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Probiotic assessment of herbal mixtures sold in Awka Metropolis

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Abstract

Herbal mixtures are among the important traditional healthcare medicines used in Awka metropolis, Nigeria, with some forms undergoing fermentation, showing the presence of microorganisms. This study aimed to assess the probiotic potential of lactic acid bacteria (LAB) isolated from 10 (ten) herbal mixture samples sold in Awka. LAB were isolated, characterized, and identified, with molecular confirmation by 16S rRNA sequencing. Their probiotic attributes were also evaluated through in vitro assays, including tolerance to acidic pH, bile, and NaCl, assessment of cell surface hydrophobicity, auto- and co-aggregation abilities, cholesterol assimilation, and antimicrobial activity against common clinical pathogens(*Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus*).Results showed the presence of *Lactobacillus guizhouensis* (MRSAI3), *Lactobacillus helveticus* (MRSAI7) and *Lactobacillus terrae* (MRSAI8).These strains demonstrated superior probiotic characteristics in the laboratory tests. They showed a high survival rate under challenging digestive conditions, such as low pH and high bile concentrations. The strains also had strong adhesion capabilities, including the ability to stick to surfaces and to other bacteria. They displayed antimicrobial activity, effectively inhibiting the growth of pathogens like *E. coli* (inhibition zones up to 18.06 mm). The strains were also able to assimilate cholesterol (up to 22.18%). The study confirmed that herbal mixtures are a promising source of beneficial indigenous bacteria with therapeutic potential for humans.

Keywords: Herbal Mixtures; Probiotics; Lactic Acid Bacteria; In Vitro; *Lactobacillus* species

1. Introduction

Herbal mixtures are complex mixtures of plant parts used for centuries across diverse cultures for their medicinal properties. In Nigeria, they are deeply rooted in traditional healthcare, treating a wide array of health conditions (Oladimeji, 2022). These complex mixtures, are consumed in various forms and are known to exert their therapeutic effects through a myriad of bioactive compounds (Rojas *et al.,* 2022). Beyond their direct phytochemical composition, the microbial ecology of these mixtures, particularly through fermentation, plays a significant role in enhancing their therapeutic value. Fermentation, a metabolic process carried out by microorganisms, can increase the bioavailability of active compounds, synthesize new bioactive molecules, and improve the overall nutritional profile of herbal preparations (Ahtesham, 2016; Cruz-Casas *et al.,* 2021). Lactic acid bacteria (LAB) are key contributors in fermentations, contributing to flavor, preservation, and health-promoting properties.

Lactic acid bacteria are a diverse group of Gram-positive, non-spore-forming, facultative anaerobic bacteria, widely recognized for their role in food fermentation and as probiotics. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (NCCIH, 2021; Gunnars, 2018). Their beneficial effects stem from various mechanisms, including enhancing gut barrier function, modulating the immune system, producing antimicrobial compounds, competing with pathogens for nutrients and adhesion sites, and contributing to host metabolism (Harikrishnan *et al.,* 2011). Given the traditional use of fermented foods and beverages in Nigeria, it is

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plausible that some herbal mixtures, especially those prepared through processes involving microbial activity, could harbor indigenous LAB strains with significant probiotic potential.

Despite the known benefits, the microbial quality of traditional herbal mixtures is often unregulated, raising concerns about the co-occurrence of pathogenic microorganisms due to unhygienic practices during preparation and handling (Olaniran *et al.,* 2022). However, while many studies focus on the contamination aspects of herbal medicines, some researchers explore their probiotic potential. Qosimah *et al.* (2023) reviewed the synergistic potential of probiotics and herbal supplements as antibacterial, antioxidant, and immunomodulatory agents. Fadare (2021) demonstrated the *in vitro* antibacterial synergy between probiotic *Lactobacillus* strains and garlic extract against *Salmonella* species. Studies on LAB isolation from traditional fermented foods in Nigeria have identified strains with promising probiotic traits, including acid and bile tolerance, adhesion to epithelial cells, and antimicrobial activity against common pathogens (Chopade *et al.*, 2019; Prabhurajeshwar and Chandrakanth, 2019). These findings showed the potential of traditional foods and, herbal preparations, as reservoirs for probiotic strains.

Therefore, this study focuses on the probiotic assessment of bacterial isolates from herbal mixtures sold in Awka metropolis.

2. Materials and Methods

2.1. Sample Collection

Ten (10) samples of various herbal mixture preparations were randomly purchased from different retail shops across Awka Metropolis, Anambra State, Nigeria. Samples were grouped as, A1 to A10, whereby A1, A2,A3,A4,A5,A6,A7,A8,A9 and A10 represent Herbal diarrhoea, Herbal cough and catarrh, Herbal stomach ulcer, Herbal pile pain management, Herbal anthritis , Herbal Man power, Herbal hypertension ,Herbal washing and setting, Herbal Herbal infection cure and Herbal malaria and typhoid respectively. which were herbal mixtures that were randomly purchased from different sellers from places (Amawbia, Ifite and Unizik temporary sites) in Awka, Metropolis.

2.2. Sterilization of Glassware and Media Preparation

All glassware were thoroughly washed, sterilized in a hot air oven at 120oC for 2 hours, and stored (Sangari *et al.,* 2022). Culture media (De Mann, Rogosa, and Sharpe (MRS) agar and broth for LAB isolation and enumeration) were prepared according to manufacturers' instructions, sterilized by autoclaving at 121oC for 15 minutes, and aseptically dispensed.

2.3. Examination of the Herbal Mixtures: pH Determination

The pH values of all collected herbal mixture samples were determined using a digital pH meter, following the protocol by Vijayakumar and Adedeji (2017).

2.4. Microbial Examination: Determination of Lactobacillus Counts

One milliliter of each herbal sample was serially diluted, and a 10−3 dilution was plated onto MRS agar using pour plate method. Plates were incubated anaerobically at 37oC for 48 hours for Total Lactic Acid Bacteria Counts (TLC) (Osuntokun *et al*., 2022).

2.5. Isolation and Identification of Lactic Acid Bacteria (LAB)

Developed colonies from MRS agar plates were subcultured by streaking on fresh MRS agar to obtain pure cultures. Pure cultures were obtained and stored on agar slants at 4^{o}C. LAB isolates were characterized based on colonial morphology, Gram staining, and biochemical tests including catalase test and sugar fermentation (glucose, lactose, sorbitol, inositol, xylitol, and mannitol) (Osuntokun *et al.*, 2022).

2.6. Molecular Identification of LAB Isolates

Genomic DNA was extracted from selected LAB isolates. The 16S rRNA gene was amplified using PCR, sequencing was performed using the BigDye Terminator kit on a 3510 ABI sequencer (Inqaba Biotechnological, Pretoria, South Africa). Obtained sequences were edited using Trace edit and compared with the NCBI database using BLASTN for species identification (Kumar *et al.,* 2016; Raheem and Adil, 2024).

2.7. In vitro Determination of Probiotic Properties

Probiotic properties were assessed following the methods of Huqin *et al.* (2018).

2.8. Tolerance to Acidic pH

LAB isolates were grown in MRS broth which was adjusted to pH 5.0, 3.0, and 2.0 with HCl, incubated anaerobically at 37°C for 48 hours. Survival rates were determined by viable counts on MRS agar.

2.8.1. Bile Tolerance

This was assessed by using MRS broth supplemented with varying concentrations of bovine bile (0.1 ml, 0.5 ml, 1.0 ml, 2.0 ml). Isolates were incubated anaerobically at 37°C for 48 hours. Survival rates were determined by viable counts.

2.8.2. NaCl Tolerance

This was done by inoculating isolates into MRS broth supplemented with 3%, 6.5%, and 10% (w/v) NaCl and observing growth after 48 hours incubation at 37oC.

2.9. Growth at Different Temperatures

The specific growth rates and duplication times were determined by monitoring optical density changes of LAB isolates in MRS broth at 30oC and 35oC over 24 hours.

2.10. Cell Surface Hydrophobicity

This was determined by measuring the adherence of bacterial cells to hexadecane/xylene (Botthoulath *et al.,* 2018). Absorbance of the aqueous phase was measured at 620 nm.

2.10.1. Auto aggregation

This was done by measuring the decrease in optical density (OD) of bacterial suspensions at 600 nm over time (3 and 24 hours) due to cell sedimentation (Chopade *et al.,* 2019).

2.10.2. Co-aggregation

The ability of LAB isolates to co-aggregate with clinical pathogens (*Escherichia coli, Staphylococcus aureus, and Salmonella* sp.) was measured by mixing bacterial suspensions and observing the reduction in OD at 570 nm after 4 hours of incubation (Prabhurajeshwar and Chandrakanth, 2019).

2.10.3. Assimilation of Cholesterol

Lactic acid bacteria (LAB) strains were grown in modified MRS broth containing 0.3% oxgall and 100 µg/mL water-soluble cholesterol. Cholesterol content in the supernatant after 12, 24, and 48 hours was determined spectrophotometrically at 550 nm after extraction and reaction with o-phthalaldehyde (Jenny e*t al*., 2023).

2.11. Assay for Antimicrobial Activity of Isolated LAB on Clinical Isolates

Cell-free supernatants of LAB isolates were obtained by centrifugation and filtration. Their antimicrobial activity was determined by the agar well diffusion assay against clinical isolates of *Escherichia coli, Staphylococcus aureus,* and *Salmonella* sp. (Santos *et al*., 2016). Inhibition zone diameters were measured after incubation.

3. Results

The result of the Probiotic Assessment of Herbal Mixtures Sold in Awka Metropolis as shown below showed the herbal mixtures purchased from Awka Metropolis,Table 1 presents the pH profile of ten different herbal samples (A1-A10), which is an important factor for assessing the chemical composition and its suitability for microbial growth. This is followed by Table 2, which showed the Total *Lactobacillus* count (TLC) in each herbal sample, providing a quantitative measure of the microbial load of these beneficial bacteria.Then table 3 revealed the morphological and biochemical characterization of the isolates whereby standard microbiological tests, such as Gram reaction, motility, and sugar fermentation, was used to confirm the isolates. The molecular analysis identified the key isolates as *Lactobacillus guizhouensis* (MRSAI3), *Lactobacillus helveticus* (MRSAI7), and *Lactobacillus terrae* (MRSAI8). Table 4 and Table 6 quantitatively assess the isolates' in vitro survival under simulated gastrointestinal stress, specifically examining their

tolerance to low pH and high bile concentrations. Table 5(Sodium chloride tolerance of the isolates) and figure 1 and 2(growth at different temperatures) further support the isolates' robustness by examining their tolerance to varying salt concentrations and temperatures. Table 8 measures the isolates' cholesterol assimilation potential, a key therapeutic trait, while Table 9 quantifies their antimicrobial activity by measuring the diameter of inhibition zones against test pathogens. Finally, Figures 3, 4, and 5 showed the isolates' cell surface hydrophobicity, autoaggregation, and coaggregation capabilities, which are essential for adherence and colonization of the intestinal mucosa.

Table 1 pH of the herbal mixtures (Mean ± SD)

Sample	Mean ± SD
A1	3.45 ± 0.025
A2	5.40 ± 0.132
A3	2.93 ± 0.076
A4	3.05 ± 0.050
A5	3.93 ± 0.076
A6	4.05 ± 0.050
A7	5.72 ± 0.076
A8	3.65 ± 0.050
A9	4.82 ± 0.104
A10	3.92 ± 0.076

Key

- A1 =Herbal diarrhoea A2 = Herbal cough and catarrh
- A3 = Herbal stomach ulcer A4 = Herbal pile pain management
- A5 = Herbal anthritis A6 = Herbal Man power
- A7 = Herbal hypertension A8 = Herbal washing and setting
- A9 = Herbal infection A10 = Herbal malaria and typhoid

Microbial Examination: Determination of Lactobacillus Counts(logcfu/ml)

Table 2 The Lactobacillus loads of herbal samples

Sample	TLC
A1	0.94±0.01
A2	1.25±0.02
A3	2.17±0.01
A4	0.88±0.03
A5	0.99±0.01
A6	1.37±0.04
A7	1.14±0.02
A8	1.19±0.01
A9	1.22±0.01
A10	1.61±0.07

Key

- A1 =Herbal diarrhoea A2 = Herbal cough and catarrh

- A3 = Herbal stomach ulcer A4 = Herbal pile pain management
- A5 = Herbal anthritis A6 = Herbal Man power
- A7 = Herbal hypertension A8 = Herbal washing and setting
- A9 = Herbal infection A10 = Herbal malaria and typhoid

Table 3 Morphological and Biochemical Characteristics of the *Lactobacillus* species

Parameter	MRSAI1	MRSAI2	MRSAI3	MRSAI5	MRSAI7	MRSAI8
Appearance	Cream	White	White	Light cream	White	Cream
Edge	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Convex	Convex	Convex	Convex	Convex	Convex
Gram Reaction	+	+	+	+	+	+
Shape	Rods	Rods	Rods	Rods	Rods	Rods
Motility	-	-	-	-	-	-
Catalase	-	-	-	-	-	-
Indole	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+
Citrate	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Sorbitol	+/-	+	+/-	+/-	+	+/-
Arabinose	+	+/-	+/-	+	+	+/-
Inositol	+	+/-	+	+	+	+/-
Xylitol	+/-	+/-	+	+	+	+

Key; - =Negative;+ = Positive;The molecular analysis identified the notable Lactobacillus isolates as Lactobacillus guizhouensis (MRSAI3), Lactobacillus helveticus (MRSAI7) and Lactobacillus terrae (MRSAI8)

Table 4 Effect of Low pH on the Growth of the Isolates

Isolate	pH = 2.0 (S% at 3h)	pH = 2.0 (S% at 5h)	pH = 3.0 (S% at 5h)	pH = 5.0 (S% at 3h)	pH = 5.0 (S% at 5h)
MRSAI1	95.88±1.18	93.03±4.35	92.98±2.09	87.59±11.95	89.48±1.94
MRSAI2	41.95±0.99	41.95±0.99	90.51±2.07	87.59±1.93	89.47±1.93
MRSAI3	86.13±1.97	88.66±2.01	92.87±2.12	90.06±2.02	91.77±2.03
MRSAI5	27.96±0.70	43.51±1.03	92.63±2.11	73.43±1.78	79.12±1.86
MRSAI7	93.58±2.04	94.00±2.17	95.81±2.22	92.72±2.16	92.72±2.16
MRSAI8	90.29±2.05	92.46±2.11	96.52±2.24	88.10±2.07	89.00±2.07

Key: S% = Percentage of Survival

Table 5 Sodium Chloride Tolerance of the Isolates

Isolates	Percentage of Nacl						
	1	2	3	4	5	6	7
MRSAI1	+	+	+	+	+	+	-
MRSAI2	+	+	+	+	+	+	+/-
MRSAI3	+	+	+	+	+	+	+
MRSAI5	+	+	+	+	+	+	-
MRSAI7	+	+	+	+	+	+	+
MRSAI8	+	+	+	+	+	+	+

+ = Presence of growth
- =No growth

3.1. Growth at Different Temperatures

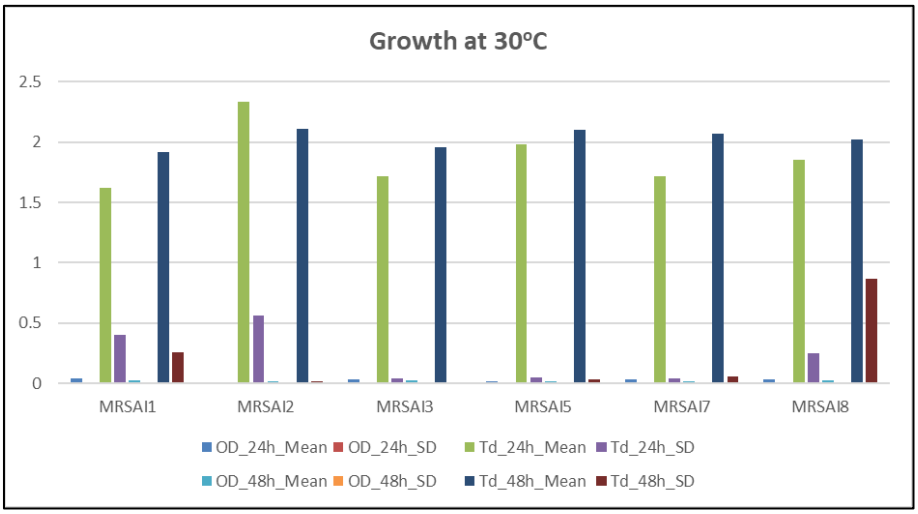


Figure 1 Growth at 30oC

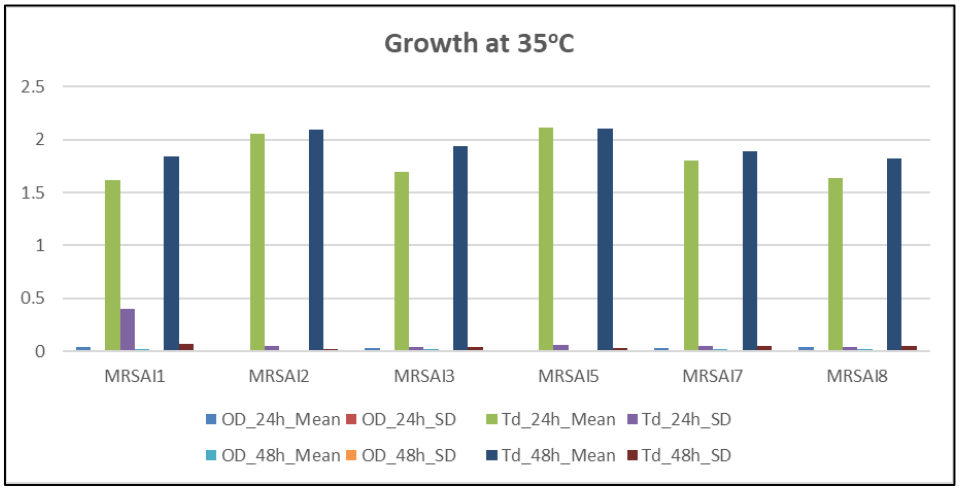


Figure 2 Growth at 35oC

Table 6 Effect of Bile on the Growth of the Isolates

Isolates	S% (0.1mL)	S% (0.5mL)	S% (1.0mL)	S% (2.0mL)
MRSAI1	93.33±2.17	92.36±2.12	91.38±2.13	90.10±2.07
MRSAI2	65.26±1.61	24.47±0.61	24.47±0.61	24.47±0.61
MRSAI3	90.78±2.20	85.67±2.02	53.38±1.29	27.96±0.69
MRSAI5	88.86±2.11	71.18±1.74	66.97±1.63	44.48±1.09
MRSAI7	94.66±2.21	90.34±2.18	88.10±2.07	85.67±2.02
MRSAI8	91.38±2.13	90.1±2.07	88.10±2.07	85.67±2.02
S%	= Percentage of Survival			

3.2. The Invitro Survival in Gastric Juice

The result of the Invitro Survival of the lactic acid bacteria isolates in Gastric Juice are shown in the table below

Table 7 The Invitro Survival in Gastric Juice

Isolate	CFU/mL after 3hours	CF/mL after 5hours
MRSAI1	78.33±1.88	40.13±0.96
MRSAI2	31.33±0.76	16.63±0.40
MRSAI3	78.33±1.88	30.37±0.71
MRSAI5	20.57±0.51	13.70±3.78
MRSAI7	16.63±0.40	10.77±0.25
MRSAI8	81.23±1.96	36.23±0.86

3.3. The Cholesterol Assimilation Potential of the Isolates

The result of the cholesterol assimilation potential of the lactic acid bacteria isolates are shown in the table below

Table 8 Cholesterol Assimilation Potential of the Isolates

Isolate	12 hours (A%)	24 hours (A%)	48 hours (A%)
MRSAI1	8.37±0.19	12.46±0.31	16.31±0.41
MRSAI2	2.48±0.06	8.13±0.19	9.95±0.25
MRSAI3	8.86±0.22	12.55±0.31	19.00±0.47
MRSAI5	5.04±0.13	5.93±0.15	11.46±0.28
MRSAI7	1.54±0.04	6.7±0.17	12.54±0.31
MRSAI8	10.01±0.25	13.94±0.35	21.69±0.56

Key: A % = Percentage Assimilation

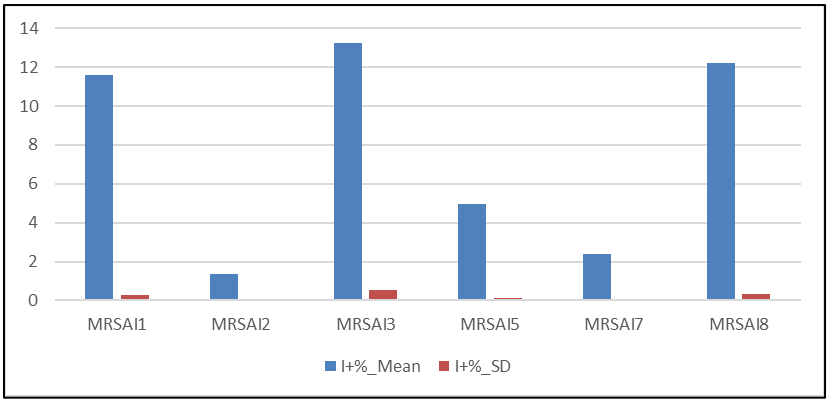
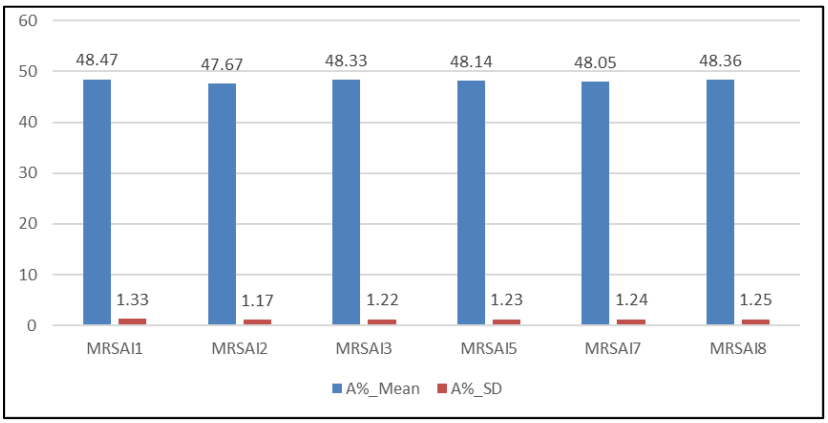


Figure 3 Cell Surface Hydrophobicity of the test isolates



A% = percentage of Absorbance

Figure 4 Auto aggregation Potential of the Isolates

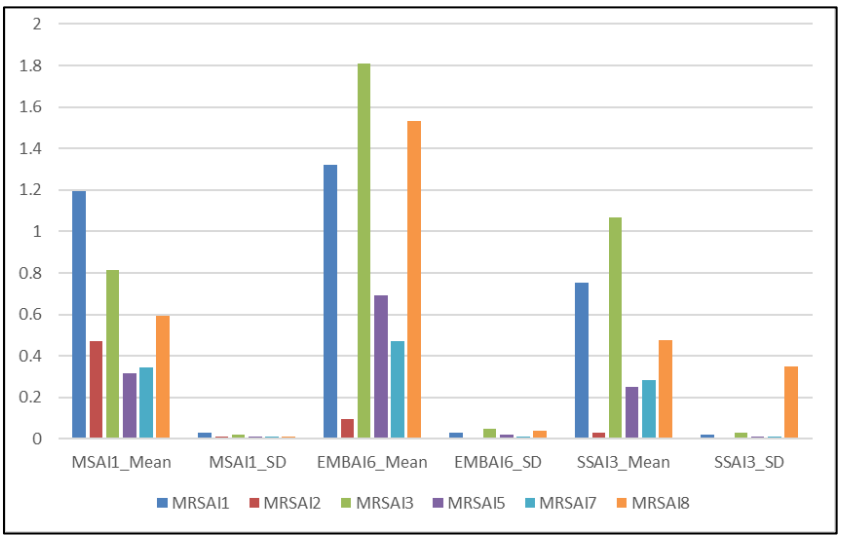


Figure 5 Coaggregation Potential of the Isolates

Table 9 Diameter Zones of Inhibition of Lactic acid bacteria Against the Test Pathogens

Lactic acid bacteria	Mean Diameter Zone ($\bar{X}$± SD) mm		
	MSAI1	EMBAI6	SSAI3
MRSAI1	11.22±0.11	17.62±0.12	14.56±0.07
MRSAI2	8.12 ± 0.07	17.08±0.33	11.74±0.12
MRSAI3	12.46±0.21	18.06±0.11	15.26±0.12
MRSAI5	9.86±0.11	15.87±0.17	13.22±0.08
MRSAI7	10.44±0.07	17.44±0.11	13.18±0.11
MRSAI8	11.82±0.17	17.88±0.22	14.37±0.81

4. Discussion

This study assessed the probiotic potential of lactic acid bacteria (LAB) isolated from herbal mixtures sold in Awka metropolis, it showed the presence of beneficial microorganisms within these herbal products. The consistent presence of LAB across various samples, as indicated by total *Lactobacillus* counts, suggests that certain herbal preparations, possibly due to their fermentation processes or raw material composition, naturally harbor these bacteria. This aligns with knowledge that traditional fermented foods and beverages are rich sources of diverse microbial strains (Cruz-Casas *et al.,* 2021).

The in vitro assessment of LAB isolates also showed that several strains possess key probiotic characteristics. Their remarkable tolerance to acidic pH (as low as pH 2.0) and bile salts is critical, as these conditions are major barriers to microbial survival in the gastrointestinal tract (Huqin *et al.,* 2018). Isolates such as MRSAI1, MRSAI3, MRSAI7, and MRSAI8 exhibited particularly robust survival rates, suggesting their ability to reach and colonize the intestines effectively. This acid and bile tolerance is a criterion for probiotic selection. Furthermore, the ability of several isolates to tolerate high NaCl concentrations indicates their resilience to various environmental stresses. The assessment of adhesion-related properties, including cell surface hydrophobicity, autoaggregation, and coaggregation, showed significant findings.Whereby high autoaggregation capacities among all LAB isolates signify their ability to self-associate and potentially form biofilms in the gut, which is important for sustained colonization and interaction with the host (Chopade *et al.,* 2019; Ivshina *et al.,* 2022). More importantly, the coaggregation abilities of isolates like MRSAI3 and MRSAI8 with common pathogens such as *E. coli*, *S. aureus*, and *Salmonella* sp. Showed a direct mechanism by which these LAB could exert antagonistic effects, preventing pathogen adhesion and colonization.

The significant antimicrobial activity demonstrated by the cell-free supernatants of the LAB isolates, especially MRSAI3, further showed their probiotic potential. The production of organic acids, bacteriocins, and other antimicrobial compounds is a primary mechanism by which LAB inhibit the growth of pathogenic bacteria (Santos *et al.,* 2016). The high inhibition zones (mm) against clinical isolates of *S. aureus*, *E. coli*, and *Salmonella* sp. suggest that these LAB strains could be effective in managing or preventing bacterial infections in the gut. Additionally, the observed cholesterol assimilation capabilities of isolates like MRSAI8 and MRSAI3 point towards an added health benefit, as certain probiotic strains can contribute to maintaining healthy cholesterol levels (Jenny *et al.,* 2023). The molecular analysis identified the notable *Lactobacillus* isolates as *Lactobacillus guizhouensis* (MRSAI3), *Lactobacillus helveticus* (MRSAI7) and *Lactobacillus terrae* (MRSAI8).

5. Conclusion

In conclusion, this study showed the ability of the indigenous LAB from herbal mixtures in Awka metropolis as promising probiotic candidates. This research contributes to the scientific understanding of traditional products and opens avenues for making use of local microbial biodiversity of herbal mixtures for health benefits.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Ahtesham, A. (2016). Fermentation, a feasible strategy for enhancing bioactivity of herbal medicines. Food Research International, 81, 1–16.

[2] Alegbeleye, O. O. (2018). Microbial contamination of medicinal plants and herbal products: A review. Journal of Food Protection, 81(12), 1989-1999.

[3] Chopade, L. R., Paradeshi, J. S., Amrukthar, K. P., and Chaudhari, B. L. (2019). Finding out potent probiotic cultures from Ayurvedic formulation Takrarishta through in vitro probiotic characterization and principal component analysis. LWT- Food Sci. Technol, 100, 205-212.

[4] Cruz-Casas, D. E., Aguilar, C. N., Ascacio-Valdés, J. A., Rodríguez-Herrera, R., Mónica, L., Chávez-González, A. C., and Flores-Gallegos. (2021). Enzymatic hydrolysis and microbial fermentation: The most favorable biotechnological methods for the release of

[5] Gunnars, K. (2018). 8 Health Benefits of Probiotics (Backed by Science). Retrieved from https://www.healthline.com/nutrition/8-health-benefits-of-probiotics

[6] Harikrishnan, R., (2011). Supplementation of probiotics and herbal mixtures in the diet of olive flounder (Paralichthys olivaceus) significantly enhanced growth performance, blood biochemical parameters, and nonspecific immune response against Streptococcus parauberis infection. Fish & Shellfish Immunology, 31(6), 1109-1117.

[7] Huqin, T., Bingjun, Q., Bei, X., Yi, Z., Yunqiu, Y., Renyou, G., and Jianhua, Z. (2018). Screening of Lactic acid bacteria isolated from fermented Cornus Officinalis fruits for probiotic potential. Journal of Food Safety.

[8] Ivshina, I. B., Krivoruchko, A. V., Kuyukina, M. S., Peshkur, T. A., and Cunningham, C. J. (2022). Adhesion of Rhodococcus bacteria to solid hydrocarbons and enhanced biodegradation of these compounds. Scientific reports, 12(1), 215-259.

[9] Jenny, S., Sathammai-Priya, N., and Priya, E. (2023). In vitro cholesterol assimilation and functional enzymatic activities of Lactobacillus sp. An International Journal of Biological Forum, 15(5a), 222-226.

[10] Kumar, S., Stechar, G., and Tamura, K (2016). Molecular Evolutionary Genetics Analysis version 7.0 for Bigger Datasets, molecular biology and evolution. Academic Oup.com 2016; 33 (7): 1870 – 1874. Lactobacillus spp. from traditional Maasai fermented milk products in report of a WHO Global Survey, WHO Geneva. 2005;30 (5)1818:30-35.

[11] Msomi, N., and Simelane, S. (2019). Traditional medicine use in South Africa. Journal of Ethnopharmacology, 233, 1-7.

[12] NCCIH. (2021). Probiotics: What You Need To Know. Retrieved from https://www.nccih.nih.gov/health/probiotics-what-you-need-to-know

[13] Oladimeji, E. (2022). What is herbal medicine and what are the benefits. Retrieved from https://www.medicalnewstoday.com/articles/herbal-medicine.

[14] Olaniran, O. B., Ajayi, S. E., Oluwatobi, O. B., and Adeleke, O. E. (2022). Assessment of microbial quality and detection of extended spectrum B-Lactamase genes in Gram negative bacterial isolates of herbal mixtures commonly isolated in Sagamu metropolis, Ogun state, Nigeria. African Journal of Clinical and Experimental Microbiology, 23(3), 257-268.

[15] Osuntokun, O.T., Thonda,O.A., Ajadi,F.A., Wilkie, E.D.and Oluwatuyi,B.B (2022). Microbiological analysis,quality and safety evaluation of commercially sold medicinal herbal cocktails from local herb seller within Akoko South West, Ondo State, Nigeria.Journal of complementary and alternative medical research.18(2): 20-36.

[16] Prabhurajeshwar, C., and Chandrakanth, K. (2019). Evaluation of antimicrobial

[17] properties and their substances against pathogenic bacteria in-vitro by probiotic

[18] lactobacilli strains isolated from commercial yoghurt. Clin. Nutr. Exp, 23, 97-115.

[19] https://doi.org/10.1016/j.yclnex.2018.10.001.

[20] Qosimah, D., Laminem, N., Mandasari, C., and Setyawati, D. (2023). Review of the Role of Probiotic and Herbal Supplements as Antibacterial, Antioxidant, and Immunomodulatory Against Aeromonas hydrophila. Jurnal Penelitian Pendidikan IPA, 9(6), 178–189.

[21] Raheem, H.A and Adil,A.H (2024). Anovel method of extraction and purification of

[22] bacterial DNA for PCR detection of meca,16srRNA,vanB. European Journal of Medical Genetics and Clinical Biology;https://doi.org/10.6179/jmgcb.vL14.403.

[23] Rojas, P., Jung-Cook, H., Ruiz-Sánchez, E., Rojas-Tomé, I. S., Rojas, C., López-Ramírez, A. M., and Reséndiz-Albor, A. A. (2022). Historical Aspects of Herbal Use and Comparison of Current Regulations of Herbal Products between Mexico, Canada and the United States of America. International Journal of Environmental Research and Public Health, 19(23), 15690.

[24] Sangari,J.S., Maduagwu,Q.C., Yilyok,C.W., Biska,L.W. and Nanbyen, D. (2022) The effect of fungi spoilage on the nutritional value of corn in langtang north local government area of plateau state.International Journal of food science and nutriton.7(3) 179 -183.

[25] Santos, T. T., Santos Ornellas, R. M., Borges Arcucio, L., Messias Oliveira, M., Nicoli, J. R., Villela Dias, C., and Vinderola, G. (2016). Characterization of Lactobacilli strains derived from cocoa fermentation in the south of Bahia for the development of probiotic cultures. LWT-Food Science and Technology, 73, 259–266.

[26] Vijayakumar, P., and Adedeji, A. A. (2017). Measuring the pH of Food Products. ResearchGate.https://www.researchgate.net/publication/330601448_Measuring_the_pH_of_Food_Products.