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VAO conceived and designed the study, under the supervision of GOA and BOO. VAO and MBM carried out the study and the drafting/proof-reading of the manuscript.

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Comparative Study of Antimicrobial Efficacy of Honey, Clove, and Ginger Extracts against Some Bacteria Isolated from Tiger Nut Milk

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Abstract:

Tiger nut milk (*kunun aya*) is a popular traditional beverage in Nigeria but is often contaminated due to unhygienic processing and handling, posing a food safety risk. This study evaluated the antimicrobial activity of honey, and aqueous and ethanolic extracts of clove (*Syzygium aromaticum*) and ginger (*Zingiber officinale*) against bacteria isolated from tiger nut milk sold in Zaria, Nigeria. A total of nine bacterial isolates—*Escherichia coli, Mannheimia haemolytica, Hafnia alvei, Staphylococcus aureus, Burkholderia cepacia, Micrococcus* spp*., Bacillus* spp*., Stenotrophomonas maltophilia,* and *Citrobacter freundii*—were tested using agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Results showed concentration-dependent inhibition by honey, clove, and ginger extracts. Honey at 90% v/v exhibited notable activity, particularly against *M. haemolytica*. Ethanolic clove extract produced the strongest inhibition zones (up to 28 mm) and lower MIC values compared to its aqueous equivalent, while ethanolic ginger showed stronger effects than aqueous ginger, though differences were not statistically significant. The findings highlight that ethanolic clove extract and high-concentration honey possess promising antibacterial activity and could serve as affordable, natural preservatives to enhance the safety of tiger nut milk. Further research is recommended to standardize extract formulations and assess their effectiveness under real storage and vending conditions.

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INTRODUCTION

Tiger nut milk (*kunun aya*), made from the tubers of *Cyperus esculentus*, is a widely consumed traditional beverage in Nigeria (Musa and Hamza, 2013; Opeyemi, 2020). It is valued for its calories and micronutrients, and is commonly sold by street vendors in both urban and rural markets. However, informal production practices, use of non-potable water, and open handling often lead to microbial contamination and spoilage (Musa and Hamza, 2013; Olofu *et al*., 2021). Tiger nut milk spoils quickly because of its high microbial load. Olofu et al. (2021) found that *E. coli* (17.8%) and *S. aureus* (10.5%) were among the most prevalent bacteria in fresh tiger nut milk. Recent survey in West Africa have reported high mesophilic counts and the presence of coliforms and other potential pathogens in tiger nut products, highlighting regional food-safety concerns (Semdé *et al*., 2024).

Growing interest in plant-derived antimicrobial agents reflects ongoing efforts to extend food shelf life naturally, while emphasizing the potential of locally available natural antimicrobials as cost-effective and sustainable means to enhance food safety and reduce antimicrobial resistance (Balouiri *et al*., 2016). In Nigeria, honey, clove (*Syzygium aromaticum*), and ginger (*Zingiber officinale*) are widely used in cuisine and folk medicine. Previous research has shown that these agents possess broad-spectrum antimicrobial and anti-inflammatory activities (Mandal and Mandal, 2011; Sebiomo *et al*., 2011; Agbagwa *et al*., 2022). Honey's antibacterial activity is attributed to its high osmolarity, low pH, generation of hydrogen peroxide, and diverse phytochemicals (Mandal and Mandal, 2011). Clove oil contains eugenol, and ginger contains phenolic compounds/gingerols, both of which are effective antibacterials. Notably, ethanolic extracts of these spices often show greater potency than aqueous extracts (Sebiomo *et al*., 2011; Mak *et al*., 2019; Shaukat *et al*., 2023; Maggini *et al*., 2024).

Although several studies have examined the microbial composition of *kunun aya* in Nigeria (Musa and Hamza, 2013; Opeyemi, 2020; Olofu *et al*., 2021; Eruteya, 2023), only a limited number have investigated the antibacterial effects of locally sourced honey or plant extracts on bacteria isolated from tiger nut milk. Evaluating such agents is pragmatic for several reasons: they are affordable and culturally accepted, could be adapted by small vendors for preserving *kunun aya*, and would add to the evidence base for food-safety interventions in low-resource settings (Balouiri *et al*., 2016; Agbagwa *et al*., 2022). This study aimed to evaluate the antimicrobial activity of honey, and aqueous versus ethanolic extracts of clove and ginger against bacteria isolated from tiger nut milk drinks.

MATERIALS AND METHODS

Sample collection and isolate identification

Tiger nut milk (*kunun aya*) samples (200–250 mL each) were purchased aseptically from multiple street vendors across the city ($n \approx 20$ samples). Samples were placed in sterile screw-cap bottles, transported in a cool box (4–8 °C), and processed in the Pharmaceutical Microbiology Laboratory, Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, within 4 hours of collection.

Tiger nut milk samples were plated on nutrient agar, and colonies of distinct morphology were purified. Nine distinct bacterial isolates were reserved for testing. Initial identification was by Gram stain and colony morphology, followed by biochemical tests (catalase, coagulase for staphylococci, oxidase, indole, MR/VP, citrate, urease, triple sugar iron, motility) as described by Cheesbrough (2006). Final identification of each isolate was confirmed with the Microbact ID kit (bioMérieux). The organisms identified include *Escherichia coli*, *Mannheimia haemolytica*, *Hafnia alvei*, *Staphylococcus aureus*, *Burkholderia cepacia*, *Micrococcus* spp.,

Bacillus spp., *Stenotrophomonas maltophilia*, and *Citrobacter freundii*.

Preparation of Test Agents

Honey: A commercially available Nigerian honey (single-origin batch) was used. Working dilutions of honey were prepared in sterile distilled water at 90%, 70%, 50%, 30%, 10%, and 5% (v/v) for testing (Mandal and Mandal, 2011; Agbagwa *et al*., 2022).

Clove and Ginger Extracts: Clove buds and ginger rhizomes were procured from local markets and authenticated by a botanist (voucher specimens deposited). Materials were air- or oven-dried (40–50 °C) and ground into fine powders. For each spice, two extract types were prepared:

Aqueous extract: 50 g of powder was boiled in 500 mL distilled water (1:10 w/v) for 30 min, and then filtered (using Whatman No. 1 filter paper). The filtrate was concentrated under reduced pressure (rotary evaporator). The resulting crude extract was reconstituted in sterile water to produce a stock (150 mg/mL).

Ethanolic extract: 50 g of powder was macerated in 500 mL of 70% (v/v) ethanol for 48 h with occasional stirring, and then filtered. Solvent was removed by rotary evaporation at 40 °C, yielding a dried crude extract. This was dissolved in 10% (v/v) dimethyl sulfoxide (DMSO) to make a 150 mg/mL stock.

Working concentrations of each extract (in both solvents) were 37.5, 30.0, 22.5, 15.0, 7.5, and 3.75 mg/mL (Sebiomo *et al*., 2011; Karuppiah & Rajaram, 2012; Balouiri *et al*., 2016). All prepared extracts were passed through a 0.45 µm filter for sterilization, where possible, and stored at 4 °C for up to two weeks. For each extract assay, 10% DMSO (when used) and sterile distilled water were included as solvent controls.

Antimicrobial Assay – Agar Well Diffusion

Antibacterial activity was assessed by agar well diffusion. Mueller–Hinton agar (MHA) plates were inoculated by swabbing with bacterial suspensions adjusted to ~0.5 McFarland (~1×10^8 CFU/mL). Wells (6 mm diameter) were punched into agar; each well received 50 µL of test solution (honey or extract at the specified concentrations). Ciprofloxacin (5 µg, disc equivalent) was used as a positive control, and sterile distilled water as a negative control. In the case of ethanolic extracts, 10% DMSO was also used as a solvent control. Plates were incubated at 37 °C for 18–24 h. Zones of inhibition around wells and control discs were measured in millimeters (mm) using meter rule in triplicate, and the mean ± standard deviation (SD) was recorded for each test.

MIC and MBC Determination

Minimum inhibitory concentrations (MICs) were determined by broth microdilution in sterile 96-well plates. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that visibly inhibits bacterial growth, and the minimum bactericidal concentration (MBC) is the lowest concentration that kills the bacteria. Two-fold serial dilutions of each agent (covering the same ranges as diffusion assays) were prepared in Mueller–Hinton broth. Bacterial inoculum (~5×10^5 CFU/mL) was added to each well. After incubation at 37 °C for 18–24 h, the MIC was recorded as the lowest concentration showing no visible turbidity. For each clear well (no growth), 10 µL was subcultured onto fresh MHA; after another 24 h incubation, the lowest concentration yielding no colony growth was taken as the minimum bactericidal concentration (MBC) (Balouiri *et al*., 2016). Each MIC/MBC test was performed in duplicate for confirmation.

Data Analysis

Data (zone diameters, MIC, and MBC values) were analyzed using SPSS v25. Mean values and SD were calculated. One-way analysis of variance (ANOVA) was used to compare mean zone diameters across concentrations for each agent (honey, clove extracts, ginger extracts). Post-hoc Tukey tests identified pairwise differences. Paired t-tests (at the highest test

concentrations) compared aqueous vs. ethanolic extracts and compared honey vs. ciprofloxacin. Significance was set at $p \leq 0.05$.

RESULTS

Nine bacterial species isolated from tiger nut milk were tested. Table 1 shows the inhibition zones for honey dilutions against each isolate. Zones increased with honey concentration. At 90% (v/v) honey, the largest inhibition was seen against *Mannheimia haemolytica* (20.0 ± 0.0 mm) and *Hafnia alvei* (18.0 ± 1.0 mm). Most other isolates (e.g. *E. coli*, *S. aureus*, *and Burkholderia cepacia*) showed zones of 16–18 mm at 90% honey. Lower honey concentrations yielded smaller or no zones; for example, at 10% or 5% (v/v), most organisms showed no inhibition. The antibiotic control (ciprofloxacin) produced larger zones (24–30 mm) against all isolates (Table 1). One-way ANOVA confirmed that honey's inhibitory zone diameters increased significantly with concentration ($F(5,33)=14.36$, $p<0.001$).

Table 1. Zone of inhibition (mm) of honey (percentage v/v) against bacterial isolates from tiger nut milk.

Organism	Zone of inhibition (mm) for various dilutions (v/v) of honey							
	90% (v/v)	70% (v/v)	50% (v/v)	30% (v/v)	10% (v/v)	5% (v/v)	Cip (mm)	Water
E. coli	16.0±1.0	12.0±0.5	12.0±0.0	10.0±0.0	10.0±0.0	—	20.0±0.0	—
M. haemolytica	20.0±0.0	16.0±0.0	14.0±0.0	14.0±0.0	12.0±0.0	10.0±0.0	26.0±0.0	—
H. alvei	18.0±1.0	15.0±0.0	13.0±0.0	12.0±0.0	10.0±0.0	—	30.0±0.0	—
S. aureus	16.0±0.0	14.0±0.0	12.0±0.0	12.0±0.0	—	—	28.0±0.0	—
B. cepacia	17.0±0.0	16.0±0.0	12.0±0.0	10.0±0.0	10.0±0.0	—	26.0±0.0	—
Micrococcus spp.	14.0±0.0	12.0±0.0	10.0±0.0	8.0±0.0	—	—	30.0±0.0	—
Bacillus spp.	16.0±0.0	14.0±0.0	12.0±0.0	—	—	—	20.0±0.0	—
S. maltophilia	20.0±1.0	16.0±0.0	16.0±0.0	12.0±0.0	10.0±0.0	—	26.0±0.0	—
C. freundii	16.0±0.0	13.0±0.0	12.0±0.0	10.0±0.0	—	—	28.0±0.0	—

Key: Values are mean ± SD; "—" = no inhibition; Cip=Ciprofloxacin.

Table 2 lists the MIC and MBC of honey against each isolate. MICs ranged from 50% to 70% (v/v). For example, honey had an MIC of 50% and MBC of 70% (MBC/MIC = 1.4) against *M. haemolytica*, while against *B. cepacia*, the (MBC/MIC = 1.4), while *B. cepacia* had MIC=MBC=50% (ratio=1.0). Generally, *E. coli* and *S. maltophilia* required higher concentrations of the honey for inhibition and killing. The MBC/MIC ratios (1.0–1.8) suggest that honey was mostly bactericidal at only slightly higher concentrations than MIC.

Table 2. MIC and MBC of honey (% v/v) against bacterial isolates from tiger nut milk.

Organism	MIC (% v/v)	MBC (% v/v)	MBC/MIC
E. coli	50	90	1.80
M. haemolytica	50	70	1.40
H. alvei	70	90	1.30
S. aureus	70	90	1.30
B. cepacia	50	50	1.00
Micrococcus spp.	70	70	1.00
Bacillus spp.	50	70	1.40
S. maltophilia	50	90	1.80
C. freundii	70	70	1.00

Tables 3 and 4 present zones of inhibition for clove extracts. The aqueous clove extract produced modest inhibition at higher concentrations (Table 3). At 37.5 mg/mL, zones ranged from 14 mm (*Micrococcus*) to 20 mm (*C. freundii*) were obtained. Most isolates showed no inhibition at the lowest concentrations (3.75 mg/mL). The ethanolic clove extract was markedly more potent (Table 4): at 37.5 mg/mL it inhibited all isolates; with zones 18–28 mm (largest against *S. aureus* and *C. freundii*, 26–28 mm). Lowering the ethanolic clove concentration to 22.5–15 mg/mL, let to reduced zones, but most organisms remained sensitive.

Table 3. Zone of inhibition (mm) of aqueous clove extract (mg/mL) against bacterial isolates from tiger nut milk.

Organism	Zone of inhibition (mm) for various concentrations of aqueous clove extract (mg/mL)							
	37.5 mg/mL	30.0 mg/mL	22.5 mg/mL	15.0 mg/mL	7.5 mg/mL	3.75 mg/mL	Cip	Water
E. coli	14.0±0.5	14.0±0.0	10.0±0.0	8.0±0.0	8.0±0.0	—	20.0±0.0	—
M. haemolytica	14.0±0.0	12.0±0.0	11.0±0.0	8.0±0.0	—	—	26.0±0.0	—
H. alvei	16.0±0.0	14.0±0.0	12.0±0.0	10.0±0.0	8.0±0.0	8.0±0.0	30.0±0.0	—
S. aureus	14.0±0.0	12.0±0.0	10.0±0.0	10.0±0.0	—	—	28.0±0.0	—
B. cepacia	16.0±0.0	14.0±0.0	14.0±0.0	12.0±0.0	10.0±0.0	8.0±0.0	26.0±0.0	—
Micrococcus spp.	14.0±0.0	12.0±0.0	10.0±0.0	8.0±0.0	8.0±0.0	—	30.0±0.0	—
Bacillus spp.	16.0±0.0	14.0±0.0	12.0±0.0	—	—	—	20.0±0.0	—
S. maltophilia	14.0±0.0	13.0±0.0	12.0±0.0	8.0±0.0	8.0±0.0	—	26.0±0.0	—
C. freundii	20.0±0.0	18.0±0.0	17.0±0.0	14.0±0.0	11.0±0.0	8.0±0.0	28.0±0.0	—

Key: Values are mean ± SD; "—" = no inhibition; Cip=Ciprofloxacin.

Table 4. Zone of inhibition (mm) ethanolic clove extract (mg/mL) against bacterial isolates from tiger nut milk.

Organism	Zone of inhibition (mm) for various concentrations of ethanolic clove extract (mg/mL)							
	37.5 mg/mL	30.0 mg/mL	22.5 mg/mL	15.0 mg/mL	7.5 mg/mL	3.75 mg/mL	Cip	Water
E. coli	18.0±0.0	14.0±0.0	12.0±0.0	8.0±0.0	—	—	20.0±0.0	—
M. haemolytica	14.0±0.0	12.0±0.0	12.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0	26.0±0.0	—
H. alvei	24.0±0.0	24.0±0.0	20.0±0.0	16.0±0.0	12.0±0.0	10.0±0.0	30.0±0.0	—
S. aureus	20.0±0.0	16.0±0.0	14.0±0.0	12.0±0.0	12.0±0.0	10.0±0.0	28.0±0.0	—
B. cepacia	22.0±0.0	20.0±0.0	16.0±0.0	14.0±0.0	14.0±0.0	12.0±0.0	26.0±0.0	—
Micrococcus spp.	14.0±0.0	12.0±0.0	12.0±0.0	8.0±0.0	—	—	30.0±0.0	—
Bacillus spp.	26.0±0.0	22.0±0.0	18.0±0.0	16.0±0.0	12.0±0.0	10.0±0.0	20.0±0.0	—
S. maltophilia	26.0±0.0	24.0±0.0	20.0±0.0	16.0±0.0	16.0±0.0	12.0±0.0	26.0±0.0	—
C. freundii	28.0±0.0	26.0±0.0	21.0±0.0	16.0±0.0	14.0±0.0	11.0±0.0	28.0±0.0	—

Key: Values are mean ± SD; "—" = no inhibition; Cip=Ciprofloxacin.

MIC and MBC values for clove extracts are shown in Table (5). For each organism, the ethanolic clove extract generally had similar or lower MIC/MBC values than the aqueous extract. For example, *E. coli* had MIC 15.0 mg/mL (MBC 22.5) with aqueous extract, versus MIC 22.5 (MBC 30.0) for ethanol (ratios 1.50 and 1.33, respectively). In most cases, the MBC/MIC ratio was close to 1 (bactericidal), except for *M. haemolytica* where aqueous extract had ratio 2.00.

Table 5. MIC and MBC of clove extracts (mg/mL) against bacterial isolates from tiger nut milk.

Organism	Aqueous MIC (mg/mL)	Aqueous MBC (mg/mL)	Ratio	Ethanol MIC (mg/mL)	Ethanol MBC (mg/mL)	Ratio
E. coli	15.0	22.5	1.50	22.5	30.0	1.33
M. haemolytica	15.0	30.0	2.00	15.0	22.5	1.50
H. alvei	22.5	37.5	1.67	22.5	30.0	1.33
S. aureus	30.0	37.5	1.25	22.5	37.5	1.67
B. cepacia	22.5	37.5	1.67	15.0	22.5	1.50
Micrococcus spp.	22.5	30.0	1.33	22.5	22.5	1.00
Bacillus spp.	22.5	30.0	1.33	22.5	30.0	1.33
S. maltophilia	30.0	30.0	1.00	15.0	30.0	2.00
C. freundii	22.5	30.0	1.33	22.5	30.0	1.33

Tables 6 and 7 present the results for ginger extracts. Aqueous ginger (Table 6) showed only moderate activity: at 37.5 mg/mL, inhibition zones ranged from 13 mm (*S. aureus*) to 17 mm (*C. freundii*), with most zones ≤15 mm. There was no inhibition for many isolates at 7.5 or 3.75 mg/mL. The ethanolic ginger extract (Table 7) was more active: at 37.5 mg/mL it inhibited *M. haemolytica* (20±0.0 mm), *C. freundii* (16±0.0 mm), and *E. coli* (18±0.0 mm), whereas *H. alvei* showed smaller zones at even 30 mg/mL (12±0.0 mm) of the ethanolic ginger extract. Lower ethanol-ginger concentrations gave smaller or no zones. Overall, ethanolic ginger tended to outperform aqueous ginger, though the difference was less marked than in the clove extracts.

Table 6. Zone of inhibition (mm) of aqueous ginger extract (mg/mL) against bacterial isolates from tiger nut milk.

Organism	Zone of inhibition (mm) for various concentrations of aqueous ginger extract (mg/mL)							
	37.5 mg/mL	30.0 mg/mL	22.5 mg/mL	15.0 mg/mL	7.5 mg/mL	3.75 mg/mL	Cip	Water
E. coli	13.0±0.0	10.0±0.0	10.0±0.0	—	—	—	20.0±0.0	—
M. haemolytica	14.0±0.0	13.0±0.0	11.0±0.0	10.0±0.0	8.0±0.0	—	26.0±0.0	—
H. alvei	14.0±0.0	13.0±0.0	11.0±0.0	9.0±0.0	—	—	30.0±0.0	—
S. aureus	13.0±0.0	11.0±0.0	10.0±0.0	8.0±0.0	—	—	28.0±0.0	—
B. cepacia	15.0±0.0	12.0±0.0	11.0±0.0	8.0±0.0	—	—	26.0±0.0	—
Micrococcus spp.	14.0±0.0	11.0±0.0	10.0±0.0	—	—	—	30.0±0.0	—
Bacillus spp.	15.0±0.0	13.0±0.0	11.0±0.0	8.0±0.0	—	—	20.0±0.0	—
S. maltophilia	13.0±0.0	11.0±0.0	11.0±0.0	8.0±0.0	—	—	26.0±0.0	—
C. freundii	17.0±0.0	15.0±0.0	12.0±0.0	11.0±0.0	8.0±0.0	—	28.0±0.0	—

Key: Values are mean ± SD; "—" = no inhibition; Cip=Ciprofloxacin.

Table 7. Zone of inhibition (mm) of ethanolic ginger extract (mg/mL) against bacterial isolates from tiger nut milk.

Organism	Zone of inhibition (mm) for various concentrations of ethanolic ginger extract (mg/mL)							
	37.5 mg/mL	30.0 mg/mL	22.5 mg/mL	15.0 mg/mL	7.5 mg/mL	3.75 mg/mL	Cip	Water
E. coli	18.0±0.0	16.0±0.0	12.0±0.0	10.0±0.0	10.0±0.0	8.0±0.0	20.0±0.0	—
M. haemolytica	20.0±0.0	16.0±0.0	16.0±0.0	12.0±0.0	8.0±0.0	8.0±0.0	26.0±0.0	—
H. alvei	12.0±0.0	10.0±0.0	—	—	—	—	30.0±0.0	—
S. aureus	14.0±0.0	12.0±0.0	12.0±0.0	8.0±0.0	—	—	28.0±0.0	—
B. cepacia	15.0±0.0	13.0±0.0	11.0±0.0	10.0±0.0	—	—	26.0±0.0	—
Micrococcus spp.	18.0±0.0	18.0±0.0	14.0±0.0	14.0±0.0	10.0±0.0	8.0±0.0	30.0±0.0	—
Bacillus spp.	14.0±0.0	10.0±0.0	8.0±0.0	—	—	—	20.0±0.0	—
S. maltophilia	14.0±0.0	12.0±0.0	10.0±0.0	10.0±0.0	—	—	26.0±0.0	—
C. freundii	16.0±0.0	14.0±0.0	12.0±0.0	10.0±0.0	10.0±0.0	—	28.0±0.0	—

Key: Values are mean ± SD; "—" = no inhibition; Cip=Ciprofloxacin.

MIC/MBC values for ginger extracts are shown in Table 8. The ethanolic ginger extract generally had similar or lower MICs than the aqueous extract for a given organism. For instance, *M. haemolytica* had MIC 15.5 mg/mL (MBC 22.5) with ethanol versus MIC 15.0 (MBC 22.5) for water. Some differences were larger: e.g., *B. cepacia* required 30.0 mg/mL (MBC 30.0) with ethanol, but only 22.5 (MBC 30.0) with aqueous. Generally, most organisms had MBC/MIC ratios of 1.00–1.67.

Table 8. MIC and MBC of ginger extracts (mg/mL) against bacterial isolates.

Organism	Ethanol MIC (mg/mL)	Ethanol MBC (mg/mL)	Ratio	Aqueous MIC (mg/mL)	Aqueous MBC (mg/mL)	Ratio
E. coli	22.5	30.0	1.33	22.5	30.0	1.33
M. haemolytica	15.5	22.5	1.50	15.0	22.5	1.50
H. alvei	30.0	30.0	1.00	30.0	30.0	1.00
S. aureus	22.5	37.5	1.67	22.5	30.0	1.33
B. cepacia	22.5	30.0	1.33	30.0	30.0	1.00
Micrococcus spp.	22.5	22.5	1.00	22.5	37.5	1.67
Bacillus spp.	15.5	30.0	2.00	22.5	30.0	1.33
S. maltophilia	15.5	22.5	1.50	22.5	30.0	1.33
C. freundii	22.5	30.0	1.33	30.0	37.5	1.25

ANOVA showed a significant dose-dependent effect on zone sizes for honey ($p \leq 0.001$), aqueous clove ($p \leq 0.001$), ethanolic clove ($p \leq 0.001$), and ethanolic ginger ($p \leq 0.001$) (Figure 1). Aqueous ginger data could not be fully tested due to many zero-inhibition values at the low concentrations. Paired comparisons at the highest concentration (37.5 mg/mL) showed that ethanolic clove produced significantly larger zones than aqueous clove (mean 21.3 vs 15.3 mm, $p=0.002$), whereas the difference between ethanolic and aqueous ginger was not significant ($p=0.17$). At 90% honey, ciprofloxacin (mean 27.2 mm) produced significantly larger zones than honey (17.8 mm, $p \leq 0.001$), indicating the antibiotic's superior activity.

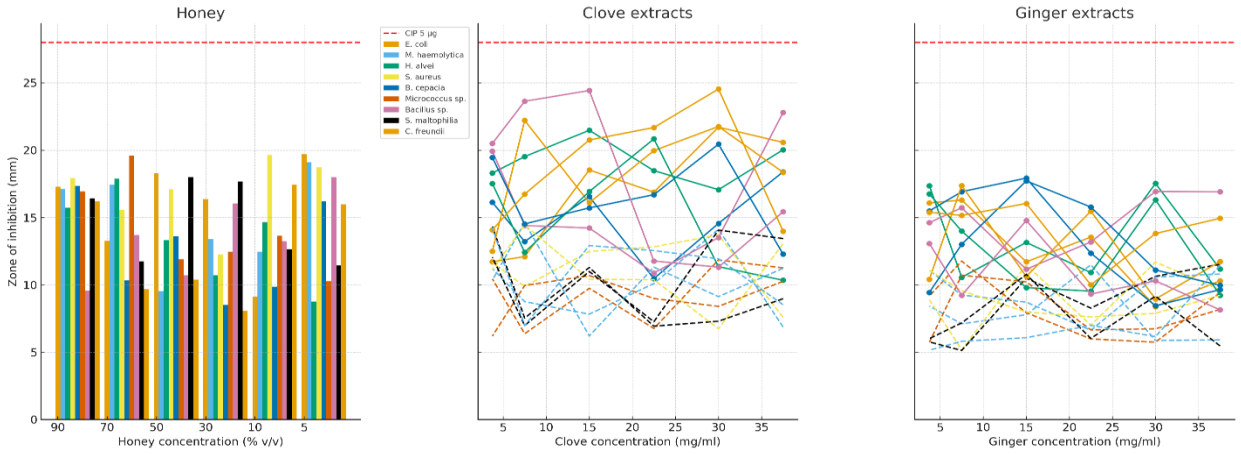

Fig. 1. Bar charts of mean inhibition zones (mm) for each extract concentration against each bacterial isolate. Each bar/point represents the mean ± SD of three independent replicates (agar diffusion assays). Separate panels are shown for honey (left), clove (middle; aqueous vs. ethanolic extracts), and ginger (right; aqueous vs. ethanolic). Ciprofloxacin (CIP, 5 µg) is shown as a dashed red line for reference.

DISCUSSION

This study demonstrates that honey and extracts of clove and ginger have concentration-dependent antibacterial effects against bacteria isolated from tiger nut milk. Among tested agents, ethanolic clove extract showed the strongest activity, producing large inhibition zones and low MICs across multiple pathogens. This aligns with literature on *Syzygium aromaticum* oil: eugenol, its main component, effectively disrupts bacterial membranes and denatures proteins (Maggini *et al*., 2024). A study by Kumar Pandey et al. (2022), notes that clove phenolics (e.g. eugenol) are more bioactive in ethanol-based extracts, consistent with our observation of larger zones for ethanolic clove (Kumar Pandey *et al*., 2022).

Ginger (*Zingiber officinale*) extracts also inhibited the isolates. Ethanolic ginger generally produced slightly larger zones than aqueous ginger, though the difference was modest. This is similar to findings by Ahmed et al. (2022), who reported that ethanol extracts of ginger inhibited Gram-positive bacteria more than aqueous extracts, in a dose-dependent manner. Our data suggest ginger's activity was lower overall than clove's activity; this might be because many isolates here required high doses of ginger for inhibition. For example, *H. alvei* was inhibited by aqueous ginger only at the highest concentration (30 mg/mL in both extracts). A study by Malu et al. (2009) also noted that aqueous ginger was less effective than other extract types against *E. coli*, *B. subtilis*, and *S. aureus*.

Honey showed broad antibacterial activity, especially at high concentration. At 90% (v/v), honey completely inhibited most of the tested isolates, with zones up to 20 mm. Its strong effect is consistent with multifactorial mechanisms: honey's high osmolarity, acidity, hydrogen peroxide content, and phytochemicals combined to inhibit bacteria (Mandal and Mandal, 2011). Nigerian honeys have been shown to possess potent antibacterial and wound-healing activity (Mshelia *et al*., 2018; Agbagwa *et al*., 2022), supporting its efficacy. However, ciprofloxacin was still more effective (27–30 mm zones) than honey (18–20 mm) against the same isolates (paired t-test $p \leq 0.001$), reflecting that conventional antibiotic remain more potent.

Some isolates, notably *E. coli* and *S. maltophilia*, generally required higher concentrations (MICs 50–70%) of honey or extracts for inhibition. *Stenotrophomonas maltophilia* is known to be intrinsically resistant to multiple agents due to efflux pumps and low membrane permeability (Looney *et al*., 2009), this could be possible justification for the higher MICs obtained. These observations underline that even natural antimicrobials may require high doses or combinations to inhibit certain pathogens.

Methodologically, the use of agar diffusion and microdilution for MIC/MBC follows standard protocols (Balouiri *et al*., 2016). Using ethanolic extracts captures lipophilic antimicrobial compounds effectively, and confirming bactericidal activity via MBC adds rigor. It is worth noting that agar diffusion assays can be influenced by compound diffusion rates and viscosity (Balouiri *et al*., 2016), which might limit some inhibition zones. Thus, MIC/MBC provides a robust comparison of potency.

Practically, these findings have implications for food safety. Tiger nut milk is often prepared under non-ideal hygienic conditions (Musa and Hamza, 2013; Opeyemi, 2020), risking microbial growth. If standardized local antimicrobials like honey or clove or ginger extracts can be incorporated safely (for example, as preservatives at acceptable doses), they could reduce bacterial loads. For instance, a diluted clove or ginger extract added at high concentration could inhibit pathogens without overly affecting taste. However, factors like sensory impact (taste, aroma) and shelf-stability must be considered before real-world use.

This study has several limitations. We used crude extracts without chemical standardization, so the exact active component concentrations are unknown and may vary by plant source and extraction conditions (Kumar Pandey *et al*., 2022). Some inhibition tests had few data points

(e.g. “no inhibition” at low doses), which limits statistical power for those comparisons. Also, *in vitro* efficacy does not guarantee effectiveness in the complex matrix of tiger nut milk, where fats, proteins, and solids may interfere with antimicrobial action. Finally, we tested a limited number of isolates; broader studies with more isolates and food matrix trials would strengthen conclusions.

CONCLUSION

High-concentration honey and ethanolic clove extract showed the strongest antibacterial activity against isolates from tiger nut milk. These locally available agents merit further study: particularly, identifying their active compounds, optimizing extraction methods, ensuring consistency (standardization), and testing their efficacy and safety in real *kunun aya* under storage or processing conditions. Such efforts could lead to affordable, culturally acceptable interventions to improve the microbial safety of this popular beverage.

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CONFLICT OF INTEREST

There is no conflict of interest.

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