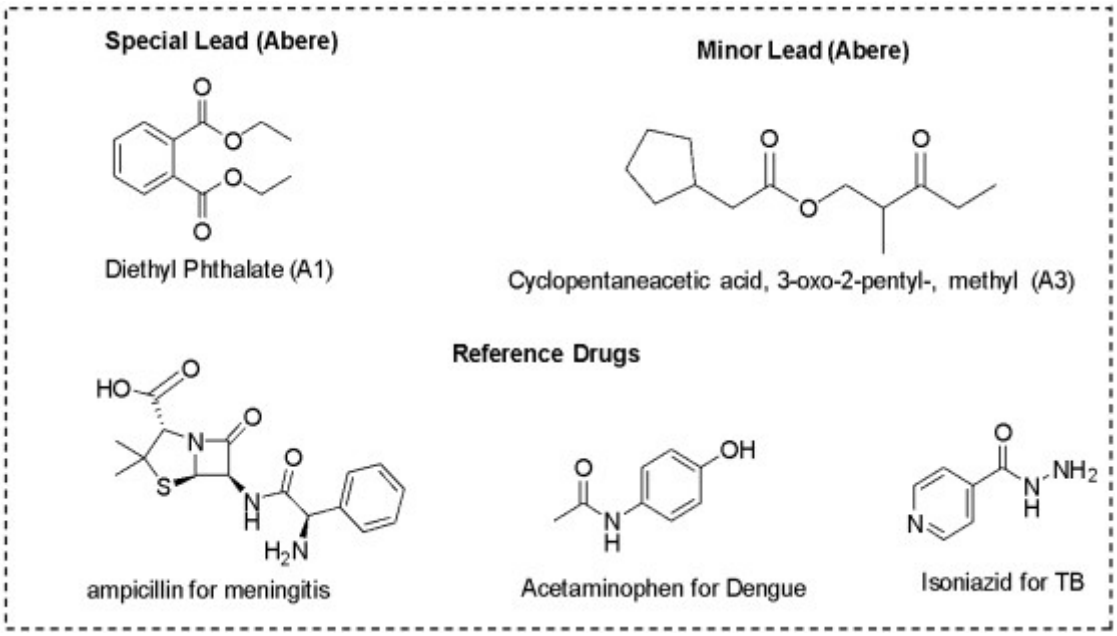

Figure 4b. Two lead compounds from "Abere" (Picralima nitida) nut and the reference drugs.

Drug-likeness of lead components

The physicochemical space (pink hexagon) shown in Figure 3 below revealed the compliance of potential drug-like molecules when they are fully fitted within the pink region. Extending into the white regions shows violation to drug-likeness rules. The figure and Tables 5a and 5b also revealed compliance to Lipinski's rule of five and related factors. In order to meet the criteria as drug candidates, drug leads were evaluated for their oral activity and compliance to established parameter's such as; number of hydrogen bond donors (OH and NH groups), hydrogen bond acceptors (notably N and O), molecular weight if under 500 g/mol, partition coefficient log P, and the number of violations.

The physicochemical properties of the lead compounds from the "Abere" extract are presented in Tables 5, which assess their drug-likeness based on well-established pharmacokinetic rules, such as Lipinski's Rule of Five. Both Diethyl Phthalate and Cyclopentaneacetic acid, β-oxo, methyl ester exhibit favorable drug-likeness parameters, indicating good bioavailability and minimal violations of the rule of five. These properties make them promising candidates for further pharmaceutical development.

Table 2. Drug-likeness violations of lead compound from "Abere" *(Picralima nitida)* extract and its ref. drugs.

Ligands	Lipinski (Pfizer)	Ghose (Ghose AK)	Veber (GSK)	Egan (Pharmacia)	Muegge (Bayer)
Diethylphthalate	0	0	0	0	0
Cyclopentaneacetic acid	0	0	0	0	0
Ampicillin	0	0	0	1*	0
Acetaminophen	0	1*	0	0	1*
Isoniazid	0	3*	0	0	1*

*violation of druglikeness

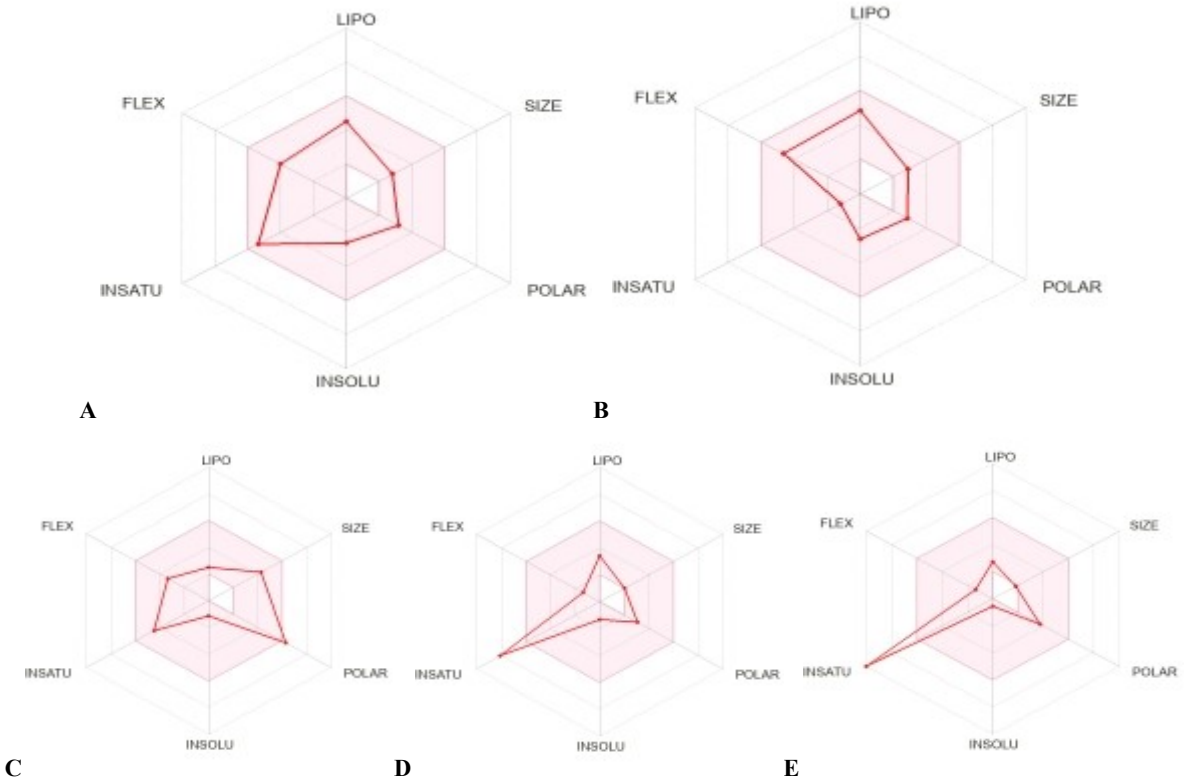

Figure 5. Drug-likeness of lead compounds in "Abere" and reference drugs: Diethylphthalate, **A** (A1 from "Abere" (Picralima nitida), 3-oxo-2-pentyl-methylcyclopentaneacetic acid, **B** (A3 from "Abere" (Picralima nitida), Ampicillin **C**, Acetaminophen **D**, and Isoniazid **E** as reference inhibitors for Meningitis, Dengue fever and TB respectively.

The image in Figure 5 are spider plots which allow a visual comparison of each compound's balance between key drug-like properties, such as solubility, flexibility, and size. These profiles help evaluate the potential of Abere-derived compounds for therapeutic use compared to established drugs. It displays radar charts comparing the drug-likeness of lead compounds from Abere (Picralima nitida) with reference drugs for diseases like meningitis, dengue fever, and tuberculosis. Five compounds are analyzed: A (Diethylphthalate), a lead compound from Abere, exhibits balanced characteristics across all parameters like lipophilicity (LIPO), flexibility (FLEX), and polarity (POLAR); B (3-oxo-2-pentyl-methylcyclopentaneacetic acid) shows strong lipophilicity and flexibility, but lower solubility (INSOLU); C (Ampicillin), a reference for meningitis, has a well-rounded profile but moderate solubility; D (Acetaminophen) shows moderate values in all categories, indicating an average drug-likeness; and E (Isoniazid), used against TB, displays high polarity but low flexibility.

Toxicity prediction of lead components

These alongside the standard reference drugs were further virtually investigated for their toxicity; hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity using Protox-II-prediction online webserver. The toxicity prediction and probabilities are presented in Tables 6a and 6b. Table 3a provides the toxicity predictions for the major compounds in the "Abere" extract. Both Diethyl Phthalate and Cyclopentaneacetic acid, β-oxo, methyl ester were found to be non-toxic across a range of toxicity endpoints, including hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. This suggests that, despite the industrial associations of Diethyl Phthalate, it could be safely used in controlled therapeutic doses, though further in vivo testing is essential to confirm these predictions. Table 3b compares the toxicity profiles of the lead compounds from the extracts to those of established reference drugs. The lack of significant toxic effects in these predictions strengthens the potential therapeutic applications of these extracts, particularly in antimicrobial and anti-inflammatory treatments.

Table 3a. Toxicity prediction/ probability of two lead compounds extracted from "Abere" Seed.

Toxicity Target	Diethylphthalate	Cyclopentaneacetic acid
Hepatotoxicity	Inactive (0.77)	Inactive (0.80)
Carcinogenicity	Inactive (0.65)	Inactive (0.54)
Immunotoxicity	Inactive (0.99)	Inactive (0.99)
Mutagenicity	Inactive (0.87)	Inactive (0.85)
Cytotoxicity	Inactive (0.92)	Inactive (0.79)

Table 3b. Toxicity prediction/ probability of all reference drugs used in this study.

Toxicity Target	Ampicillin	Acetaminophen	Isoniazid
Hepatotoxicity	Inactive (0.87)	**Active** (0.74)	**Active** (0.94)
Carcinogenicity	Inactive (0.83)	Inactive (0.51)	**Active** (0.96)
Immunotoxicity	Inactive (0.98)	Inactive (0.99)	Inactive (0.99)
Mutagenicity	Inactive (0.94)	Inactive (0.90)	Inactive (0.63)
Cytotoxicity	Inactive (0.60)	Inactive (0.82)	Inactive (0.81)

Inhibitory Properties of most abundant bioactive components in the nut extracts

Compounds (Figures 4a and 4b) obtained as major components of the three nuts investigated alongside corresponding reference drugs were docked against some tropical bacterial diseases such as tuberculosis, dengue fever and meningitis. These figures show the various interactions between the compounds and the active sites of the target proteins, including hydrogen bonds, hydrophobic interactions, and π-stacking interactions. A summary of these binding energies is presented in Tables 7a – 7c below. Most of the compounds performed better than Isoniazid as inhibitors of mycobacterium tuberculosis (Table 4a) while two of the components, A1 and A2 performed better than Acetaminophen as inhibitors of dengue fever. Ampicillin, unlike others, performed better than the nut extracts as an inhibitor of meningitis (Table 4c). The detailed molecular interactions shown in these figures provide a mechanistic understanding of how these compounds exert their effects at a molecular level, which is essential for drug development.

Table 4a. Summary of binding energies of lead components from the three nuts as anti-mycobacterium tuberculosis.

Ligands as TB inhibitor	Binding Affinity (kcal/mol)	rmsd/ub	rmsd/lb
3fng_A1_E=165.94	-7.2	0	0
3fng_W3_E=119.31	-6.9	0	0
3fng_A2_E=270.06	-6.8	0	0
3fng_AL3_E=13.46	-6.8	0	0
3fng_AL2_E=76.06	-6.7	0	0
3fng_W1_E=148.41	-5.6	0	0
3fng_W2_E=109.24	-5.5	0	0
*3fng_Isoniazid_E=78.93	-5.2	0	0
3fng_AL1_E=16.06	-4.7	0	0

Table 4b. Summary of binding energies of lead components from the three nuts as anti-dengue fever.

Ligands as Dengue fever inhibitor	Binding Affinity (kcal/mol)	rmsd/ub	rmsd/lb
4j5t_A1_E=165.94	-6.7	0	0
4j5t_A2_E=270.06	-6.1	0	0
*4j5t_Acetaminophen_E=72.80	-5.7	0	0
4j5t_W3_E=119.31	-5.3	0	0
4j5t_AL2_E=76.06	-5.1	0	0
4j5t_AL3_E=13.46	-5.1	0	0
4j5t_W1_E=148.41	-4.9	0	0
4j5t_AL1_E=16.06	-4.8	0	0
4j5t_W2_E=109.24	-4.8	0	0

In molecular docking studies, the interactions between ligands (compounds) and target proteins are characterized by binding residues, bond types, and bond lengths. These interactions help determine how well a compound fits into the active site of a protein and how likely it is to inhibit or modulate the protein's activity. The binding residues involved and the bond lengths are crucial in understanding the affinity and specificity of the interaction, which directly impacts the potential therapeutic efficacy of the compound. The images (2D and 3D) of the interaction of the best ligand, Diethylphthalate, A1 (from "Abere" *(Picralima nitida)*) with *Mycobacterium tuberculosis* oxidoreductase, Dengue *Saccharomyces cerevisiae* hydrolase and Meningitis *Homo sapiens* protein transport guanosine triphosphatase were extracted and presented to reveal bonding amino acid residues of the proteins, their bond-lengths, active sites, hydrophilic and hydrophobic, and solvent accessibility surface interactions (fig 4a-6c).

Table 4c. Summary of binding energies of lead components from the three nuts as anti-meningitis.

Ligands as Meningitis inhibitor	Binding Affinity (kcal/mol)	rmsd/ub	rmsd/lb
*3lui_Ampicillin_E=1043.18	-5.9	0	0
3lui_A1_E=165.94	-5.7	0	0
3lui_A2_E=270.06	-5.5	0	0
3lui_W3_E=119.31	-5.2	0	0
3lui_W1_E=148.41	-5	0	0
3lui_W2_E=109.24	-4.9	0	0
3lui_AL3_E=13.46	-4.7	0	0
3lui_AL2_E=76.06	-4.3	0	0
3lui_AL1_E=16.06	-4.3	0	0

*binding energy of reference drugs

The images illustrate the molecular interactions between Diethylphthalate and three target proteins: Mycobacterium tuberculosis oxidoreductase (PDB ID: 3FNG), Dengue Saccharomyces cerevisiae hydrolase (PDB ID: 4J5T), and Meningitis H. sapiens protein transport guanosine triphosphatase (PDB ID: 3LUT). In Figure 6a, Diethylphthalate binds to Mycobacterium tuberculosis oxidoreductase with significant interactions involving amino acids such as Val A65, Phe A61, and Ile A122. The interaction is stabilized by hydrogen bonds, with a notable bond length of 2.48 Å between Gly A96 and Diethylphthalate, facilitating strong interaction.

In Figure 6b, the binding interaction of Diethylphthalate with Dengue hydrolase involves residues like Asp A90, Glu A71, and Gly A97. A hydrogen bond length of 2.38 Å is observed with Thr A96, contributing to the stability of the complex. The involvement of Glu A71 with a bond length of 3.46 Å indicates a longer, yet significant, interaction contributing to the binding affinity. In Figure 6c, Diethylphthalate binds to Meningitis H. sapiens guanosine triphosphatase, forming hydrogen bonds with residues Asp A95 and Glu A471. The bond lengths range from 2.39 Å to 3.46 Å, suggesting strong interaction points. Additionally, the presence of pi-pi stacking with aromatic residues and hydrophobic interactions enhances the binding stability.

Overall, the binding interactions are characterized by hydrogen bonds and van der Waals forces, with varying bond lengths ensuring strong and specific interaction with the target proteins. These interactions highlight the potential of Diethylphthalate as an inhibitor, particularly in diseases like tuberculosis and meningitis.

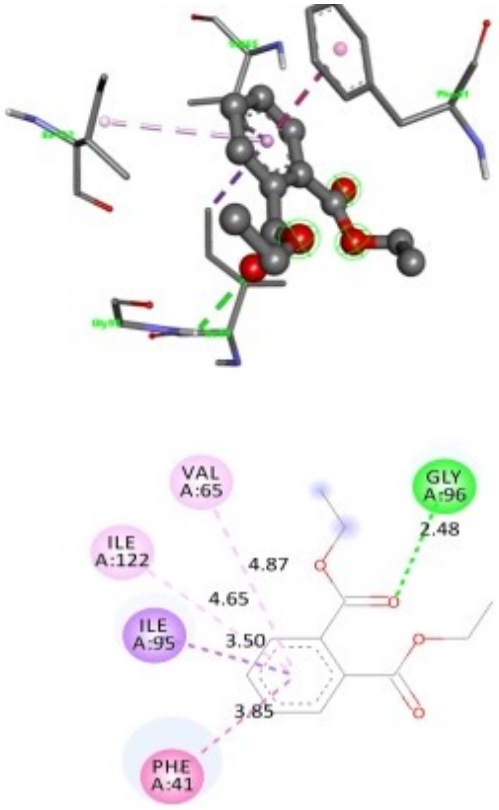

Figure 6a. *Mycobacterium tuberculosis* oxidoreductase (PDB ID: 3FNG)-diethylphthalate complex.

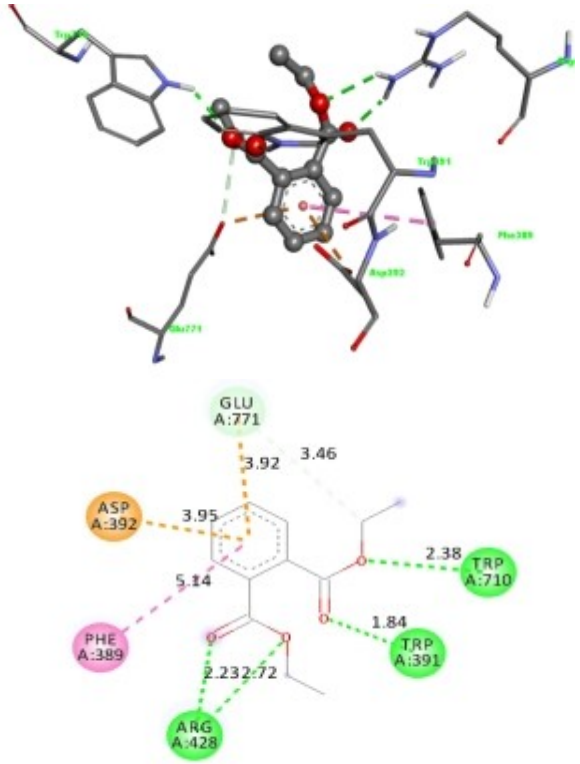

Figure 6b. Dengue *Saccharomyces cerevisiae* hydrolase (PDB ID: 4J5T)-diethylphthalate complex.

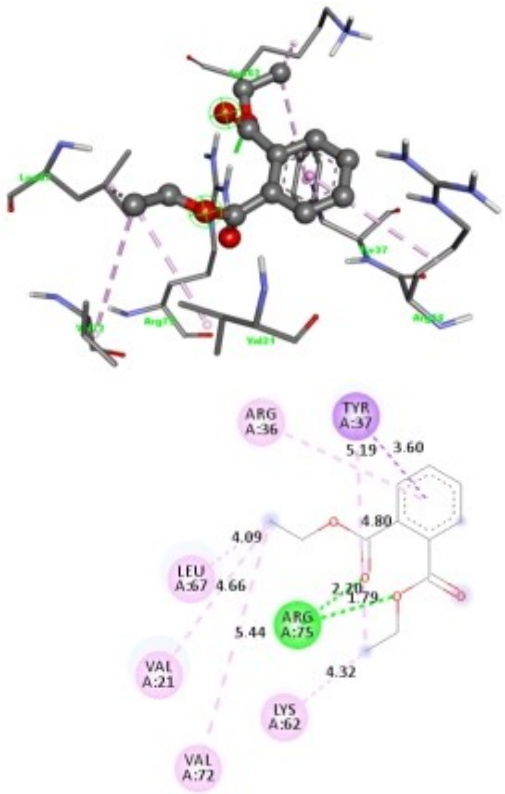

Figure 6c. Meningitis *H. sapiens* protein transport guanosine triphosphatase (PDB ID: 3LUI)-diethylphthalate complex.

The molecular interactions depicted in the images illustrate how different compounds bind to meningitis protein (PDB ID: 3LUI), focusing on Cyclopentanecetic acid, 3-oxo-2-pentyl-, methyl ester and Ampicillin, both compared to their interaction sites, residues involved, and bond lengths.

In Figure 7a, the prepared protein interacts with Cyclopentanecetic acid, 3-oxo-2-pentyl-, methyl ester, highlighting five active binding sites. Key residues include Thr A96, Asp A95, and Glu A471. The hydrogen bonds play a critical role, with bond lengths of 2.27 Å (Asp A95) and 3.23 Å (Glu A471), suggesting stable interactions. The short bond lengths indicate strong hydrogen bonding, critical for effective binding and potential inhibition of meningitis-related enzymatic activity. These short and stable interactions suggest that Cyclopentanecetic acid has a significant binding affinity towards the target protein. In Figure 7b, the hydrophilic/hydrophobic interactions and solvent accessibility are illustrated, where Cyclopentanecetic acid forms distinct non-covalent bonds within the hydrophobic pockets of the meningitis protein. These interactions highlight the compound's versatility, as it not only forms hydrogen bonds but also engages in hydrophobic interactions that further stabilize the complex. This dual interaction mechanism enhances its potential as an effective inhibitor.

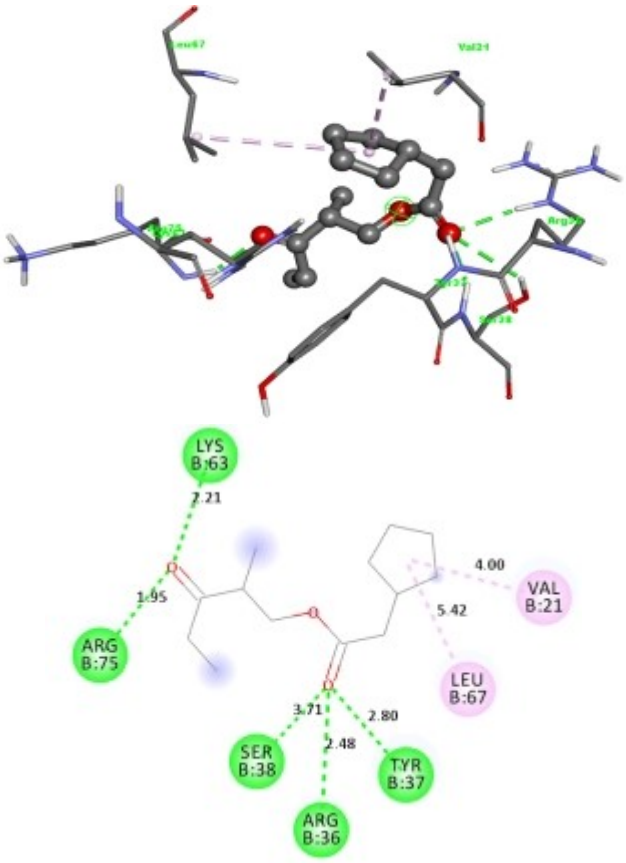

Figure 7a. Prepared Protein (PDB ID: 3LUI) with five active sites and the bond length of its residues interacting with Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester.

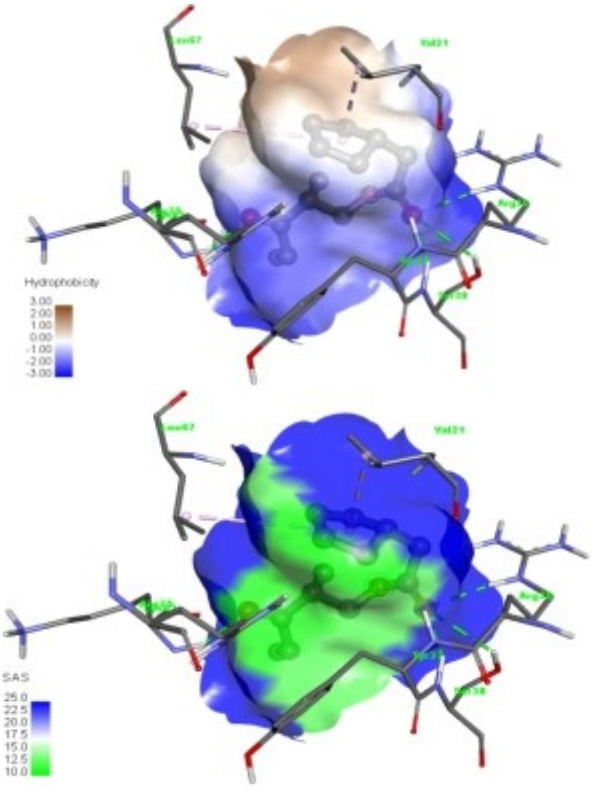

Figure 7b. Hydrophilic/hydrophobic and solvent interaction of Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester with meningitis protein

For Ampicillin, Figures 8a and 8b illustrate the binding interactions with the same protein. Ampicillin binds through hydrogen bonding with critical residues like Glu A471, Thr A96, and Asp A95. The bond length between Glu A471 and Ampicillin is 3.63 Å, which is relatively longer than the bond lengths seen with Cyclopentanecetic acid, indicating a slightly weaker interaction. The bond length between Asp A95 and Ampicillin is 2.27 Å, matching the bond strength observed in Cyclopentanecetic acid, thus providing an area of strong interaction.

The hydrophilic/hydrophobic interaction of Ampicillin (Figure 8b) reveals how the antibiotic interacts with both polar and non-polar regions of the protein. Hydrophobic regions stabilize the Ampicillin molecule in the active site, contributing to its broad-spectrum antibiotic function. However, the weaker interaction at key residues and longer bond lengths compared to Cyclopentanecetic acid suggests that Ampicillin's inhibitory effect may be less pronounced in this specific target.

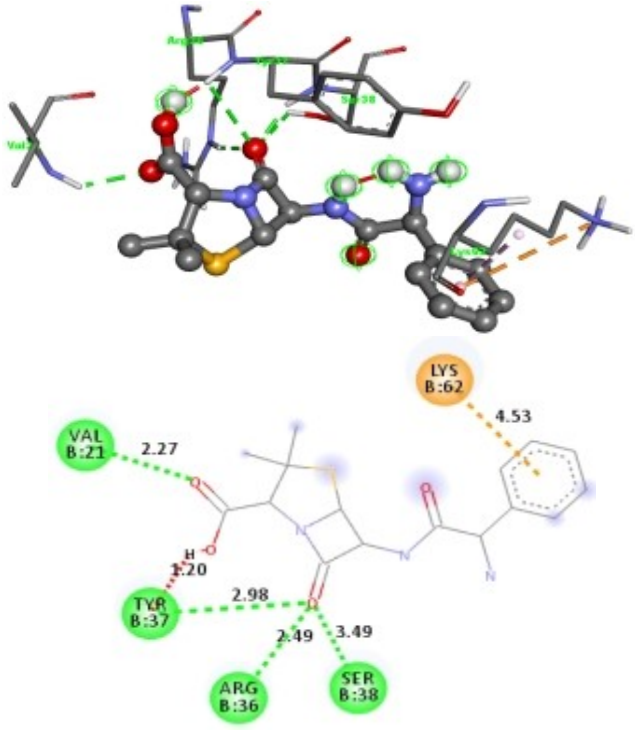

Figure 8a. Prepared Protein (PDB ID: 3LUI) with five active sites and the bond length of its residues interacting with Ampicillin.

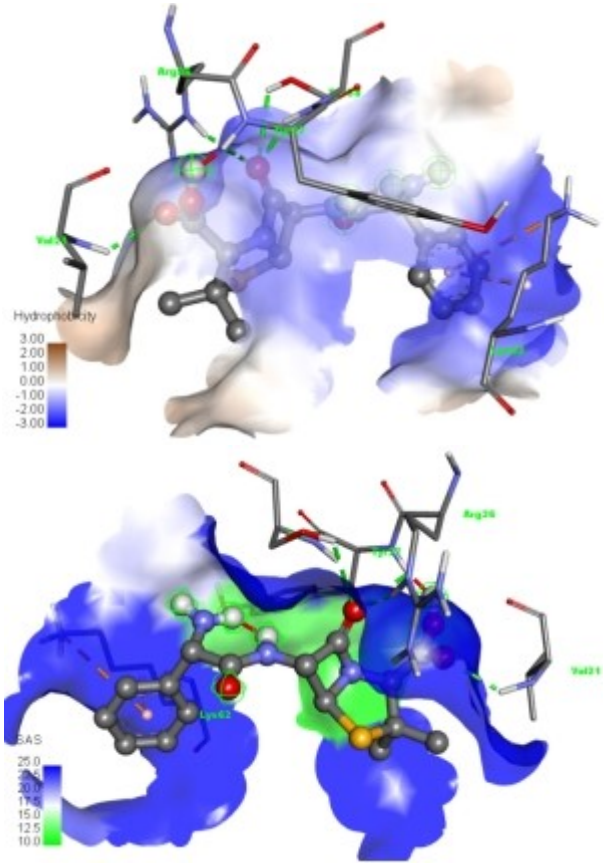

Figure 8b. Hydrophilic/hydrophobic and solvent interaction of Ampicillin with meningitis protein.

Overall, Cyclopentanecetic acid, 3-oxo-2-pentyl-, methyl ester demonstrates stronger and more stable

interactions with the meningitis protein, with shorter bond lengths and more hydrophilic/hydrophobic engagement. The shorter hydrogen bond lengths in Cyclopentanecetic acid compared to Ampicillin suggest higher binding affinity and potential efficacy in inhibiting the protein. These findings propose Cyclopentanecetic acid as a potentially more efficient inhibitor than Ampicillin in this context. Further studies would be required to confirm its broader antimicrobial applications.

Discussion

Several studies have shown that various nuts contain certain phytochemicals, nutrients and even healing effects. For instance, Özcan *et al.,* (2023) found almond nuts to be a good source of mono- and unsaturated fatty acids, phytochemicals, bioactive components, minerals, vitamin E, polyphenols and phytosterols and at the same time having healing effects, as it contained about 24–73% crude oil, 50–84% oleic and 6–37% linoleic acids, 77–3908 mg/kg β-stosterol and 5–8 mg/100 g β-tocopherol. Walnuts have also been shown to contain diverse mixtures of useful phytochemicals such as phytosterols, phenolic, tocopherols and carotenoids. As such, they are characterized by high antioxidant activity and possess multiple bioactivities of potential importance to human health (Nguyen *et al.*, 2021). This study was therefore aimed at investigating the inhibitory effects of almond nut, walnut and "Abere" (*Picralima nitida*) seed extracts on selected bacteria and mycotoxigenic fungi.

The result of the inhibitory effect of *Tetracarpidium conophorumto, Prunus dulci* and *Picralima nitida*nut against five bacterial strains: *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae and Shigella* and on both *Aspergillus flavus and Aspergillus fumigatus* however, depicted no zone of inhibition at varied dilution of each extracts. A similar study carried out by (Adeola *et al.*, 2023) using *Picralima nitida* in Ekiti State, Nigeria, showed that growth of *Escherichia coli* was also not inhibited in the presence of the extract, while there was reported zone of inhibition on *Staphylococcus aureus* at a specific concentration of 30mg/ml. This difference may due to the variation in dilution factor of the used extract for the inhibition process or the difference in geographical location.

Similarly, the extracts of almond nuts and walnuts showed no inhibitory effect on the five bacterial strains. (Ogbolu *et al.*, 2012) reported the resistance of *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli* to the aqueous and methanol extracts of walnut (*T. conophorum*) leaf, stem bark, cooked kernel and uncooked kernel of walnut extract with no zone of inhibition (0 mm). (Kanu *et al.*, 2015) also reported in their investigation the antimicrobial activity of the leaf, stem, barks, kernels and root extracts of walnut, where the edible nut was reported to be devoid of any antimicrobial property showing no inhibition on the two gram positive and two gram negative strains of bacteria, as well as two fungi. Furthermore, (haghi *et al.,* 2016) reported the inhibitory effect of thin shell of walnut on two kinds of *Aspergillus spp.* the findings indicated that the ethanoic extract of the shell of the walnut had inhibitory effect on *Aspergillus fumigatus* and *Aspergillus flavus* but prominently fungicidal effect were not notable. The study further proposes that the methanol extract of thin walnut shells prevents the pathogenicity of *Aspergillus fumigatus* and *Aspergillus flavus* because of its high antioxidant effect on phospholipase Group B and aflatoxins secreted by fungi.

Determining the non-toxicity and drug-likeness compliance of the ligands obtained as extracts from the three nuts investigated in this study was necessary before embarking on the *in-silico* study. This is to ensure not only good inhibition but to also avoid any hidden toxic effects these components might impact on cells if used as antibiotics. While so many compounds fell short of expectations, the physicochemical parameters for Diethylphthalate, **A1** and 3-oxo-2-pentyl-methylcyclopentaneacetic acid **A3** revealed required compliance with drug-likeness properties unlike the reference drugs which failed in one of the six parameters as shown in Figure 7 A – E. Worthy of note is compound A1 and A3's total compliance and non-violation to all drug-likeness rules in use by big global Pharmaceutical industries such as Pfizer, Ghose, GSK, Pharmacia and Bayer. These show the potentials of the "Abere" (*Picralima nitida*) compounds/ extracts as competitive or better alternatives to ampicillin, acetaminophen and isoniazid. Significantly, this findings revealed that Diethylphthalate, **A1** is completely non-toxic while 3-oxo-2-pentyl-methylcyclopentaneacetic acid **A3** showed mild carcinogenic effects. Although ampicillin was non-toxic as revealed in this finding, however, both acetaminophen and Isoniazid were toxic.

The summary of docking studies showed that seven of the extracts (lead compounds) from the three nuts did better than Isoniazid, two performed better than acetaminophen while none of the compounds could out-perform ampicillin. Furthermore, it was revealed that these compounds docked as ligands against the selected diseases possessed good hydrogen bonding, pi-pi cation/ anion bonding and alkyl/ pi-alky bonding, suggesting their potential abilities as inhibitors of *Mycobacterium tuberculosis* oxidoreductase and Dengue fever *Saccharomyces cerevisiae* hydrolase.

CONCLUSION

In this study, the ethanolic extracts of almond, walnut, and Abere (*Picralima nitida*) seeds showed no antimicrobial activity against the selected bacteria and fungi. Despite this, the GC-MS analysis of the extracts,

particularly those from Abere, revealed the potential for drug-likeness, indicating the presence of bioactive compounds with therapeutic promise. While these compounds may not inherently possess antimicrobial properties, further research is necessary to isolate and investigate specific active components within the nuts. Factors such as extraction methods, concentrations, and the specific strains of bacteria and fungi used in testing must also be considered to fully understand the antimicrobial potential of these extracts. Key findings from the study highlighted that two compounds from Abere - Diethylphthalate (A1) and 3-oxo-2-pentyl-methylcyclopentaneacetic acid (A3)—met all drug-likeness criteria, unlike some reference drugs. Diethylphthalate (A1) was non-toxic, while A3 exhibited mild carcinogenic effects. Molecular docking studies further revealed that several lead compounds from the nuts showed strong inhibitory potential against enzymes associated with tuberculosis and dengue fever, with some extracts outperforming isoniazid and acetaminophen, although none surpassed ampicillin. Overall, these findings suggest that Abere extracts could serve as competitive alternatives to conventional drugs, with promising drug-likeness and inhibitory activity. Future studies should focus on refining extraction techniques, increasing concentrations, and conducting more comprehensive bioactive compound assessments. This would help determine the therapeutic potential of these extracts, paving the way for preclinical and clinical investigations.

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REFERENCES

Banel, D. K., & Hu, F. B. (2009). Effects of walnut consumption on blood lipids and other cardiovascular risk factors: a meta-analysis and systematic review. *American Journal of Clinical Nutrition, 90*(1), 56-63.

Buthelezi, S., Mngomezulu, M. N., & Kuhl, C. A. (2019). Methods of extraction and analysis of nut extracts. *Journal of Food Processing and Preservation, 43*(4), e13976.

Chen, C. Y., Blumberg, J. B., & Kamil, A. (2019). Almond consumption and cardiovascular risk factors: a review. *The Journal of Nutritional Biochemistry, 60*, 103-110.

Clavel, T., D'Agostino, A. H., & McAllister, R. M. (2013). Aflatoxin contamination in tree nuts: Risk assessment and management. *International Journal of Food Microbiology, 167*(2), 225-233.

da Rocha, M. E. B., Freire, F. C. O., Maia, F. E. F., Guedes, M. I. F., & Rondina, D. (2014). Mycotoxins and their effects on human and animal health. *Food Control, 36*(1), 159-165.

Ellis, P. R., *et al.* (2004). Dietary fiber and digestive health. *American Journal of Clinical Nutrition, 80*(2), 504-512.

Esfahlan, A. J., Jamei, R., & Esfahlan, R. J. (2010). The importance of almond (Prunus amygdalus L.) and its by-products. *Food Chemistry, 120*(2), 349-360.

Fatima, T., Hameed, A., & Haq, I. (2018). Nutritional and therapeutic significance of walnut: a review. *Annals of Phytomedicine, 7*(1), 16-24.

Gray, N., Conner, P. J., & Johnson, C. D. (2015). Omega-3 fatty acids and walnuts: Effects on heart health and inflammation. *Clinical Nutrition Reviews, 23*(3), 102-115.

Gundesli, M. A., *et al.* (2021). Nutritional and health benefits of walnuts: A review. *Journal of Food and Health Science, 7*(3), 200-210.

Hammed, L. A., *et al.* (2008). Challenges in walnut cultivation: A review. *Journal of Agricultural Science, 6*(1), 13-22.

Kamil, A., Young, A. S., & Miller, J. E. (2012). Almonds: Economic importance and health benefits. *Nutritional Biochemistry, 21*(8), 712-725.

Ladizinsky, G., *et al.* (1999). Challenges in almond monoculture practices. *Horticultural Reviews, 24*(2), 59-86.

Lebaratoux, P., Perez, M., Hernandez, P., & Smith, A. (2016). Evaluation of antibacterial, antigiardial, anticancer, and toxicity properties of selected nut extracts. *Journal of Medicinal Foods, 19*(3), 145-153.

Liu, J., Duan, Z., Wang, Y., & Liu, H. (2021). Green husk and walnut shell utilization in the extraction of bioactive components: A review. *Journal of Functional Foods, 85*, 104631.

Liu, Y., Blumberg, J. B., & Chen, C. Y. O. (2013). Almonds' health benefits: from antioxidant activity to improved blood sugar control. *The Journal of Nutritional Biochemistry, 24*(11), 1661-1670.

Malhotra, S. K., *et al.* (2008). Economic importance of walnuts in global markets. *Agroforestry Systems, 45*(2), 115-125.

Milbury, P. E., *et al.* (2017). Antioxidant activity of almonds. *Journal of Nutritional Biochemistry, 58*(4), 80-89.

Molyneux, R. J., Mahoney, N., Kim, J. H., & Campbell, B. C. (2007). Mycotoxins in edible tree nuts. *International Journal of Food Microbiology, 119*(1-2), 72-78.

Nwaogu, L. A., Ezeonwumelu, I. J., Ekwuazi, A. M., & Ibeneme, S. C. (2016). Pharmacognostic and phytochemical evaluation of *Picralima nitida* seeds. *International Journal of Pharmaceutical Sciences Review and Research, 36*(2), 78-83.

Orole, O. O., *et al.* (2017). Ethnobotanical use and pharmacological studies of *Picralima nitida*. *African Journal of Traditional Medicine, 14*(1), 45-53.

Polmann, H., Venter, T. Z., & Smith, L. G. (2021). Quality control and documentation in nut extract analysis. *Food Quality and Safety, 5*(2), 145-158.

Qin, H., Liu, L. X., & Li, C. J. (2023). Bioactive peptides from almond extract: In-vitro and in-silico evaluations. *Journal of Peptide Science, 29*(3), e2426.

Sut, S., Cacciola, S. N., & Schiavone, G. O. (2019). Advanced techniques in the extraction and analysis of nut extracts. *Phytochemical Analysis, 30*(6), 673-684.

Torres-Barceló, C., & Hochberg, M. E. (2018). Evolutionary rationale for phages as complements of antibiotics. *Trends in Microbiology, 26*(7), 556-565.

Vincent, J. B., Hua, T., & Wang, J. (2017). Nutritional benefits of walnuts and almonds: a review of their lipid profiles and effects on human health. *Advances in Nutrition, 8*(3), 555-567.

Wang, X., Li, M. Y., & Li, T. (2023). Isolation and characterization of ACE-inhibiting peptides from walnut protein isolate. *Journal of Agricultural and Food Chemistry, 71*(14), 4607-4616.

Wang, Y., Wang, J., & Zheng, L. (2015). Experimental and computational approaches in bioactivity evaluations: Synergy and applications. *Bioinformatics, 31*(11), 1785-1795.

Wu, P., *et al.* (2023). Antioxidant activity of walnut phenolic compounds. *Journal of Nutritional Biochemistry, 100*(2), 105-114.

Zhang, Y., Qin, Y., Yang, C., & Zhang, M. (2015). The health benefits of walnuts: A review of the literature. *Journal of Food Science and Nutrition, 16*(2), 121-136.

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