

## Potential Biopreservative Effect of Antimicrobial Peptides of Seeds of *Vigna Unguiculata* (Cow Pea) on *Hibiscus Sabdariffa* L. (Roselle) Calyces (Zobo) Drink

Sani Hauwa Muhammad<sup>1\*</sup>, Bukar Aminu<sup>2</sup>, Magashi Abdulqadir Magaji<sup>3</sup>

### Abstract

Natural and efficient food preservatives are in greater demand because of growing concerns about the safety and quality of processed beverages. Antimicrobial peptides have emerged as promising natural substitutes for synthetic preservatives, boasting advantages like wide-ranging activity and reduced toxicity. In this research, we investigate the inhibitory effects of these peptides against common spoilage microorganisms in 'Zobo' drink. Peptides were extracted and purified from germinated *Vigna unguiculata* seeds harvested on days 2, 4, 6, and 8 using a combination of ammonium sulfate precipitation at 70% saturation, dialysis, and gel filtration, resulting in distinct peaks. SDS-PAGE was used to determine the peptides' molecular weights. Additionally, six bacterial and four fungal species were isolated and identified following standard protocols. The highest protein concentration was observed in germinated seeds harvested on day four (B4) as 1.8060mg/ml on fraction 6 while the highest zone diameter of inhibition was recorded on germinated Bean seed day two fraction three (B4:3) The zone diameter measured  $21.333 \pm 0.8819$  mm, while none of the four fungal species exhibited any activity or zone of inhibition. After being adjusted to a pH of 2.3, the peptides' ability to sanitize "zobo" drink was assessed. was conducted at 0 hours, 24 hours, 48 hours, and 72 hours, and all of the pure peptides increased the drink's shelf life. According to the sensory evaluation, the judges preferred the drink maintained with 10% (B4:3) at pH 2.3, with a percentage likeness of 83.33% after 72 hours of preservation. The results of this study may aid in the creation of innovative and secure preservation methods for the beverage sector.

**Keywords:** *Vigna unguiculata* (cow pea), Rosell flower drink (Zobo), plant antimicrobial peptides, food microbiology, biopreservatives

### INTRODUCTION

The preservation of beverages remains a critical challenge in the food industry, primarily due to the rapid proliferation of spoilage microorganisms.

These microorganisms can quickly compromise the safety and sensory qualities of beverages, leading to undesirable changes in taste, aroma, texture, and overall product quality [1]. Ensuring the microbiological safety of food and beverages is particularly important because contamination by pathogenic bacteria can pose significant health risk.

Foodborne illness outbreaks are commonly associated with pathogens such as *Salmonella* spp., *Shigella* spp., *Micrococcus* spp., Among these are *Clostridium botulinum*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Bacillus licheniformis*, *Enterococcus faecalis*, and *Escherichia coli* [2,3]. and [4].

#### \*Author For Correspondence

Sani Hauwa Muhammad  
E-Mail: Qanwasani37@Gmail.Com

<sup>1</sup>Assistant Chief Scientific Officer, Department of Planning, Research and Policy Analysis, National Board for Technology Incubation, Abuja, Nigeria.

<sup>2-3</sup>Research Scholar, Department of Microbiology, Bayero University Kano, Kano, Nigeria

Received Date: November 27, 2024

Accepted Date: December 27, 2024

Published Date: January 10, 2025

**Citation:** Sani Hauwa Muhammad, Bukar Aminu, Magashi Abdulqadir Magaji. Potential Biopreservative Effect of Antimicrobial Peptides of Seeds of *Vigna Unguiculata* (Cow Pea) on *Hibiscus Sabdariffa* L. (Roselle) Calyces (Zobo) Drink. Research & Reviews: Journal of Food Science & Technology. 2025; 14(1): 26–41p.

Given these challenges, there has been increasing interest in identifying natural antimicrobial agents that can serve as effective preservatives. Natural compounds exhibiting antimicrobial activity present a viable alternative to synthetic preservatives, in response to the increasing consumer preference for cleaner and more sustainable food products [5]. Antimicrobial peptides, in particular, have garnered significant attention for their potential to inhibit a wide range of foodborne pathogens ([2], [3], [4]). Antimicrobial peptides, sometimes called cationic host defense peptides [6,7], anionic peptides/proteins [8], cationic antimicrobial peptides, or  $\alpha$ -helical antimicrobial peptides [9], can be produced either ribosomally or non-ribosomally.

In eukaryotic and prokaryotic cells, ribosomally synthesized peptides are formed in the ribosome, whereas non-ribosomal peptides are synthesized in the cytosol of certain fungi and bacteria [10,11]. For instance, lipopeptide—a non-ribosomally synthesized peptide—exhibits broad-spectrum activity against microorganisms resistant to multiple drugs [12, 13]. Several researchers have highlighted the effectiveness of antimicrobial peptides in combating various food-borne pathogens, suggesting their potential role in food preservation [2,3,4]. These antimicrobial compounds, including proteins and peptides secreted by animals, plants, and bacteria, are utilized for preserving food. Fermentation, a prominent method of food preservation, leverages the growth of specific microorganisms. Furthermore, Peptides are recognized for their significant biological activities, including their roles in maintaining organic homeostasis, modulating the immune system, and protecting the body from oxidative stress and other detrimental processes. These properties are extensively documented in scientific literature [14]. The global market for peptide therapy is expected to rise at an average annual rate of 7.7% by 2021, reflecting the steady increase in approval of peptide-based medications over the past several decades [15]. Antimicrobial peptides are particularly advantageous due to their compatibility with biological systems, biodegradability, thermal stability, and high specificity—qualities that make them highly desirable for food preservation [16]. Peptides completely degrade in the body without any negative side effects, in contrast to antibiotics. For example, *nisin*, a peptide produced by *Streptococcus lactis*, is commercially available and widely used as the food additive E234 [17]. Growing consumer preference for foods free from synthetic preservatives has driven the search for natural antimicrobial agents with broad-spectrum efficacy and superior properties. Notably, lactic acid bacteria are key agents in fermentation, producing organic acids, various metabolites, and antimicrobial peptides [18]. Lactic acid bacteria serve as bio-preservatives in fermented foods by producing lactic acid, hydrogen peroxide, and small peptides that inhibit microbial growth. The United States Food and Drug Administration (USFDA) has recognized lactic acid bacteria as a safe preservative for fermented foods [19,20]. Recently, Bacilli have also been used in food preservation due to their secretion of antimicrobial peptides [21]. This method of bio-preservation maintains the food's flavor, texture, and aroma without altering its physical, chemical, or biological properties. Additionally, these peptides not only provide microbial control but also contribute additional health benefits, including antioxidant, antihypertensive, and immunomodulatory effects [22]. hence, this dual functionality makes them valuable candidates for use in functional beverages. The current study focuses on the preservative properties of plant-derived antimicrobial peptides, specifically those isolated from germinated seeds of *Vigna unguiculata* (cowpea), and their application in preserving 'Zobo' drink, a traditional Nigerian beverage made from hibiscus flowers. By assessing the inhibitory effects of these peptides on spoilage microorganisms typically found in beverages, we aim to explore their potential as natural, clean-label preservatives. In addition to enhancing the shelf life and safety of 'Zobo' drink, the use of such natural preservatives aligns with the increasing preference for sustainable and health-promoting food products.

## MATERIALS AND METHODS

Collection and Identification of Plant Materials Using conventional keys and descriptions based on the most recent phylogenetic classification, all plant samples were first identified in the field [19]. At the Department of Plant Biology's herbarium at Bayero University Kano, Nigeria, additional verification and authentication of the beans (*Vigna unguiculata*), BUKAN 0099, and Hibiscus *sabdariffa* L. (Roselle) calyces, BUKAN 0040, were carried out.

### **Seed Germination**

The optimal germination process was carried out in accordance with the International Seed Testing Association's rules [20], using 800 visibly healthy *Vigna unguiculata* seeds for the germination assay. Following a 12-hour soak in distilled water and surface sterilization, the seeds were divided among ten trays. They were put on a damp filter paper sheet, covered with another layer of damp filter paper, and kept in a germination chamber with eight hours of light exposure every day at 20°C.

Germinated seeds were collected on days 2, 4, 6, and 8. The seeds were then freeze-dried, milled, and sieved through a 0.5 mm mesh. Until additional examination, the resultant germinated pea flour was kept at 4°C.

### **Protein Extraction to Produce Antimicrobial Peptides**

At 4°C, 100 ml of phosphate buffer (pH 7.5, 0.1M) was added in a 1:10 ratio to 10 grams of dried, ground seeds. After three cycles of freezing and thawing, the mixture was centrifuged for ten minutes at 10,000 rpm. At 4°C, the supernatant was kept.

### **Total Protein Estimation Using Lowry's Method**

0.2 ml of the peptide solution was pipetted into separate test tubes after various dilutions of bovine serum albumin (BSA) were made. After adding two milliliters of the alkaline copper sulfate reagent to each tube, the tubes were allowed to sit at room temperature for ten minutes. After that, each tube received 0.2 ml of Folin-Ciocalteu reagent, which was then incubated for 30 minutes.

Absorbance was measured at 660 nm after adjusting the colorimeter to zero. Plotting absorbance against protein content allowed for the creation of a calibration curve [22].

### **Purification and Characterization of Antimicrobial Peptides Using Ammonium Sulfate Precipitation Method**

Ammonium sulfate precipitation is commonly used to concentrate antimicrobial peptides via a salting-out technique. The soluble parts of the milled seeds were present in the supernatant from samples treated with Phosphate Buffered Saline. This was treated with 70% ammonium sulfate for 20 hours at 4°C.

The precipitate was collected by centrifugation, and the supernatant was further centrifuged at 10,000 rpm for 20 minutes to remove residual ammonium sulfate [23].

### **Dialysis**

A membrane filter with hole sizes ranging from 2 to 50,000 kDa was used to dialyze the precipitated samples in order to eliminate contaminants [24]. Filtration Chromatography with Gel Using sodium acetate buffer (pH 5), gel filtration chromatography with Sephadex G-75 was used to further purify the ammonium sulfate precipitate. The presence of peptides in eluted samples was measured by spectrophotometric analysis at 280 nm. Peak concentrations were calculated by plotting protein concentrations against the number of eluted fractions [24].

### **SDS-PAGE**

SDS-PAGE was performed using ExpressPlus™ PAGE Gels following the manufacturer's instructions to determine the molecular weight of the isolated peptides [24].

Bacterial and Fungal Species Isolation and Identification from Spoiled Foods Six bacterial and four fungal species were isolated from spoiled local delicacies in Nigeria, including white rice, 'zobo' drink, 'kunun zaki' (fermented millet drink), and 'jollof' rice. The organisms were physically examined for their characteristic appearance and identified microscopically. Biochemical tests were conducted for bacterial species, and lactophenol cotton blue mounts were prepared for fungal isolates. The isolates were confirmed by molecular analysis and genetic sequencing [25].

### Susceptibility Test

Antimicrobial activity was evaluated using the agar well diffusion method. To standardize the culture to  $10^8$  cfu/ml, 0.1 ml of an inoculum of overnight cultures of each organism was added to 20 ml of sterile lactose broth and cultured for 8 hours. Wells (4 mm diameter) were made in Mueller-Hinton agar plates, and 100  $\mu$ l of the culture was spread evenly. Extracted peptides, at 100% concentration from gel chromatography, were inoculated into the wells. The zone diameter of inhibition was evaluated after the plates were incubated for 24 hours at 37°C. Every peptide and bacterial/fungal species combination was examined three times [26].

### Culture Media's Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of peptides isolated from *Pisum sativum* seeds was determined using the broth dilution method. Fresh cultures of spoiled food isolates were inoculated into LB broth, and 1 ml of antimicrobial protein preparation was added at concentrations of 12.5% (0.125 ml protein + 0.875 ml saline), 25% (0.25 ml protein + 0.75 ml saline), 50% (0.5 ml protein + 0.5 ml saline), and 100% (no saline) and incubated at 37°C for 24 hours. The results were expressed in protein concentration (mg/mL) [26].

### Determination of Physical, Chemical, and Biochemical Properties of Extracted Peptides pH and Heat Treatment

The method described by [26] was followed. After the peptides were poured into test tubes, NaOH and HCl were used to bring their pH down to between 3 and 11.

The samples were incubated at 30°C for 3 hours, and residual antimicrobial activity was measured using agar well diffusion. Heat stability was tested by treating the peptide preparation at various temperatures (30°C to 121°C) for 15 minutes, followed by evaluation of antimicrobial activity using agar well diffusion.

### Effect of Enzymes and Surfactants

Peptide sensitivity to enzymes was tested by treating the cell-free supernatants (pH 7) with 0.1 mg/ml and 1.0 mg/ml concentrations of trypsin (Hi Media Laboratory Pvt Ltd, India) for 2 hours at 30°C [26].

### Preservative Studies

#### Preparation of roselle calyces drink

The 'zobo drink' was prepared as described by [27], with modifications from [28]. Dried *Hibiscus sabdariffa* calyces were washed, weighed, and boiled in 1 liter of water for 10 minutes. After cooling, the juice was extracted, and the mixture was sieved. Sugar (60 g) was added, stirred, and cooled. No preservatives or flavoring agents were added.

#### 'Zobo Drink' Treatments

A set of treatments for the 'zobo drink' was performed as follows [29]:

- 20 ml of 'zobo' drink + 2 ml (10%) peptide
- 20 ml of 'zobo' drink + 1 ml (5%) peptide
- 20 ml of 'zobo' drink + 0.5 ml (2.5%) peptide
- 20 ml of 'zobo' drink + 0.25 ml (1.25%) peptide
- 20 ml of 'zobo' drink + 0.1 mg (0.5%) sodium benzoate
- 20 ml of untreated 'zobo' drink

### Aerobic Mesophilic Bacterial Count Measurement for Sanitizing Activity Assessment

Serial dilutions of the different 'zobo' drink treatments were prepared up to a  $10^{-5}$  dilution at 0 hr, 24 hrs, 48 hrs, and 72 hrs. One ml of the  $10^{-3}$  dilution was transferred to petri dishes, followed by 15 ml of molten nutrient agar. The plates were incubated for 24 hours at 37°C after solidifying. The value was doubled by the dilution factor after the colonies were counted [30].

### Calculating Temperature and pH

The pH of the different 'zobo' drink samples was measured using a pH-meter (Jenway), calibrated with a pH 7.0 solution. Temperature was recorded using a standard thermometer [30].

### Determination of Titratable Acidity

The acidity of the 'zobo' drink was determined by titration using 0.1 N NaOH, as described by [24]. The acidity of the sample was expressed as a percentage of lactic acid after it was titrated until a steady blue color developed [23].

### Extension of Roselle Calyx "Zobo" Drink's Shelf Life

The shelf-life of the 'zobo' drink was studied by replicating the experimental set-up and recording the number of days until deterioration for each treatment (A-F) [24].  
Sensory Analysis of the "Zobo" Drink by Roselle Calyx

A panel of ten judges used a hedonic scale to rate organoleptic attributes like flavor, color, texture, and odor [31].

### Analysis of Data

Microsoft Corporation's statistical software was used to examine data from sensory evaluations and sanitizing activity tests using Analysis of Variance (ANOVA) at a 5% probability level.

## RESULTS

### The Percentage of Seeds That Germinate

Figure 1 presents the germination results for *Vigna unguiculata*, designated as B. The number of germinated seeds increased progressively with the number of days, along with the corresponding percentage of seeds germinated out of the initial 800 seeds. This resulted in a percentage germination of 89.25%. The eighth (8th) day saw the greatest number of seeds germinate, with 714 seeds out of the original 800 seeds sprouting, or an 89.25% germination rate.

### Crude Sample

Table 1 presents the protein concentrations for the germinated *Vigna unguiculata* on days 2, 4, 6, and 8, labeled as B<sub>2</sub>, B<sub>4</sub>, B<sub>6</sub>, and B<sub>8</sub>, respectively. The absorbance of the unidentified samples at 660 nm was used to calculate these concentrations. The concentration was determined using a straight-line graph ( $y = mx + c$ ), where "c" stands for the intercept (which was 0), "m" for the slope (0.454), "y" for the average absorbance at 660 nm, and "x" for the concentration.

The highest protein concentration was observed in the crude sample of germinated beans on day 8, followed by the concentration on day 4.

### Gel Filtration Chromatography

Gel filtration chromatography was used to further purify the ammonium sulfate precipitate, yielding fifty (50) fractions for each of the four (4) partially pure samples. Using spectrophotometry, the eluted fractions' peptide concentrations were determined at 280 nm. The highest protein concentration was observed for the germinated *Vigna unguiculata* on day 4 (B<sub>4</sub>), with a value of  $1.8060 \pm 0.0030$ , while the lowest concentration was found in the germinated beans on day 8 (B<sub>8</sub>), with a value of  $0.0173 \pm 0.0026$ . All peak fractions were then put through antimicrobial tests after an absorbance versus fraction count graph was created.

### Molecular Weight Determination Using SDS-PAGE

Molecular Weight Determination Using SDS-PAGE The results of the SDS-PAGE analysis are shown in Figure 4.6. Lane PL (Protein ladder) displays the molecular weight markers, while lanes 2-9 show the eight extracts, which appear as bands in the SDS-PAGE corresponding to different molecular weights when compared with the markers. The protein extracts exhibited varying molecular weights. The molecular weight of the protein from the germinated *Vigna unguiculata* seed on day two, fraction four (B<sub>2,4</sub>), was approximately 7.5 kDa. For the germinated *Vigna unguiculata* seeds on day four,

fraction three (B<sub>4.3</sub>), the molecular weight was approximately 3.2 kDa. The protein from the germinated *Vigna unguiculata* seed on day six, fraction three (B<sub>6.3</sub>), was found to be approximately 10 kDa, while the protein from the germinated *Vigna unguiculata* seed on day eight, fraction three (B<sub>8.3</sub>), had an approximate molecular weight of 8 kDa.

Chart Flow of Strategy for the Isolation of Plant Antimicrobial Peptide from *Vigna unguiculata* Seeds

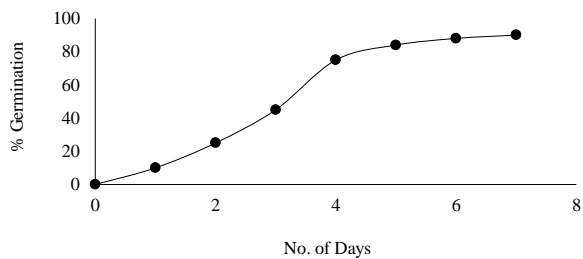
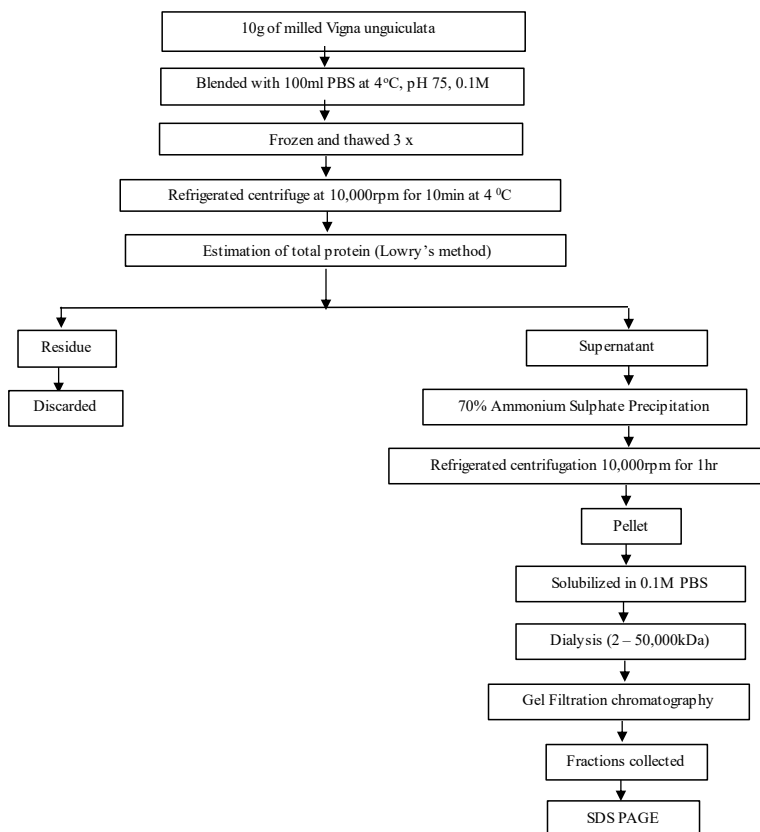
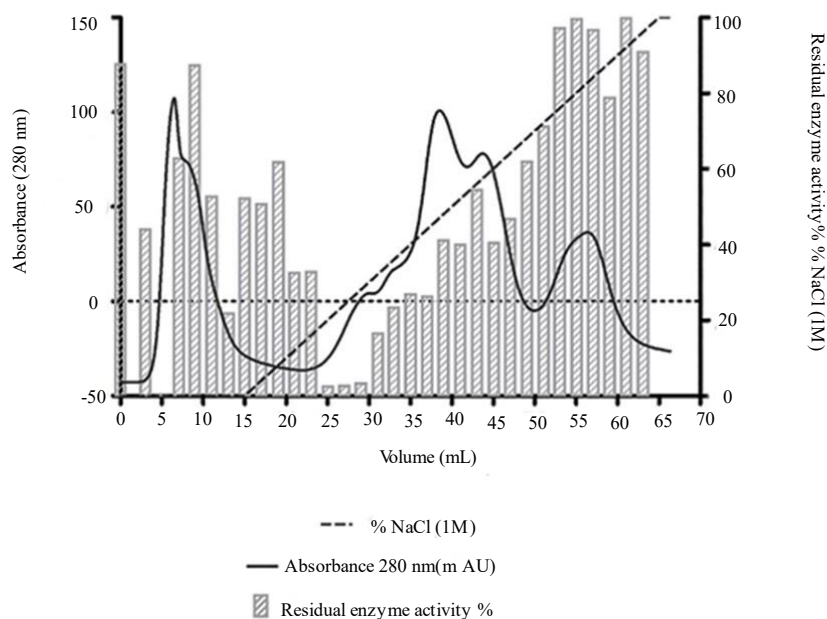


Figure 1. Percentage of bean seeds germinated in eight (8) days.

**Table 1.** Protein Concentration of Crude Sample.

Beans	Average absorbance	Protein conc(mg/ml)
B <sub>2</sub>	0.926	2.0396
B <sub>4</sub>	1.074	2.3656
B <sub>6</sub>	1.027	2.2621
B <sub>8</sub>	1.080	2.3788



**Figure 2.** A spectrophotometric analysis showing number of Fractions (50), Protein concentration (mg/mL) and Peak concentrations of the Purified Peptides of Germinated *Vigna unguiculata* at day four (B<sub>4</sub>), c = day two (B<sub>6</sub>) and d = day two (B<sub>8</sub>).

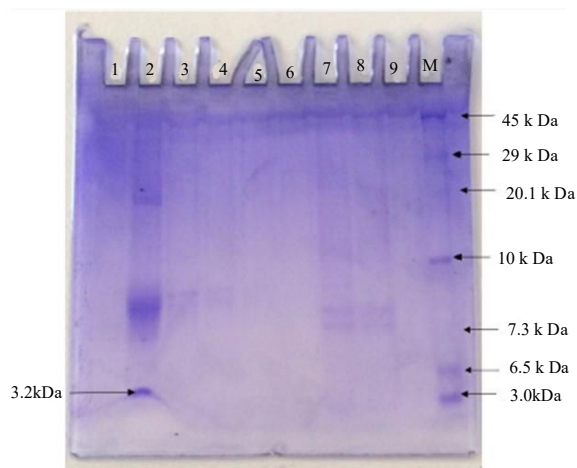
**Commented [s1]:** <Author> Please Check Fig 2,3 Text is Type

#### Bacterial Foodborne Pathogenic/Spoilage Microorganisms: Isolation and Identification

Using molecular, biochemical, and culture methods, bacterial foodborne pathogens and spoilage microorganisms were isolated and identified. The results revealed the presence of five (5) distinct bacterial species and three (3) fungal species. The bacterial species identified include *Salmonella*, *Staphylococcus aureus*, *Listeria*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas*. *Aspergillus niger*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, and *Candida albicans* were the fungal species that were found.

#### Antimicrobial activity of purified peptide of germinated *Vigna unguiculata* seeds

The antibacterial activity of the purified peptides from germinated beans on day two (B<sub>2</sub>) against foodborne and spoilage bacteria and fungi showed varying zones of inhibition. The largest inhibition zone was observed with the peptides from germinated beans on day four (B<sub>4</sub>), measuring  $21.333 \pm 0.8819$  mm against *Salmonella typhi*. The smallest zone,  $8.00 \pm 0.000$  mm, was recorded against *E. coli*. No inhibitory activity was observed against fungi, as no zones of inhibition were detected for any of the peptides derived from germinated bean seeds.



**Figure 3.** The Protein Band and Molecular Weight (kDa) of the fractions of germinated Beans and Peas using SDS-PAGE Key: PL = Protein Ladder, B2:4=Germinated Beans day two, fraction four, B4:3= Germinated Beans day eight, fraction three, B6:4=Germinated Beans day two, fraction four, B8:3= Germinated Beans day eight, fraction three.

**Table 2.** Summary of 16S rRNA/18S rRNA sequencing results of the Isolates to identify the Closest Homologs

Sample ID	Sequence bp	Identity of the closest homologs of the sequence	% identity	Accession N° of the closest homolog
E	727	Escherichia coli strain N4	93.01 %	MF754146.1
B1	752	Bacillus cereus OOA	99.73 %	OR702892.1
S	754	Salmonella enterica subsp. Enteric serovar typhi	100 %	OR078809.1, AB855737.1
Sa	763	Staphylococcus aureus B3	98.69%	MT154229.1
P2	997	Pseudomonas aeruginosa	96.66%	MH470331.1
5-H	545	Aspergillus niger	98.82%	MT620753.1
7-H	541	Aspergillus fumigatus	97.97%	MT645322.1
J	471	Saccharomyces cerevisiae isolate HAM3	99.79%	OQ318157.1

**Purified Peptides' Minimum Inhibitory Concentration (MIC) against Test Organisms**

The greatest MIC values were found with *Listeria* spp., according to the minimum inhibitory concentration (MIC) of the peptides from germinated beans on day four (B4) against test organisms. in fractions 1, 3, 6, and 7, and *E. coli* in fractions 1 and 7

**Table 3.** Antimicrobial Activity of Crude Extract of Germinated *Vigna unguiculata* Seeds.

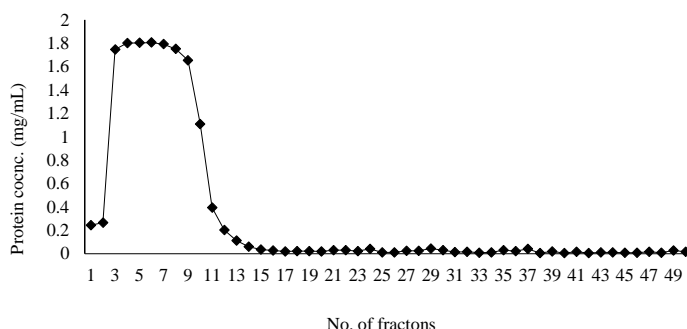
Organisms	B <sub>2</sub>	B <sub>4</sub>	B <sub>6</sub>	B <sub>8</sub>
<i>S. aureus</i> SD	8.000±0.3333	10.000±0.0000	8.000±0.0000	10.000±0.0000
<i>S. typhi</i> SD	11.000±0.6667	14.000±0.6667	8.000±0.0000	12.000±0.3333
<i>E. coli</i> SD	7.000±0.0000	11.000±0.5773	10.000±0.0000	8.000±0.0000
<i>Listeria</i> sppSD	6.000±0.6667	12.000±0.3333	12.000±0.3333	8.000±0.0000
<i>A. fumigatus</i> SD	4.000±0.0000	4.000±0.0000	4.000±0.0000	4.000±0.0000

Key: B<sub>2</sub>= *Vigna unguiculata* day 2, B<sub>4</sub>= *Vigna unguiculata* day 4, B<sub>6</sub>= *Vigna unguiculata* day 6, B<sub>8</sub>= *Vigna unguiculata* day 8. Size of well = 4mm. SD= Standard Deviation

**Table 4.** Antimicrobial activity of purified peptide of germinated *Vigna unguiculata* day four (B<sub>4</sub>).

Organisms	10.243 0mg/ MI	20.266 mg/mL	31.7466 mg/mL	41.803 mg/mL	51.806 mg/mL	61.806 mg/mL	71.792 mg/mL	81.751 mg/mL	91.655 mg/mL
S. typhi SD	15.333 3 <sup>a</sup> ±0.3 334	19.3333 <sup>a</sup> ±0.666 7	21.3333 <sup>a</sup> ±0.8819	20.3333 <sup>a</sup> ±0.881 9	18.3333 <sup>a</sup> ±0.333 4	19.6667 <sup>a</sup> ±0.333 4	14.6667 <sup>a</sup> ±0.333 3	15.0000 <sup>a</sup> ±0.577 3	15.0000 <sup>a</sup> ±0.577 3
S.aureusSD	15.333 3 <sup>a</sup> ±0.3 333	12.3333 ±0.6667	19.0000 <sup>ab</sup> ±0.5773	16.6667 ±0.3333	16.6667 <sup>a</sup> ±0.666 7	18.0000 <sup>a</sup> ±0.000 0	12.6667 <sup>a</sup> ±0.333 4	13.3333 <sup>a</sup> ±0.333 3	12.6667 <sup>ab</sup> ±0.881 9
Listeria sppSD	10.000 0±0.57 77	11.6667 ±0.3334	15.3333± 0.8819	18.6667 <sup>a</sup> ±0.333 4	11.0000 ±0.5773	10.3333 ±0.3334	9.3333± 0.3334	9.3333± 0.3333	9.3333± 0.3333
E. coli SD	8.0000 ±0.000 0	17.3333 <sup>ab</sup> ±0.666 7	17.0000± 0.5773	16.6667 ±0.3334	12.6667 ±0.6667	13.0000 ±0.5773	11.3333 <sup>ab</sup> ±0.333 4	10.3333 ±0.3333	10.3333 ±0.3334
Paeruginos a SD	10.666 7±0.66 67	11.6667 ±0.8819	12.0000± 0.5773	10.3333 ±0.3334	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000
B.cereusSD	10.666 7±0.66 67	14.3333 ±0.6667	18.3333 <sup>a</sup> ±0.8819	12.0000 ±0.0000	12.6667 ±0.6667	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000
Afumigatus SD	4.0000 ±0.000 0	4.0000± 0.0000	4.0000±0 .0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000
A. niger SD	4.0000 ±0.000 0	4.0000± 0.0000	4.0000±0 .0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000
Candida sppSD	4.0000 ±0.000 0	4.0000± 0.0000	4.0000±0 .0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000
Scerevisiae SD	4.0000 ±0.000 0	4.0000± 0.0000	4.0000±0 .0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000
(1% ) Sodium Benzoate) SD	4.0000 ±0.000 0	4.0000± 0.0000	4.0000±0 .0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000	4.0000± 0.0000

The Table above shows the antimicrobial activity of the peptides of *Vigna unguiculata* at day four of germination against some Bacterial and Fungal isolates, for fractions 1-9. Size of well = 4mm. SD = Standard Deviation



**Figure 4.3:** A spectrophotometric analysis showing number of Fractions (50), Protein concentration (mg/mL) and Peak concentrations of the Purified Peptides of Germinated *Vigna unguiculata* day two (B<sub>4</sub>)

**Table 5.** Minimum Inhibitory Concentration (MIC) in mg/ml of purified peptide of germinated *Vigna unguiculata* day four (B<sub>4</sub>).

Organisms	10.243 mg/mL	20.266 mg/mL	31.746 mg/mL	41.803 mg/mL	51.803 mg/MI	61.806 mg/MI	71.792 mg/mL	81.751 mg/mL	91.655 mg/mL
S. typhi	0.1215	0.133	0.218	0.4507	0.4507	0.2253	0.224	0.875	0.206
S. aureus	0.1215	0.133	0.109	0.9015	0.9015	0.4500	0.448	0.437	0.413
Listeria spp	0.243	0.133	1.746	0.9015	0.9015	1.8060	1.792	0.437	0.827
E. coli	0.243	0.133	0.873	0.4507	0.9015	0.4507	1.792	0.218	0.413
P.aeruginosa	0.243	0.266	0.873	1.803	-	-	-	-	-
B. cereus	0.1215	0.133	0.873	0.9015	0.9015	-	-	-	-

The Table above shows the minimum inhibitory concentration expressed in percentage of the peptides of *Vigna unguiculata* day 4, for fractions 1-9.

**Table 6.** Effect of pH on the purified Peptides of Germinated *Vigna unguiculata* seed

Organisms	pH 3	4	5	6	7	8	9	10	11
S. typhiSD	8.6667±0.6667	10.0000±1.1547	9.0000±0.0000	10.6667±0.6667	11.6667±0.3333	9.3333±0.6667	7.3333±0.3333	8.0000±0.0000	7.6667±0.3333
S. aureus SD	8.0000±0.0000	12.6667±0.6667	11.3333±0.6667	10.0000±0.5773	11.3333±0.3333	8.6667±0.3333	8.0000±0.0000	8.0000±0.0000	7.0000±0.0000
Listeria sppSD	4.0000±0.0000	9.3333±0.6667	10.6667±0.3333	10.6667±0.6667	12.0000±0.0000	8.6667±0.6667	9.3333±0.6667	4.0000±0.0000	4.0000±0.0000
E. coli SD	10.6667±0.6667	12.0000±0.0000	9.3333±0.3333	7.6667±0.3333	10.0000±0.0000	8.3333±0.3333	8.6667±0.6667	9.3333±0.6667	8.0000±0.0000
P. aeruginosa SD	11.6667±0.3333	13.3333±0.6667	12.0000±0.0000	10.0000±0.0000	10.0000±0.0000	11.3333±0.6667	8.6667±0.6667	7.3333±0.3333	7.6667±0.3333
B. cereus SD	9.3333±0.6667	12.0000±0.0000	9.6667±0.3333	8.6667±0.6667	11.3333±0.3333	8.0000±0.0000	8.0000±0.0000	7.6667±0.3333	7.3333±0.6667

The Table above shows the effect of varying pH on the purified peptides of germinated *Vigna unguiculata* at day 4, fraction 3. Zone diameter of well = 4mm. SD = Standard Deviation

**Table 7.** Effect of temperature on the purified Peptides of Germinated *Vigna unguiculata* seeds.

Organisms	30° C	40° C	50° C	60° C	70° C	80° C	90° C	100° C	121° C
S. typhi SD	14.6667±0.6667	11.3333±0.6667	8.6667±0.6667	8.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000
S. aureus SD	13.3333±0.6667	10.6667±0.6667	9.3333±0.6667	7.3333±0.6667	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000
Listeria spp SD	13.0000±0.0000	11.3333±0.3333	10.0000±0.0000	8.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000
E. coli SD	11.0000±0.5773	8.6667±0.6667	9.3333±0.6667	8.0000±0.0000	7.3333±0.6667	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000
P. aeruginosa SD	13.0000±0.5773	9.3333±0.6667	8.0000±0.0000	6.6667±0.6667	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000
B. cereus SD	13.3333±0.6667	10.0000±0.0000	8.0000±0.0000	8.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000

The Table above shows the effect of varying Temperature on the purified peptides of germinated *Vigna unguiculata* at day 4, fraction 3. Zone diameter of well = 4mm. SD = Standard Deviation

**Effect of Proteolytic Enzyme Trypsin on Germinated *Vigna Unguiculata* Seeds**

The effect of the proteolytic enzyme trypsin on the antibacterial activity of the germinated beans seeds at day 2, 4,6, and 8 at different concentrations of 1.0 mg/ml and 2.0mg/ml on *S. aureus* and *E. coli*, shows no zone of inhibition.

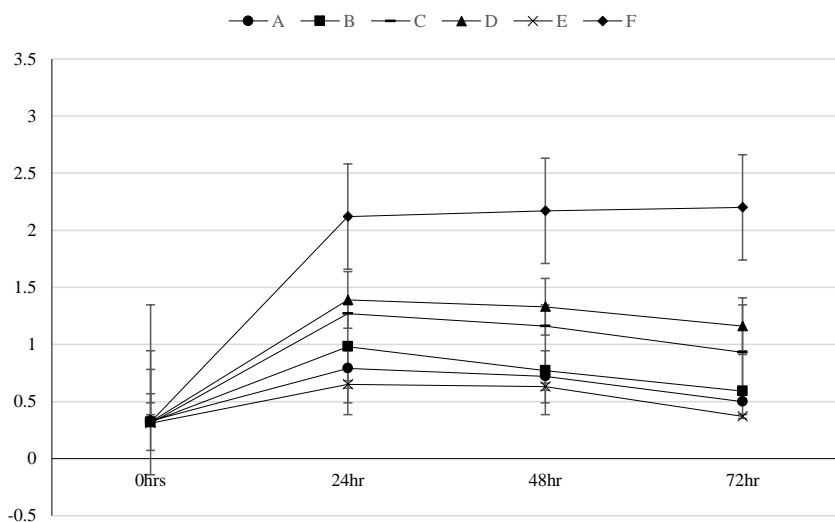
**Table 8.** Effect of Proteolytic Enzyme Trypsin on fractions of Germinated *Vigna unguiculata* seeds.

Trypsin	B <sub>2:4</sub>		B <sub>4:3</sub>		B <sub>6:3</sub>		B <sub>8:3</sub>	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
1.0%	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000
2.0%	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000	4.0000±0.0000
Control	11.3333±0.6667	12.6667±0.6667	12.0000±1.1546	12.6667±0.6667	11.3333±0.3333	9.0000±0.0000	8.6667±0.6667	11.3333±0.6667

KEY: B<sub>2:4</sub> = *Vigna unguiculata* day 2 fraction 4, B<sub>4:3</sub> = *Vigna unguiculata* day 4 fraction 3, B<sub>6:3</sub> = *Vigna unguiculata* day 6 fraction 3, B<sub>8:3</sub> = *Vigna unguiculata* day 8 fraction 3. SD = Standard Deviation

### Sanitization Activity of ‘Zobo’ Drink

The sanitization activity of Laboratory produced ‘zobo drink’ at pH 2.3, the treatment of *zobo* drink with the peptide from germinated *Vigna unguiculata* seeds was monitored at 0hour, 24hours, 48hours, and 72 hours. At 0 hour, the log count for the *zobo* drink was recorded as... was generally found to be low. After 24hrs of storage, the log count was found to have increased for all the treatments but after 48hrs of storage, there was log reduction of count for treatment A to 0.7781log, treatment B reduced its log count to 0.842log, while treatment C reduced its log count to 1.337log. Treatment D reduced its log count to 1.526log, while treatment E (sodium benzoate) reduced its count to 0.7193log. Treatment F (the untreated ‘zobo drink’) had an increase log count to 2.509log, respectively in the case of (B<sub>4:3</sub>). After 72hrs of storage, there was further decrease in the log count of treatment ABCDE, while treatment F which is the untreated had a log increase to 2.535log. Same pattern was observed with the three other fractions.



**Figure 4.** a, b, c, d above shows Sanitizing activity of purified Peptides of germinated *Vigna unguiculata* at day 2, fraction four (B<sub>2:4</sub>), day 4, fraction 3 (B<sub>4:3</sub>), day 6, fraction 3 (B<sub>6:3</sub>), day 8, fraction 3 (B<sub>8:3</sub>) at pH 2.3, at 0hr, 24hr, 48hr and 72hrs respectively. Key: A =Treatment with 10%, B = Treatment with 5%, C = 2.5% D = 1.25%, E = Treatment with 0.5% Sodium Benzoate and F= Untreated/control.

### Biopreservative Activity of Peptides Extract on “Zobo Drink”

The mean and percentage similarity of the zobo drink treated with peptides from germinated *Vigna unguiculata* seeds at a pH of 2.3 were evaluated. For (B<sub>4:3</sub>), at 0hr, the mean likeness for the treated and untreated ‘zobo’ drink was found to be 8.5±0.221 which was equivalent to (94.44%), while treatment A with the highest concentration of the peptide (10%) had a lower mean likeness of 8.3±0.213 (92.22%). After 24hrs of storage, treatment A and F had the least likeness by the Judges. By 48hrs of storage, treatment A had the highest percentage and mean likeness of 8.6±0.163 (95.55%). While the untreated ‘zobo’ had the least mean and percentage likeness of 5.3±0.152 (58.88%). At 72hrs, treatment A (10% B<sub>2:4</sub>) still had the highest percentage likeness of 7.5±0.166 (83.33%) while the untreated ‘zobo’ had mean and percentage likeness of 3.3±0.152(36.67%) respectively and hence was rejected.

**Table 9.** Sensory Evaluation of “Zobo drink” treated with purified Peptides of Germinated Bean seeds at 0hr, 24hr, 48hr, 72hr, pH 2.3.

Time (hrs)	A	B	C	D	E	F
0 SD	8.3 (92.22%)± 0.152	8.5 (94.44%)±0.166	8.5 (94.44%)±0.166	8.5(94.44%)± 0.166	8.5 (94.44%)±0.166	8.5(94.44%)±0 .166
24 SD	8.3 (92.22%)± 0.152	8.4 (93.33%)±0.163	8.4(93.33%)± 0.163	8.4(93.33%)± 0.163	8.4(93.33%)± 0.163	7.6(84.44%)± 0.163
48 SD	8.6 (95.55%)± 0.163	8.0(88.88%)± 0.298	8.0(88.88%)± 0.298	7.6(84.00%)± 0.452	7.5(83.33%)± 0.166	5.3(58.88%)± 0.152
72 SD	7.5(83.33%)± 0.166	6.9(76.66%)± 0.276	6.7(74.44%)± 0.300	6.3(70.00%)± 0.395	6.2(68.88%)± 0.133	3.3(36.67%)± 0.152

Key: A = Treatment with 10%, B = Treatment with 5%, C = 2.5% D = 1.25%, E = Treatment with 0.5% Sodium Benzoate and F = Untreated. B<sub>2:4</sub> = Beans day 2 fraction 4, Purified peptide extract at pH of 2.3. Hedonic scale score: 9=Like Extremely, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much, 1=Dislike Extremely. SD = Standard Deviation. Values are mean ± SD Values are means of sensory scores while values in ( ) are percentage acceptability scores

### DISCUSSION

The highest germination rate of 89.25% was observed with *Vigna unguiculata* seeds at the end of the 8th day of germination. These findings align with studies that suggest that when seeds are placed in conditions conducive to germination, the metabolic rate significantly increases [32] (Figure 1). Protein extraction and purification showed varying protein concentrations. This observation supports the findings of [33,34], who indicated that plants store proteins in seeds, and that during germination, specific endopeptidases break down storage proteins. These smaller peptides exhibit antimicrobial activity, helping to protect the seedling from surrounding microbial threats. Initial purification of the small peptides was carried out using ammonium sulfate precipitation at 75% saturation, followed by dialysis, consistent with the methods employed by [35] for partially purifying peptides from both germinated and ungerminated seeds. Peaks of different protein concentrations (Figure 2) and molecular weight (Figure 3) obtained, were checked for antimicrobial activity against six (6) bacterial and four (4) fungal isolates and these peptides have shown strong antimicrobial activity against a number of bacteria (Table 4). This is in line with study by [36]. were two peptides were isolated from seed and two peptides from pod of *Pisum sativum* L. (garden pea) respectively. The purified peptides of *Vigna unguiculata* were subjected to SDS PAGE along with protein ladder of 3.8-97kDa. From the result, germinated beans day 4 fraction 3 (B<sub>4:3</sub>) was found to be approximately 3.2kDa (Figure 3). Similar to this study, [37]. isolated antimicrobial peptide with molecular weight of approximately 9. Antimicrobial peptides were isolated from bitter melon seeds using techniques such as affinity chromatography, ion exchange chromatography, and gel filtration. In a separate study, [38] reported the isolation of a 10kDa antimicrobial peptide from chili pepper seeds. Potential pathogens and spoilage organisms recovered from the spoiled foods include *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus cereus* and *Listeria* spp, while Fungal isolates recovered from the spoiled

foods include *Aspergillus niger*, *Aspergillus fumigatus*, *Candida* spp and *Saccharomyces cerevisiae*. Report by [39,40], indicated that most of these organisms are major contaminants in most types of foods and drinks involved with handling and could easily cross-contaminate a whole production batch unnoticed (Table 3). Agar well diffusion method using extracted AMPs, was used in this study. Against both gram-positive and gram-negative bacteria, a clear and noticeable zone of inhibition was seen. Fractions of each germinating seed sample with varying protein concentrations at 100% concentration were used for antimicrobial activities. The antimicrobial activity of the purified peptides exhibited varying levels of effectiveness, ranging from high to low activity, and in some cases, no activity. This aligns with the findings of [33], who reported that *S. aureus* and *S. typhi* displayed the highest antimicrobial activity, and that the eluted fractions demonstrated superior activity compared to the crude seed extracts, suggesting that the peptides/proteins isolated from the crude extracts had undergone successful purification (Table 4). No antifungal activity was observed against two species of mold (*A. niger* and *A. fumigatus*) and two yeast species (*Candida* spp. and *Saccharomyces cerevisiae*), which contrasts with the study by [41], where antifungal peptides were isolated from *Phaseolus vulgaris* cv. 'Spotted beans' with a molecular weight of 7.3kDa. Temperature effects on the peptides revealed the highest zones of inhibition at 30°C and 40°C, indicating that peptides from *Vigna unguiculata* are most effective at moderate or possibly lower temperatures (Table 6). These results are consistent with the findings of [36], who reported similar effects with peptides extracted from *Pisum sativum* seeds. The pH dependency of the peptides was also evident, with activity varying across different microbes; the peptides showed the most activity between pH 4 and 7 (Table 7) [36]. Using the proteolytic enzyme trypsin against *S. aureus* and *E. coli*, it was determined that the extracted peptides from *Vigna unguiculata* were proteinaceous (Table 8).

The sanitization activity test, which analyzed the physicochemical parameters of the laboratory-prepared zobo drink at pH 2.3 at 0 hour, 24 hours, 48 hours, and 72 hours using the peptides from *Vigna unguiculata* (B<sub>4:3</sub>), showed an increase in log count after 24 hours for all treatments (A–F), including treatment E, which contained the conventional preservative (0.5% sodium benzoate). Consistent with the results of [39], there was a notable drop in log count after 48 hours of storage (day 2), with the exception of treatment F (untreated zobo drink), which acted as the negative control. Furthermore, the titratable acidity, temperature, and pH fluctuations showed that the zobo drink's pH at 0 hours had significantly increased, particularly in treatment A (10% concentration). Additionally, there was no discernible difference between treatment A (10%) and treatment E (0.5% sodium benzoate) in terms of titratable acidity, and there were minor temperature differences across the treatments, which is consistent with research by [39].

After 24, 48, and 72 hours of storage, there was a further decline in pH and temperature, while titratable acidity continued to rise. This is consistent with the findings of [42], who observed that the titratable acidity of zobo drink (whether stored at ambient or refrigerated temperatures) showed a steady increase as storage time progressed (Figure 4). Sensory evaluation was conducted over 72 hours of storage at room temperature. The evaluation's findings showed that the zobo drink's shelf life could be successfully increased to 72 hours while maintaining a high degree of similarity (83.22%). In comparison to the positive control (treatment E, 0.5% sodium benzoate), the best treatment was B<sub>4:3</sub> (germinated bean seed day two fraction three at the greatest concentration of 10%) (Table 9). At the five percent significance level, a general analysis of variance (One-Way ANOVA) on the percentage acceptance of the zobo drink with various treatments (A–F) showed statistically significant differences between the treatments.

## CONCLUSION

To sum up, the investigation into the preservative qualities of peptides obtained from *Vigna unguiculata* in Zobo drink shows that these organic peptides have the potential to be useful preservatives. Strong antibacterial action was demonstrated by the peptides against a variety of toxic microbes and spoiling, extending the beverage's shelf life.

Furthermore, their ability to prevent oxidative processes and preserve the sensory qualities of the *Zobo* drink enhances their potential as preservatives. The potential of plant-derived peptides as safer and more environmentally friendly substitutes for artificial preservatives in the food and beverage sector is highlighted by this study. The successful integration of these peptides into commercial food items will be facilitated by additional research aimed at refining extraction techniques and thorough safety and stability evaluations. In addition to ensuring better product quality and safety, the use of these natural preservatives satisfies customer desire for clean-label, environmentally friendly food solutions.

### Recommendations

1. More investigation is required to identify the peptides recovered from the *Vigna unguiculate* germinating seeds using LC-MS, as well as to ascertain their molecular structures and amino acid sequence.
2. Other testing of the peptides' preservation qualities on other slightly and completely processed food models is required.

### REFERENCES

1. Pisoschi, A. M., Pop, A., Georgesa, C., Turcus, V., Olah, K.N. and Mathe, E. (2018): An Overview of Natural Antimicrobial Roles in interaction dynamics with membrane. *Biochem. Biophysical Res. Commun.* 473, 642–647.
2. Elayaraja S, Annamalai N, Mayavu P, Balasubramanian T (2014): Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. *Asian Pac J Trop Biomed*, 4:305–311
3. Hintz T, Matthews KK, Di R (2015) The use of plant antimicrobial Compounds for food preservation. *Bio Med Res Int*. doi:10.1155/2015/246264
4. Kraszewska J, Beckett MC, James TC, Bond U (2016) Comparative analysis of the antimicrobial activities of plant defensin-like and ultrashort peptides against food-spoiling bacteria. *Appl Environ Microbiol*. doi:10.1128/AEM.00558-16
5. Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., & Givskov, M. (2002). Food spoilage-interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 78(1-2), 79-97.
6. Abraham P, George S, Santhosh K (2014): Novel antibacterial peptides from the skin secretion of the Indian bicoloured frog *Clinotarsus curtipes*. *Biochimie*, 97:144–151.
7. Bala P, Kumar J (2014): Antimicrobial peptides: a review. *Int J Life Sci Biotechnol Pharm Res*, 3(1):61–71
8. Harris F, Dennison S, Phoenix DA (2009): Anionic antimicrobial peptide from eukaryotic organisms. *Curr Protein Pept Sci*, 10(6):585–606
9. Riedl S, Zweytick D, Lohner K (2011): Membrane-active host defense peptides challenges and perspectives for the development of novel anticancer drugs. *Chem Phys Lipids*, 164:766–781
10. Ribeiro A, Carrasco L (2014): Novel formulations for antimicrobial peptides. *Int J Mol Sci*, 15:18040–18083
11. Rea MC, Ross RP, Cotter PD, Hill C (2011): Classification of Bacteriocins from Gram-positive bacteria. In Drider D, Rebuffat S (eds) *Prokaryotic antimicrobial peptides: from genes to application*, 1st edn. Springer, New York, pp. 29–47
12. Pasupuleti M, Schmidtchen A, Malmsten M (2012): Antimicrobial peptides: key components of the innate immune system. *Crit Rev Biotechnol* 32:143–171
13. De Zoysa GH, Cameron AJ, Hegde VV, Raghobama S, Sarojini V (2015): Antimicrobial peptide with potential for biofilm eradication: synthesis and structure activity relationship studies
14. Bellaver, E.H. Kempka, A.P. (2023): Potential of milk-derived bioactive peptides as antidiabetic, antihypertensive, and xanthine oxidase inhibitors: A comprehensive bibliometric analysis and updated review. *Amino Acids*, 55, 1829–1855.
15. Muttenthaler, M., King, G.F., Adams, D.J., Alewood, P.F. (2021): Trends in peptide drug discovery. *Nat. Rev. Drug Discov*, 20, 309–325.

16. Kamal, I., Ashfaq, U.A., Hayat, S., Aslam, B., Sarfraz, M.H., Yaseen, H., Rajoka, M.S.R., Shah, A.A., Khurshid, M. (2023): Prospects of antimicrobial peptides as an alternative to chemical preservatives for food safety. *Biotechnol. Lett.*, 45, 137–162.
17. Barbosa, A.A.T., Mantovani, H.C., Jain, S. (2017): Bacteriocins from lactic acid bacteria and their potential in the preservation of fruit products. *Crit. Rev. Biotechnol.*, 37, 852–864.
18. Abdelbasset M, Djamila K (2008): Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk “Raib”. *Afr J Biotechnol.*, 7(16):2908–2914
19. Meza J, Zarzosa A, Corona J, Bideshi D (2015): AMPs: current and potential applications in biomedical therapies. *Biomed Res Int.*, doi:10.1155/2015/367243
20. Upendra RS, Khandelwal P, Jana K, Ajay Kumar N, Gayathri Devi M, Stephaney ML (2016): Bacteriocin production from indigenous strains of lactic acid bacteria isolated from selected fermented food sources. *Int J Pharma Res Health Sci.*, 4(1):982–990
21. Sumi CD, Yang BW, Yeo IC, Hahm YT (2015): Antimicrobial peptides of the genus Bacillus: a new era for antibiotics. *Can J Microbiol.*, 61:93–103
22. Pihlanto, A. (2018). Bioactive peptides derived from bovine whey proteins: Opioid and ACE-inhibitory peptides. *Trends in Food Science & Technology*, 84, 45-53
23. Chase, M.W., Christenhusz, M.J.M., Fay, M.F., Byng, J.W., Judd, W.S., Soltis, D. E., Mabberley, D.J., Sennikov, A.N. and Soltis, P.S. (2016): An update of the angiosperm phylogeny group classification for the orders and families of flowering plants (APG IV). *Botanical Journal of the Linnean Society*, 181 (1) 1-20.
24. Anon. (1999). International Rules for Seeds Testing. Published by The International Seed Testing Association (ISTA), Switzerland.
25. Pasha, C., Golla, K., Vutukuru, S.S., Rani-Usha, J., and Meghanath, P. (2016): Screening of small peptides from various germinating seeds having Antimicrobial activity. *Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*. 11(1), Pp 52-60.
26. Lowry, O.H., Nira, J.R., Lewis, F.A., and Rose, J.R., (1951): Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* (193) 265-275
27. Anna, K.K., and Sanaa, B.A. (2014): Purification, characterization of Thermostable Amylopullulanase from Bacillus licheniformis (BS18) by using solid state fermentation (SSF), *J. Baghdad for Sci.* Vol.11 (2).
28. Valery, B., Vladimir, S., and Ivan, D., (2014): Obtaining Bacteriocins by Chromatographic Methods. *Advances in Bioscience and Biotechnology*, 446-451.
29. Bukar, A. Uba, A. and Oyeyi, T.I. (2010): Phytochemical Analysis and Antimicrobial activity of Parkia biglobosa (jacq) benth extract against some Foodborne Microorganisms. *Advances in Environmental Microbiology*, 4(1): 74 – 79.
30. Murugan, S., Anand, R. Uma Devi, P., Vidhya, N. and Rajesh, K.A. (2007): Efficacy of Euphobiamilli and E.pulcherrima on aflatoxin producing fungi (Aspergillus flavus and A.parasiticus). *African Journal of Biotechnology*. 6(6):718-719.
31. Aramide, A.A., Abiose, S.H., and Adeniran, A.H (2009): Microbial evaluation of probiotic beverage from Roselle extract. *African Journal of Food Science*.1 (2): 385-392
32. Bukar, A. (2012). Preservative properties of extracts of Parkia biglobosa (JACQ) Benth. Anogeissus leiocarpus L. and Moringa oleifera Lam On some minimally and fully processed foods. PhD. Thesis Department of Microbiology, Bayero University, Kano 116 – 121 Pp. (ISBN: 3659268291). LAP Lambert Academic Publishing
33. Ali, M. and Bukar, A. (2018): Evaluation of Preservative Properties and Antimicrobial Activities of Anogeissus leiocarpus Extract on Food Pathogen of Hibiscus sabdariffa calyx (zobo) drink. *Bayero Journal of Pure and Applied Science*, 11(1), 153-160
34. Food and Agricultural Organization/World Health Organization (2010). Codex Standard for Fermentedmilks (2nd ed.). Codex Stan 243-2003. Gourama H (1997). Inhibition of growth and Mycotoxin Production of Penicillium by Lactobacillus species. *Lebensm-Wiss u-Technol*, 30: 279-283.
35. Peryam, D.R., (1998): The “9” point hedonic scale. Peryam and knoll research corporation Pp. 4 – 13.

36. Sanchez, M.C., Attares, P., Pedrosa, M.M., Burbano, C., Cuadrado, C., Goyoaga, C., Muzquiz, M., Jimenez-Martinez, C., and Davila Ortiz, G. (2005): Alkaloid variation during germination in different lupin species. *Food chemistry*. (90) 347-355.
37. Rehma, S., and Khanum, A. (2011): Isolation and characterization of peptides from *Pisum sativum* having antimicrobial activity against various bacteria. *Pak. J. Bot.* 43, 2971-2978.
38. Saad, S.D., MirNaiman, A., Hajera, T., and Mazharuddin, K. (2010): Studies on Antibacterial and Antifungal Activity of Pomegranate (*Punicagranatum L.*). *American-Eurasian J. Agric. & Environ. Sci* 9 (3), 273-281.
39. Bhavith, K. P., NarayanaSwamy, M., RamachandraSwamy, N., Chandrashekharaiiah, K. S. (2014): Purification and Characterization of esterase from the seeds of *Caesalpiniamimosoides*, *Journal of Experimental Biology and Agricultural Sciences*, Volume – 2(6).
40. Jabeen, U., and Khanum, A. (2014): Isolation and characterization of potential food preservative peptide from *Momordica charantia L.* *Arabian Journal of Chemistry*
41. Parkash, A., Ng, T.B., Tso, W.W. (2002): Purification and characterization of charantin, a napin-like ribosome- inactivating peptide from bitter gourd (*Momordica charantia*) seeds. *J. Pept. Res.* 59, 197-202.
42. Diz, M.S., Carvalho, A.O., Rodrigues, R., Neves-erreira, A.G., Dacunha, M., Alves, E.W., Okorokova-Facanha, A.L., Oliveira, M.A., Perales, J., Machado, O.L., Gomes, V.M. (2006): Antimicrobial peptide from chilli pepper seeds cause yeast plasma membrane permeabilization and inhibits the acidification of medium by yeast cells. *Biochem. Biophys. Acta* 1760, 1323-1332.
43. Bukar, A. Uba, A. and Oyeyi, T.I. (2009): Phytochemical Analysis and Antimicrobial activity of *Parkia biglobosa* (jacq) benth extract against some Foodborne Microorganisms. *Advances in Environmental Microbiology*, 4(1): 74 – 79.
44. Mailafia, S., Okoh, R.G., Olabode, O.H., and Osanupin, R. (2017): Isolation and Identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Vet world*. 10 (4): 393-397.
45. Wang, J., Wang, H.X., Ng, T.B. (2007): A peptide with HIV-1 reverse transcriptase inhibitory activity from the medicinal mushroom (*Russula paludosa*) *Peptides*. Vol 28 (3) 560-565
46. Venuti I., Marina C., Tiziana M., Carmela V., Paolo S., Giovanni B. V., Tiziana P. (2024): Safety and Quality Assessment of Hot Drinks Vending Machines in Southern Italy. *Food Control*. (161) 110376