ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antibacterial Activity and Molecular Docking Analysis of the Stem Bark Extracts of *Persea americana* Mill (Lauraceae)

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ARTICLE INFO

Article history:
Received 23 April 2022
Revised 25 May 2022
Accepted 15 June 2022
Published online 02 July 2022

ABSTRACT

Persea americana is utilized as food in most countries. Its excellent nutritional benefit and bioactivities have been extensively evaluated. Therefore, this study aims to determine the *in-silico* and *in-vitro* bioactivity of its bark extracts against multidrug-resistant (MDR) bacteria isolated from fish pond effluents. Extraction of the plant was done using the maceration method in ethanol, methanol, acetone, cold water, and hot water for 7 days and screened against MDR bacteria. Phytochemical screening was carried out on all the plant extracts. One hundred microliters of the crude extract were used for the minimum inhibitory concentration (MIC) test. Different bioactive molecules in the hot-water extract were identified using Gas chromatography-mass spectrometry (GC-MS) method and evaluated using molecular docking tools to inhibit penicillin-binding protein (PBP) and DNA gyrase (DNAg). Phytochemicals such as tannins, flavonoids, saponins, steroids, alkaloids, and glycosides were present in all the extracts. Inhibition zones ranging from 0-20mm for methanol bark (MB), hot-water bark (HWB), and cold-water bark (CWB), ethanol bark (EB) (0-18mm), acetone bark (AB) 0-13mm at 100 mg/mL of the crude extract were observed. The MIC of the extracts against multi-drug resistant isolates in EB, MB, and HWB were at 6.25 mg/mL, while those of AB and CWB were at 12.5 mg/mL and 12.5 mg/mL, respectively. GC-MS analysis showed the presence of 105 compounds. Likewise, molecular docking revealed that certain phytoconstituents examined in this study had higher binding affinity compared to conventional antibiotics. Therefore, *P. americana* bark possesses promising bioactive chemicals that can be used as antibacterial agents.

Keywords: Antibacterial activity, Molecular docking, *P. Americana*, Multidrug resistant bacteria

Introduction

Traditional antibiotics' effectiveness against hazardous germs has declined in the last decade due to the rise in bacterial resistance.[1] However, there has been an increasing interest in discovering plant components having antibacterial effects for several decades. During the bacterial division cycle, these chemicals influence the outer membrane, proteolysis, and Gene expression.[2] Several studies have found that certain plant components have a strong mutagenesis effect.[3-4] As a result, researching plant substances' antimicrobial properties is critical. Computational techniques are most often used to pinpoint new therapeutic strategies. Researchers frequently use molecular docking, a sophisticated bioinformatics tool for drug development. Molecular docking can be used to determine a ligand's affinity for a target protein. This approach is commonly used to find plant phytochemicals with antibacterial activity.[5] As a result, we were eager to investigate *Persea americana's* antibacterial activities. *Persea americana*, a tropical and Mediterranean tree and shrub found in Mexico, Central America, and South America, have historically been known for its therapeutic benefits. It is a healthy source of energy for diabetics due to its low sugar level and a high amount of carbohydrates, nutrients, cellulose, and necessary vitamins for humans.[6] Because of its importance in improving and sustaining a safe heart and circulatory system, as well as the existence of unsaturated lipids in it, it has a higher global intake.[6-7] Avocado extracts have long been recognized to inhibit bacteria, yeast, fungus, and protozoa growth, and they are an effective antibacterial agent with unique properties.[7] Avocado extracts are said to prevent the growth of several human pathogens such *as Bacillus cereus, Bacillus subtilis, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Salmonella serovar Typhi, Shigella dysenteriae, Candida albicans, Saccharomyces cerevisiae, Leishmania donovani, and Mycobacterium tuberculosis.* molecular docking is the process of creating surroundings that could model the conditions found in the human body which is used to examine the reactions between a target protein and phytoconstituents under simulated defined circumstances in a reliable and reproducible and cost-effective manner.[2] Therefore, this article aims to determine the in silico and in vitro bioactivity of *P. americana* (Mill) bark extracts against bacteria containing resistance genes obtained from fish ponds.

Materials and Methods

Plant material collection and identification

The bark of *Persea americana* was collected on 16th October 2019 at Awotan, Ibadan, Oyo State, Nigeria, where it was found growing naturally. It was thoroughly washed, cut into little pieces, and dried at room temperature for one month. The identification and authentication of the plant were carried out at the Herbarium of the Forest Research Institute of Nigeria, Ibadan and a voucher specimen number FHI 11262 was assigned. The dried material was pulverized, weighed, and kept for active component extraction in a sterile hand grinder.

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Citation: Olatayo AO, Ayansina ADV, Dahunsi SO. Antibacterial Activity and Molecular Docking Analysis of the Stem Bark Extracts of *Persea americana* Mill (Lauraceae). Trop J Nat Prod Res. 2022; 6(6):980-990. doi.org/10.26538/tjnpr/v6i6.27

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Extraction of plants
For seven (7) days, fifty grams of the powdered bark was processed with 500 mL each of ethanol, methanol, acetone, cold water, and hot water using the maceration process. The powder was placed into pre-sterilized wide-mouthed bottles containing the solvents for the prescribed number of days and was regularly shaken and stirred (using a rotary shaker and a glass rod). The extracts were then sieved and filtered using a Whatman number 1 filter paper after which vacuum concentration was done in a rotary evaporator. The crude extracts were kept refrigerated in an airtight glass jar before use.

Phytochemical analysis
Established phytochemical approaches such as those used by Trease and Evans[9] and Sofowora[10] were used to screen for glycosides, steroids, flavonoids, tannins, alkaloids, and saponins to determine the components of the crude extract.

Test organisms
Seven bacteria isolates were obtained from Bowen University's Microbiology Program, including five Gram-negative bacteria *Escherichia coli* (OM135507), *Kosakonia oryzae* (OM135508), *Stenotrophomonas maltophilia* (OM135509), *Elizabethkingia anopheles* (OM135510), *Stenotrophomonas maltophilia* strain 1D-2A (OM135512), and two Gram-positive i.e., *Bacillus sp.* (OM135511), *Lysinibacillus sphaericus* (OM135513). The bacteria were cultivated at 37°C in nutrient agar (NA) plates.

Preparation of stock solutions of plant extract and various concentrations for Minimum Inhibitory Concentration (MIC)
This was accomplished by re-constituting 100 mg of the crude extract in 1 mL of Dimethyl Sulphoxide (DMSO). Various additional dilutions used for the determination of MIC were obtained from the most active stock solution. The dilutions are 50, 25, 12.5 and 6.25 mg/mL respectively.

Antibacterial assay of the extracts
The antibacterial action of the extracts was tested utilizing the agar well diffusion method, according to the National Committee for Clinical Laboratory Standards. Bacteria were swabbed with cotton swabs on agar plates, and wells of 8 mm were formed with a Pasteur pipette. In the created wells, a hundred microliter of each extract (100 mg/mL) was impregnated, with control of 100L of DMSO. The plates were then incubated in an upright posture at 37°C for 24 hours. All experiments were repeated in duplicate, and the antibacterial potential was determined by measuring the inhibition zones with a ruler.

Determination of Minimum Inhibitory Concentration
Using Doughari[11] technique, bark extracts were prepared and examined at four different concentrations (50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL). The cultures were incubated at 370°C for 24 hours. The MIC was defined as the concentration of the extract below where no inhibition occurred.

GC-MS analysis
A fractionating column was used for the GC-MS analysis of hot-water bark crude extracts. The carrier gas was helium, with a flow rate of 1 mL min^{-1}. A computer algorithm that compared the mass spectra to the National Institute of Standards and Technology (NIST 11) data set is also used to characterize the peaks.

Docking study of selected compounds identified by GC-MS
For this work, 21 active phytoconstituents selected based on quality from *Persea americana* bark were put through molecular docking tests to see if they might inhibit antibiotic target proteins. Two target proteins, DNA gyrase (PDB entry 1KZN) and Penicillin Binding Protein (PBP1a) (PDB entry 2OQO) were chosen for docking studies in this investigation. As a positive control, molecular docking was done between five conventional antibiotics (cefepime, meropenem, imipenem, ciprofloxacin, and nalidixic acid) and the mentioned targets.

Ligand and receptor preparation
In the docking process, three-dimensional (3D) structures of the target protein (i.e., DNA gyrase and Penicillin Binding Protein (PBP1a) are downloaded PDB format from the Protein Data Bank (http://www.rcsb.org/pdb) serve as receptors. pyMOL (version 1.7.4.5) was used to open the files. Water molecules and ligands that remained bound to the protein were detached and saved in the Pdf file format for further study. The protein was transformed into a macromolecule using PyRex software (version 0.8). Following that, the files were saved in pdbqt format. The PubChem site was used to obtain ligand structures (active compound structures). The search was carried out by simply typing the ligand's name into the search box. The files for each ligand were downloaded and saved. Open Babel (version 2.3) was used to convert SDF files to PDB files. Auto-dock Vina (version 1.1.2) was used to open the ligand's PDB format. Torque was adjusted by sensing the root and adjusting as needed. The pdbqt format was used to save the file.

Silico ADME study
Utilizing Lipinski's rule of five, the ADME characteristics of the better phytoconstituents were determined by employing a web-based application Swiss ADME. According to Lipinski, a chemical can have antibiotic behavior if it does not breach more than one of the following requirements: (i) Molecular Weight must be less than 500; (ii) hydrogen bond donors must be less than 5; (iii) hydrogen bond acceptors must be less than 10; (iv) lipophilicity must be less than 5; and (v) molar refractivity must be between 40 and 130. These chemicals are thought to be great therapeutic candidates because they follow the Lipinski rule.

Determination of active site
The binding sites of the amino acid residues in the receptor domain where the ligand is to be attached were found using Auto-dock Vina (version 1.1.2). As a result, a 3-dimensional grid box model of the receptor region was generated.

Protein-phytoconstituents Docking
The interaction was carried out utilizing Auto-dock Vina (version 1.1.2). Molecules and targets saved in pdbqt form were copied to the Vina folder. The Vina file system was then copied and saved as "conf.txt" in notepad. The Vina program was executed using PyRx. The docked complexes were examined to identify ligands with higher interaction affinity and antagonistic constant with the target protein. The interactions of the ligand-protein were investigated using the Discovery Studio 2020 program.

Statistical analysis
Antibacterial activity and minimum inhibitory concentration results are reported as means ± Standard deviation. All experiments were repeated in duplicate

Results and Discussion

Phytoconstituents analysis
The preliminary phytoconstituents screening revealed that steroids, tannins, flavonoids, saponins, steroids, alkaloids, and glycosides were present in all extracts. Alkaloids and glycosides were absent in ethanol, cold-water, and hot-water extracts. The antibacterial activity of the extracts could be due to these bioactive components, as shown in Table 1.
The discovery of novel sources of therapeutically and industrially essential compounds is mainly reliant on the phytochemical screening of medicinal plants. It is crucial to begin screening plants for secondary metabolites as soon as possible. Plants are valuable in medicine because they contain chemical components that help the human body fulfill various physiological processes.[12-13] Phytochemical study of *P. americana* bark extracts revealed flavonoids, alkaloids, saponins, tannins, steroids, and glycosides, which is consistent with previous research findings.[14-16]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Concentration of phytochemicals

Extract	Tannin	Flavonoid	Saponin	Steroid	Alkaloid	Glycoside
Ethanol bark	+	+	+	+	-	+
Methanol bark	+	+	+	+	+	+
Acetone bark	+	+	+	+	+	+
Hot-water bark	+	+	+	+	-	-
Cold-water bark	+	+	+	+	+	-

Key: +: presence of phytochemicals, and -: absence of phytochemicals

Table 2: Antibacterial activity (Zone of inhibition in mm)

Isolate code	Bacteria Name	EB	MB	AB	HWB	CWB
ATB1d	*Bacillus cereus*	13 ± 1.41	20.5 ± 0.70	13.5 ± 0.70	22.5 ± 3.53	11.5 ± 2.12
ATB1a	*Lysinibacillus sphaericus*	-	21 ± 1.41	-	22 ± 2.82	-
ID2A	*Stenotrophomonas maltophilia strain 1D-2A*	-	-	-	-	-
IBC2a	*Stenotrophomonas maltophilia strain 1BC-2A*	-	-	-	-	-
IBC1c	*Elizabethkingia anopheles*	-	-	10.5 ± 2.12	-	-
AF3D	*Escherichia coli*	-	-	-	-	-
OYE2c	*Kosakonia oryzae*	-	-	-	-	-

*Each value is expressed in mean ± standard deviation
Key: EB: ethanol bark, MB: methanol bark, AB: acetone bark, HWB: hot-water bark, CWB: Cold-water bark, and -: not detec

Plant substances known as alkaloids have been shown to have antibacterial effects. Alkaloids are by far the most powerful and pharmacologically relevant natural compounds.[17] Because of their analgesic, antispasmodic, and antibacterial characteristics, pure natural, and synthetic alkaloids are utilized as fundamental therapeutic agents.[17] Flavonoids, which are free radical scavengers and super antioxidants that protect cells from oxidative damage and have anticancer characteristics, were also found in the plant extracts. Flavonoids, among other things, defend against allergies, inflammations, platelet aggregation, bacteria, ulcers, viruses, and cancer.[18] Due to the presence of flavonoids, plant extracts from *P. americana* might be employed as antispasmodic, antifungal, and antibacterial medications. This explains why the locals use these herbs to treat diarrhea, spasmodic bronchitis, and other microbial illnesses.[19] Tannins in *P. americana* extracts may explain its broad usage in herbal medicine for wound healing, ulcer healing, hemorrhoids, frostbites, and burn to heal.[20] Tannins are metal chelators that can combine with macromolecules to produce compounds. This process depletes microorganisms' vital substrates, co-factors, and enzymes, resulting in cell death. Tannins contain astringent qualities that help wounds and irritated mucous membranes heal.[20]
The occurrence of saponins provides strong evidence that *P. americana* has cytotoxic effects, such as intestinal permeabilization.[18] The presence of saponin is assumed to be responsible for the cell's hemolytic characteristics, which allow harmful chemicals to enter and important components to depart.[21] Antibacterial properties of steroids have been discovered, and their interactions with sex hormones make them extremely significant substances. [22-23] Steroids have also been demonstrated to bind in the digestive tract, limiting cancer cell growth and decreasing blood cholesterol levels. [24] Glycosides are a kind of steroidal chemical that can improve cardiac output, change the electrical activity of the heart, and have been found to reduce blood pressure in various studies.[18-20, 22-24] They have an impact on smooth muscle and other tissues in addition to blocking Na+-K+ ATPase in cardiac muscle cell membranes.[25] The study's findings suggest that the discovered phytochemical substances have bioactive elements and that these plants are asserting to be a vital pool of phytochemical compounds with great medicinal significance.

Antibacterial assay
On all of the test organisms, the ethanol extract produced inhibition zones ranging from 0–12 mm, Methanol extract (0–20 mm), acetone (0–13 mm), hot-water (0-20), and cold-water (0–10 mm). Methanol and hot-water extracts were only effective against *Bacillus cereus* and *Lysinibacillus sphaericus* (20 mm and 20 mm, respectively), whereas ethanol, acetone, and cold-water extracts were effective against *Bacillus cereus* at 12 mm and 10 mm, while acetone was effective against *Bacillus cereus and Elizabethkingia anopheles* at 13 mm and 09 mm. *Stenotrophomonas maltophilia* strain 1D-2A, *Stenotrophomonas maltophilia strain 1BC-2A*, *Escherichia coli*, and *Kosakonia oryzae* were resistant to all of the extracts as shown in Figure 1 and Table 2. In this investigation, the bark of *P. americana* was discovered to exhibit antibacterial action against gram-negative bacteria. This might be related to cytoplasmic component leaking caused by a breakdown in cell wall construction.[26] Several other investigations have found antibacterial activity in *P. americana* bark.[14-16] The antibacterial activity of *P. americana* was shown to be dependent on the extraction solvent employed in the bacterial test. The higher activity of the methanol and hot-water extracts in comparison to the ethanol, acetone, and cold-water extracts might be explained by the fact that different solvents have diverse degrees of polarity, resulting in varying degrees of solubility for the various bioactive components.[27] Because the other extracts (ethanol, acetone, and cold-water extracts) have a narrower range of showed that the active component must be soluble in methanol and hot-water extracts.[12, 28] Methanol and hot-water bark extracts were shown to be more effective against *Bacillus cereus* and *Lysinibacillus sphaericus* than other extracts.

Minimum inhibitory concentration
The minimum inhibitory concentration (MIC) zone for all of the bark extracts ranged between 3 and 17 mm (Table 3). After overnight incubation with a medium, the minimum inhibitory concentration (MIC) is the lowest concentration capable of totally inhibiting any visible microbial growth.[26] The MIC result showed that as concentration grew, the organisms tested got more inhibited.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Minimum inhibitory concentrations (MIC) of *Persea americana* bark extract in mg/mL

Bacteria isolate	**EB**				**MB**				**AB**				**HWB**				**CWB**			
	a	**B**	**C**	**d**	**a**	**b**	**c**	**d**	**a**	**b**	**c**	**d**	**A**	**b**	**c**	**d**	**a**	**b**	**c**	**d**
Bacillus cereus	19±1.14	14.5±0.70	13±0.00	11±0.00	17.5±0.70	15±0.70	11±1.41	7.5±0.70	9.5±0.70	14.5±0.70	12.5±0.70	11±0.00	16.5±0.70	8±0.00	6.5±0.70	-	6.5±0.70	4±0.00	-	-
Lysinibacillus sphaericus	-	-	-	-	16±0.00	13±0.00	11.5±0.70	8.5±0.70	-	-	-	-	15.5±0.71	13.5±0.69	09±1.41	4.5±.070	-	-	-	-
Stenotrophomonas maltophilia strain 1D-2A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stenotrophomonas maltophilia strain 1BC-2A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Elizabethkingia anopheles	6±0.00	3.5±0.70	-	-	-	-	-	-	8.5±0.71	6±0.00	3.5±0.63	-	-	-	-	-	-	-	-	-
Kosakonia oryzae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Each value is expressed in mean ± standard deviation
Key: EB: ethanol bark, MB: methanol bark, AB: acetone bark, HWB: hot-water bark, CWB: Cold-water bark, a: 50 mg/mL, b: 25 mg/mL, c: 12.5 mg/mL, d: 6.25 mg/mL and -: not detected

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

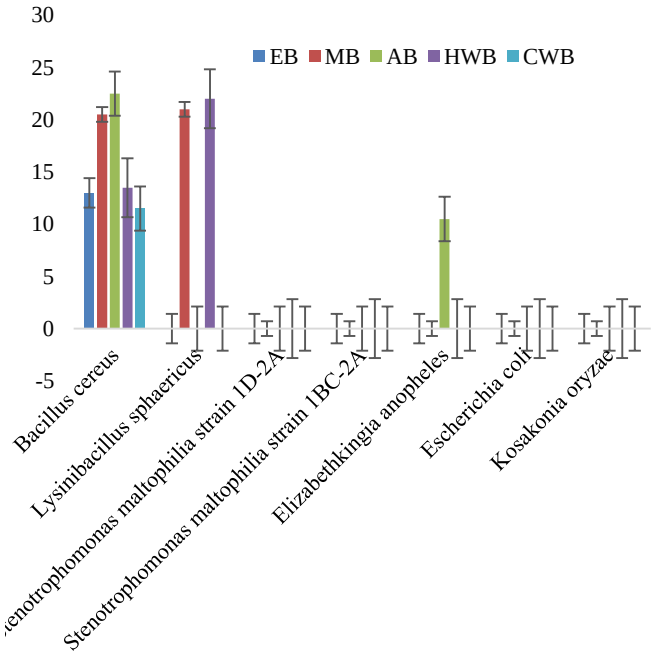

Figure 1: Antibacterial activity (zone of inhibition in mm)

Because the MIC values showed the unique nature of this plant's antibacterial action, the inhibition zone values just demonstrated the amount of the extract's efficacy as the concentration grew. The fact that hot-water bark has a low MIC value (5 mm) against *Lysinibacillus sphaericus* suggests that it is effective against these bacteria, which indicates that a lower concentration of extract is needed to keep the organism from growing.[26] This is significant because infectious diseases caused by *Lysinibacillus sphaericus* are on the rise in immunocompromised individuals.[29] Differences in minimum inhibitory concentration, on the other hand, might be explained by strains' varying susceptibility to chemical compounds as a result of various resistance levels.[26]

Gas Chromatography-Mass Spectroscopy (GCMS) Analysis

The GC-MS chromatogram of hot-water bark extract showed 105 peaks, which indicated the presence of 105 different bioactive/phytochemical compounds (Figure 1).

The majority of compounds present in the hot-water bark extract were 2-furan methanol, 2-methoxy-4vinyl phenol, 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta [c] pyran-1-yl) ethanone, 13-Tetradecenal, Tetradecanoic acid, Neophytadiene, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Tetradecanoic acid, 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-, Methyl 8,11, 14-heptadecatrienoate, Butyl citrate, Cyclododecyne, 2-Methyl-Z, Z-3, 1octadecadienoic, alpha-Tocospiro A, Tricyclo [20.8.0.0 (7,16)] 3-(hexahydro-azepine-1-yl)-, 1,1-dioxide (Table 4).

Table 4: Bioactive compound identified and selected based on quality from hot-water bark extract of *P. americana*

S/N	Peak	Retention time	Area %	Compound name	Molecular formula	Molecular weight	Quality
1	5	4.202	0.07	2-Furanmethanol	$C_5H_6O_2$	98.09	92
2	23	9.492	0.16	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	150.17	96
3	26	10.306	0.09	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethenone	$C_{13}H_{18}O_2$	206.28	97
4	37	12.368	0.16	13-Tetradecenal	$C_{14}H_{26}O$	210.35	91
5	44	13.933	0.11	Tetra-decanoic acid	$C_{14}H_{28}O_2$	228.37	97
6	47	14.470	0.82	Neophytadiene	$C_{20}H_{38}$	278.5	91
7	51	15.209	0.30	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	98
8	52	15.729	2.35	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256.46	99
9	52	15.729	2.35	Tetra-decanoic acid	$C_{14}H_{28}O_2$	228.37	93
10	59	17.156	5.24	9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-	$C_{19}H_{32}O_2$	292.5	98
11	59	17.156	5.24	Methyl 8, 11, 14-heptadecatrienoate	$C_{18}H_{30}O_2$	278.4	96
12	64	17.872	1.68	Butyl citrate	$C_{18}H_{32}O_7$	360.4	90
13	65	18.022	0.62	Cyclododecyne	$C_{12}H_{20}$	164.29	90
14	76	20.852	1.19	2-Methyl-Z, Z-3,13-octadecadienoic	$C_{19}H_{36}O$	280.5	91
15	81	21.689	0.48	alpha. -Tocospiro A	$C_{29}H_{50}O_4$	462.7	95
16	85	22.486	0.27	Tricyclon [20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy-	$C_{30}H_{52}O_2$	444.7	90
17	87	22.856	0.37	gamma. -Tocopherol	$C_{28}H_{48}O_2$	416.7	97
18	89	23.364	1.96	Vitamin E	$C_{29}H_{50}O_2$	430.7	99
19	93	23.947	0.11	Oleic Acid	$C_{18}H_{34}O_2$	282.5	90
20	95	24.282	0.44	Stigmasterol	$C_{29}H_{48}O$	412.7	92
21	103	26.130	0.05	1, 2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	$C_{13}H_{16}N_2O_2S$	264.35	91

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The hot-water bark extract GC-MS screening revealed 105 peaks, indicating the presence of 105 distinct bioactive or phytochemical substances. Retention duration, purity, molecular weight, and formulas were all used to confirm the compounds.

11-Dodecenol, 2-Methyl-Z, Z-3,13-octadecadienoic, Octadecane, 2-methyl, Methoxyacetic acid, 2-tetradecyl ester, Dodecane has been reported to possess antimicrobial and anti-inflammatory activities.[30] Hexadecanoic acid, 14-methyl-, methyl ester, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12-octadecadienoic acid (z, z) has been reported by previous researchers to have anti-inflammatory, nematicide, cancer preventive, hypo-cholesterolemic, and hepato-protective activities.[31-32] Similarly, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl) has been reported to possess antimicrobial and anti-inflammatory activity. [33]

Rule of five

Table 5 displays the docking rules of five (Ro5) docking compounds determined using the SWISSADME predictor. The Ro5 is not violated by any of the chemicals utilized in this investigation.

A range of bioactive chemicals derived from various plants has been shown to have antibacterial action. [34-35] The rule of five is one method for determining if a chemical has therapeutic potential. As shown by this guideline, orally administered herbal remedies must not violate more than one of the established criteria.[36] As a consequence, each docking chemical was examined to check if it met Lipinski's RO5, and none failed to meet the rule of five (Table 5). The rule is used to establish if a chemical molecule possesses the properties and characteristics necessary to be utilized as an efficient medication that may be consumed orally in humans, as well as to assess its drug-likeness.[36-37]

Molecular docking

The prediction of the binding energy among prospective ligands and proteins was conducted utilizing molecular docking simulation. The docking study findings for the chosen phytoconstituents with PBP and DNAg are shown in Table 6. Docking analysis showed that certain substances from the hot-water extract had better binding positions with PBP than imipenem cefepime and Meropenem, including 1-(3,6,6-tetracycline). -Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethenone, n-Hexadecanoic acid, (Z, Z, Z)-, Cyclododecyne, alpha. -Tocospiro A, Tricyclo [20.8.0.0 (7,16)] triacontane, 1(22), 7(16)-diepoxy-gamma. -Tocopherol, Vitamin E, Stigmasterol and 1,2-Benzisothiazole, 3-(hexahydro-1H-azepine-1-yl)-, 1,1-dioxide. 1,2Benzisothiazole, 3-(hexahydro-1-azepine-1-yl) and 1,1dioxide, Tricyclo [20.8.0.0 (7,16)] triacontane, 1 (22) and 7 (16)-diepoxy (Figure 2) were high affinity to DNA gyrase that were more efficient or similar to Nalidixic acid and Ciprofloxacin. Several of the greatest computational methods for drug engineering and antimicrobial agent mechanism determination is molecular docking.[37] Although, *P. americana's* antibacterial ability has been proven in multiple investigations, the antibacterial processes of the active phytoconstituents of these plants remain unknown. The docking of 21 known compounds from *P. americana* hot-water bark extract, and bacterial proteins (penicillin-binding protein and DNA gyrase) was done in this study. The results revealed Tricyclo [20.8.0.0 (7,16)] The binding affinity of 1,2-benzisothiazole, 3-(hexahydro-1H-azepine-1-yl)-, and 1,1-dioxide with penicillin-binding protein (Table 6 and Figure 2a-h) and DNA gyrase were much higher. Inhibition of this enzyme inhibits DNA synthesis and cell division, resulting in cell death.

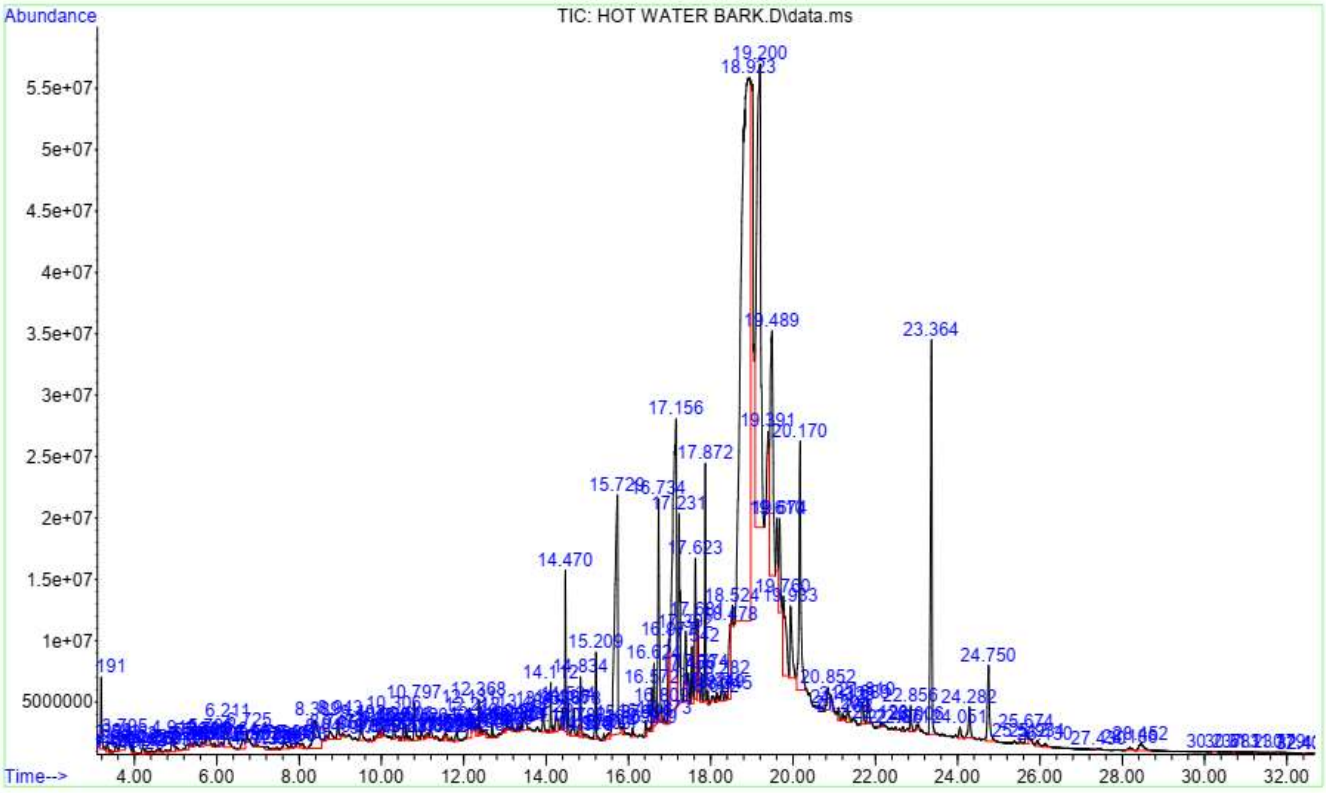

Figure 2: GC-MS chromatogram of hot-water bark extract of *P. americana*

Table 5: Lipinski's rule of five (RO5) of hot-water bark Compounds as potential protein inhibitors.

Compounds	Molecular formula	Molecular weight (<500 g/mol)	LogP (<5)	H-bond donor (<5)	H-bond acceptor (<10)	LogS (solubility)	Violations
2-Furanmethanol	$C_5H_6O_2$	98.10 g/mol	0.62	1	2	-1.09 (s)	0
2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	150.17 g/mol	2.14	1	2	-2.81 (s)	0
1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethenone	$C_{13}H_{18}O_2$	206.28 g/mol	2.53	0	2	-2.54(s)	0
13-Tetradecenal	$C_{14}H_{26}O$	210.36 g/mol	4.48	0	1	-3.79(s)	0
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37 g/mol	4.45	1	2	-4.31(ms)	0
Neophytadiene	$C_{20}H_{38}$	278.52 g/mol	7.07	0	0	-6.77(ps)	1
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	5.20	1	2	-5.02(ms)	1
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{19}H_{32}O_2$	278.43 g/mol	5.09	1	2	-4.78(ms)	1
Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	278.43 g/mol	5.19	0	2	-4.33(ms)	1
Butyl citrate	$C_{18}H_{32}O_7$	360.44 g/mol	2.95	1	7	-2.67(s)	0
Cyclododecyne	$C_{12}H_{20}$	164.29 g/mol	4.21	0	0	-4.21(ms)	0
2-Methyl-Z,Z-3,13-octadecadienoic	$C_{19}H_{36}O$	280.49 g/mol	5.91	1	1	-5.20(ms)	1
.alpha.-Tocospiro A	$C_{29}H_{50}O_4$	462.70 g/mol	6.37	1	4	-6.53(ps)	1
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	$C_{30}H_{52}O_2$	444.73 g/mol	7.50	0	2	-8.56(ps)	1
gamma.-Tocopherol	$C_{28}H_{48}O_2$	416.68 g/mol	7.95	1	2	-8.29(ps)	1
Vitamin E	$C_{29}H_{50}O_2$	430.71 g/mol	8.27	1	2	-8.60(ps)	1
Oleic Acid	$C_{18}H_{34}O_2$	282.46 g/mol	5.71	1	2	-5.41(ms)	1
Stigmasterol	$C_{29}H_{48}O$	412.69 g/mol	6.97	1	1	-7.46(ps)	1
1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	$C_{13}H_{16}N_2O_2S$	264.34 g/mol	2.19	0	3	-2.65(s)	0

KEY: logP: Partition coefficient between *n*-octanol and water, logS: Aqueous solubility, s: soluble, ms: moderately soluble, Ps: poorly solubility

Table 6: Binding energy (kcal/mol) of selected compounds derived from P americana hot-water bark extract docked against PBP 1a (2oqo) and DNA GYRASE (1kzn)

S/N	LIGAND	BPBP 1a (2oqo)	DNA GYTASE (1kzn)
1	2-Furanmethanol	-3.6	-4.1
2	2-Methoxy-4-vinyl phenol	-4.7	-5.5
3	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone	-5.7	-6.3
4	13-Tetradecenal	-4.2	-5.0
5	Tetradecanoic acid	-4.2	-5.2
6	Neophytadiene	-5.0	-5.6
7	n-Hexadecanoic acid	-5.3	-5.3
8	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	-5.6	-5.2
9	Methyl 8,11,14-heptadecatrienoate	-4.8	-5.8
10	Butyl citrate	-4.8	-5.4
11	Cyclododecyne	-5.4	-5.7
12	2-Methyl-Z,Z-3,13-octadecadienoic	-5.1	-5.2
13	alpha.-Tocospiro A	-6.1	-5.9
14	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	-8.3	-7.3
15	gamma.-Tocopherol	-6.6	-6.4
16	Vitamin E	6.1	-6.6
17	Oleic Acid	-5.0	-5.1

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18	Stigmasterol	7.8	-6.7
19	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	-6.2	-7.3
20	Cefepime	-6.8	-
21	Meropenem	-6.1	-
22	Imipenem	-5.2	-
23	Ciprofloxacin	-	-7.6
24	Nalidixic acid	-	-7.1

Figure 3a: Binding interaction between PBP and gamma. –Tocopherol

Figure 3b: Binding interaction between PBP and 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide

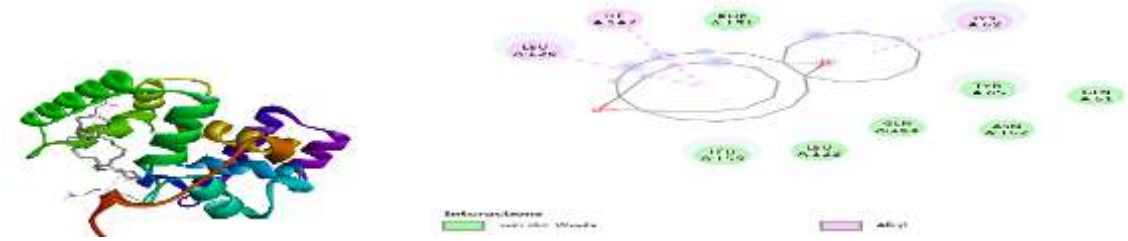

Figure 3c: Binding interaction between PBP and Tricyclo [20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy

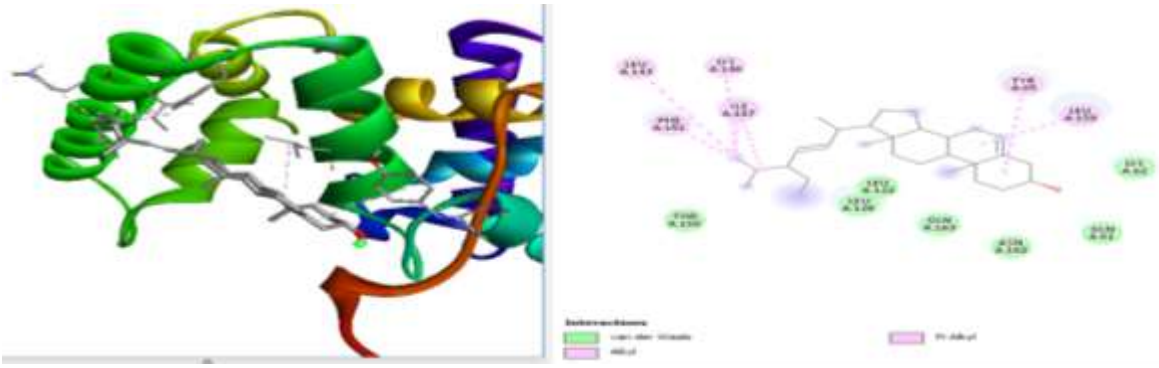

Figure 3d: Binding interaction between PBP and Stigmasterol

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ISSN 2616-0692 (Electronic)

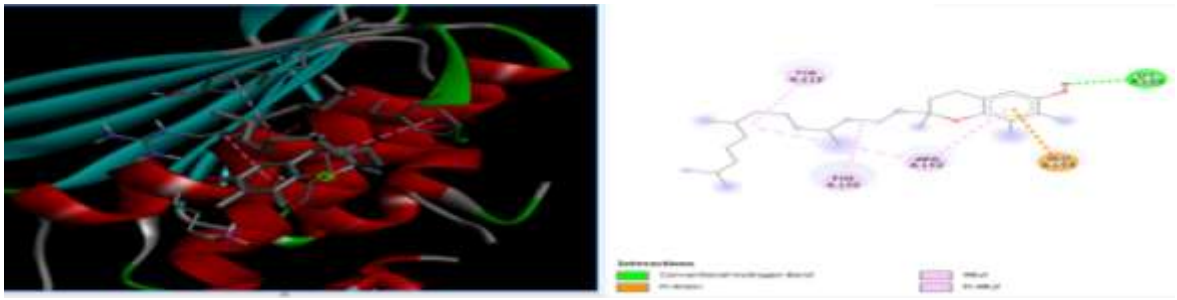

Figure 3e: Binding interaction between DNA Gyrase and gamma.-Tocopherol

Figure 3f: Binding interaction between DNA Gyrase and 1,2-Benzisothiazole, 3-(hexahydro-1H-azepine-1-yl)-, 1,1-dioxide

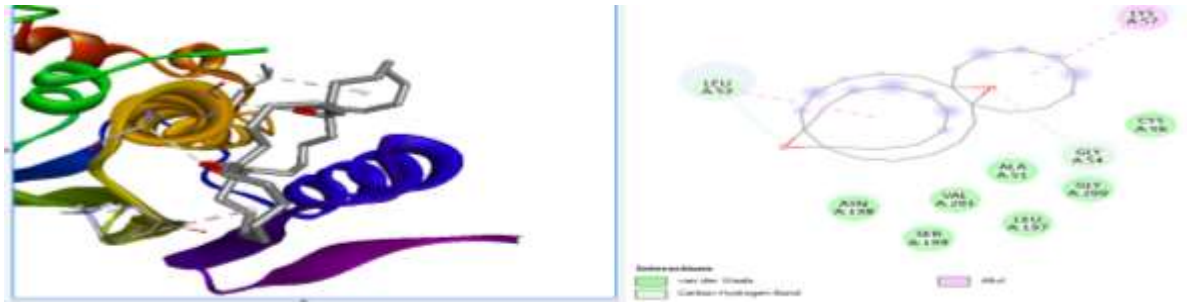

Figure 3g: Binding interaction between DNA Gyrase and Tricyclo [20.8.0.0(7,16)] triacontane, 1(22),7(16)-diepoxy

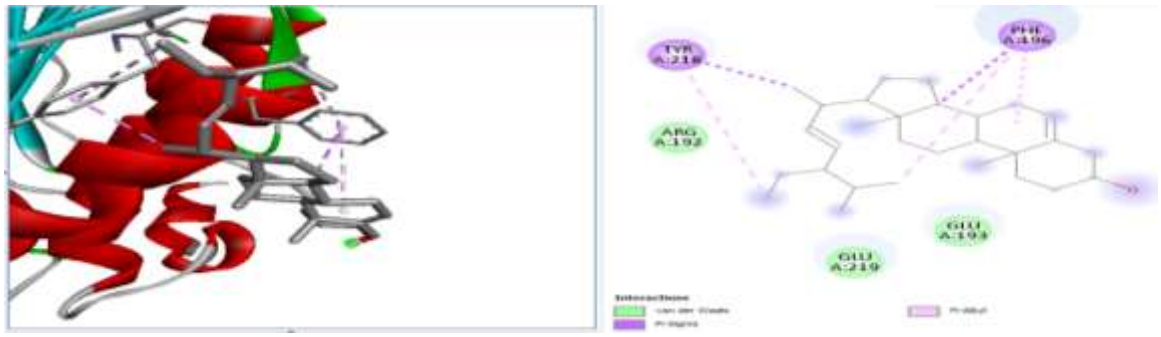

Figure 3h: Binding interaction between DNA Gyrase and Stigmasterol

Conclusion

The hot-water bark extract of *Persea americana* and its constituents show antibacterial action against the Gram-negative bacteria employed in this investigation. In silico results showed that Tricyclo [20.8.0.0(7,16)] and 1,2-Benzisothiazole, 3-(hexahydro-1H-azepine-1-yl)-, 1,1-dioxide exhibits better selectivity towards the PBP and DNA gyrase binding sites and might be a powerful antibacterial agent. Several research papers, on the other hand, demonstrated that based on binding efficiency indices, lead-drug discovery initiatives would provide bioactive molecules with higher pharmacokinetic results. In the age of antimicrobial drug research, isolated bioactive chemicals should be used to build more reasonable structure-activity connections.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors appreciate BOWEN University for providing a fund to publication, purchase reagents and other necessary materials used in this work. We are also grateful to the University management for providing Laboratory space for this research work.

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