ChemSearch Journal 15(2): 98 – 110, December, 2024
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 05/11/2024 Accepted: 19/11/2024
http://www.ajol.info/index.php/csj

Chemical, Antibacterial and Antioxidant Assessment of *Persea americana* Seeds, Leaves, and Stem Bark Extract and *in-silico* Pharmacokinetic Properties of the Prominent Compounds

[1]Ukoha, P. U. and [2]Igwe, O. U.

[1]Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, P.M.B 1007 Afikpo, Ebonyi State, Nigeria

[2]Department of Chemistry, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria

***Correspondence Email:** patrickukoha@yahoo.com

ABSTRACT

This work aims to identify the bioactive compound in the seeds, leaves, and stem bark of *P. americana* (avocado pear) responsible for some of its medicinal properties, as well as to examine the pharmacological properties. The phytochemical analyses confirmed the presence of alkaloids, flavonoids, tannins, saponins, and steroids in the seeds, leaves, and stem bark of *Persea americana* (avocado pear) at variable compositions. From the GC-MS analysis, fifteen, twenty-three, and twenty-four compounds were identified in the seeds, leaves, and stem bark of which dodecanoic acid (33.326%), phytol (27.583%), and cis-verbenol (8.927%) as the most prominent compounds respectively. The extracts showed potent growth inhibition against five pathogenic bacteria organisms (*Escherichia* coli, *Klebsiella pneumoniae*, *Salmonella typhi*, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis*) in comparison with gentamicin used as a standard antibacterial agent. The free radical scavenging activity of the extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method with ascorbic acid as a reference standard. The extracts showed significant antioxidant activity at minimum and maximum concentrations of 25 and 400 µg/ml. The stem bark extract (24.445 – 94.025 %) showed the highest antioxidant activity followed by the seeds extract (23.445 – 91.575 %) before the leaves extract (22.530 – 89.775 %). Comparatively, the highest antibacterial and antioxidant activity exhibited by the stem bark extract could be attributed to the amount of bioactive compounds present in it as shown by the GC/MS analysis. The docking result of the prominent compounds showed that all of the test compounds had negative binding affinities, indicating that the compounds had been successfully docked to the receptors. The compounds showed good pharmacokinetic properties, such as high blood-brain barrier absorption, oral bioavailability, and water solubility, in the *in-silico* ADME and drug-likeness predictions. The findings in this research give credence to the compounds as promising potential drug candidates for the treatment and management of diseases and infections in herbal medicine.

Keywords: Antibacterial, Antioxidant, Molecular Docking, *Persea americana*, Phytochemical

INTRODUCTION

Persea americana (avocado pear) parts have been used in traditional herbal medicine across the globe for the treatment of diarrhea, toothache, diabetes, asthma, high blood pressure, rheumatism, skin rashes, and dysentery. Some of these claims are yet to be fully documented. Plants are sources of materials used as food and drugs and many of these plants have been found to possess this dual usefulness (Igwe and Okwu, 2013a; Igwe, 2014a). Medicinal plants have continued to attract attention in the global search for alternative medicine for the treatment of many diseases affecting humans (Sofowora *et al.*, 2013; Sofowora, 1993). Many important drugs used today are directly or indirectly derived from plants due to their bioactive constituents such as; alkaloids, steroids, tannins, etc. (Oniyangi and Cohall, 2020; Codeiro and Oniyangi, 1998). In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents (Krishnaraju *et al*., 2005). Thus, according to Balandrin *et al.*, (1985). It is anticipated that phytochemicals with adequate antimicrobial efficacy will be used for the treatment of bacterial infections.

Persea americana is a medium to large tree of 9-20m in height and it is classified as an evergreen although some varieties lose their leaves for a short time before flowering, leaves are 7-41cm in length and variable in shape (elliptic, oval and lanceolate). They are often pubescent and reddish when young, becoming smooth, leathery, and dark green when mature. Flowerings are yellowish green and 1-13cm in diameter, the many

flowering inflorescences are borne in a pseudo-terminal position, with the central axis of the inflorescence terminates in a shoot. The fruit is a berry, consisting of a single large seed surrounded by a buttery pulp which contains 3-30% oil. The skin is variable in thickness and texture, fruit color at maturity is green-black, purple, or reddish depending on the variety, and fruit shape ranges from spherical to pyriform.

Avocado pear is a good source of fiber and high fiber intake lowers the risk of cardiovascular diseases, cancer, hypertension, as well as obesity. The seed is also rich in tannins and carotenoids which were shown to inhibit the in-vitro growth of prostate cancer cell lines as reported by (Artega *et al.,* 2005; Adeyemi *et al.,* 2002 and Saver, 1993). *Persea americana Mill*. (*Lauraceae*) commonly known as avocado pear originally solely native to humid tropical areas of Mexico which were later cultivated and extended to other regions of Latin America, Europe, and the USA (United States of America). Today, fruits are widely grown worldwide on a large scale in various subtropical countries and are generally recognized as a popular and healthy food source that supplies proteins and lipids to the human diet. The three principal cultivars of avocado pear are Mexican, West Indian, and Guatermalan with obvious differences in their fruit skin structure and size.

MATERIALS AND METHODS

Sample collection.

The seeds, leaves, and Stem bark of *P. americana* (avocado pear) were obtained from a tree in Umuekwerede Eziudo Ezinihitte Mbaise's Local Government Area of Imo State Nigeria. The plant material was taken to the Department of Forestry, Michael Okpara University of Agriculture Umudike, Umuahia Abia State, Nigeria for identification and authentication.

Sample preparation

The inner seeds were removed from the fruit by cutting with a knife. The seeds, leaves, and Stem bark were air-dried separately at room temperature and ground into powder using an electric blender, after which they were weighed using an electric weighing balance (850g for seeds, 950g for leaves, and 670g for Stem bark of the grounded sample was obtained respectively). The ground samples were stored in an air-tight bag till required for use.

Sample Extraction

The extraction process followed the procedure as reported by Igwe and Echeme (2013), with minor modifications. The powdered sample (300 g) was weighed into a 2 L beaker. It was extracted with methanol (1.2 L) using cold maceration procedure for a period of 72 hours. It was then filtered using Whatman No 1 filter paper and the filtrate was concentrated in a rotary evaporator at a temperature of 40℃. The various extracts obtained were stored separately in an air-tight container and kept in a refrigerator before further use.

Phytochemical screening.

Phytochemical screening of the crude extracts obtained was carried out to identify the constituents, using standard phytochemical methods as described by Onwuka, (2005); The analysis involves the detection of the presence of Alkaloids, Flavonoids, Tannins, Saponins, and Steroids.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the methanolic extracts of *P. americana* (avocado pear) seeds, leaves, and stem bark was carried out at BGI Laboratory Limited, Port-Harcourt, Rivers State, using GC-MS machine model: GC system-6890 and employing the protocol reported by Igwe and Abii (2014).

Preparation of Receptors and Ligands

The crystal structure of porcine pancreatic alpha-amylase complexed with acarbose (PDB ID: 1OSE) and the crystal structure of human angiotensin-converting enzyme (PDB 1D: 1O8A) were downloaded from the RCSB Protein Databank. Molecular Molero viewer software was employed to remove water molecules and the substrate ligands. The 3D structures of the prominent compounds, dodecanoic acid (seed), phytol (leaves), and cis-verbenol (bark), were downloaded from PubChem and subsequently converted to PDB format using ArgusLab 4.0.1 software. The PDB structures of acarbose and lisinopril, which serve as the reference drugs, were also downloaded from PubChem. The reference drugs were also docked as controls to validate the docking procedure employed.

Docking Protocol

The docking was performed using Auto Dock Vina embedded in the PyRx virtual screening tool (Dallakyan & Olsonn, 2015). The ligands were added using Open Babel in the PyRx virtual screening tool. The energies of the ligands were minimized and transformed into PDBQT format. The visualization of the docked complexes was conducted using Biovia Discovery Studio.

Prediction of ADME Properties

The ADME profiles such as Absorption, Distribution, Metabolism, and Excretion of the ligands were predicted using the SwissADME server (SwissADME, 2024).

Antibacterial Activity Screening

The bacteria organisms used for the *in vitro* antibacterial screening were *Escherichia coli*

(Gram-negative), *Klebsiella pneumonia* (Gram-negative), *Salmonella typhi* (Gram-negative), *Streptococcus pneumonia* (Gram-positive) and *Staphylococcus epidermidis* (Gram-positive). The test organisms were clinical isolates of human pathogens obtained from stock cultures at the Federal Medical Centre, Umuahia, Abia State, Nigeria. With the aid of a single-hole punch office paper perforator, circular discs of 5 mm diameter were cut from the Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminum foil and sterilized in an autoclave at 121 ℃ for 15 minutes. They were however used within 48 hours of production. The sensitivity of each test microorganism to the sample was determined using the Disc Diffusion Technique (Cheesbrough, 2000). A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the innoculeum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract-bearing paper discs were carefully placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 hours in an incubator at 37 ℃. They were examined for growth and the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimeter rule. Gentamicin was used as a standard antibacterial agent. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the sample having different zones and selecting the lowest concentration.

Antioxidant Activity Determination

The free radical scavenging activity of the sample was determined using the 1,1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picrylhydrazyl; DPPH) method as reported by Igwe and Onuoha, (2016). DPPH (1.0 g), a stable radical was dissolved in 100 ml of methanol. Different concentrations of the test sample (3.0 ml each) were added to 3.0 ml of a 0.004 % methanol solution of DPPH and incubated for 30 minutes at room temperature. The decrease in absorbance of the solution brought about by the test samples was measured at 517 nm using a spectrophotometer. Ascorbic acid, which is a known antioxidant (Igwe, 2014b) was used as a reference standard. The radical scavenging activity was calculated as the percentage inhibition of DPPH discoloration using equation 1:

$$\%\ DPPH\ = \left[\frac{\left(A_{blank} - A_{sample}\right)}{A_{blank}}\right] \times 100 \qquad (1)$$

Where; A_{blank} is the absorbance of the control reaction solution (containing all reagents except the test sample); A_{sample} is the absorbance of the test sample.

RESULTS AND DISCUSSION

The phytochemical analyses in Table 1: confirmed the presence of alkaloids (0.845 – 1.905 mg/100 g), flavonoids (1.630 – 2.870 mg /100 g), tannins (1.965 -2.470 mg/100 g), saponins (1.770 – 2.465 mg/100 g) and steroids (0.495 – 0.845 mg/100 g) in the seeds, leaves and stem bark at variable compositions. From the GC-MS analysis of the methanol extracts shown in Figures 1 to 3, fifteen, twenty-three, and twenty-four compounds were identified in the seeds, leaves, and stem bark of dodecanoic acid, phytol, and cis-verbenol as the most prominent compounds respectively. The extracts from Tables 2 to 4: showed potent growth inhibition against five pathogenic bacteria organisms (*Escherichia* coli, *Klebsiella pneumoniae*, *Salmonella typhi*, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis*) in comparison with gentamicin used as a standard antibacterial agent. The free radical scavenging activity of the extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method with ascorbic acid as a reference standard. The extracts showed significant antioxidant activity in Table 5: at minimum and maximum concentrations of 25 and 400 µg/ml. The stem bark extract (24.445 – 94.025 %) showed the highest antioxidant activity followed by the seeds extract (23.445 – 91.575 %) before the leaves extract (22.530 – 89.775 %). The search for organic antioxidants has become necessary since most of the synthetic antioxidants have been reported to be mutagenic (Igwe and Okwu, 2013a; Igwe and Akabuike, 2016). Comparatively, the highest antibacterial and antioxidant activity exhibited by the stem bark extract could be attributed to the amount of bioactive compounds present in it as shown by the GC/MS analysis.

Table 1: Phytochemical Screening of Methanol Extract of Avocado Pear (mg/100g)

Sample	Alkaloids	Flavonoids	Tannins	Saponins	Steroids
Seeds	0.845±0.005	1.63±0.01	2.47 ±0.02	2.17±0.01	0.495 ±0.005
Leaves	1.685±0.005	2.38±0.02	2.14±0.04	2.465±0.015	0.845±0.005
Stem bark	1.905±0.015	2.87±0.03	1.965±0.005	1.77±0.01	0.72±0.00

Data are mean ± standard deviation of triplicate determinations

Table 2: Result of the Anti-bacterial Activity of Methanol Seeds Extract of *P. americana* (avocado pear)

Pathogens	Concentration %				MIC%	Gentamicin
	25	50	75	100		(100%)
E. Coli	8.325±0.005	11.115±0.115	13.335±0.005	15.495±0.015	25	18.28
K. Pneumonia	7.535±0.195	10.555±0.275	12.07±0.07	13.935±0.065	25	19.50
S. typhi	7.955±0.035	11.065±0.065	12.795±0.035	14.815±0.065	25	17.00
S. Pneumonia	---	8.435±0.005	11.085±0.085	13.32±0.01	50	18.23
S. epidermidis	5.54	9.82±0.05	12.45±0.01	14.325±0.055	50	19.48

Data are mean ± standard deviation of triplicate determinations

Table 3: Result of the Anti-bacterial Activity of Methanol Leaves Extract of *P.* americana (avocado pear)

Pathogens	Concentration %				MIC%	Gentamicin
	25	50	75	100		(100%)
E. Coli	7.4±0.15	10.32±0.1	12.35±0.21	14.85±0.03	25	18.28
K. Pneumonia	6.63±0.01	9.54±0.32	12.0±0.11	13.3±0.17	25	19.50
S. typhi	6.655±0.225	10.12±0.12	12.2±0.16	14.51±0.01	25	17.00
S. Pneumonia	---	7.165±0.115	9.95±0.03	13.045±0.065	50	18.23
S. epidermidis	---	6.14±0.08	8.52±0.36	11.085±0.085	50	19.48

Data are mean ± standard deviation of triplicate determinations

Table 4: Result of the Anti-bacterial Activity of Methanol Stem bark Extract of *P. americana* (Avocado pear)

Pathogens	Concentration %				MIC%	Gentamicin
	25	50	75	100		(100%)
E. Coli	9.395±0.065	12.345±0.115	14.085±0.055	16.29±0.37	25	18.28
K. Pneumonia	8.87±0.01	12.35±0.11	12.945±0.055	15.8±0.25	25	19.50
S. typhi	10.1310.13	12.495±0.15	13.815±0.175	15.385±0.495	25	17.00
S. Pneumonia	4 .36±0.08	9.63±0.14	13.545±0.025	14.29±0.15	25	18.23
S. epidermidis	6.42±0.2	10.4±0.28	13.79±0.21	15.1±0.1	50	19.48

Data are mean ± standard deviation of triplicate determinations

Table 5: Result of the Anti-oxidant Activity (%) of Methanol Extract of *P. americana* (Avocado pear)

Concentration	Seeds	Leaves	Stem bark	Vitamin C
25	23.445±0.115	22.53±0.79	24.445±0.365	76.76±0.1
50	66.615±0.635	64.555±0.445	69.565±0.405	93.905±0.555
100	77.74±0.1	73.45 ±0.14	87.375±0.375	95.31±0.42
200	85.6±1.05	84.16±0.08	87.375±0.3755	96.655±0.005
400	91.575±0.055	89.775±0.215	94.025±0.195	97.625±0.145

Data are mean ± standard deviation of triplicate determinations

Table 6: Docking score and interaction of the compounds with α-amylase

Complex	Docking score (kcal/mol)	Amino acid residues
Cis-verbenol/α-amylase	-6.0	Alkyl/pi-alkyl: LEU 165A, TRP 58A, HIS 299A van der Waals: ASP 300A, HIS 305A, GLN 63A, VAL 163A, LEU 162A Pi-sigma: TYR 62A
Docecanoic acid/α-amylase	-4.8	Alkyl/pi-alkyl: LEU 162A, VAL 163A, TRP 58A van der Waals: ASP 197A, ALA 198A, ASP 300A, HIS 305A, LEU 165A, GLU 60A, GLN 63A, TRP 59A Pi-sigma: TYR 62A
Phytol/α-amylase	-6.0	Alkyl/pi-alkyl: LEU 162A, VAL 163A, TRP 59A, HIS 305A, TYR 62A, HIS 101A van der Waals: ASP 197A, ALA 198A, HIS 201A, HIS 299A, LEU 165A, GLU 233A, GLN 63A, TRP 58A Conventional hydrogen bond: ASP 300A
Acarbose/ α-amylase (Standard)	-8.0	van der Waals: GLY 283A, GLY 281A, PRO 405A, TYR 333A, ARG 421A, ARG 252A, ARG 398A, PHE 335A, PRO 4A, THR 11A, ASP 290A, HIS 331A, PHE 406A, ASN 279A Conventional hydrogen bond: TRP 280A, LYS 278A, SER 289A, ASP 402A, GLY 334A, PRO 312A, GLU 404A, GLU 282A

Table 7: Docking score and interaction of the compounds with ACE

Complex	Docking score (kcal/mol)	Amino acid residues
Cis-verbenol/ACE	-6.1	Alkyl/pi-alkyl: TYR 69A, LEU 81A, LEU 139A, LEU 140A van der Waals: ASN 136A, SER 516A, GLU 143A, ASN 85A, ARG 124A, TYR 62A, ASN 70A Conventional hydrogen bond: ASN 66A
Docecanoic acid/ ACE	-5.1	Alkyl/pi-alkyl: PHE 457A, TYR 523A, PHE 527A, VAL 385A van der Waals: GLU 411A, HIS 353A, GLN 251A, THR 282A, ASP 415A, SER 355A Pi-sigma: HIA 383A
Phytol/ ACE	-6.2	Alkyl/pi-alkyl: LEU 139A, PHE 512A, VAL 518A van der Waals: LEU 82A, ARG 124A, ASN 66A, SER 516A, ARG 522A, TYR 523A, HIS 353A, HIS 513A, SER 355A, VAL 351A, ASN 70A, GLU 143A, LEU 140A, LEU 81A, TYR 69A Conventional hydrogen bond: TYR 62A
Lisinopril/ ACE (Standard)	-7.6	Alkyl/pi-alkyl: HIS 513A, VAL 518A, HIS 387A, ALA 63A van der Waals: HIS 383A, GLU 384A, GLU 411A, ALA 354A, HIS 353A, TYR 523A, ASP 358A, TYR 360A, LYS 368A, PHE 512A, PHE 391A, TYR 394A, VAL 351A, ASN 70A Conventional hydrogen bond: SER 355A, ALA 356A, HIS 410A Pi-Pi stacked: TRP 357A

Table 8: Drug-likeness prediction of prominent compounds present in *P. americana* seeds and Stem bark

Compound	Mol. Wt.(g/mol)	HB Acceptor	HB Donor	Lipophilicity LogP	Molar Refractivity	No. of Violations
Dodecanoic acid	200.32	2	1	3.51	61.57	Yes 0
Cis-Verbenol	152.23	1	1	2.32	46.38	Yes 0

Table 9: Some ADME Parameters prediction of prominent compounds present in *P. americana* seeds and Stem bark

Compound	TPSA(A^2)	Water Solubility Log S (ESOL)	Bio. Avail. Score	Med. Chem. (PAIN) Alert	Synthetic Accessibility
Dodecanoic acid	37.30	-3.07	0.85	0	1.87
Cis-Verbenol	20.23	-2.77	0.55	0	4.47

Table 10: Interaction of prominent compounds present in *P. americana* seeds and Stem bark with Cytochromes P450 (CYP)

Compound	CYP1A2	CYP2C19	CYP2C9 (Inhibitors)	CYP2D6	CYP3A4	Log Kp(Skin permeation) (cm/s)
Dodecanoic acid	No	No	No	No	No	-4.54
Cis-Verbenol	No	No	No	No	No	-4.99

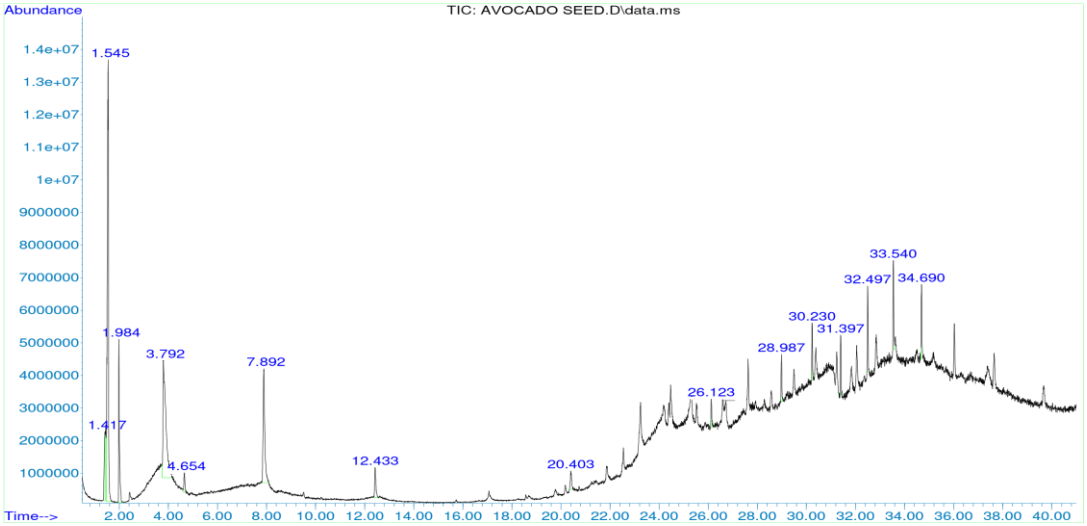

Figure 1: GC-MS *P. americana* (avocado pear) Seeds

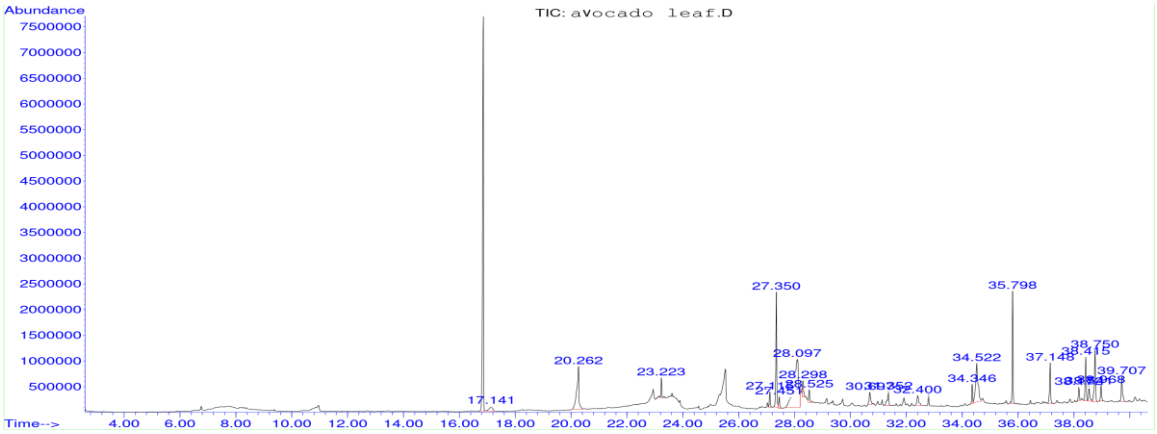

Figure 2: GC-MS *P. americana* (avocado pear) Leaves

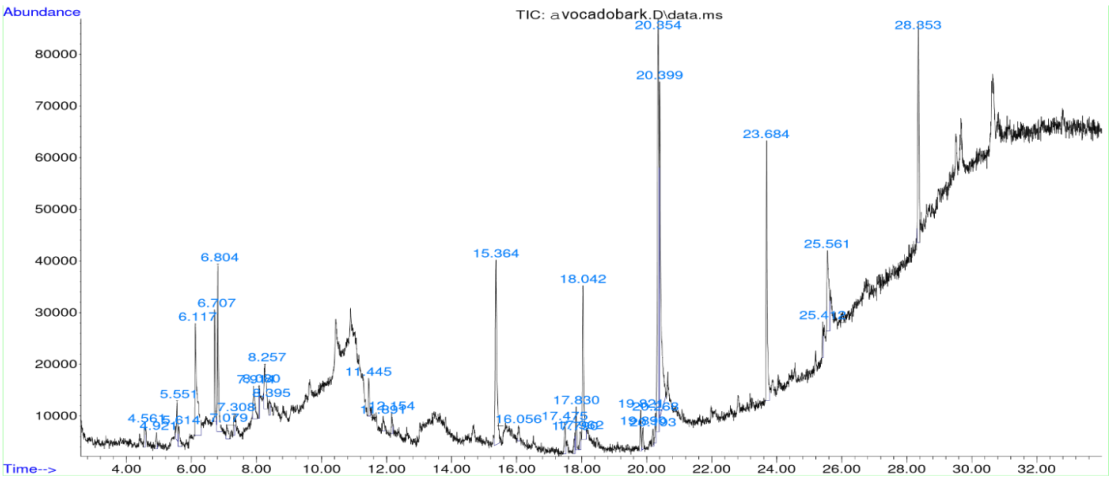

Figure 3: GC-MS *P. americana* (avocado pear) Stem bark

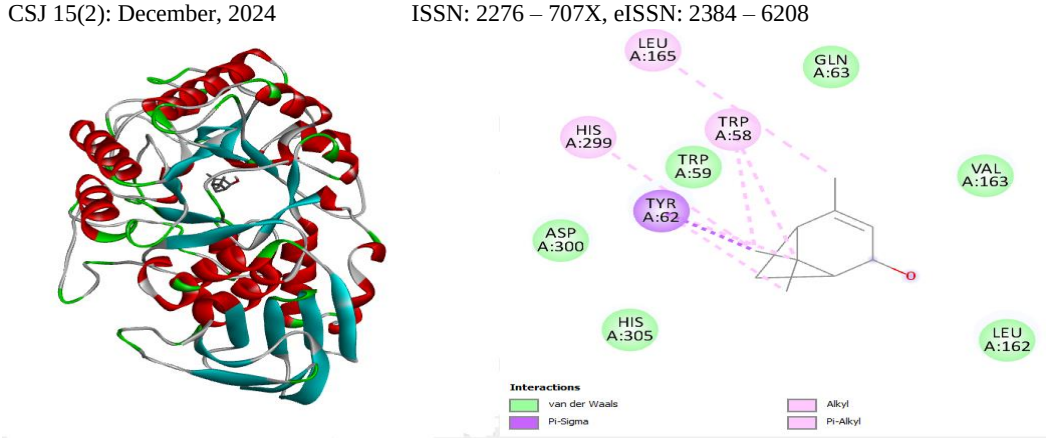

Figure 4: Docking view and 2D interactions of cis-verbenol/α-amylase complex

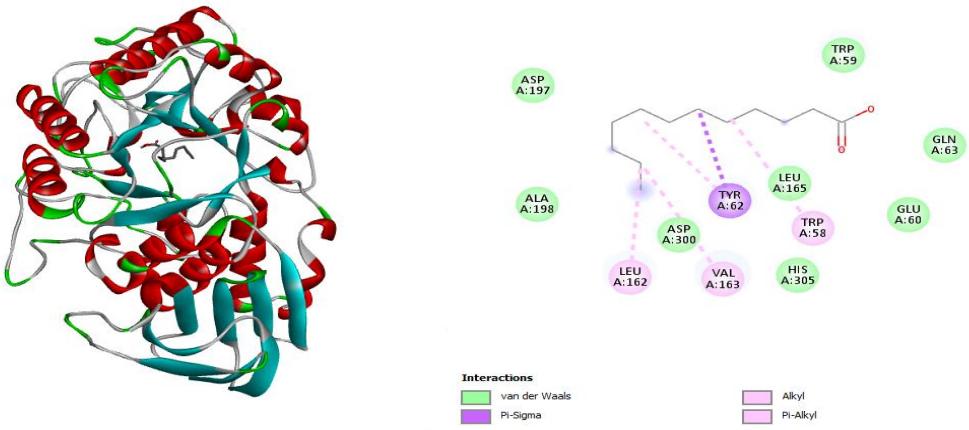

Figure 5: Docking view and 2D interactions of dodecanoic acid/ α-amylase complex

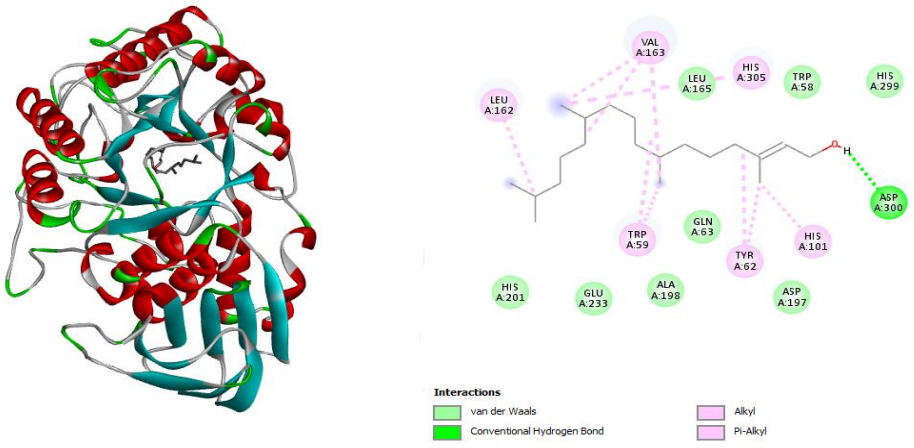

Figure 6: Docking view and 2D interactions of phytol/ α-amylase complex

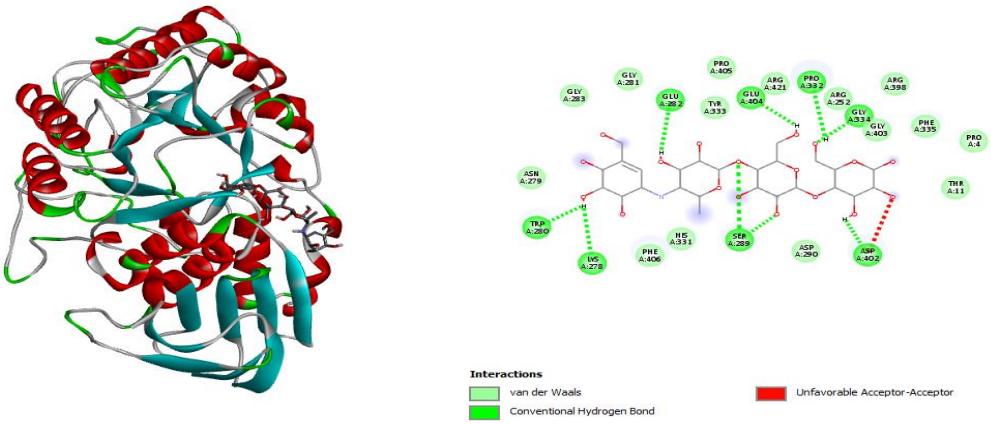

Figure 7: Docking view and 2D interactions of acarbose/α-amylase complex

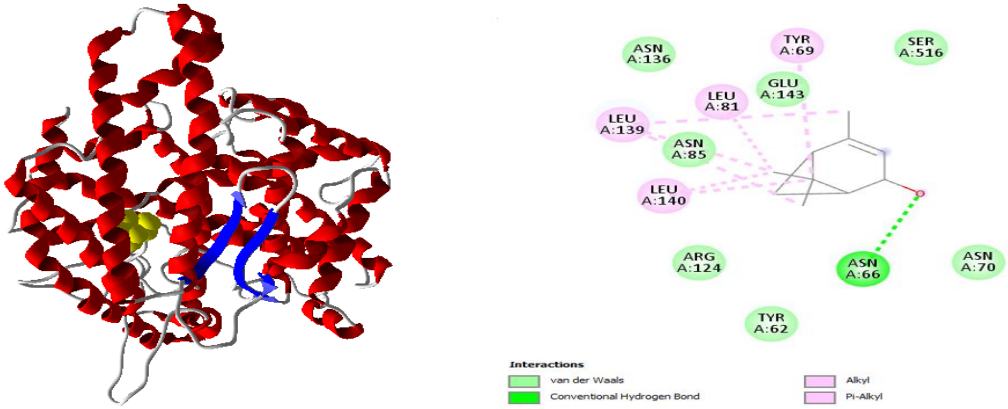

Figure 8: Docking view and 2D interactions of cis-verbenol/ ACE complex

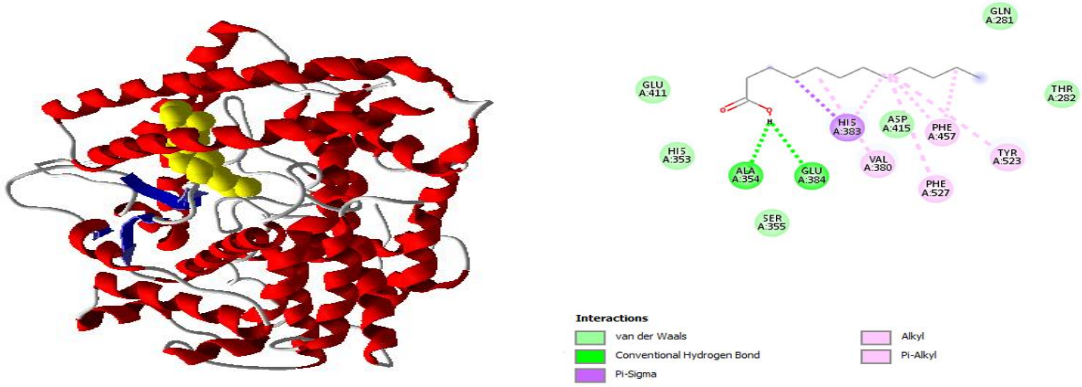

Figure 9: Docking view and 2D interactions of dodecanoic acid/ACE complex

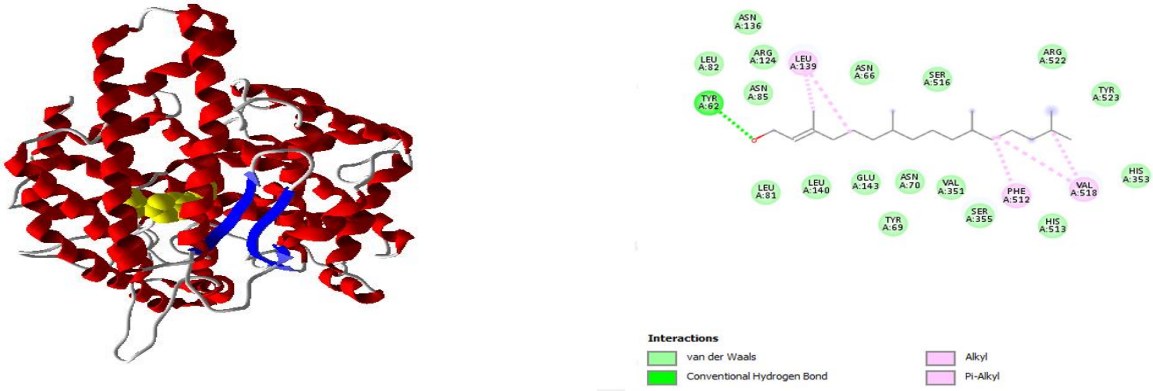

Figure 10: Docking view and 2D interactions of phytol/ACE complex

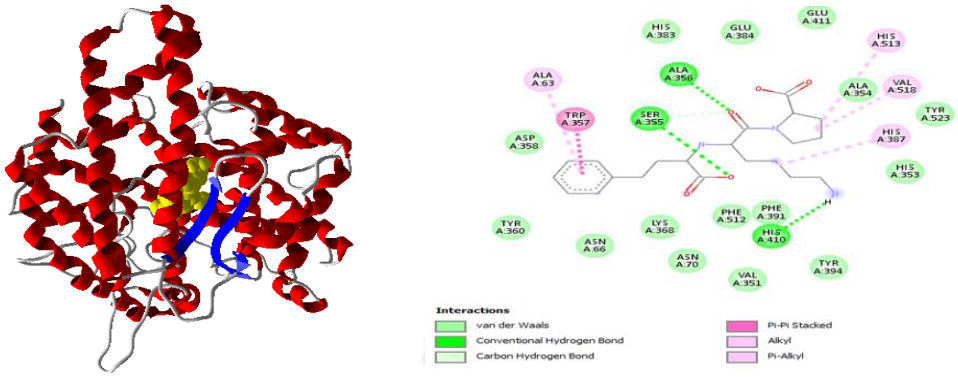

Figure 11: Docking view and 2D interactions of lisinopril/ACE complex

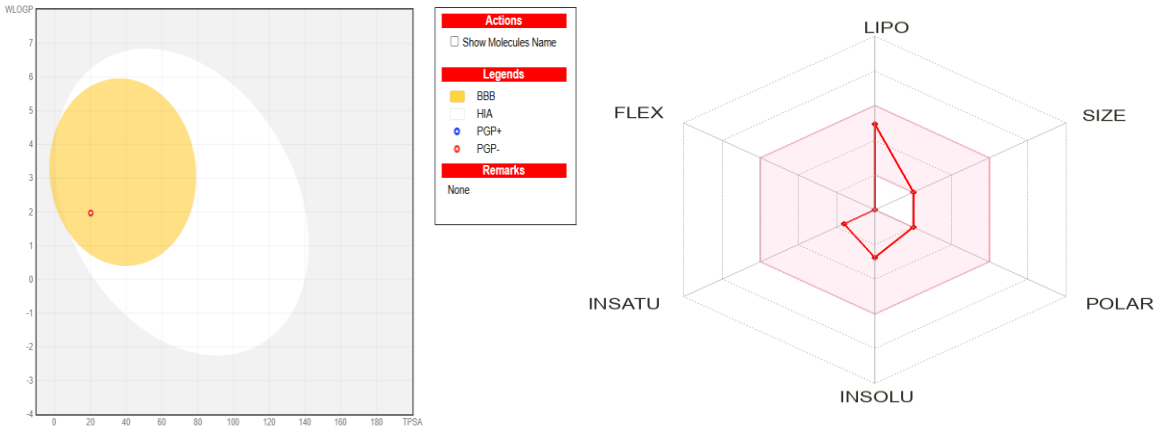

Figure 12: (a) BOILED Egg and Bioactivity radar of cis-verbenol

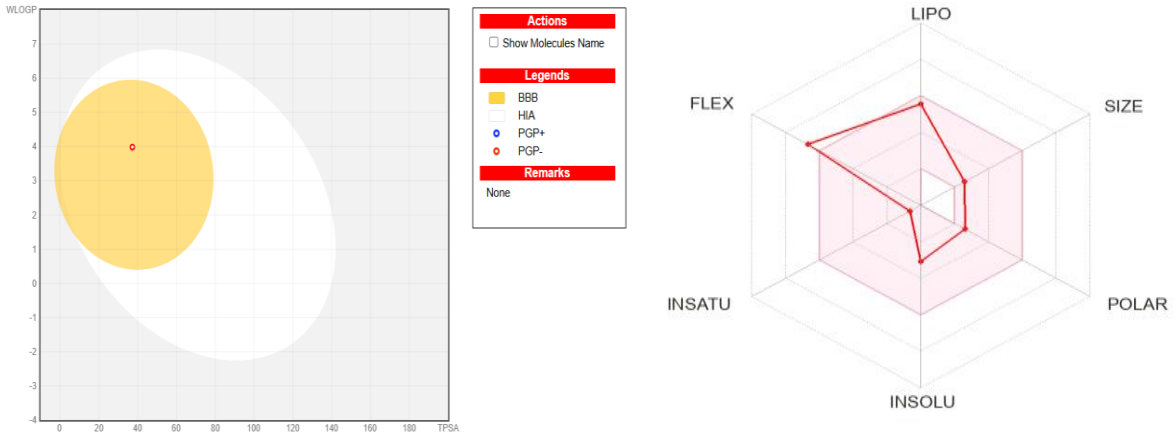

Figure 13: (a) BOILED Egg and Bioactivity radar of dodecanoic acid

Table 6: shows the docking score and amino acid residues. It was observed that the ligands/α-amylase complex gave favorable binding affinity of -6.0, -4.8, and -6.0 kcal/mol for cis-verbenol, dodecanoic acid, and phytol, respectively. The standard drug acarbose has a binding affinity of -8.0 kcal/mol. This suggested that the ligands may not be as potent as the standard drug, acarbose, in the management of diabetes. For the cis-verbenol /α-amylase (Figure 4), alkyl/pi-alkyl interactions were observed with amino acid residues LEU 165A, TRP 58A, and HIS 299A. The van der Waals were observed with amino acids ASP 300A, HIS 305A, GLN 63A, VAL 163A, and LEU 162A. Pi-sigma interactions occurred through TYR 62A. Dodecanoic acid/ α-amylase docked complex (Figure 5) showed alkyl/pi-alkyl interactions with LEU 162A, VAL 163A, and TRP 58A. Interactions of van der Waals with ASP 197A, ALA 198A, ASP 300A, HIS 305A, LEU 165A, GLU 60A, GLN 63A, TRP 59A, and Pi-sigma bonding with TYR 62A. Phytol/α-amylase docked complex (Figure 6) showed alkyl/pi-alkyl interactions with amino acid residues LEU 162A, VAL 163A, TRP 59A, HIS 305A, TYR 62A, and HIS 101A. Interactions of van der Waals were observed with ASP 197A, ALA 198A, HIS 201A, HIS 299A, LEU 165A, GLU 233A, GLN 63A, and TRP 58A. Conventional hydrogen bonding occurred through ASP 300A. The

interactions of the acarbose/α-amylase complex showed the involvement of hydrogen bonds with TRP 280A, LYS 278A, SER 289A, ASP 402A, GLY 334A, PRO 312A, GLU 404A, and GLU 282A. Hydrogen bonding is a distinctive indicator of strong interactions between proteins and ligands, often leading to increased binding affinity (Otuokere et al., 2024). Typically, the inhibitory potential towards the target is enhanced by an increase in the amount of H-bonds in such interactions. These findings underscore their unique binding characteristics, providing valuable insights for their potential development as promising drug discovery and optimization candidates.

Table 7: shows the docking score and amino acid residues of the ligands/ACE complex. It was observed that the ligands/ACE complex gave favorable binding affinity of -6.1, -5.2, and -6.2 kcal/mol for cis-verbenol, dodecanoic acid, and phytol, respectively. The standard drug lisinopril gave a binding affinity of -7.6 kcal/mol. This suggested that the ligands may not be as potent as the standard drug, lisinopril, in the management of hypertension. For the cis-verbenol/ACE complex (Figure 8), alkyl/pi-alkyl interactions were observed with amino acid residues TYR 69A, LEU 81A, LEU 139A, and LEU 140A. The van der Waals interactions were observed with amino acids ASN 136A, SER 516A, GLU 143A, ASN 85A, ARG 124A, TYR 62A, and ASN 70A. A conventional hydrogen bond was observed through ASN 66A. Docecanoic acid/ACE docked complex (Figure 9) showed alkyl/pi-alkyl interactions with PHE 457A, TYR 523A, PHE 527A, and VAL 385A. Pi-sigma interaction was observed with HIA 383A. Phytol/ACE docked complex (Figure 11) showed alkyl/pi-alkyl interactions with amino acid residues LEU 139A, PHE 512A, and VAL 518A. Interactions of van der Waals were observed with LEU 82A, ARG 124A, ASN 66A, SER 516A, ARG 522A, TYR 523A, HIS 353A, HIS 513A, SER 355A, VAL 351A, ASN 70A, GLU 143A, LEU 140A, LEU 81A, and TYR 69A. Conventional hydrogen bonding occurred through TYR 62A. The interactions of the lisinopril/ACE complex showed the involvement of hydrogen bonds with SER 355A, ALA 356A, and HIS 410A. Hydrogen bonding is a distinctive indicator of strong interactions between proteins and ligands, often leading to increased binding affinity (Otuokere et al., 2024). Typically, the inhibitory potential towards the target is enhanced by an increase in the amount of H-bonds in such interactions. These findings underscore their unique binding characteristics, providing valuable insights for their potential development as promising drug discovery and optimization candidates.

According to Lipinski *et al.*, (1997), they posit that optimal absorption is observed when the MW is less than 500 g/mol and the number of H bond acceptors is less than 10. According to Tables 8, 9, and 10, the molecular weights were 152.23 and 200.32 g/mol. Furthermore, all of the compounds examined exhibited H-bond acceptors of less than 10 and H-bond donor counts of less than five. This indicated that the chosen compounds fell within the acceptable range according to Lipinski's criterion. The RO5 is a heuristic devised by Lipinski to ascertain whether a molecule possesses specific attributes that are anticipated to render it suitable for oral administration as a pharmaceutical agent. Cis-verbenol and dodecanoic acid exhibited a drug-likeness prediction with zero violations. It has been hypothesised that all of the ligands satisfy the criteria of RO5. Previous research (Nwankwo *et al*., 2022; Otuokere *et al*., 2022a; Otuokere *et al*., 2022b; Ikpeazu *et al*., 2020; Igwe *et al*., 2020) has anticipated that compounds that obey RO5 will have a low attrition rate during the drug development process.

The topological surface area (TPSA) is considered a significant chemical descriptor that exhibits a strong correlation with pharmacokinetic parameters, as stated by the Lipinski rule (Lipinski *et al*., 1997). It is recommended that a high-quality drug should possess a TPSA value below 140 $Å^2$. According to Tables 8, 9, and 10, the TPSA values are below 140 $Å^2$, indicating their potential as effective pharmaceutical agents. The presence of a soluble molecule significantly enhances several drug development projects, particularly in terms of facilitating easier handling and formulation processes (Ritchie *et al*., 2013). Additionally, in the context of oral delivery research projects, the property of solubility plays a significant role in determining absorption (Ottaviani, 2010). In addition, a drug intended for parenteral administration must possess a high degree of solubility in water to facilitate the delivery of an adequate amount of the active component within the limited volume of the pharmaceutical dosage (Savjani *et al*., 2012). The ESOL model water solubility profile for the test compounds predicted that the ligands were soluble in water (Delaney, 2004). The bioavailability score, as proposed by Martin (2005), aims to estimate the likelihood of a compound exhibiting a minimum oral bioavailability of 10% in rats or Caco-2 permeability. The ligands exhibited favorable bioavailability scores of 0.55 and 0.85. To be considered a therapeutic candidate, a molecule typically needs to have a bioavailability score of at least 0.10. This observation indicated that all the ligands are anticipated to possess oral bioavailability. PAINS, often referred to as pan assay interference compounds, frequent hitters, or promiscuous compounds, are a class of chemicals that possess substructures that exhibit a strong reaction in assays, regardless of the specific protein being targeted. According to our findings, there exists no promiscuous molecule that has the

potential to induce medication interferences. Synthetic accessibility is assessed by normalizing the score on a scale ranging from 1 (indicating easy synthesis) to 10 (indicating highly difficult synthesis). According to Tables 6 and 7, the synthetic accessibility test score of the ligands indicated that the test compounds possessed can be easily synthesized. Potts & Guy, 1992, proposed that a molecule with a greater negative log Kp (with Kp measured in cm/s) exhibits lower skin permeability. The log Kp values for all the IDL derivatives were all negatives. This implies that the IDL ligands were non-skin permeable.

Furthermore, it is crucial to possess an understanding of the interaction between drugs and cytochromes P450 (CYP). The isoenzyme superfamily plays a crucial role in the process of drug elimination via metabolic biotransformation. According to Van Waterschoot and Schinkel (2011, CYP and P-gp can work together to process tiny compounds in a way that enhances the protection of tissues and organisms. It has been estimated that a significant proportion, ranging from 50% to 90%, of medicinal compounds interact with five prominent isoforms, namely CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 (Di, 2014). The inhibition of these isoenzymes is undeniably a significant factor in drug-drug interactions linked to pharmacokinetics (Hollenberg, 2002), resulting in hazardous or undesirable side effects caused by reduced elimination and buildup of the drug or its metabolites (Kirchmair *et al*., 2015). The identification of several inhibitors targeting the CYP isoforms has been documented. Certain drugs exhibit selectivity towards certain isoenzymes, whereas others have an impact on other CYP isoforms (Veith *et al*., 2009). Hence, it is crucial for drug development to predict the likelihood of the molecule causing substantial drug interactions by inhibiting CYPs, and to ascertain the specific isoforms that are impacted. The results of our study indicated that the ligands did not exhibit inhibitory effects on CYP P450 enzymes. The bioavailability radar, as depicted in Figures 12 and 13, is presented to provide a prompt evaluation of drug-likeness. The analysis considered six physicochemical properties: lipophilicity, size, polarity, solubility, flexibility, and saturation. The physicochemical range on each axis was established using descriptors derived from previous works, Ritchie *et al*., 2011. These descriptors were shown as a pink region, within which the radar plot of the molecule must completely fall to be classified as a drug-like compound. In our research study, it has been observed that dodecanoic exhibited limited oral bioavailability because it was slightly flexible, while cis-verbenol exhibited excellent oral bioavailability.

The utilization of the BOILED-Egg (Figures 12 and 13) enables a straightforward assessment of human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) based on the spatial arrangement of molecules within the WLOGPversus-TPSA reference. The white zone is indicative of a heightened likelihood of passive absorption through the gastrointestinal tract, whereas the yellow region (yolk) signifies a heightened likelihood of BBB penetration. It is not mutually exclusive for yolk and white zones to coexist (Ritchie *et al*., 2011; Lovering et al., 2009). Furthermore, the points are depicted in blue when they are anticipated to be actively effluxed by the permeability glycoprotein, P-gp (PGP+), and in red when they are anticipated to be non-substrate of P-gp (PGP-). The ligands exhibited significant BBB penetration in this study. They were anticipated to be non-substrate of P-gp (PGP-). However, the ligands are unsuitable substrates for P-glycoprotein (P-gp), a protein that facilitates the efflux of drugs and other compounds for subsequent metabolism and clearance (Amin, 2013). This leads to therapeutic effectiveness because the drug concentration is not lower than anticipated (Levin, 2012).

CONCLUSION

The phytochemical analysis results revealed the presence of Alkaloids, Flavonoids, Tannis, Saponins, and Steroids in the seeds, leaves, and stem bark extracts of *P. americana* (avocado pear), hence the presence of these secondary metabolites is responsible for its medicinal potency and the highest antibacterial and antioxidant activity exhibited by the stem bark extract could be attributed to the amount of bioactive compounds present in it as shown by the GC/MS analysis. The result from the *in-silico* studies of the prominent compounds showed that dodecanoic acid/α-amylase, pyhtol/α-amylase and *cis*-verbenol/α-amylase had binding energies of -4.8, -6.0, and -6.0 Kcal/mol while dodecanoic acid/ACE, phytol/ACE and *cis*–verbenol/ACE had binding energies of -5.1, -6.2 and -6.2 Kcal/mol respectively. When comparing the result with the standard control, it was observed that *cis*-verbenol and phytol had excellent binding energies less than the standard drug. However, the pharmacokinetic (ADME) properties of the drug proved that *cis*-verbenol, dodecanoic acid, and phytol can easily be absorbed, distributed, and properly metabolized in the kidney and liver and excreted. The drug-likeness test according to Lipinski's rule of five proved that *cis*-verbenol, dodecanoic acid, and phytol can be used as drugs, hence it can be regarded as a potential drug candidate for the management and treatment of diabetes mellitus and hypertension. The findings in this research give credence to the use of the extracts from the seeds, leaves, and stem bark of *P. americana* in the treatment and management of diseases and infections in herbal medicine.

REFERENCES

Amin, M.L. (2013). P-glycoprotein inhibition for optimal drug delivery. Drug Target Insights 7:27-34.20.

Artega, J.D., Zhang, Q., Huerta, S., Go, V.I., Herber, D. (2005). Inhibition of Prostate Cancer cell growth by an avocado extract: role of lipid-soluble bioactive substances *J. Nutr. Biochem 16:23-30.*

Adeyemi, O.O., Okpo, S.O., Ogunti, O.O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Presea Americana* Mill (Lauraceae). Fitoterapia 73: 375-380.

Batandrin, M.F., Kjocke, A.J., Wurtele, D.F. (1985). Natural plant chemical sources of industrial and Pharmaceutics. Humana Press, New Jersey USA. Pp 137-141.

Codeiro, N.J.V. and Oniyangi, O. (2020). Phytomedicines. 2nd Edition. Pp 200-2005.

Cheesbrough, M. (2000). Medical Laboratory Manual for Tropical Countries, Educational Publisher, Pp 447.

Dallakyan, S. and Olsonn, A.J. (2015). Small-molecule library screening by docking with PyRx Methods Mol Bio. 1263: 243-250.

Di, L. (2014). The role of drug-metabolizing enzymes in clearance. Expert Opin Drug Metab Toxicol 10: 379-393.

Delaney, J.S. (2004). ESOL: Estimating Aqueous Solubility Directly from Molecular Structure. *J. Chem. Inf. Model*.44: 1000-1005.

Hollenberg, P.F. (2002). Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. Drug Metab. Rev.34: 17-35.

Ikpeazu O.V., Amaku, F.J., Otuokere, I.E., Igwe, K.K. (2020). Using the Pharmacophoric features of Azithromycin to design a potential SARS-CoV-2 inhibitor. Eur J Engr Res Sci. 5(9) 1037-1042.

Igwe, O.U., Otuokere, I.E., Ohwimu, J.G., Amadi, K.C., Nwadire, F.C. (2020). Synthesis, characterization and molecular docking studies of Co(II) metal complex of sulfathiazole. *Bulletin of the Chemical Society of Ethiopia*. Vol. 34(1):83-92.

Igwe, O.U. and Akabuike, H.C. (2016). Free radicals scavenging activity, phytochemistry and antimicrobial properties of *Tetrepleura tetraptera* seeds. *Int. Res. J. Chem. Che. Sci.*, 3(2):037-042.

Igwe, O.U. and Onuoha, P.U. (2016). Potentials of *Citrullus Ianatus* seeds as antioxidant and antimicrobial agents and a probe of their phytochemicals. *International Journal of Chemical, Material and Environmental Research*, 3(3), 62-67.

Igwe, O.U. and Abii, T. (2024). Characterization of bioactive sesquiterpenes, organic acids and their derivatives from the leaves of *Psidium guajava* Linn. *Int. J. Pure Appl. Chem.*, 4(4):456-467.

Igwe, O.U. (2014a). Chromatographic and spectrometric characterization of bioactive compounds from the leaves of *Hyptis lanceolate* Poir. *International Journal of Chemistry and Pharmaceutical Sciences*, 2(1):547-553

Igwe, O.U. (2014b). Quantitative estimation of ascorbic acid levels in citrus fruits at variable temperatures and phytochemical properties. *International Journal of Chemical and Biochemical Sciences*, 5:67-71, ISSN 2226-9614.

Igwe, O.U. and Echeme, J.O. (2013). Isolation, characterization and antibacterial activity of 4-(4-phenyl-1,4-dihydronaphthalen-1-yl) pentenoic acid from the stem bark of *Brachystegia eurycoma* Harms. *Int. J. Drug Dev. Res.*, 5(2):335-340.

Igwe, O.U. and Okwu, D.E. (2013a). Isolation, characterization and antibacterial activity of 3-hydroxy-2,2-bis(6-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl) propanal from the stem exudates of *Brachystegia eurycoma* Harms. *Der Pharma Chemica*, 5(2): 39-44.

Igwe, O.U. and Okwu, D.E. (2013b). Isolation, characterization and antioxidant activity of a furo-chromen-4-one from the seeds of *Brachystegia eurycoma* Harms. *Int. J. Chem. Sci.*, 11(1): 121-128.

Kirchmair, J., Goller, A.H., Lang, D., Kunze, J., Testa, B., Wilson, I.D., Glen, R.C., Schneider, G. (2015). Predicting drug metabolism experiment and/or computation? *Nat Rev Drug Discoy*. 14(6): 387-404. Doi:10. 1038/nrd4581

Krishnaraju, A.V., Rao, T.V., Rao., Sundararaju, A. (2005). Assessment of Bioactivity of Indian Medicinal plants using Brine shrimp (*Alternaria solania*) Lethality Assay. *International Journal of Applied Science and Engineering* 2:125-134.

Levin, G.M. (2012). P-glycoprotein: why this drug transporter may be clinically important Curr Psychiatry 11: 38-40.

Lovering, F., Bikker, J., Humblet, C. (2009). Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J. Med. Chem.* 52: 6752-6756.

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Advanced Drug Delivery Reviews*, 23: 3. https://doi.org/10.1016/S0169-409X(96)00423-1.

Martin, Y.C. (2005). A Bioavailability Score. *J. Med. Chem.* 48: 3164-3170.

Nwankwo, C.I., Omeh, T.N., Omodamiro, O.D., Otuokere, I.E., Alaebo, P.O., Atasie, O.C., Ekwuribe, G.A. (2022). Phenolics of Abelmoschus esculentus Pods. HPLC Identification and in-Silico Studies to Identify Potential Anti-inflammatory Agents, *Trop J Nat Prod Res*. 6(8): 1311-1319. doi.org/10.26538/tjnpr/v618.28

Otuokere, I.E., Igwe, K.K., Nwankwo, C.I., Achi, N.K., Asuquo, I.G, Eberendu, K.O. (2024). Electrostatic Potential Mapped onto Electron Density Surface, ADME, Molecular Docking and Molecular Dynamics Simulations of Some Indolin-2-one Analogues as Cytochrome C Peroxidase inhibitors, Research Square, 1-20. https://doi.org/10.21203/rs.3.rs-4200649/v1

Otuokere, I.E., Akoh, O.U., Echeme, J.O., Nwankwo, C.I., Egbucha, J.N., Ammasai, K. (2022a). GC-MS Analysis and Molecular Docking Studies to Identify Potential SARS-CoV-2 Nonstructural Protein Inhibitors from Icacina trichantha Oliv Tubers, *Trop J Nat Prod Res*. 6(8): 1336-1342. Doi.org/10.26538/tjnpr/v6i8.29

Otuokere, I.E., Akoh, O.U., Nwadire, F.C., Nwankwo, C.I., Egbucha, J.N., Wilson, C., Okwudiri, O.A. (2022b). GC-MS Profiling and In-Silico Studies to Identify Potential SARS-CoV-2 Nonstructural Protein Inhibitors from Psidium guajava *Afri Sci Res*. 161-173

Oniyangi, Oluseyi and Cohall, Damian. H. (2020). Phytomedicines (medicines driven from plants) for sickle cell disease. Cochrane Library.

Onwuka, G.I. (2018). *Food Analysis and Instrumentation (Theory and Practice)* second edition. Naphthali Printers Nigeria, Pp 299-314.

Ottaviani, G. (2010). What is modulating solubility in simulated intestinal fluids? *Eur. J Pharm Sci* 41. 452-457.

Potts, R.O. and Guy, R.H. (1992). Predicting Skin Permeability. *Pharm. Res*. 09: 663-669.

Ritchie, T.J., Macdonald, S.J.F., Peace, S., Pickett, S.D., Luscombe, C.N. (2013). Increasing small molecule drug development ability in suboptimal chemical space. *Med. Chem. Commun*. 4: 673

Ritchie, T.J., Ertl, P., Lewis, R. (2011). The graphical representation of ADME-related molecule properties for medicinal chemists. *Drug Discov. Today* 16: 65-72

Swiss ADME. Available online: http//www.swissadme.ch/index.php Accessed May 2024.

Sofowora, A., Ogunbodede, E., Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *Afr. J. Tradit Complement Altern. Med.* 10(5):210-229

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa* 2nd edition. Spectrum Book Limited, Ibadan. Pp6-188.

Sayjani, K.T., Galiar, A.K., Sayjani, J.K. (2012). Drug solubility: importance and enhancement techniques. ISRN Pharm. 2012: 195727. doi: 10.5402/2012/195727

Saver, J.D. (1993). Historical Geography of crop plants- A selected rooster. CRC Press, Boca Raton, Florida Pp 1014-1017.

Van Waterschoot, R.A.B. and Schinkel, A.H. (2011). A critical analysis of the interplay between cytochrome P450 3A and P-glycoprotein recent insights from knockout and transgenic mice. *Pharm Rev*.63: 390-410

Veith, H., Southall, N., Huang, R., James, T., Fayne, D., Artemenko, N., Shen, M., Inglese, J., Austin, C.P., Lloyd, D.G., Auld, D.S. (2009). Comprehensive characterization of cytochrome P450 isozyme selectivity across chemical libraries. *Nature Biotechnology* 27(11):1050-1055. https://doi.org/10.1038/nbt.1581