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Original Research Article

Chitosan Nanoparticles-Mediated Treatment for Artisanal Tiger Nut (*Cyperus esculentus* L.) Beverages Sold in Selected Northern Nigeria Markets against some Resistant Microbes

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Abstract: ***Background*:** *Cyperus esculentus* L. is a herbaceous plant which grows well in Northern Nigeria and other parts of the world. The rhizomes among other uses are used to process an artisanal beverage called 'kunun-aya' in Northern Nigeria while the leaves have other medicinal uses. ***Objective*:** This study was aimed at assessing the chitosan nanoparticles treatment approach for artisanal 'kunun-aya' beverages sold in some Northern Nigeria markets against resistance microorganisms found in the drink. ***Method*:** Phytochemical contents, metallic elements, and physicochemical analysis of the rhizome extract were assessed following standard procedures. The artisanal 'kunun-aya' beverage was prepared by fermentation process following local methods of preparation in large basin. The preparation of chitosan nanoparticles was carried out using nano spray drying technique while microbial analysis and susceptibility testing were carried out using standard methods. ***Results*:** The results showed that there are *Escherichia coli*, coliform bacteria, *Bacillus* spp., *Aspergillus* sp., *Penicillium* spp., *Streptococcus* spp., *Salmonella-Shigella* and *Staphylococcus aureus* in all the artisanal kunun-aya beverages obtained from all the local markets. The presence of these microorganisms was prevalent in those obtained from the Kano, Bauchi and Sokoto markets while those obtained from the Jos markets were the lowest. The study did not show heavy presence of heavy metals such as cadmium, arsenic and lead. The study further showed that kunun-aya beverage mediately prepared using chitosan nanoparticles did not show any microbial growth on culture media after 48 hours. Similarly, there was no presence of either heavy or trace metals when the sample was analyzed using atomic absorption spectrophotometry. Finally, there were no significant differences ($p < 0.05$) among the artisanal kunun-aya beverages from various northern Nigeria markets. ***Conclusion*:** The study, therefore, showed that chitosan nanoparticles-mediated treatment for kunun-aya (*Cyperus esculentus*) beverages is a promising treatment strategy against microorganisms found in artisanal kunun-aya local beverages sold in northern Nigeria.

Keywords: Kunun-Aya, *Cyperus Esculentus*, Artisanal Beverages, Microorganisms, Chitosan Nanoparticles.

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INTRODUCTION

There are many beverages produced locally in Nigeria that are not regulated by regulating bodies such as the National Agency for Food Drug Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON). These beverages are produced locally using the process of fermentation by microbial agents [1]. These drinks produced artisanally are sold in income markets and have contributed to the economies of the states where they were sold.

In Nigeria, these beverages are also found in Southern Nigeria markets as well, and examples of such beverage drinks are kunun-aya, kunun-zaki, zobo, ginger drink, burukutu, soybean milk, local yoghurts, nono and palm wine [1]. These drinks are produced from plant parts except local yoghurts and nono which are produced from cow kinds of milk, and sugar is often added to add some taste [2]. Due to unhygienic ways of producing these drinks, their consumption has resulted in one or several outbreaks of diseases, especially in Northern Nigeria where a cholera outbreak has been reported previously [3]. Similarly, the incidence of methanol poisoning by microbial contamination has been reported previously in Southern Nigeria where many lives were lost from the artisanal production of methanol [1]. Microbial contamination can occur during the production and storage of beverage drinks in Northern Nigeria [3]. Many microorganisms such as *E. coli*, *Staphylococcus aureus*, *Bacillus spp*., *Streptococcus pyogenes*, *Candida albicans*, *Saccharomycetes*, *Aspergillus*, *Penicillium* and *Lactobacillus* have been reported to contaminate locally (artisanal) processed tiger nut beverage in Northern Nigeria but E. coli was the most prevalence of these microbes [1]. Some of these mentioned microorganisms have been reported to be resistant towards antimicrobial drugs when they get into humans and are very difficult to eliminate from their hosts.

The use of chitosan nanoparticles in various aspects of human endeavors is gaining much popularity. Chitosan nanoparticles are used as carriers of various drugs to deliver drugs specifically to target organs or tissues. They are today employed as carriers for anticancer, antimicrobial, anti-inflammatory, analgesic and antidiabetic drugs [4]. Chitosan nanoparticles have also been employed in drug delivery as mediation for multi-resistant microorganisms such as *Staphylococcus aureus*, *Shigella dysenteriae*, and other resistant species as well as in pollution control and water purification. They are also used in food processing industries as adsorbents for various forms of contamination [5]. The small particle size (1-100 nm), bioavailability, controlled release, specificity, zero toxicity, and ability to overcome blood-barrier to deliver its content are some of the advantages chitosan nanoparticles have over other drug delivery tools and nanoparticles [6]. Many diseases have been successfully treated using nanoparticulate drug delivery. This aspect has been extended to food processing where it has drastically helped to remove contamination of processed and stored foods for a very long period [7].

The beverage drink 'kanun-aya' is popular in Northern Nigeria and made from the rhizomes of the tiger nut plant (*Cyperus esculentus* L.). The processed kunu-aya is usually packaged in used plastic bottles from cocoa cola products and bottled water. These used rubber bottles are mostly poorly washed before usage which serves as an agent of distribution of many microorganisms [1]. *Cyperus esculentus* is a herbaceous plant resembling grass belonging to the Family *Cyperaceae*. The plant is distributed in Northern Nigeria, South America, Asia and Europe as well as some African countries. It is cultivated on a large scale in the Northern Nigeria States like Jigawa, Bauchi, Kano, Borno, and Katsina, where it is grown for commercial purposes. The rhizomes can be eaten fresh, dried or processed into a 'kunun-aya' beverage drink.

This present study was carried out to assess the chitosan nanoparticles treatment approach for locally processed tiger nut beverages sold in some markets in Northern Nigeria against resistant microorganisms found in the beverage.

MATERIALS AND METHODS

Collection, Identification, and Preparation of Plant Material

Fresh rhizomes of *Cyperus esculentus* were harvested from the wild at a forest in Fori, Maiduguri, Nigeria. It was identified by Dr. C.A. Ukwubile of the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Nigeria. A voucher number of UMM/FPH/CYR/001 was deposited in the herbarium of the Department for the plant. The rhizomes weighing 1500 kg were air-dried under shade for two weeks and reduced into a coarse powder using a local pestle and mortar and then weighed. Exactly 400 g of the powdered rhizomes were extracted in 800 mL absolute methanol (Sigma Aldrich St. Louse Mo, USA) and concentrated in vacuo using a rotary evaporator (ThermoFisher, UK) to obtain a brown-coloured jelly-like tiger nu extract which was weighed and stored in clean sample bottle for further use.

Phytochemical Analysis of the Extract

The phytochemical contents of the tiger nut extract were determined for the presence of some metabolites such as alkaloids, saponins, flavonoids,

phenols, terpenoids/steroids, tannins, etc. using previously described methods [8].

Physicochemical Analysis of the Extract

The physicochemical analysis of the tiger nut rhizomes was carried out to determine the moisture content, acid insoluble and total ash values as well as extractive values using standard methods [9].

Preparation and Characterization of Chitosan Nanoparticles (CSNPs)

Chitosan nanoparticles were prepared by nano spray drying techniques with modifications. Briefly, chitosan nanoparticles obtained from crab shells weighing 200 g were dissolved in 1000 mL of deionized water, stirred at 3000 rpm for 20 min, and freeze-dried into small-sized microspheres. They were then characterized in terms of percentage yields, particle size, *in vitro* release, swelling index and entrapment efficiency. The freeze-dried CSNPs were then stored in clean bottles for further use [10].

Preparation of Chitosan Nanoparticles-Mediated Artisanal 'Kunun-Aya' Beverages

Powdered tiger nut rhizomes weighing 1000 g were mixed with 2000 mL of water in a basin according to how it is being prepared locally in Northern Nigeria. After this, 40 g of prepared chitosan nanoparticles were dissolved in the basin to form chitosan-beverage complexes. The mixture was then stirred properly for 20 min to mix. The blended mixture was then poured into a clean sieve cloth with 0.5 mm mesh size to separate the shaft from the filtrate, and further strained with a muslin cloth to get the final kunun-aya. To compare with the locally prepared ones, samples of already prepared kunun-aya beverages were bought from selected markets in Zaria, Kano, Kaduna, Birni-Kebbi, Jalingo, Yola, Gombe, Maiduguri, Lafiya, Bauchi, Jos and Sokoto. In each town, five kunun-aya samples were bought from five markets. A total of 300 samples were bought. All the samples were stored with ice blocks at 4 ^{o}C for further analysis.

Quality Analysis of CSNPs-Mediated Treated and Artisanal 'Kunun-Aya' Beverages

Analysis of Microbial Density:

To determine the presence of various microorganisms in both samples (chitosan-mediated treated and artisanal kunun-aya beverages), different media such as nutrient agar, Mueller-Hinton agar (MHA), mannitol salt agar, peptone water broth, eosin methylene blue (EMB), MacConkey agar, *Salmonella-Shigella* agar and Sabauroud dextrose agar were used for specific bacteria and fungi. The media were prepared following the instructions of the manufacturers. The emerged microbial colonies were then isolated, identified by biochemical tests and preserved for onward analysis of various parameters such as total viable bacteria, fungi, coliforms, *Salmonella-Shigella* and counts [1].

Metallic Analysis of Samples:

The kunun-aya samples were analyzed for the presence of some metallic elements such as lead, chromium, iron, cadmium, arsenic, zinc, potassium, nickel, manganese, magnesium, copper, and aluminum. using the Agilent technologies atomic absorption spectrophotometry (AAS).

Antimicrobial Susceptibility Testing of Isolated Microbes from 'Kunun-Aya'

The isolated microorganisms from the artisanal kunun-aya beverages were subjected to antimicrobial susceptibility testing against various antibiotics and antibiotic-loaded chitosan nanoparticles. This was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar medium [11].

Statistical Analysis

Data obtained for microbial counts was subjected to one-way ANOVA followed by Dunnett's post hoc test analyzed using GraphPad Prism statistical software version 9. Results were expressed as mean ± SD (n = 3) while $p < 0.05$ was considered statistically significant.

RESULTS

The qualitative phytochemical screening of the tiger nut methanol extract revealed the presence of alkaloids, flavonoids, tannins, fats/oils, phenols, and cardiac glycosides while anthraquinones, triterpenes/steroids and saponins were absent (Table 1).

Table 1: Phytochemical contents of avocado seed methanol extract

Constituents	Test	Inference
Alkaloids	Dragendorff	+
	Wagner	+
Flavonoids	Shinoda's	+
	NaOH	+
Tannins	$FeCl_3$	+
	Goldbeater's	+
Triterpenes/Steroids	Liebermann's	-
Anthraquinones	Bontrager	-
Fats/Oils	Spot	+
	Sudan III	+
Phenols	$FeCl_3$	+
Saponins	Frothing	-
Cardiac glycosides	Salkowski	+

Note: + present, and – absent

The result also showed that moisture content was 7.01 ± 0.01 % w/w, water extractive value of 6.56 ± 0.01 % w/w, ash value of 5.88 ± 0.01 % w/w and total ash value of 12.02 ± 2.02 % w/w. All the parameters fall within the normal ranges (Table 2).

Table 2: Physicochemical parameters of tiger nut rhizome

Parameter (% w/w)	Value (mean ± SD)
Moisture content	7.01 ± 0.01
Ash value	2.11 ± 0.01
Total ash	12.02 ± 2.02
Water soluble ash	1.22 ± 0.01
Alcohol insoluble ash	0.15 ± 0.01
Water extractive	6.56 ± 0.01
Alcohol extractive	5.88 ± 0.01
Dry matter	8.76 ± 1.01

Data are expressed as mean ± SD (n = 3).

The prepared chitosan nanoparticles showed the following characteristics such as minimal particle size of 202.08 ± 2.01 nm, zeta potential of 25.01 ±1.12 V, cumulative drug release of 92.32 %, and swelling index of 68.22 ± 0.01 % (Table 3).

Table 3: Characterization of chitosan nanoparticles

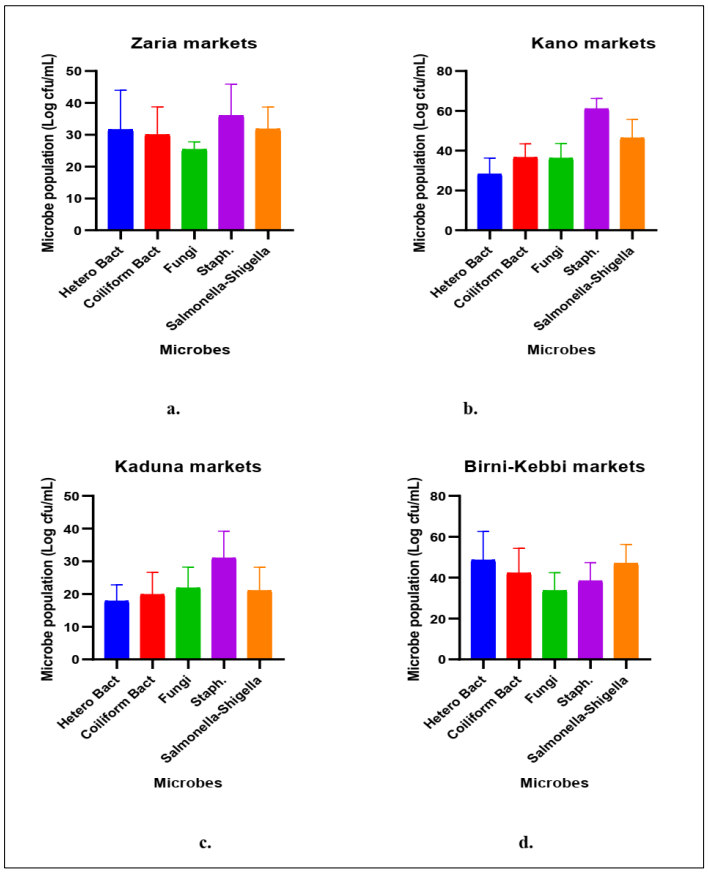
Parameter	Value/Unit
Yield	76.08 %
Particle size	202.08 ± 2.01 nm
Zeta potential	25.01 ± 1.12 V
CDR (8 h)	92.32 %
Swelling index	68.22 ± 0.01 %

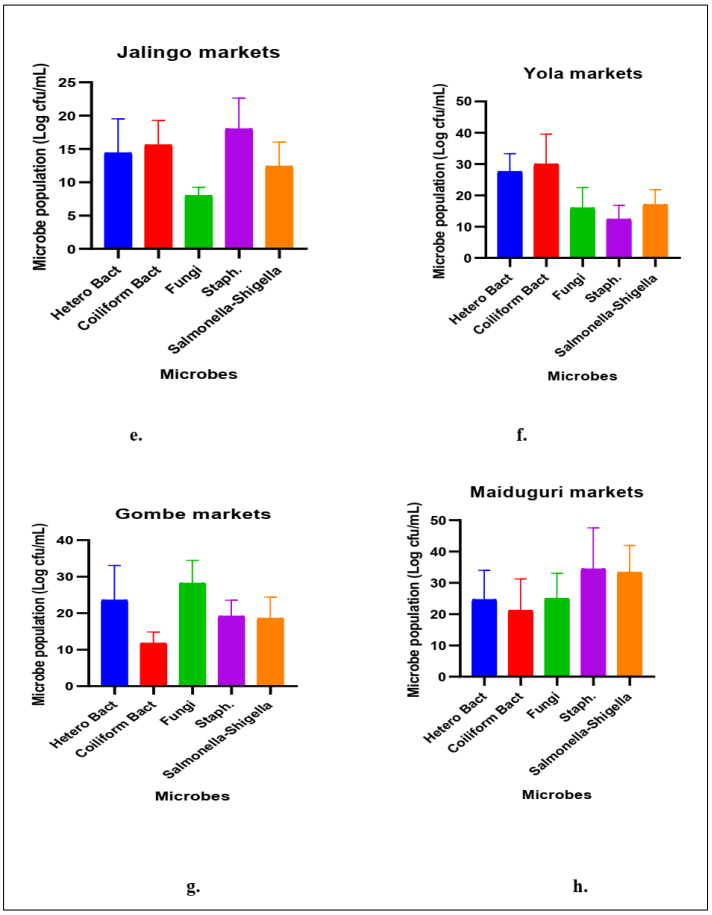
CDR denotes cumulative drug release in 24 hours, n = 3

The kunun-aya prepared by mediating with chitosan nanoparticles (CS/KA) was not too turbid when to those purchased from the local markets (Plate I a). It also showed the highest diameter zones of inhibition against the microbes (Plate I b).

Plate I: Artisanal kunun-aya beverages purchased from various markets in northern Nigeria (a), and cultured artisanal kunun-aya beverages (b). Note: CS/KA is a chitosan nanoparticle-mediated treatment for kunun-aya beverages

The result further showed that Staphylococcus spp. had the highest density in all the kunun-aya surveyed from these markets (Figure 1 a-l). However, other microbes especially fungi were also noticed in kunun-aya sold in these markets. The kunun-aya (KA) obtained from local markets in Jos showed the lowest distribution of these microbes (Figure 1 k) while those obtained from Kano and the Sokoto local markets were the highest in terms of microbial densities (Figure 1 b and l)


Zaria markets
Microbe population (Log cfu/mL)
Hetero Bact
Coliform Bact
Fungi
Staph.
Salmonella-Shigella
Microbes
a.
Kano markets
b.
Kaduna markets
c.
Birni-Kebbi markets
d.


Jalingo markets
Microbe population (Log cfu/mL)
Microbes
e.
Yola markets
f.
Gombe markets
g.
Maiduguri markets
h.

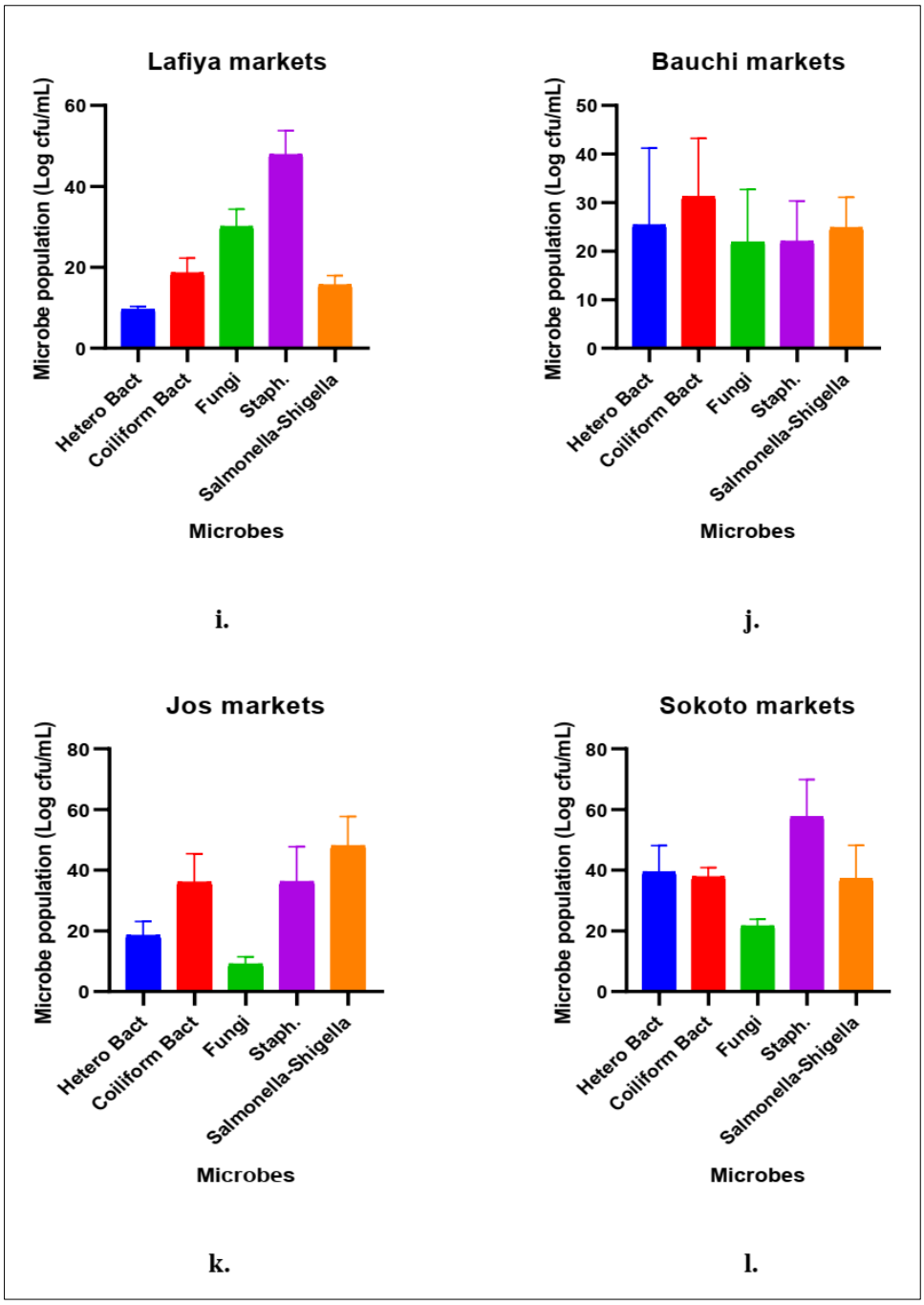


Fig. 1: Microbial distribution in kunun-aya beverages from major markets in Northern Nigeria. Results are expressed as mean ±SE (N = 300, n = 5).

The chitosan nanoparticle-mediated treatment (CSNP-KA) does not show any significant growth of microorganisms after 48 hours of culturing (Figure 2). The result showed that *Bacillus spp*. and *E. coli* had the highest growth rate on the culture media.

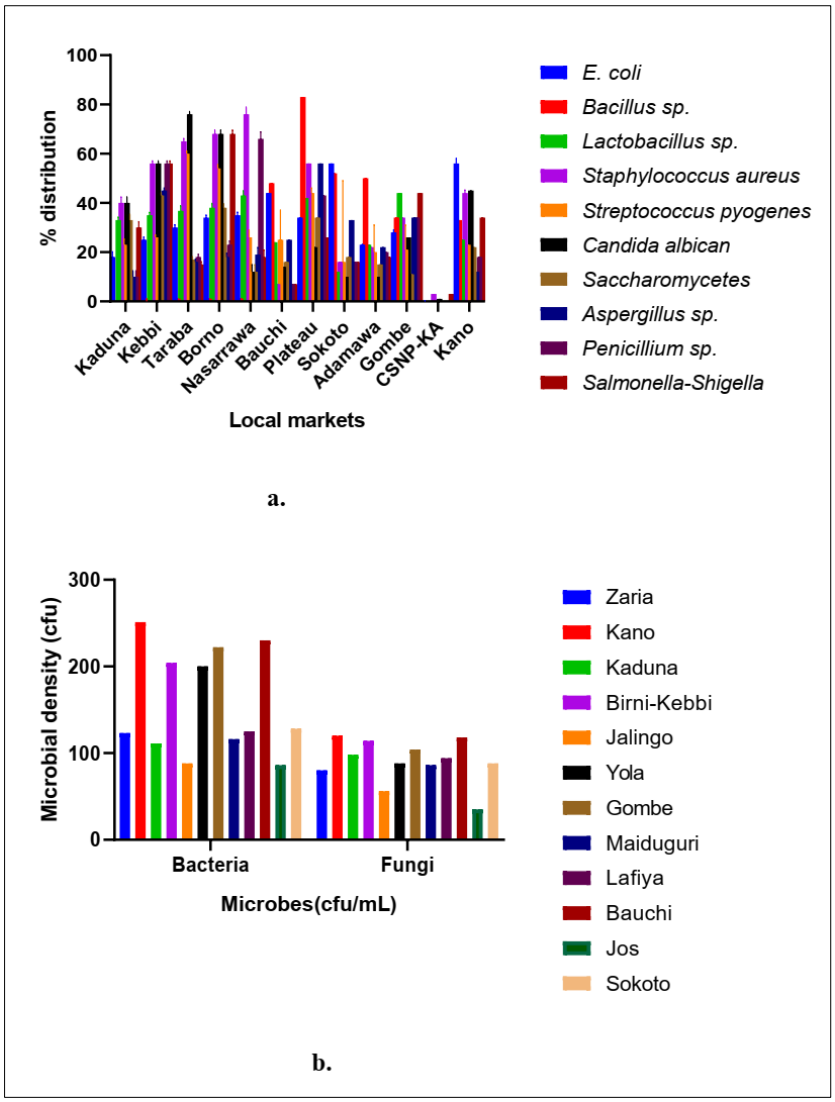


Fig. 2: Microbial distribution in artisanal kunun-aya beverages obtained from various markets in Northern Nigeria

The presence of some heavy metals has also been revealed in kunun-aya from all the zones as opposed to CSNP-KA (Figure 3 a and b). The highest microbial density was observed in kunun-aya obtained from markets in North-west (NW) Nigeria while North-central (NC) had the lowest (Figure 3 b).

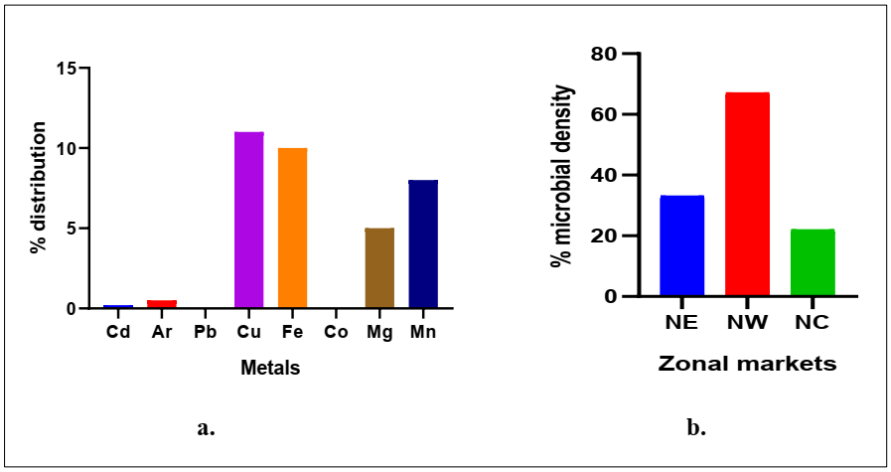


Fig. 3: Distribution of heavy metals in kunun-aya obtained from some northern Nigeria local markets

Finally, our result showed that chitosan nanoparticles treated kunun-aya when cultured did not show growth of any microorganism unlike those purchased from artisanal vendors from local markets in selected northern Nigeria states (Table 4).

Table 4: Effects of various media on microbial growths of cultured kunun-aya drinks

MO	ZA	KN	KD	BK	JG	YL	GB	MG	LF	BA	JS	CS-KA
Ba	+	++	+	+	+	+	+	+	+	+	+	-
Ec	+	++	+	++	+	+	++	+	+	++	+	-
Lb	++	+	+	++	+	+	+	++	+	+	+	-
Sa	++	++	+	++	+	+	++	+	++	+	+	-
Sp	+	-	+	+	+	+	+	+	+	+	+	-
Cab	++	++	++	++	+	++	++	++	++	++	+	-
Sac	+	++	+	+	+	+	+	+	+	+	+	-
As	+	+	+	+	+	+	+	+	+	+	+	-
Pe	-	+	+	+	+	+	+	+	+	+	+	-
S-S	-	++	+	+	++	+	++	-	-	+	+	-

+: Moderate growth, ++: Dense growth, -: No growth, MO: microorganisms, ZA: Zaria markets, KN: Kano markets, KD: Kaduna markets, BK: Birnin kebbi markets, JG: Jalingo markets, YL: Yola markets, GB: Gombe markets, MG: Maiduguri markets, LF: Lafiya markets, BA: Bauchi markets, JS: Jos markets, CS-KA: Chitosan nanoparticles-kunun-aya, Ba: *Bacillus*, Ec: *E. coli*, Lb: *Lactobacillus*, Sa: *Staphylococcus*, Sp: *Streptococcus pyogenes*, Cab: *Candida albican*, Sac: *Saccharomyces's*, As: *Aspergillus*, Pe: *Penicillium*, S-S: *Salmonella-Shigella*.

DISCUSSION

In northern Nigeria, kunun-aya is a locally made non-alcoholic beverage with a sweet taste that consumed in all parts of the country [1]. The beverage is made by artisanal villagers from the rhizomes of tiger nut in most cases under unhygienic conditions [12]. In most cases, the production of this beverage is not regulated by agencies saddled with the responsibility of quality control. There has been several reports about contamination of kunun-aya by microorganisms such as *Escherichia* coli, *Staphylococcus aureus*, *Bacillus spp*., etc. and some fungi like *Saccharomyces spp*., *Aspergillus spp.*, *Penicillium spp.* etc. [12].

From the current study, phytochemical screening of the rhizomes showed that they contain alkaloids, flavonoids, tannins, fats/oil, phenols, and cardiac glycosides. These secondary metabolites play crucial roles in the pharmacological activities of the plant. For instance, flavonoids and phenols have been reported to possess antioxidant, anti-inflammatory and antimicrobial properties [8]. The roles of these metabolites in this current study were not different from the aforementioned. Similarly, tannins have been shown to inhibit the synthesis of glycans by heterotrophic bacteria such as *E. coli* thereby preventing their general metabolism in their hosts [13]. The study also showed that moisture content of 7.01 ± 0.01 % (w/w), total ash 12.02 ± 0.01 % w/w, and water extractive value of 6.56 ± 0.01 % w/v are within the normal range [14]. Too much water in crude drugs makes the drug susceptible to microbial contamination, as such the moisture level must not be above 10.0 % w/w [15].

Targeting of microorganisms by drug agents with the conventional drug delivery procedure has yielded little result. This is because, most drugs fail to reach the site of action or reduce efficacy or potency due to enzymatic action and the host's immune systems [16]. To overcome this, drugs are now being delivered to organs and tissues using nanocarriers such as chitosan nanoparticles. In the current study chitosan nanoparticles (CSNPs) were used as mediation for the preparation of kunun-aya beverage. The prepared CSNPs were able to mediate in the preparation of kunun-aya due to their small particle size of 202.08 ± 2.01 nm, swelling index value of 68.22 ± 0.01 %, percentage yield value of 76.08 % and drug release value of 92.32 % (in 24 hour). These parameters are essential conditions for accurate delivery of drug or mediation using chitosan nanoparticles [6]. Chitosan nanoparticles have been reported to have shown antimicrobial activity against multi-drug resistance organisms such as *E. coli*, *Staphylococcus aureus, Aspergillus niger* and *Shigella dysenteriae* [17]. In this study, there were no microbial growths in kunun-aya prepared from CSNPs mediated treatment unlike the kunun-aya bought from selected local markets in northern Nigeria.

The kunun-aya beverages obtained from Kano, Sokoto, and Kebbi markets recorded the highest prevalence of *E. coli* and *Staphylococcus*

species. The lowest microbial densities were witnessed in kunun-aya obtained from Jos markets. The presence of these microorganisms in artisanal kunun-aya beverages sold in these markets has resulted in various types of food-borne diseases previously witnessed among the people in these states. This is also because most of these microorganisms thrived in unhygienic environments, especially *E. coli*, *Staphylococcus*, *Candida* and *Salmonella-Shigella*. The study showed that markets within the northwest and northeast harbors kunun-aya with the highest microbial densities. Similarly, atomic absorption spectroscopy (AAS) showed the presence of copper, iron and manganese in kunun-aya obtained from these local markets, but no metal was detected in kunun-aya treated with CSNPs. This is because CSNPs can absorb metals due to their charges [6].

Currently, chitosan nanoparticles have been used as a mediator in various food and beverage processing industries. They have been employed mainly in waste recovery from discarded foods, food preservation from microbial contamination, water purification, wine purification and storage, as well as fruit juice deacidification [18]. In this study, we applied chitosan nanoparticles as treatment mediation for the production of artisanal kunun-aya beverages because of their incomparable characteristics some of which are small particle sizes, zeta potential, antimicrobial, non-toxic, anticoagulant, biodegradable, chelating, antioxidant and biocompatible. It has been reported that chitosan-made antimicrobial films for packaging foods are now been developed by food industries globally [19]. Also, nano-films entrapping chitosan nanoparticles are being produced because of chitosan's ability as a food preservative as well as coating materials from natural sources [20].

Therefore, the use of chitosan nanoparticles in this research was due to its numerous advantages highlighted above and shown by the results obtained from the study. Chitosan can be made from natural sources from the shells of crabs, chitin of insects (like grasshoppers, locusts, etc.), crayfish and shrimps. These sources are readily available in almost every part of northern Nigeria, and they can used during the production of kunun-aya, and other artisanal beverages sold in local markets.

CONCLUSION

The study has shown that the antimicrobial effects of chitosan nanoparticles against microorganisms such as *E. coli*, *Staphylococcus spp*., *Bacillus spp*., *Aspergillus*, *Candida spp*., etc. have made it a good preservative agent for kunun-aya beverages promoting the shelf-life. Its ability to remove heavy metals from the kunun-aya beverages also suggests that it has excellent chelating ability. Finally, chitosan nanoparticles should be used as bio-degradable antimicrobial processing and packaging materials to improve the preservation and storage of perishable beverages like kunun-aya and foods since it has been shown from the current study that it is antimicrobial against resistant and non-resistant microorganisms.

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Competing Interest: We have none to declare.

REFERENCES

1. Nwaiwu O, Aduba CC, Igbokwe VC, Sam CE, Ukwuru MU. Traditional and artisanal beverages in Nigeria: Microbial diversity and safety issues. Beverages 2020;6:1–22. https://doi.org/10.3390/beverages6030053.
2. Olivia NU, Goodness UC, Obinna OM. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves. Futur J Pharm Sci 2021;7. https://doi.org/10.1186/s43094-021-00208-4.
3. Fagbamila IO, Abdulkarim MA, Aworh MK, Uba B, Balogun MS, Nguku P, et al. Cholera outbreak in some communities in North-East Nigeria, 2019: an unmatched case–control study. BMC Public Health 2023;23:1–11. https://doi.org/10.1186/s12889-023-15332-4.
4. Ukwubile CA. Tumor Targetability and Anti-Ovarian Cancer Effect of Docetaxel-Loaded Folate Modified <I>Annona</I> <I>muricata</I> Linn. (Annonaceae) Chitosan Nanoparticles. Nanomedicine and Nanobiology 2015;1:57–63. https://doi.org/10.1166/nmb.2014.1004.
5. Mahmoud Abbas AO. Chitosan for biomedical applications. Univ Iowa 2010:1–329.
6. Guo H, Li F, Qiu H, Liu J, Qin S, Hou Y, et al. Preparation and Characterization of Chitosan Nanoparticles for Chemotherapy of Melanoma Through Enhancing Tumor Penetration. Front Pharmacol 2020;11:1–8. https://doi.org/10.3389/fphar.2020.00317.
7. Jafernik K, Ładniak A, Blicharska E, Czarnek K, Ekiert H, Wiącek AE, et al. Chitosan-Based Nanoparticles as Effective Drug Delivery Systems—A review. Molecules 2023;28:1–17. https://doi.org/10.3390/molecules28041963.
8. Abubakar B, Yahaya Z, Alhaji SA, Giaze RT, Aminu N. Phytochemical Screening and Toxicity Study of Methanol Stem Bark Extract of <i>Prosopis africana<i> (African Mesquite) In Albino Wistar

Rat. Dutse J Pure Appl Sci 2023;9:117–26. https://doi.org/10.4314/dujopas.v9i2b.13.
9. Anes C, Salvia T, Oise E, Modu B, Amarachukwu V. Evaluation of physicochemical parameters , acute and subchronic toxicities , and anti-diabetic activity of Spondias venulosa (Engl .) Mart . ex Engl . leaf extract on alloxan-induced diabetic rats. J Ethnopharmacol 2023;306:116169. https://doi.org/10.1016/j.jep.2023.116169.
10. Kumar DA, Dharmendra S, Jhansee M, Shrikant N, P PS, S VB. Development and Characterization of Chitosan Nanoparticles. Int Res J Pharm 2011;2:145–51.
11. Armansyah T, Siregar TN, Suhartono, Sutriana A. Phytochemicals, characterization and antimicrobial tests of red betel leaves on three solvent fractions as candidates for endometritis phytotherapy in Aceh cattle, Indonesia. Biodiversitas 2022;23:2111–7. https://doi.org/10.13057/biodiv/d230446.
12. Ekanem J., Mensah B., Marcus N., Ukpe B. Microbial Quality and Proximate Composition of Kunu Drinks Produced and Sold in Ikot Ekpene Metropolis, Akwa Ibom State, Nigeria. J Appl Sci Environ Manag 2019;22:1713. https://doi.org/10.4314/jasem.v22i11.1.
13. Akinduti PA, Emoh-Robinson V, Obamoh-Triumphant HF, Obafemi YD, Banjo TT. Antibacterial activities of plant leaf extracts against multi-antibiotic resistant Staphylococcus aureus associated with skin and soft tissue infections. BMC Complement Med Ther 2022;22:1–11. https://doi.org/10.1186/s12906-022-03527-y.
14. (Melastomataceae). African J Biotechnol 2009;8:113–5. https://doi.org/10.5897/AJB07.478.
15. Alamgir ANM. Introduction BT - Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy. In: Alamgir ANM, editor., Cham: Springer International Publishing; 2017, p. 1–17. https://doi.org/10.1007/978-3-319-63862-1_1.
16. Jones PW, Chin Y-W, Kinghorn DA. The Role of Pharmacognosy in Modern Medicine and Pharmacy. Curr Drug Targets 2006;7:247–64. https://doi.org/http://dx.doi.org/10.2174/138945006776054915.
17. El-Zehery HRA, Zaghloul RA, Abdel-Rahman HM, Salem AA, El-Dougdoug KA. Novel strategies of essential oils, chitosan, and nano- chitosan for inhibition of multi-drug resistant: E. coli O157:H7 and Listeria monocytogenes. Saudi J Biol Sci 2022;29:2582–90. https://doi.org/10.1016/j.sjbs.2021.12.036.
18. Manigandan V, Karthik R, Ramachandran S, Rajagopal S. Chapter 15 - Chitosan Applications in Food Industry. In: Grumezescu AM, Holban AMBT-B for FD, editors. Handb. Food Bioeng., Academic Press; 2018, p. 469–91. https://doi.org/https://doi.org/10.1016/B978-0-12-811449-0.00015-3.
19. Jha R, Mayanovic RA. A Review of the Preparation, Characterization, and Applications of Chitosan Nanoparticles in Nanomedicine. Nanomaterials 2023;13. https://doi.org/10.3390/nano13081302.
20. No HK, Meyers SP, Prinyawiwatkul W, Xu Z. Applications of chitosan for improvement of quality and shelf life of foods: A review. J Food Sci 2007;72. https://doi.org/10.1111/j.1750-3841.2007.00383.x.