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Therapeutic Potentials of *Persea americana* Peptide: In Silico and Experimental Studies

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A B S T R A C T

Objective: Salmonella infections constitute a growing healthcare concern ranging from gastroenteritis to severe invasive diseases that require hospitalization. Persea americana is valued for its high nutritional content and health benefits. This study evaluated the therapeutic effects of *Persea americana* derived seed peptide (PASP) on *Salmonella*-infected Wistar rats.

Methods: Peptide from the plant material was obtained via aqueous extraction. Susceptibility testing of PASP against the test multi-drug-resistant Salmonella typhi was performed using standard microbiological assays. The constituents in PASP were measured via high performance liquid chromatography (HPLC) analysis, and virtually screened against DNA gyrase B. Therapeutic effect of PASP was evaluated in-vivo using murine model, comprising seven different groups (n=3).

Results: PASP elicited potent activity against the tested organism producing inhibition zone of: 22 mm at 100 mg/mL, and MIC value of 25 mg/mL. The pharmacodynamics of PASP revealed a time dependent decline in microbial cells. Amongst the constituents of PASP, Phenylalanine, Cystine, Histidine, Aspartic acid, Glutamic acid had the highest binding scores, with the free binding energy of these compounds' superior to Ofloxacin. PASP had appreciable therapeutic effect on the *S. typhi* infected rats via alleviating signs and symptoms of infection, modulating hematological parameters PCV (44.00±0.57%), WBC (6.93±2.08x109/L), Lymphoctyes (8.46±4.08%); and had no pronounced detrimental impact on the rats' biomarkers ALP (44.33±3.17 U/L), AST (184.66±4.05 U/L), ALT (108.33±13.17 U/L) (group administered with 25 mg/mL PASP).

Conclusion: These findings provide substantial evidence on the safety and anti-Salmonella efficacy of bioactive peptide from P. americana seeds.

Keywords: Persea americana seeds, Peptide, *Salmonella typhi*, Antimicrobial resistance, DNA gyrase, virtual screening, Wistar rats

➢ **How to cite this paper**

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Introduction

Salmonella is a genus of Gram-negative, rod-shaped bacteria that causes salmonellosis, an infection primarily targeting the digestive system. It is one of the leading causes of bacterial foodborne illnesses globally. Infections can range from mild gastroenteritis, which typically resolves on its own, to more severe, invasive forms that may necessitate hospitalization [1]. The infection is primarily acquired by consuming contaminated food or water, particularly raw or undercooked animal-derived products, including poultry, eggs, and dairy. Additionally, contact with infected animals or their environments can lead to infection. Due to its ability to thrive in various environmental conditions, *Salmonella* remains a persistent challenge in the food production industry [2].

The annual incidence of *Salmonella*-related diseases is substantial, contributing to millions of cases each year and leading to significant morbidity and mortality worldwide [3]. These infections result in tens of millions of cases annually, leading to hundreds of thousands of deaths. The most severe complications, such as sepsis and organ failure, are particularly threatening to vulnerable groups, including children, the elderly, and individuals with weakened

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immune systems [1,4]. In light of this, effective treatments are critical, especially for more severe cases. Treatment often involves fluid replacement for dehydration and, in certain cases, antibiotics. However, antibiotic use must be approached with caution due to the growing issue of antibiotic resistance.

Salmonella infections can be categorized into two primary forms is typhoidal and non-typhoidal salmonellosis. Non-typhoidal salmonellosis is the more common form, generally presenting as acute gastroenteritis. Symptoms include diarrhea, abdominal pain, fever, nausea, and vomiting. While most individuals recover without medical intervention, severe cases can lead to dehydration and systemic infection [6]. On the other hand, typhoidal salmonellosis, caused by Salmonella enterica serotypes Typhi or Paratyphi, leads to more serious systemic infections. This form is marked by prolonged fever, abdominal discomfort, and systemic symptoms, such as hepatosplenomegaly (enlarged liver and spleen). If left untreated with antibiotics, typhoid fever can be fatal [7]. *Salmonella* infections remain a major public health concern due to their widespread occurrence, significant morbidity, and economic burden. This issue is particularly acute in low-resource regions where limited access to clean water, sanitation, and healthcare exacerbates the problem. In these areas, inadequate food safety practices increase the risk of foodborne transmission [4,8].

One of the growing challenges in managing Salmonella infections is the increasing prevalence of antibiotic resistance. The misuse and overuse of antibiotics, both in healthcare and agricultural settings, have led to the development of multidrug-resistant strains of Salmonella [9-12]. These resistant strains complicate treatment, resulting in longer durations of illness, higher healthcare costs, and increased mortality. As such, addressing antibiotic resistance in Salmonella is a critical component of public health efforts, underscoring the need for improved antibiotic stewardship, enhanced sanitation measures, and better food safety practices [10,12].

Persea americana, or avocado, is a tropical fruit celebrated for its rich nutritional content and wide-ranging health benefits. While the fruit is widely appreciated, the avocado seed, often discarded, has a long history in traditional medicine, where it has been used to address various health concerns, including digestive disorders, hypertension, inflammation, and infections [15]. In many cultures, the seed is considered a powerful natural remedy, with folkloric traditions often citing its role in treating bacterial infections, such as those caused by *Salmonella*. The healing properties attributed to avocado seeds in these traditions are believed to stem from their rich composition of bioactive compounds, including polyphenols, flavonoids, tannins, and bioactive peptides. These compounds are thought to contribute to the seed's antimicrobial, anti-inflammatory, and antioxidant effects, which may help in combating infections and promoting overall health. Recently, scientific research has begun to lend support to these long-standing beliefs, providing evidence that avocado seeds contain potent compounds capable of inhibiting bacterial growth, including that of Salmonella [16]. This growing convergence of folkloric knowledge and modern science underscores the potential of avocado seeds as a promising therapeutic option for infectious diseases.

The emergence of multidrug-resistant *Salmonella* strains has increased the need for alternative treatment options capable of overcoming the limitations of existing antimicrobial strategies [17,18]. This research explored the therapeutic potential of bioactive peptides extracted from *P. americana* seeds in Salmonella-infected Wistar rats.

Materials and Methods

Microbial Strain and Inoculum Standardization Procedures

The test organism (multidrug resistant Samonella typhi) utilised for this study was obtained from the Laboratory of Microbiology at our institution. The virulence and antibiotic resistance profile of the organism were confirmed via standard microbiological techniques [19]. A zero-point-five (0.5) McFarland standard was achieved by combining 0.05 mL of 1% barium-chloride dihydrate with 9.95 mL of 1% sulfuric-acid, forming a 1.0% w/v solution of barium sulfate. To prepare the inoculum, 18-hour bacterial colonies were transferred to sterile saline and modified to conform to the 0.5 McFarland standard. The final bacterial suspension was then diluted to 10^6 CFU/mL [19].

Collection of Plant Material and Preparation of Extract

Fresh matured seeds of *P. americana* used for the study, were authenticated at the Plant Science and Biotechnology Departmental Herbarium and Taxonomic Unit. Voucher specimen of the seeds designated as PSBHT-283 were deposited at the Herbarium. The seeds were extracted by mixing similar volumes of methanol and dichloromethane, and agitated for 18 to 24 hours at room temperature. Distilled water was then added, the mixture vortexed, and left for 24 hours. The resulting aqueous amino acid/peptide-rich supernatant was decanted, concentrated using a rotary

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evaporator to remove methanol, and prepared for C18 flash pre-purification [20].

The sterility of the extracts was tested by adding 2 mL of the sterile extract to 8 ml of sterile Mueller-Hinton broth and Salmonella-Shigella broth. The mixture was incubated at 37°C for 24 hours. A sterile extract was confirmed if there was no turbidity or cloudiness in the broth after incubation.

Antimicrobial Susceptibility Testing

The anti-*Salmonella* activity of PASP was evaluated via the agar well diffusion technique [21]. One milliliter of each standardized test organism suspension was evenly spread on dried sterile Mueller Hinton agar (Oxoid Ltd. Basingstoke, UK) plates. After the plates dried, uniform wells (6mm) were created using a cork borer and labeled. Fifty microliters of PASP (100 mg/mL in 5% dimethyl-sulfoxide, Sigma Aldrich -Germany) were added to the wells, with ofloxacin as a control in one well. The plates were left at room temperature for 15 minutes to allow diffusion before being incubated at 37°C for 24 hours. Zones of inhibition were measured after incubation. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were ascertained via tube dilution and plating assays [21], respectively. For the MIC, the extract was prepared in concentrations ranging from 100 to 3.125 mg/mL, and 0.5 mL of the test inoculum was liquidated into each tube. Negative controls (MHB alone) and positive controls (MHB with test organisms) were included. After 24 hours incubation at 37°C, the MIC was identified as the least concentration showing no turbidity. For the MBC, subcultures from the MIC tube and other non-turbid tubes were plated on fresh Mueller Hinton agar, incubated, and the concentration with no visible growth was identified as the MBC.

Time-Kill Kinetics Assay of Persea americana Essential Oil

Time-kill assay as described by [22] was used to probe the microbicidal activity of the extract. Standard inoculum of the bacterial culture in Mueller Hilton Broth containing the extract at different concentrations of MIC, 2xMIC, 4xMIC were incubated at 37˚C. Aliquots were withdrawn from the culture at 6 hours, 18 hours and 24 hours and cultured on Mueller Hilton Agar (Oxoid Ltd. Basingstoke, UK), followed by incubation at appropriate temperature. Tubes containing sterile broth plus inoculum served as control. After incubation at 37˚C, the number of colonies were counted and expressed as CFU/mL. Time–kill curves were then constructed by plotting the log10 CFU/mL against the exposure time (hours).

High Performance Liquid Chromatography (HPLC) Screening of P. americana

Amino acid analysis of *P. americana* was conducted using an Agilent 1260 Infinity II series system (Agilent Technologies, Santa Clara, CA, USA), equipped with a DAD WR G71115A detector, a G7130 column oven, and a Quat Pump VL G711A (Agilent Technologies). The HPLC analysis was performed using a 120 EC-C18 4µm Poroshell column (4.6x150 mm, Agilent Technologies), with slight modifications. For HPLC detection, an electron ionization system operated in electron impact mode with DAD UV light ionization energy was engaged. A 100 µL sample was mixed with 1 mL of methanol for HPLC analysis, following a previous procedure [23]. The mobile phase comprised: acetonitrile (ACN), methanol, and 0.1% formic acid (45:45:10), with a flow rate of 0.6 mL/min, 20 µL injection volume, and a detection wavelength of 210 nm. The column oven temperature was set at 40°C [23].

Generation and Preparation of Compound library

The HPLC identified compounds from P. americana were downloaded from the PubChem (https://pubchem.ncbi.nlm.nih.gov) repository alongside with the protein standard drug in structure data file (sdf) format. These molecules were exported onto Schrodinger workspace (Schrodinger, 2021) and prepared using Ligprep tool for the in-silico study.

Protein Preparation

The research collaborator for structural bioinformatics protein databank (RCSB PDB) [www.rcsb.org] website provided the x-ray crystallographic structure of DNA gyrase subunit B complex with ATP from *Salmonella typhi* having PDB ID of 6J90. The missing residues and loop in the protein and other side chain anomalies were resolved, followed by energetic optimization with force field OPLS4 using protein preparation wizard of Schrodinger suit 2021. The receptor grid generator was used to generate glide grid on the co-ligand attached site with glide coordinate of x = -12.97; y = 51.4 and z = 28.69. The prepared protein crystallographic structure and Ramachandran residues' distribution are shown in Figure 1.

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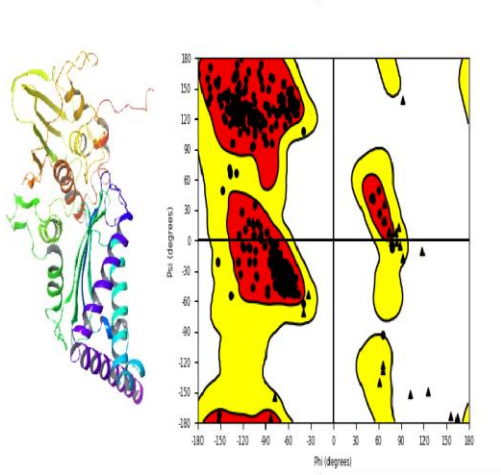

Figure 1: The crystal structure of DNA gyrase subunit B and the distribution plot of Ramachandran residues

Structure-based virtual screening

The prepared compounds from P. americana and the standard ligand were screened against DNA gyrase subunit B (gyrB) using the extra precision (XP) GLIDE docking filtering procedure in Maestro Schrodinger suite (v 2021). This scoring function is recognized for its reliability and ability to differentiate, though it requires a longer runtime [24].

Prime/MM-GBSA calculations

The gyrB-ligand complexes were refined using the local optimization feature in Prime, and the binding energy (Δbind) for the complexes was calculated using the OPLS4 force field. MM/GBSA calculations were performed on the docking complexes to determine the binding free energy, using the following equation:

$$\Delta GBind = \Delta EMM + \Delta GSolv + \Delta GSA$$

ADME/Tox screening

The pharmacokinetic profile, drug-likeness, and toxicity of the hit compounds were determined using the SwissADME (http://www.swissadme.ch) and Pro-Tox II online servers (https://tox-new.charite.de/protox II) online servers.

Experimental Animals

The experiment was executed using 8–12-week-old Wistar rats, which were acclimatized prior to the start to ensure physiological and homeostatic stability. The rats were housed under controlled environmental conditions, including a temperature of 25–27°C, humidity between 40-60%, and a 12-hour light/dark cycle. They were provided with ad libitum access to commercial rat feed and drinking water [25].

Grouping of Animals and Treatments

The animals were grouped into experimental units after acclimatization. The infectivity dose (2.0 × 108 cfu/mL) was derived [26] and infection was confirmed by symptomatology and culturing of stool samples from inoculated animals in growth media, such as Salmonella-Shigella agar, Nutrient agar and MacConkey agar; and checking for the types and dominance of microbes.

Therapeutic assay was performed according to the procedures outlined by Oluyele et al. [25], with some modifications. The animals were assigned into seven different groups of 3 rats per group. The first four groups were orally infected with the test agent. Treatments were initiated at the establishment of infection and terminated on the 7th day of administration. The first and second groups received treatments of 25 mg/mL and 50 mg/mL of PASP respectively, the third group were treated with antibiotics (Ofloxacin 2 mg/mL), the fourth group were treated untreated, the fifth and sixth groups were respectively given 25 mg/ml and 50 mg/mL of PASP only, while the sixth group (uninfected) were given sterile water.

Haematological and Biochemical Analyses

At the conclusion of the assay, the rats were anesthetized, and blood samples were collected via cardiac puncture. Blood was collected into two types of bottles: EDTA bottles for immediate processing to measure hematological features like red blood cell count (RBC), packed cell volume (PCV), white blood cell count (WBC), and differential leukocyte counts. Blood in plain bottles was allowed to clot for 2 hours, then centrifuged to separate the serum. Biochemical markers, like alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were analyzed in the serum [25].

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Data Analysis

The data collected were analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0, developed by IBM Corp. (Armonk, NY, USA). To assess the significance of the results, the data underwent one-way analysis of variance (ANOVA), which allows for the comparison of means across multiple groups. Following this, Duncan's New Multiple Range Test was applied to determine where significant differences between group means occurred. Statistical significance was set at a p-value of 0.05 or less ($p \leq 0.05$), indicating that differences between groups were considered meaningful if the p-value was below this threshold.

Results

Anti-Salmonella activity and Compounds Present in P. americana seed peptides

The results of the in vitro anti-Samonella activity of PASP is presented in Table 1. The zone of inhibition of PASP against the test organism ranged from 15mm to 22mm at the evaluated concentrations. The minimum inhibitory concentration value revealed the potency of PASP at 25mg/ml. The killing-kinetics revealed a concentration and time dependent microbicidal effect against the pathogen, the results are presented in Figure 2. High performance liquid chromatography (HPLC) analysis revealed fourteen amino acids in PASP, amongst which include: Alanine, Phenylalanine, Valine, Cystine, Histidine, and Isoleucine. The data are presented in Table 2 and Figure 3.

Table 1: Anti-Salmonella activity of *P. americana* peptides

	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
ZOI	22mm	17mm	15mm	ND	ND
MIC	+	+	+	-	-
MBC	+	+	+	-	-

Legend: ND= not determined, ZOI = zone of inhibition, + = No growth, - = Growth,

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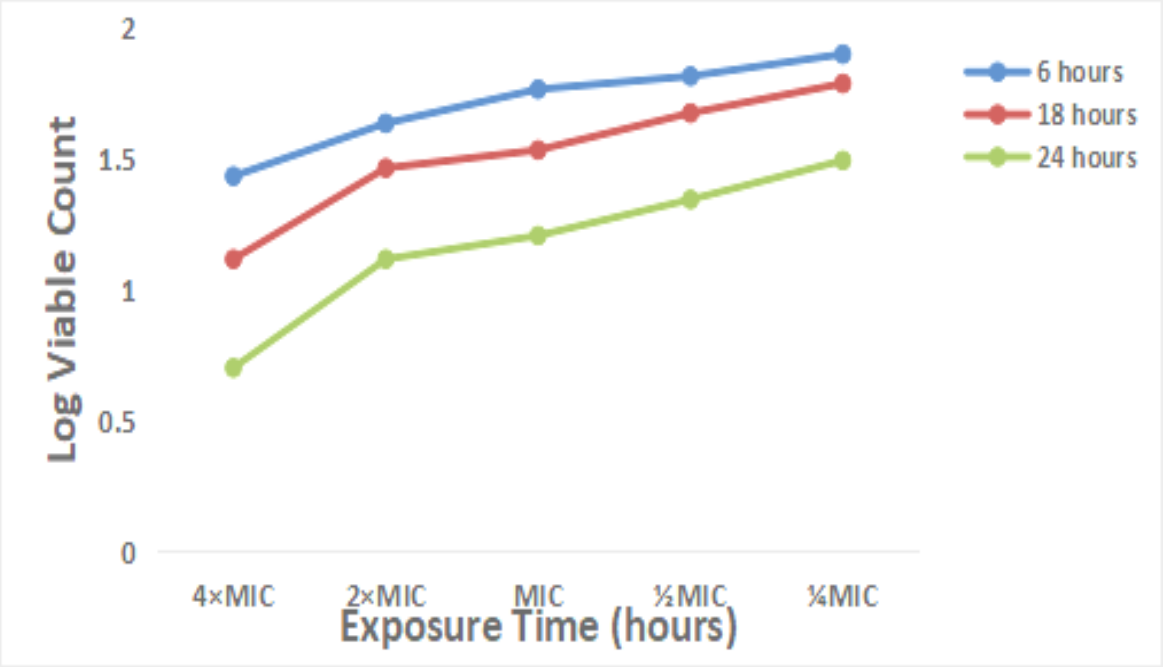

Figure 2: Time Kill Kinetics of *P. americana* seeds against S. typhi

Table 2: Components present in *P. americana* seed peptide

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/L]	Name
1.363	VV E	1.21343e4	1.03867e-4	1.26036	Alanine
1.772	VV E	1.39199e4	5.61437e-5	7.81517e-1	Arginine
1.990	VV E	1.24511e4	3.30157e-5	4.11080e-1	Aspartic Acid
5.954	VV E	713.45398	1.34497e-4	9.59575e-2	Cystine
7.333	VV E	155.08902	2.44361	378.97720	Leucine
8.192	VV E	134.99869	4.41668	596.24533	Serine
10.063	VV E	34.17072	5.24087e-1	17.90843	Threonine
13.090	VV E	4958.28027	1.53947	7633.12985	Valine
14.499	VV E	148.59344	4.48122	665.88040	Histidine
15.178	VV E	62.40528	1.82689	114.00763	Isoleucine
15.811	VV E	90.67102	5.11915	464.15818	Methionine
16.709	VV E	138.44759	40.64557	5627.28146	phenylalanine
16.918	VV E	66.31350	45.81028	3037.83968	Tyrosine
17.232	VV E	83.42548	14.11938	1177.91638	Glycine

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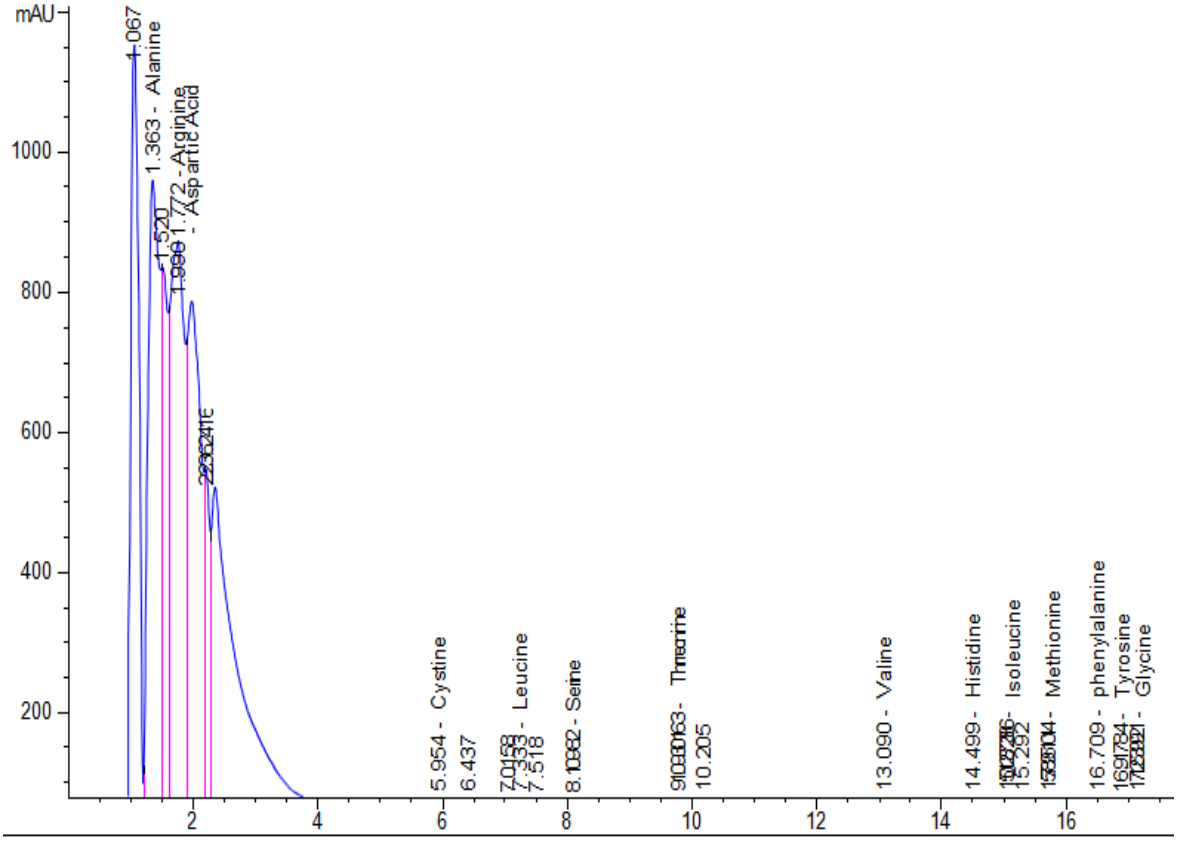

Figure 3: Chromatograph of HPLC identified components of *P. americana* seeds

Molecular Docking and MM/GBSA of the bioactive compounds against the target

As depicted in Figure 4, the top active compounds (Phenylalanine, Cystine, Histidine, Aspartic Acid, Glutamic Acid) had varying binding affinities against DNA gyrase B subunit (gyrB) ranging from -6.177 to -6.482 kcal/mol which are on par with that of the benchmark drug (Ofloxacin = -7.001 kcal/mol). The binding free energies of the complex were determined by calculating the molecular mechanics generalized born surface area (MM/GBSA). As shown in Figure 4, the entire set of compounds had binding free energy above -30 kcal/mol, which were better than the binding free energies of the standard drug (Ofloxacin = -25.03 kcal/mol). From Table 3 and Figure 5, the active compounds from P. americana interacted with various amino acid present at the pocket of the protein through various molecular interactions like van der waals, alkyl bond, hydrogen bond, and pi-alkyl bonds. Cystine and Glutamic acid formed six (6) hydrogen bonds, Histidine and Aspartic acid formed five (5) hydrogen bonds, while Phenylalanine formed three (3) hydrogen bonds, with other hydrophobic interactions; whereas Ofloxacin (standard drug) formed a single hydrogen bond at the active site.

ADMET profile of Top Ligands

All top ligands of PASP with the highest docking scores were found to be non-inhibitors of CYP1A2, CYP2C19, CYP2C9 oxidase enzymes. Majority of the compounds had high gastrointestinal absorption, and bio-availability value above 0.5. The compounds' Ilog P values ranged from -0.09 to 1.08. None of the compounds breached the Lipinski rule of five. TPSA values indicated that all the ligands, except for Cystine, had scores below 140 Ao. The results are presented in Table 4.

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Docking RESULTS

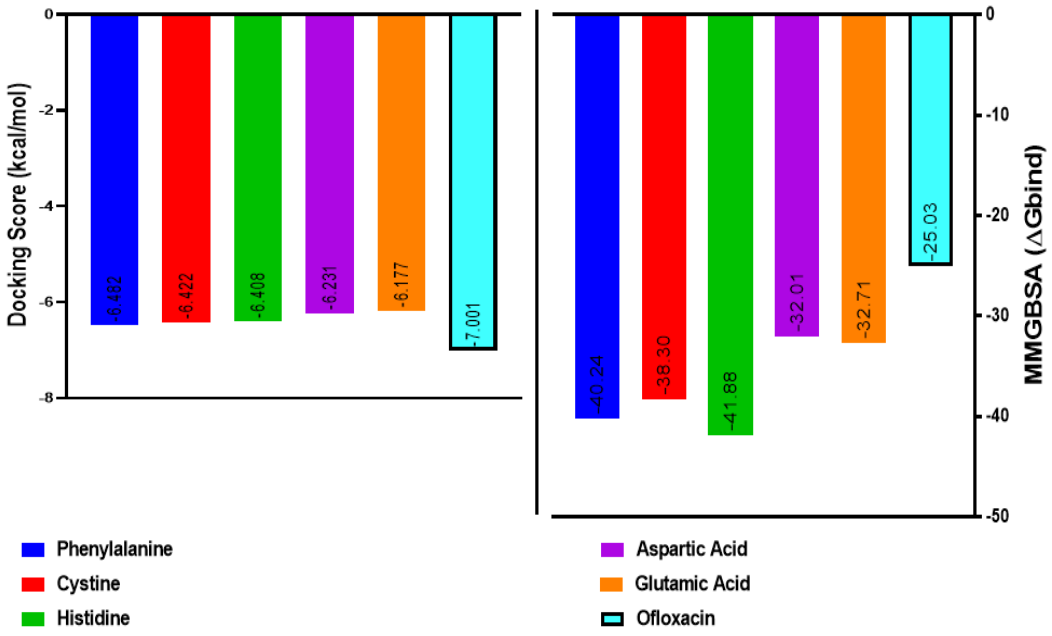

Figure 4: Graphical representation of the binding affinity and binding free energy calculation of bioactive peptides of P. americana against gyrB

Table 3: Hydrogen Bonds and Hydrophobic interactions of the hit phytochemicals of P. americana seeds

Compound Name	H-Bond	Hydrophobic interactions	Other Interactions
Phenylalanine	GLY 117, ASN 46, VAL 120,	TYR 109, ALA 100, LEU 115, VAL 118, VAL 120, ILE 94, ILE 78	Pi-citation: LYS 103, ARG 76
Cystine	ALA 100, GLY 102, TYR 109, ASN 46, SER 121, VAL 120	ALA 100, TYR 109, VAL 118, VAL 120, ILE 78, ILE 94, PRO 79,	NONE
Histidine	ASN 46, VAL 120, GLU 42, VAL 118, GLY 117	ALA 100, LEU 115, VAL 118, VAL 120	NONE
Aspartic Acid	ASN 46, VAL 118, VAL 120, GLY 117, GLY 119	ILE 94, VAL 118, VAL 120, LEU 115, ALA 100	NONE

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Glutamic Acid	ASN 46, VAL 120, GLY 117, HIE 116, LEU 115, GLU 42	LEU 115, VAL 118, VAL 120, ILE 94, ALA 100	NONE
Ofloxacin	ASN 46	PRO 79, ILE 78, VAL 167, VAL 71, VAL 43, VAL 120, ALA 47, ILE 94, TYR 109	Pi-citation: LYS 103

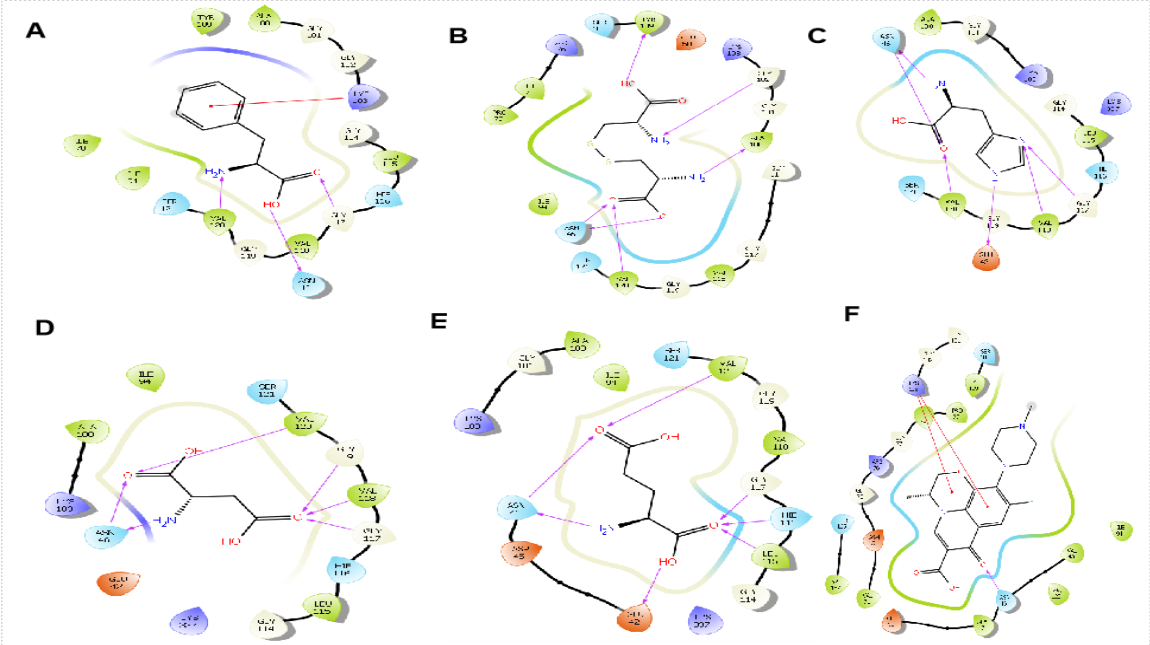

Figure 5: 2D-Molecular interactions of amino-acid residues of DNA gyrase subunit B with *P. americana* top compounds and standard drug. Legend: A-Phenylalanine, B-Cystine, C-Histidine, D-Aspartic Acid, E-Glutamic Acid, F-Ofloxacin

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Table 4: Druglikeness and ADMET profile of the Top compounds from *P. americana* seeds

Compound Name	MW	HBA	HBD	TPSA	iLOGP		ESOL Log S	GIA	CYP1A2	CYP2C19 inhibitor	CYP2C9 inhibitor	BA
Phenylalanine	165.19	3	2	63.32	1.08	0	-0.08	High	No	No	No	0.55
Cystine	240.3	6	4	177.24	0.51	0	2.33	Low	No	No	No	0.55
Histidine	155.15	4	3	92	-0.03	0	1.09	High	No	No	No	0.55
Aspartic Acid	133.1	5	3	100.62	-0.09	0	1.98	High	No	No	No	0.56
Glutamic Acid	147.13	5	3	100.62	0.4	0	1.84	High	No	No	No	0.56

Legend: MW= molecular weight, HBA = hydrogen bond acceptor, HBD = hydrogen bond donor, ROV = rule of five, GIA= gastrointestinal absorption, BA= bioavailability.

Effect of Administration of PASP on Haematological and Biochemical Parameters of Wistar Rats

As presented in Figure 6, the outcome of the haematological assay of the infected and treated rats showed that PASP modulated haematological parameters with profound effect on packed cell volume and lymphocytes. The administration of PASP resulted in a reduction of the biochemical parameters ALT and AST, with the most significant effect observed in the group administered 25 mg/kg of PASP. The results are showcased in Figure 7.

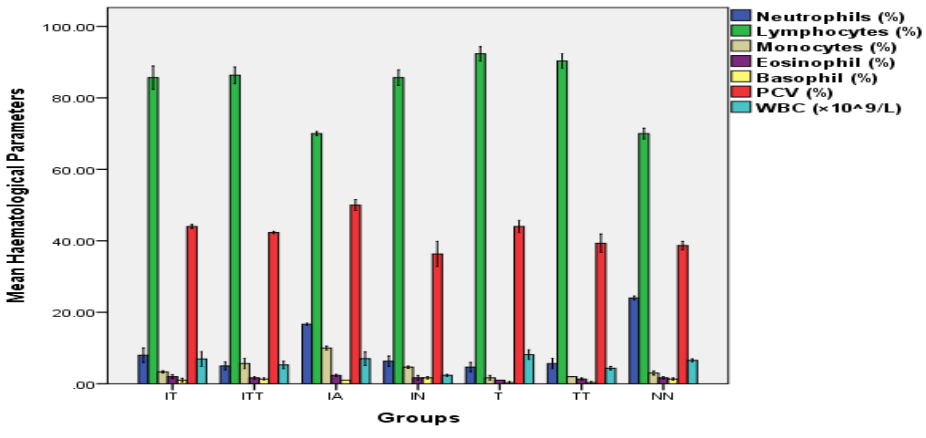

Figure 6: Effect of repeated oral administration of PASP on hematological features of Wistar rats

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Legend: IT = Infected, administered 25 mg/kg of PASP; ITT = Infected, administered 50 mg/kg of PASP; IA = Infected, treated with Ofloxacin; IN = Infected, not treated; T = Not infected, given 25 mg/kg of PASP; TT = Not infected, given 50 mg/kg of PASP; NN = Not infected, given sterile water.

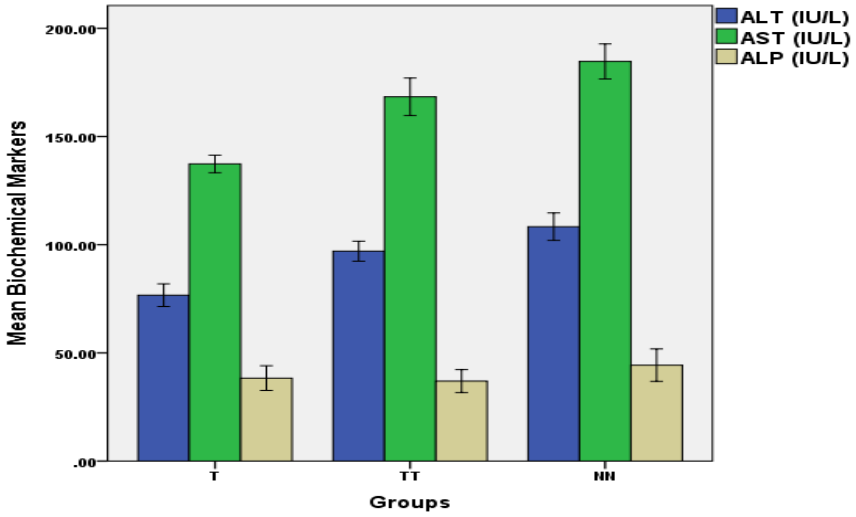

Figure 7: Effect of repeated oral administration of PASP on biochemical markers of Wistar rats

Legend: T = Not infected, given 25 mg/kg of PASP; TT = Not infected, given 50 mg/kg of PASP; NN = Not infected, given sterile water.

Discussion

Bioactive peptides found in natural sources like plants have gained interest for their antimicrobial properties and therapeutic potential. In this study, *P. americana* seed peptides (PASP) was investigated for its therapeutic role in Salmonella-infected Wistar rats. The test organism showed substantial susceptibility to PASP, with inhibition zone (IZ) of 22 mm recorded at 100 mg/mL, lower concentrations resulted in lower IZs which were equally remarkable. This observation is supported by the results obtained in the MIC and MBC assays, with PASP displaying cidal action at 25 mg/mL. This suggests the potential of PASP as a viable agent that can combat infections due to *S. typhi*. Our findings align with documented reports on peptides from several plants which demonstrated notable antimicrobial activity [27].

Antimicrobial peptides exert their effects through interactions with microbial cell membranes, leading to membrane disruption and the inhibition of protein and nucleic acid synthesis [28]. Peptides can damage membranes through several main mechanisms. In the barrel-stave mechanism, antimicrobial peptides (AMPs) insert vertically into the membrane, creating pores that span the membrane. In the carpet mechanism, peptides lie parallel to the lipid bilayer, disrupting the membrane's structure. A combination of these two mechanisms results in toroidal pores. Additionally, disordered toroidal pores can form with fewer peptides, stabilized by others. Lastly, in the aggregate or detergent-like model, peptide-lipid complexes form micelles that create channels, allowing cellular contents to leak out [29,30].

Research indicates that the primary structural attributes influencing the activity of these peptides are their net charge and hydrophobicity. For instance, Yang et al. [31] found that antimicrobial peptides typically possess cationic amino acids, giving them a cumulative positive charge between +2 and +9, along with an abundance of hydrophobic residues such as leucine, phenylalanine, valine, tryptophan and isoleucine. The positive charge enables these peptides to bind to the negatively charged phospholipids on pathogen membranes via electrostatic interactions, ultimately forming pores or channels, or even disrupting the membrane structure by converting it into micelles [32,28].

Understanding the killing kinetics of a substance is crucial for determining how rapidly it can reduce bacterial populations over time. The effectiveness of PASP against S. typhi was evaluated at varying concentrations (¼×MIC to

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4×MIC). The results demonstrated that PASP exerted a time- and concentration-dependent microbicidal effect on the pathogen. At the highest concentration, the reduction in viable counts was most significant at 18 and 24 hours. These findings are consistent with previous studies, which suggested that plant extracts exhibit microbicidal activity against pathogens [33,34].

Virtual screening of the active molecules from the HPLC screening of PASP was performed against the DNA gyrase B subunit (gyrB) binding site. DNA gyrase, a type II topoisomerase, utilizes ATP to add negative supercoils into closed circular double-stranded DNA, maintaining an underwound state of chromosomes. This negative supercoiling aids in events such as DNA transcription, replication, recombination, and repair, which require DNA strand separation. In addition to introducing negative supercoils, DNA gyrase can also convert other topological forms of DNA, such as catenanes and knotted rings [35]. Ofloxacin is a broad-spectrum antibacterial drug in the fluoroquinolone family that exhibits a bactericidal effect by binding to and inhibiting bacterial DNA gyrase. These inhibitory effects interrupt DNA replication, transcription, and repair, thereby preventing cell division in bacterial cells [36].

Molecular docking-based virtual screening is a key method in drug design, engaged in forecasting the association of small molecules with the amino acid residues at the target's binding location. Complementary to the observed in vitro antibacterial activity of the plant, inhibition of gyrB by bioactive compounds from PASP was selected to study the anti-Salmonella mechanism of the compounds from the plant. The binding affinity results obtained in this study suggest that the top five active compounds (Phenylalanine, Cystine, Histidine, Aspartic Acid, Glutamic Acid) from *P. americana* were potent inhibitors of gyrB, exhibiting superior binding affinity compared with Ofloxacin (conventional drug). The high binding affinities might be adduced to the formation of diverse interactions between the functional groups of the bioactive compounds and the amino acid residues at the binding site of gyrB. This observation correlates with the findings of Oyedemi et al. [37], who observed that compounds from Ligustrum lucidum and Lobelia inflata were potent inhibitors of DNA-gyrase A of *Staphylococcus aureus* when compared to the conventional Norfloxacin. Furthermore, according to another docking studies [38], HPLC identified compounds of Diospyros malabarica showed impressive propensity for binding to DNA gyrase. Of interest in their study is Kaempferol, reported to have the strongest binding affinity (-8.9 kcal/mol), this is on par with the binding score for same compound (-8.809 kcal/mol) from our study.

ADMET prediction showed that the top five ligands of PASP with the highest docking scores were non-inhibitors of the CYP1A2, CYP2C19 and CYP2C9 oxidase enzymes. With the exception of Cystine, all top ligands showed high gastrointestinal absorption. The ligands Phenylalanine, Cystine and Histidine had a bioavailability score of 0.55, while Aspartic Acid and Glutamic Acid sparkled a slightly higher score of 0.56. These values suggest that the compounds have potential as oral drug candidates. Bioavailability refers to the quantity of an administered drug that reaches the systemic circulation in an unchanged form, regardless of the route of administration [39]. A bioavailability score below 0.5 typically indicates low oral bioavailability, while a score above 0.5 suggests high oral bioavailability. The Ilog P values for these compounds ranged from -0.09 to 1.08, indicating that they are relatively water-insoluble but still able to penetrate cell membranes to some extent, a desirable trait for drugs intended for oral administration. Moreover, all the top bioactive ligands complied with Lipinski's Rule of Five, further supporting their potential for oral bioavailability. According to this rule, drugs intended for oral use should have a molecular weight of < 500 g/mol, < 10 hydrogen bond acceptors, < 5 hydrogen bond donors, and a log P value < 5 [40]. The Topological Polar Surface Area (TPSA) scores for most of these ligands were below 140 Ao, suggesting that their absorption potential is high, as a lower TPSA value is typically associated with better cell membrane penetration.

The observed inhibitory activity of compounds from PASP against gyrB supports the results obtained from our in vitro experiments, which prompted us to expedite further studies on the plant using in-vivo murine model. The results of the hematological assay conducted on infected and treated rats revealed that PASP significantly increased the packed cell volume (PCV) in the treated groups (44.00±0.57%; 42.33±0.33%), while the untreated infected group showed a notable decrease in PCV (36.33±3.52%). These findings suggest that PASP may contain bioactive compounds that enhance the activity of erythropoietin, a hormone responsible for regulating red blood cell production, thereby promoting erythrocyte production. This is consistent with studies on various medicinal plants, such as Phyllanthus emblica, Spinacia oleracea, Ficus carica, Bidens pilosa, and Phoenix dactylifera, which have been shown to support hemoglobin synthesis and red blood cell formation [41,42,25]. Plant extracts with such properties could serve as effective blood supplements and potential treatments for anemia in animals [42,25].

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White blood cells (WBCs) exert an essential role in safeguarding the body from foreign pathogens and producing antibodies as vital part of the immune response. In this study, PASP was observed to influence WBC counts in rats. When compared to the infected untreated group (2.37×10^9/L), WBC counts were significantly higher in both infected-treated groups (6.93×10^9/L and 5.27×10^9/L) as well as in groups treated with PASP alone (8.16×10^9/L and 4.37×10^9/L). No major differences were seen in the levels of eosinophils and basophils across the different groups. However, the group receiving PASP alone showed a notable increase in lymphocyte count (92.3% and 90.3%), compared to the infected untreated group (85.6%). Other plant extracts, such as those from Phoenix dactylifera, Bidens pilosa, and Telfairia occidentalis, have also been reported to promote lymphocyte proliferation in animal models, potentially enhancing the animal's resistance to infections [25,42-44].

Administration of PASP caused a decline in biochemical parameters of ALT and AST, which was most pronounced in the group administered with 25mg/kg PASP. However, these values returned closer to normal levels in the cohort treated with 50 mg/kg PASP. Although ALP levels were lower in the treated cohorts relative to the control, no significant changes were observed, and the decrease was less pronounced than the reductions seen in ALT and AST levels. Surge in the plasma levels of liver enzymes like AST, ALT, and ALP indicate liver damage or dysfunction. These biomarkers are involved in amino acid metabolism, with ALP also serving as a marker for cholestasis, a condition of impaired bile flow [25].

Amino acids like Leucine, Isoleucine, Valine, Threonine, Phenylalanine, Cystine, Histidine and Methionine which are present in PASP in our study, have been reported as constituents of peptides known to elicit broad spectrum beneficial physiological effects [45,46], thus supporting the observed bioactivity of PASP.

Conclusion

The findings from this study provide substantial evidence supporting the efficacy of bioactive peptides derived from *P. americana* seeds, highlighting their significant potential as a promising anti-*Salmonella* agent. The data suggest that these bioactive compounds exhibit notable antimicrobial properties, capable of inhibiting the growth and spread of Salmonella bacteria. This offers exciting possibilities for developing novel, plant-based therapeutic options to combat Salmonella related illnesses. Furthermore, the study underscores the therapeutic potential of avocado seeds, which have long been used in traditional medicine for their healing properties, particularly in addressing infections. As the scientific community continues to explore natural alternatives to conventional antibiotics, these findings not only validate the folkloric uses of avocado seeds but also pave the way for further research into their broader applications in infectious disease treatment. Ultimately, the integration of these bioactive peptides into therapeutic strategies could contribute to the development of more sustainable, plant-derived solutions for managing bacterial infections, particularly in the face of growing antibiotic resistance.

Future directions

While this study focused primarily on *S. typhi*, it is essential to test PASP against a broader range of clinically relevant bacterial strains to provide a more comprehensive understanding of its antimicrobial spectrum. Further research should aim to elucidate the molecular and biochemical interactions between PASP and bacterial cells, which could offer deeper insights into how PASP might be optimized for therapeutic use. In addition, investigating whether PASP can enhance the efficacy of existing antibiotics would be a significant step forward in combating multidrug-resistant bacteria. Future clinical trials should be considered to assess the safety and efficacy of PASP in human populations, particularly in combination therapies with current antibiotics. Efforts should also focus on developing pharmaceutical formulations that maximize the stability, bioavailability, and therapeutic efficacy of PASP. A detailed investigation into the pharmacokinetics and potential side effects in clinical settings will be crucial for transitioning from animal models to human trials, ultimately advancing PASP as a viable therapeutic option.

Declaration

Conflict of interest

Authours declare no conflicting interest.

Ethical considerations

Ethical approval was obtained for the study.

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