Research Article

DOI: 10.22146/ifnp.91418 https://journal.ugm.ac.id/ifnp
ISSN 2597-9388

Journal of Indonesian Food and Nutrition Progress 61

Lemongrass-Synthesized Silver Nanoparticles as Preservatives of Fermented Locust Beans

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Submitted: December 5th, 2023; Revised: May 1st, 2024; Accepted: May 7th, 2024; Published: November 15th, 2024

ABSTRACT: Fermented locust beans (FLB), produced from *Parkia biglobosa* (Mimosaceae-Fabaceae), are used in many parts of West Africa, including Nigeria, as condiments to enhance the taste and nutritional quality of foods. These benefits are, however, marred by the short shelf-life of FLB. Traditional preservative methods and the use of chemicals have their shortcomings. This study, therefore, investigated the effectiveness of lemongrass-synthesized silver nanoparticles (LSSNP) as a preservative of FLB. The LSSNP was prepared, characterized, and used to treat fresh FLB at 10% v/w. Dynamic light scattering analysis revealed a mean hydrodynamic size of 89 nm for the LSSNP, while transmission electron micrograph showed roughly spherical particles with an average size of 100 nm. *Bacillus licheniformis* KGEB16, *B. licheniformis* APBSWPTB167, *B. licheniformis* PS4, *B. subtilis* CICC10148, and *Enterobacter xiangfangensis* M5S2B6 isolated were susceptible to LSSNP with comparable zones of inhibition to reference antibiotics. A significant reduction of the microbial load of FLB by up to 63.7% due to LSSNP treatment was achieved. The organoleptic and proximate properties of LSSNP-treated FLB were preserved. A histo-morphological study showed normal hepatic architecture in rats fed with LSSNP-treated FLB. This study showed that LSSNP possesses antimicrobial properties and can be employed as a green and safe alternative for the preservation of FLB.

Keywords: lemongrass, silver nanoparticles, fermented locust beans, preservation

INTRODUCTION

Parkia biglobosa (Mimosaceae-Fabaceae), also known as African locust beans, is used in many parts of West Africa to produce fermented locust beans (FLB), commonly referred to as *iru* and *dawadawa* in Nigeria. The inedible seed of African locust beans is processed via a solid-state fermentation described in our previous study (Saliu *et al*., 2019) into edible FLB, a highly valuable condiment with improved nutritional composition, by microorganisms predominantly from the genus *Bacillus*, although other bacteria genera, including *Staphylococcus*, *Leuconostoc*, and *Lactobacillus*, have also been reported to participate in the fermentation (Olasupo *et al*., 2010). The processes that lead to the production of edible fermented locust beans include.

Production of FLB is a non-standardized and uncontrolled process that allows growth of organisms indefinitely (Nwamaka *et al*., 2010), leading to spoilage and undesirable conversion of many of the beneficial primary components to non-nutritive constituents and making it highly perishable with a very short shelf life at room temperature (28±2 °C). Traditional preservation strategies employ drying and salting methods to extend the shelf life of FLB. Previous studies reported that excess salting of FLB increased acidity and peroxide values, resulting in poor organoleptic properties (Ojewumi, 2018). Sun drying and elevated oven temperature also significantly affected protein contents and nutritive indices of the FLB (Ojewumi, 2018). Refrigeration would be a viable approach to improve storage quality. However, epileptic electricity supply in Nigeria and other developing countries in sub-Saharan Africa underscore the need for alternative and economical preservation methods, such as green nanoparticles, to eliminate fermenting microorganisms at the end of fermentation while maintaining the organoleptic properties of FLB.

Nanoparticles are any material on the nanoscale with a size of less than one micron. Nano-synthesis of antimicrobial materials enhances their effectiveness, lending credence to health, agriculture, and food applications. There is empirical evidence of the antimicrobial activities of metal nanoparticles (Baranwal *et al*., 2018), and more so for silver nanoparticles (AgNPs). Silver in nanoparticulate forms induces microbicidal activity via reactive oxygen species-induced cell membrane disruption and DNA transformation (Das *et al*., 2017). These inherent properties make AgNPs extremely attractive as

antimicrobial agents in food packaging, safety, and preservation. The synthesis of AgNPs using the green biosynthetic route is eco-friendly, involves a single-step, is easy to scale up, and does not require high temperature, pressure, or any toxic reagent. Hence, it is cost-effective.

Extracts from Nigerian medicinal plants such as *Cymbopogon citratus* (lemongrass) have been used for the synthesis of AgNPs and have demonstrated activities against some spoilage and pathogenic organisms (Masurkar *et al*., 2011; Ajayi & Afolayan, 2017). The use of AgNPs in polymeric matrices for food packaging is well documented (Carbone *et al*., 2016). A few reports exist on the direct use of biosynthesized AgNPs for preserving fruits such as cabbage (Khan *et al*., 2016). However, to our knowledge, there is no report on its use as a preservative of fermented foods, particularly FLB. Thus, this study was designed to investigate the potential of lemongrass-synthesized silver nanoparticles (LSSNP) to preserve FLB.

MATERIALS AND METHODS

Samples

Lemongrass was collected in the Ilorin metropolis, Nigeria (8.4799° N, 4.5418° E). The leaf sample was identified, authenticated, and assigned voucher no. UILH/001/800 at the Herbarium unit of the Department of Plant Biology, University of Ilorin. Freshly produced fermented locust beans (FLB) were also purchased from a local producer in Ilorin.

Qualitative determination of phytochemicals of the lemongrass extract:

Lemongrass extract was screened for tannins, phenolics, terpenoids, flavonoids, steroids, and alkaloid contents following methods previously described by Ezeonu and Ejikeme (2016). Aqueous extract was prepared as described by Dubey et al. (2010). Briefly, properly washed leaves were added to distilled water at 20% w/v in an Erlenmeyer flask, boiled for 10 minutes, and filtered through Whatman filter paper (No. 40). For tannins and phenolics, 3 drops of 0.1% ferric chloride were added to 5 ml of the extract. A brownish-green coloration shows a positive result. A mixture of chloroform (2 ml) and concentrated tetraoxosulphate (IV) acid (2 ml) were added to 5 ml of aqueous extract to detect terpenoids. A reddish-brown coloration at the interface was positive. The addition of 5 ml of 0.1 M ammonia solution followed by 5 ml concentrated tetraoxosulphate (IV) acid to 10 ml of the aqueous extract to give yellow coloration was used for flavonoid detection. For steroids, lemongrass was extracted with absolute ethanol 20% w/v and filtered after 2 hours. The filtrate was added to 2 mL acetic anhydride, followed by 2 mL of concentrated tetraoxosulphate (VI) acid. A violet-to-blue or green coloration indicates the presence of steroids. To detect alkaloids, 20 ml of 5% tetraoxosulphate (IV) acid in 50% ethanol was used for extraction. The filtrate was made alkaline by adding 28% ammonia solution in a separating funnel. Chloroform (5 ml) was added to 5 ml of the filtrate for further extraction, and finally, 5 ml of 1 M dilute tetraoxosulphate (IV) acid was used twice. The final acid extract (2 ml) was mixed with 0.5 ml of Draggendorff's reagent. Orange precipitate indicates the presence of alkaloids.

Lemongrass-synthesized silver nanoparticles

Synthesis

The lemongrass extract was synthesized following the protocol described by Dubey et al. (2010). The extract was mixed with 0.02 M $AgNO_3$ (Sigma Aldrich, MO, USA) solution at 1:4 (v/v) and allowed to stand for 5 minutes while the color was monitored for a change from yellow to brown to signify silver nanoparticle formation, using a UV-Vis spectrophotometer (UV5, Mettler Toledo, Columbus, OH, USA) at its characteristic wavelength (λ = 420 nm).

Characterization

The size distribution profile was determined via dynamic light scattering (DLS) using a Nano Zetasizer (Malvern Instruments, WR, UK). Chemical interaction between plant phytochemicals and the AgNPs was examined with Fourier transform infrared (FTIR) using Spectrum 100 FTIR spectrometer (Perkin Elmer Inc., CT, USA), and FTIR spectrum was analyzed with Spectragryph v1.2.12 software (Menges, 2017). Scanning and transmission electron microscopy (SEM and TEM) were used to determine the particle surface morphology, percentage silver content, and size using Zeiss Gemini 2 Crossbeam 540 FEG SEM (Carl Zeiss, Oberkochen, Germany) and Jeol 2100F FEG TEM (Jeol Ltd, Tokyo, Japan) respectively.

Sterility testing

Sterility was determined by direct inoculation of Fluid Thioglycollate Medium (10 ml) and Tryptone Soya Broth (10 ml) in separate McCartney bottles with 100 µL of LSSNP for bacterial and fungal growth, respectively, and incubation at 37 °C for 14 days (Bugno et al., 2022). Turbidity was used as an index of growth and confirmed on nutrient agar and sabouraud dextrose agar.

Fermented locust beans

Treatment with lemongrass-synthesized silver nanoparticles

Lemongrass-synthesized silver nanoparticles (absorbance at 420 nm ~1) at 10% (v/w) were added to each of 25 sterile bottles containing FLB (5 g) and properly mixed to integrate the nanoparticles into the food matrix as a direct usage (Biswas et al., 2022). Untreated FLB samples were also prepared in 25 bottles. All bottles were stored at room temperature, away from sunlight, and samples were withdrawn periodically for analyses.

Organoleptic, pH, and proximate analyses

Samples of LSSNP-treated FLB were analyzed for changes in color, odor, and texture by a 5-man panel on a 5-point hedonic scale over 58 days. The panelists comprised individuals who were very familiar with the sensory qualities of FLB and were trained on how to score the parameters on the form provided. Changes in pH were monitored using the CLIDA 25C precision tabletop pH meter calibrated using buffer solutions 4, 7, and 9 after mixing the crushed FLB with deionized water at 10% w/v (Zebedee *et al*., 2022). The moisture, protein, ash, crude fiber, carbohydrate, lipid contents, and calorific values of the untreated FLB and the LSSNP-treated samples were analyzed before and at the end of the experimental period as previously described (Ajayi et al., 2015).

Isolation and characterization of organisms

Organisms were isolated on Nutrient Agar (NA), MacConkey Agar (MA), De Man, Rogosa, and Sharpe (MRS) Agar, and Sabouraud Dextrose Agar (SDA) using the pour plate techniques (Sanders, 2012). Plates were incubated at 37 ± 2 ℃ for 18-24 hours except SDA plates (28 ± 2 ℃ for 72 hours). After incubation, colonies were counted, and organisms were subcultured on the respective agar medium to obtain pure cultures.

Following Lear et al. (2018), QIAamp DNA Mini Kit (250) cat No. 51306 was used to extract genomic DNA, and the 16SrRNA genes were amplified using the 16SF (5'AGAGTTTGATCCTGGCTCAG 3') and 16SR (5' TACCTTGTTACGACTT 3') primers. The PCR product was purified from contaminants by electro-elution of the gel slice containing the excised desired fragments with a Qiaquick gel extraction kit (Qiagen, USA). The elution was carried out in 300 µL of nuclease-free water in order to enhance the purification of the PCR products. Sequencing was performed using an automated sequencer (ABI PRISM 310, Applied Biosystems, USA). Translated nucleotide sequences were analyzed for similarities using the BLASTN tool (www.ncbi.nlm.nih.gov:80/BLASTN/). ClustalW Multip le Sequence Alignment was constructed using BioEdit v7.2.5 for Windows.

Antimicrobial susceptibility test

The agar well diffusion method (Balouiri et al., 2016) and Kirby-Bauer disc diffusion method (Hudzicki, 2009) were used to test for the Susceptibility of isolates to LSSNP and some conventional antibiotics. The nanoparticle, LSSNP, was loaded in soft agar, which was used to fill holes on seeded Mueller Hinton agar plates while the antibiotics were in paper disks. The minimum inhibitory concentration was determined by varying the volume of LSSNP mixed with agar to obtain 50%, 25%, and 12.5%. The diameter of inhibition zones was measured in five planes, and means were used to represent antimicrobial activity.

Histo-morphological assessment of lemongrass-synthesized silver nanoparticles

Ten Wistar rats, with an average weight of 150 g, were acclimatized under standard laboratory conditions for two weeks and fasted for 12 hours before treatment. The rats were randomly divided into 2 groups and fed with either LSSNP-treated FLB or untreated FLB for 21 days. The rats were sacrificed, and liver tissues were harvested for histological examination (Ibrahim et al., 2018).

Statistical Analysis

Data were recorded as means with standard deviations of quintuplicate measurements. ANOVA test for significance ($p < 0.05$) with post hoc were obtained using SPSS 20 software

Ethical approval

Due to the use of laboratory animals, an ethical certificate with approval number UERC/ASN/2021/2226 was duly obtained from the University of Ilorin Ethical Committee. In addition, the ARRIVE guideline for reporting in vivo animal experiments was adhered to.

RESULTS AND DISCUSSION

Phytochemical components of aqueous lemongrass extract

The phytochemicals found in the aqueous lemongrass extract were terpenoids, flavonoids, and alkaloids, each of which plays a beneficial role in health and well-being. Terpenoids possess antioxidant, anti-tumor, anti-microbial, hepatoprotective, and immuno-modulatory functions (Yende *et al*., 2014). Flavonoids function as

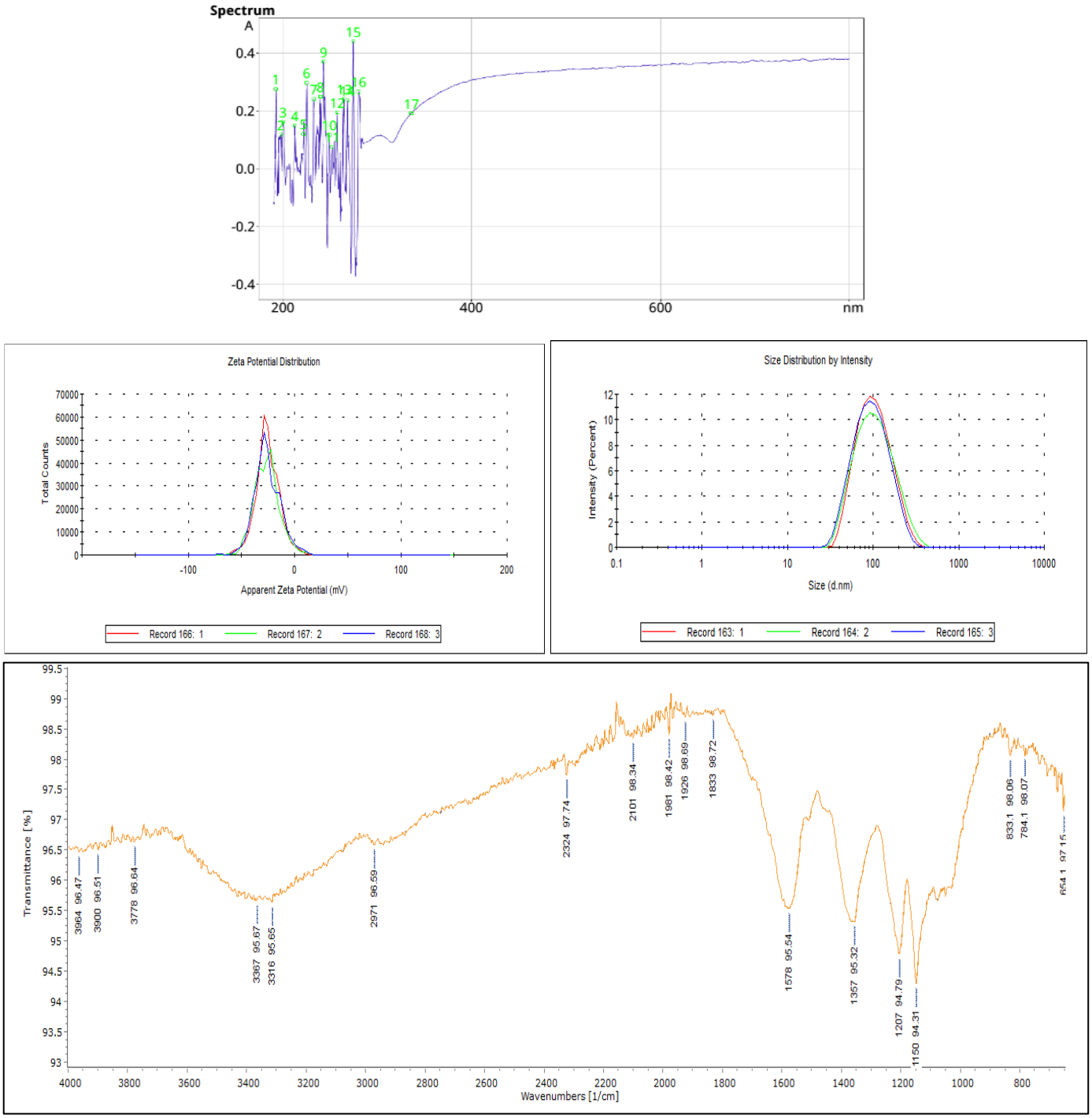

Figure 1. Characteristics of lemongrass-synthesized nanoparticles
A: UV-vis absorbance spectra; B: Particle size distribution of LSSNPs based on intensity; C: Zeta potential distribution of LSSNPs; D: FTIR Spectra of LSSNPs

antimicrobial, antipyretic, and antioxidant, while alkaloids serve as astringents and neuro-pharmacological modulators (Ekpenyong *et al*., 2015).

Characteristics of Lemongrass Synthesized Silver Nanoparticles

A change in the color of the aqueous lemongrass extract from pale yellow to dark brown occurred. In line with the submissions of Roy *et al*. (2017) and Kumar *et al*. (2017), this signified synthesis of nanoparticles and indicates the bio-reduction of silver ions (Ag^+) from the silver salt ($AgNO_3$) into the silver atom by the flavonoids, terpenoids, and alkaloids present in the extract. Green silver nanoparticles have demonstrated antibacterial activities against foodborne pathogens and food spoilage organisms, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* (Prakash *et al*., 2019). The synthesis, therefore, serves an important role in enhancing their preservative

potential. The absorbance spectra measured between the wavelength of 190 nm and 1100 nm at an interval of 0.2 is presented in Figure 1A. The peak surface plasmon resonance absorbance value was 366.2 nm (peak 15). A higher signatory peak absorbance for AgNPs has been reported earlier (Oluwaniyi *et al.*, 2016; Kumar *et al*., 2017; Roy *et al*., 2017). Dynamic Light Scattering (DLS) characterization (Figure 1B) showed LSSNP resolved virtually at a single peak at 27 °C, implying that the particles were stable and the right temperature helped control size formation. Particle hydrodynamic diameter averaged 89.18 nm, with peaks resolving around 100 nm and a poly-dispersity index average of 0.185. This further confirms the formation of nanoparticles and particle stability as a poly-dispersity index above 0.7 implies a very broad size (Roy *et al*., 2017). Zeta potential distribution analysis (Figure 1C) reiterates this at -25.7±0.306. Fourier Transform infrared (FTIR) spectra (Figure 1D) ranged from 784 to 3964 cm^{-1}. Spectra between 3550-3200 cm^{-1} is indicative of a strong, broad O-H stretching for alcohols, suggesting the presence of the flavonoid group, flavonols, and implying that LSSNP will possess antimicrobial, antioxidant, and antipyretic activity following the report of Ekpenyong *et al*. (2015). The bands between 2145 and 2120 cm^{-1} suggests a strong N=C=N (carbodiimide) bonding; bands around 2,931 cm^{-1} suggests C–H stretching of aldehydes (Roy *et al*., 2015); 2000-1900 cm^{-1} is signatory for C=C=C (allene) stretching; 1342-1266 cm^{-1} suggests a strong C-N stretching for aromatic amine; between 750 to 840 cm^{-1} for para- and meta-di-substituted aromatic compounds.

The transmission electron micrograph (Figure 2A, B, and C) revealed that the LSSNP is spherical in shape with a single shell possessing an approximate size of 50 nm with agglomeration and uniform distribution. This was confirmed by scanning electron imaging (Figure 2D and E) while highlighting the crystalline morphology of the LSSNP surface. The slight distortion in the structure is attributable to a capping effect by biomolecules such as proteins from the aqueous extract (Lateef *et al*.,2015; Oluwaniyi *et al.*, 2016; Kumar *et al*., 2017), which influence the stability, morphology, dimensions, and toxicity of silver nanoparticles (Stevanović *et al*., 2011). A spherical shape for AgNPs synthesized using leaf extracts, including lemongrass leaves, has been previously reported (Masurkar et al., 2011; Oluwaniyi *et al.*, 2016; Kumar *et al*., 2017; Roy *et al*., 2017). The scanning electron micrograph at the two different magnifications used underscores the neat and uniform crystalline structure of LSSNP.

Sterility of lemongrass-synthesized silver nanoparticles

The broth media used to determine the sterility of LSSNP maintained a clear translucency through the 14-day incubation, and no growth occurred on the agar media used to confirm the sterility (Table 1). This underscores the aseptic condition under which the nanoparticle was produced, the importance of which was earlier stressed by Hall *et al*. (2007).

Effect of Treatment with lemongrass-synthesized Silver Nanoparticles on the Fermented Locust Beans

pH

The pH of FLB during the period of the study was generally neutral (Figure 3A) and did not differ significantly ($p < 0.05$) between treated and untreated samples. This indicates that neither acid nor alkali was produced during processing, which may account for the poor shelf quality of FLB as most organisms thrive at pH within neutral. In contrast to our result, Olajuyigbe and Ajele (2008) reported a pH range of 5 to 11 for protease and esterase activity on FLB and attributed it to its spoilage.

Table 1. Sterility Testing of LSNPs

Media	Incubation Period (Days)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fluid Thioglycollate Medium (FTM)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tryptone Soy Broth (TSB)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nutrient Agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sabouraud Dextrose Agar	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Legend: "-" denotes a negative result i.e., no growth

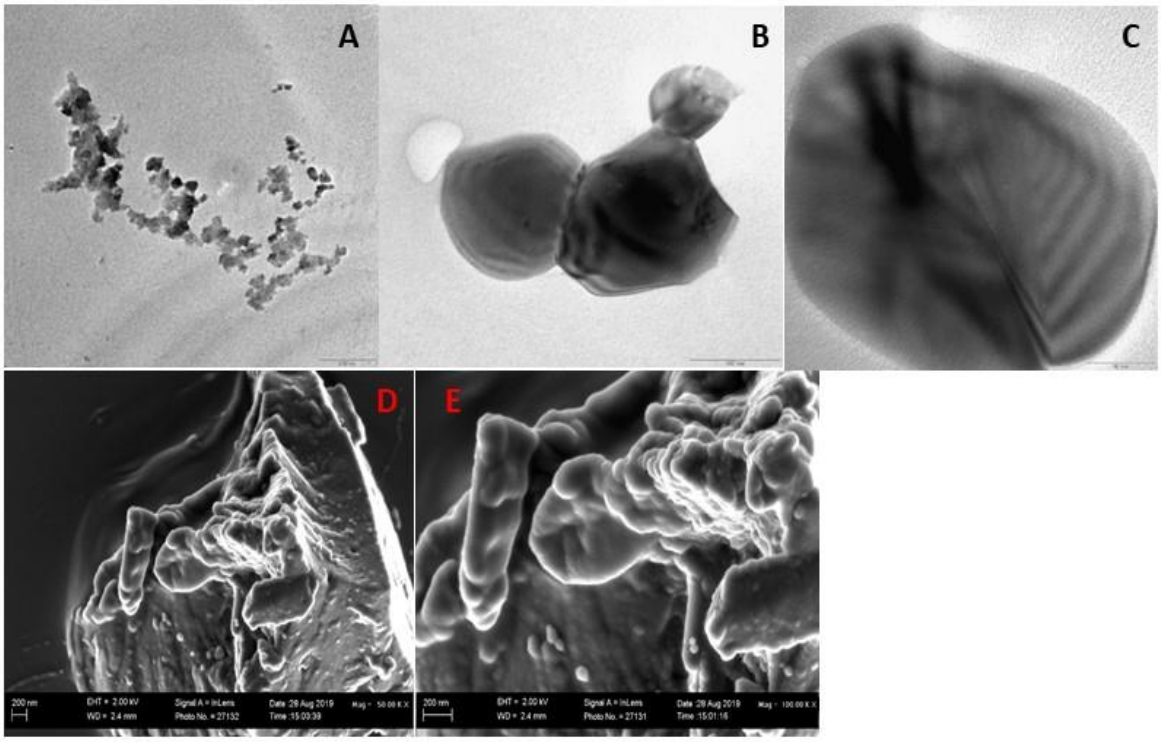

Figure 2. Micrograph of lemongrass-synthesized nanoparticles

A: Transmission Electron Micrograph of agglomerate lemongrass-synthesized nanoparticles;
B: Transmission Electron Micrograph of attached lemongrass-synthesized nanoparticles shells;
C: Transmission Electron Micrograph of single lemongrass-synthesized nanoparticles shell;
D: Scanning Electron Micrograph of lemongrass-synthesized nanoparticles;
E: Scanning Electron Micrograph of lemongrass-synthesized nanoparticles (x100)

Organoleptic Properties

The acceptance index did not differ significantly (p<0.05) between the treated and untreated samples during the initial period of storage (Figure 3B). However, the effect of treatment became evident in the later period, underscoring the importance of preservation in combination with production under hygienic conditions. The ability of nanoparticles to improve safety and preserve the nutritional value of food without impacting negatively on the sensory quality has been reported (Das *et al*., 2017).

Proximate Composition

The protein content of the LSSNP-treated sample (34.424%) compared favorably with that of the untreated (30.856%). The other components were not significantly ($p \leq 0.05$) affected by the treatment. The ash, crude fiber, carbohydrate, and calorific values for the LSSNP-treated and untreated samples were 1.22/2.20; 1.48/2.08; 19.98/20.24; and 978.98/994.47, respectively. The lipids content was, however, highly depleted from 3.75% prior to LSSNP treatment to 0.4% after. This may imply that the LSSNP reacts with lipids in the sample. Nano preservation has been shown to enhance physical properties and nutritional quality and protect against the chemical deterioration of foods (Bajpai *et al*., 2018).

Microbial Load

Colony counts on Nutrient Agar, Sabouraud Dextrose Agar, MacConkey Agar, and de Mann Rogossa and Sharpe Agar plates were used to assess the effect of LSSNP treatment on the microbial load of fermented locust beans. The organisms were inhibited by LSSNP on first contact. However, the nature of inhibition was static as the organisms recovered and started growing by the second day of incubation. The significant difference ($p \leq 0.05$) in colony counts between the treated and untreated samples observed in the first 13 days of incubation (Figure 3B) is an indication that the LSNNP has the capacity to improve the shelf-life of fermented locust beans. Recent studies have reported the use of essential oil from lemongrass as a preservative of FLB (Saliu & Idowu, 2023), and tofu (Hamad et al., 2019).

Microbial Isolates

Table 2 shows the analysis of the 16SrRNA sequence of bacteria isolated from the FLB with the ascension numbers. In all the isolates, the sequence showed more

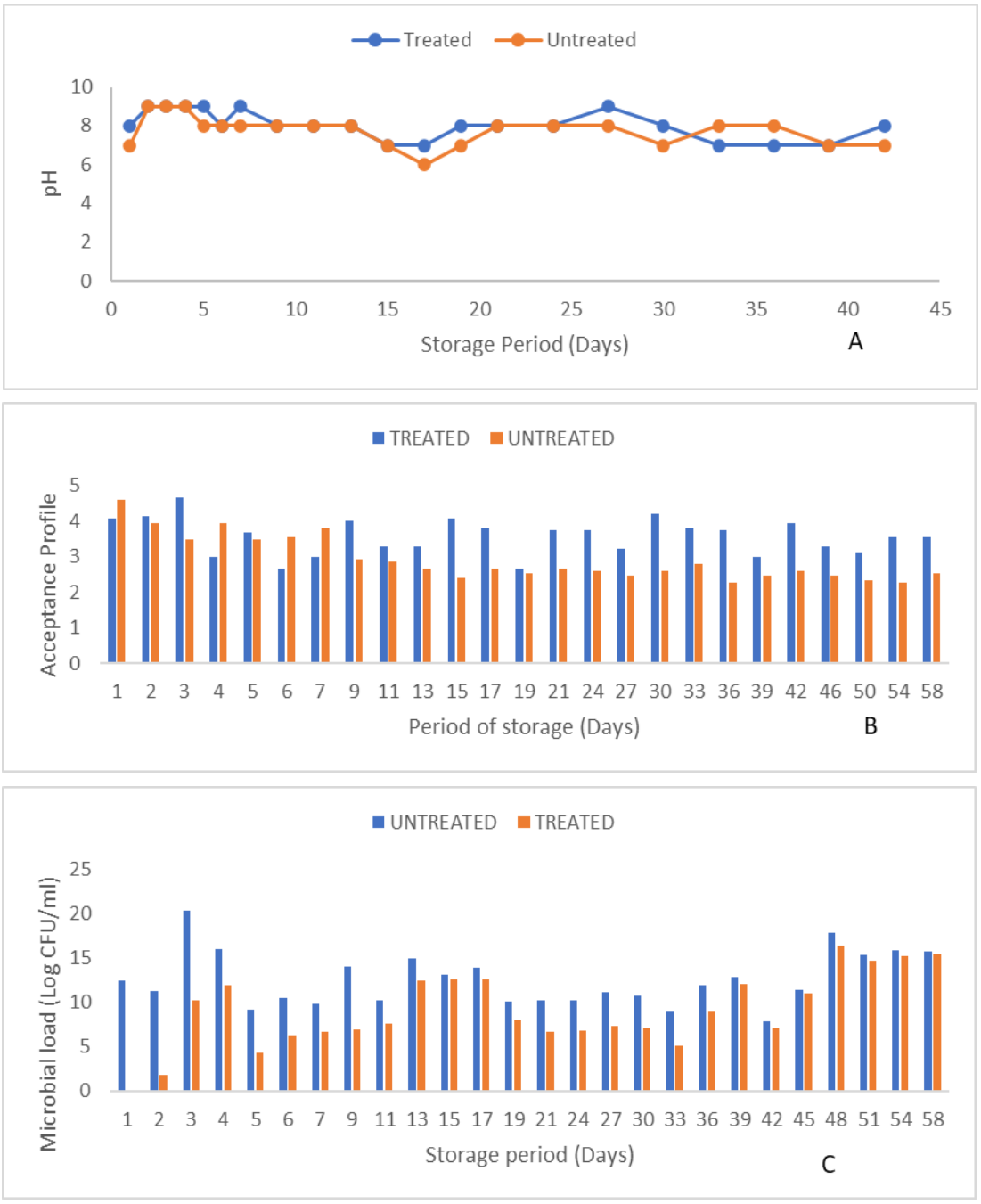

Figure 3. Effect of treatment with lemongrass-synthesized nanoparticles on the fermented locust beans

A: pH (Measured after crushing the sample in deionized water at 10% w/v) B: Sensory evaluation (The acceptance profile is a combination of three sensory properties i.e., color, odor, and texture of the Fermented Locust Beans. The sensory quality was scored on 5 hedonic scale by a 5 man panel) C: Microbial load

than the 70% minimum required for identity. Most of the isolates belonged to the genus *Bacillus*. This supports earlier assertions that *Bacillus* spp. dominates the fermentation of locust beans (Ojewumi, 2017). The coliforms (*Enterobacter xiangfangensis* M5S2B6 and *Serratia marcescens*) were isolated only on Day 7 from the untreated sample, laying credence to the hygienic nature of the production process that reduced contamination to the barest. The presence of *E. xiangfangensis* M5S2B6, though not known to be pathogenic (Gu *et al*., 2014), and *S. marcescens*, a nosocomial pathogen (Buffet-Bataillon *et al*., 2019) is a cause for concern as it stresses the danger of contaminated FLB to food safety. The lack of coliforms in the treated sample implies that the LSSNP inhibited the organisms, which was confirmed in the sensitivity test.

Table 2. Molecular characteristics of isolates

Isolate Annotation	Putative Organism Identity	Number of bases/Query Cover	Probable Identity (%)	Accession Number
N1	*Bacillus licheniformis* strain KGEB16	1395	91.69	KF303790.1
N4	*Bacillus subtilis* strain CICC10148	1480	93.01%	AY971358.1
N5	*Bacillus licheniformis* strain APBSWPTB167	1446	92.09%	MG733640.1
N8	*Bacillus licheniformis* strain PS4	942	83.15%	JN411561.1
MAC 1	*Enterobacter xiangfangensis* strain M5S2B6	1444	92.65%	MG928407.1
S1	*Bacillus subtilis* subsp. *subtilis* strain E2	1202	93.36%	KX002006.1
S2	*Bacillus siamensis* strain HQB720	1043	95.02%	KT758584.1
S3	*Bacillus licheniformis* strain PS4	942	83.15%	JN411561.1
S4	*Bacillus tequilensis* strain JS5 6	1040	94.23%	MK110355.1

Susceptibility of Isolates to lemongrass-synthesized silver nanoparticles

The LSSNP inhibited all the isolated organisms from FLB with the minimum inhibitory concentration ranges of 50 to 100% and compared favorably with reference antibiotics both in terms of the inhibition zones and spectrum of activity (Table 3). This agrees with earlier reports on the antimicrobial activity of biologically synthesized AgNPs (Ahmed *et al*., 2016). However, its use in preserving Nigerian foods has not been studied extensively, hence the paucity of information on that aspect. As suggested (Ahmed *et al*., 2016), the biocidal effect of silver nanoparticles could be a result of the interaction between the positively-charged silver ion and the negatively-charged bacterial cell membrane and the free radicals' formation due to the xenobiotic nature of the accumulated nanoparticles (Prabhu and Poulose, 2012.). In addition, the shape and size of nanoparticles are believed to influence their biocidal activity (Altinsoy et al., 2018).

Effect of lemongrass-synthesized silver nanoparticles on the histopathological properties of Wistar rats

The histopathological properties of the liver section of Wistar rats presented here as photomicrographs (Figure 4) showed liver tissue with preserved lobular architecture, no significant inflammation, and no feature of acute or chronic injury. No significant difference was observed between the treated and untreated, as there was only mild periportal infiltration by chronic inflammatory (lymphatic) cells. This is at variance with some earlier research which reported histopathological changes such as aneurism, collapse, subepithelial edema, and epithelial cell necrosis in the tissues and cells of African catfish, *Clarias gariepinus* (Naguib *et al*., 2020).

CONCLUSION

This current study successfully synthesized nanoparticles with lemongrass extract as the reducing agent. The surface plasmon resonance, DLS, particle hydrodynamic

Table 3. Susceptibility of Isolates to lemongrass-synthesized silver nanoparticles and some reference antibiotics

			Diameter of zone of inhibition (mm)							
Isolates	LSSNP 100 µL	LSSNP MIC	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
N1	30.75±3.35^{a}	50%	11.08±0.82^{b}	10.75±0.96^{b}	13.51±0.58^{b}	-	11.75±1.71^{b}	-	27.75±2.63^{a}	-
N4	10.5±1.29^{b}	50%	11.25±0.50^{b}	10.08±0.05^{b}	13.02±0.02^{b}	-	13.18±1.11^{b}	-	24.25±0.51^{a}	-
N5	-	ND	-	-	13.75±0.96^{b}	-	-	-	23.01±4.09^{a}	-
N8	17.02±2.48^{a}	50%	9.01±0.82^{b}	8.52±1.00^{b}	10.50±1.35^{b}	-	-	-	15.25±1.35^{a}	-
MAC 1	31.25±7.32^{a}	25%	-	-	13.03±0.82^{b}	-	-	-	30.05±0.02^{a}	-
S1	15.75±1.51^{a}	50%	-	-	10.02±1.15^{b}	-	-	-	-	-
S2	10.5±0.58^{b}	100%	-	-	11.25±0.50^{b}	-	9.52±0.58^{b}	-	16.51±0.25^{a}	-
S3	44.25±3.18^{a}	25%	-	-	12.05±0.04^{c}	-	8.56±0.58^{d}	-	25.75±0.50^{b}	-
S4	7.22±0.04^{c}	50%	-	-	16.04±1.42^{b}	-	-	-	23.25±2.75^{a}	-

Note: Values are means of five *independent* measurements (±SD) of the diameter of inhibition representing the antibiotic activities. Means with different superscripts in a row are significantly different ($p < 0.05$). CAZ: Certazidine 30µg; CRX: Cefuroxime 30µg; GEN: Gentamycin 10µg; CTR: Ceftriaxone 30µg; ERY: Erythromycin 5µg; CXC: Cloxacillin 5µg; VAN: Vancomycin 30µg; OFL: Ofloxacin 5µg; AUG: Amoxycillin/Clavulinate 30µg.

diameter, Zeta potential, and FTIR spectra of the LSSNP indicate stable particles. The LSSNP demonstrated antimicrobial activities against microorganisms isolated from FLB, significantly reducing its microbial load. Also, LSSNP did not exhibit any toxic effect on the liver tissues of Wistar rats. This study, therefore, recommends LSSNP as a preservative of FLB after a more comprehensive toxicological study to ascertain its safety.

ACKNOWLEDGEMENT

This research is funded by the authors and did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors, however, acknowledge the management of the Department of Microbiology, University of Ilorin, for the use of laboratory space and facilities.

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