

Weissella Paramesenteroides' Effects on Two Neglected Wild Food Plants (*Hydrocotyle bonariensis* and *Garcinia kola*) Used in the Treatment of Hypertension in Côte d'Ivoire

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Abstract

Lactic acid fermentation is a promising approach for enhancing the nutritional and functional properties of certain underutilised food plants. In this study, *Hydrocotyle bonariensis* and *Garcinia kola* were subjected to controlled fermentation by a probiotic lactic acid bacteria strain identified as *Weissella paramesenteroides* by 16S ribosomal gene sequencing. The resulting matrices were analysed to assess their antioxidant and anti-inflammatory activities using the DPPH and protein denaturation inhibition tests, respectively. The fermentation process significantly increased the content of proteins and minerals while reducing anti-nutritional factors such as phytates and oxalates. These biochemical changes correlated with enhanced antioxidant activity and anti-inflammatory potential in vitro. The antioxidant activity of *Hydrocotyle bonariensis* increased from 60.74±4.09% to 83.55±0.68%. Anti-inflammatory activity also increased after fermentation, reaching 72.96±0.89% for *Hydrocotyle bonariensis* and 98.11±2.67% for *Garcinia kola*. Fermentation also significantly increased levels of protein, vitamins (A, D and E) and essential minerals, such as magnesium, zinc, sodium and potassium. These nutritional and functional improvements suggest that incorporating *Hydrocotyle bonariensis* and *Garcinia kola* based fermented products into a diet for the prevention of hypertension and other metabolic diseases could improve intestinal health due to the beneficial effects of lactic fermentation.

Keywords: hypertension, *Weissella paramesenteroides*, *Hydrocotyle bonariensis*, *Garcinia kola*, fermentation

1. Introduction

Hypertension is high pressure in the blood vessels. Its incidence is high, but the consequences can be serious if left untreated. Since, people with high blood pressure can be asymptomatic, the only way to find it out is to regularly check blood pressure. Hypertension is a major cause of premature mortality worldwide. Worldwide, nearly 1.28 billion people between the ages of 30 and 79 are affected by high blood pressure (Lumbantoruan et al., 2023). It is important to note that 46% of these people are unaware of their condition. The majority (two-thirds) live in low- and middle-income countries. There are several reasons for the appearance of hypertension, including poor diet (too much salt, saturated and trans-fatty acids, and too little fruit and vegetables), physical inactivity, smoking, alcohol consumption, obesity, etc. Current medical treatment of this condition with synthetic drugs (diuretics; beta-blockers; calcium channel blockers; converting enzyme inhibitors and angiotensin-2 antagonists) is costly and often associated with side effects (Coulibaly, 2022). In addition to medication, lifestyle risk factors need to be systematically addressed, in particular lifestyle changes (healthier diet, smoking cessation and increased physical activity). According to the WHO, one of the alternatives is to promote the consumption of fruit and vegetables. As well as being economical, fruit and vegetables are an important source of bioactive molecules such as prebiotics, vitamins, minerals, antioxidants and anti-inflammatories. Fruit and vegetables help to improve the health of the gut microbiota. Scientific studies

show that adequate fruit and vegetable consumption reduces the risk of metabolic diseases (Sodjinou, 2008). In Côte d'Ivoire, certain neglected wild fruits and vegetables are used for food and as part of traditional treatments for metabolic diseases. This is the case of the research carried out by (N'Dri et al., 2023) on *Garcinia kola* seeds, which has been shown to have an antihypertensive effect. However, the way in which these plants are used (by cooking or decoction) reduces the health benefits. That's why the search for new alternatives is growing worldwide, including the consumption of fermented foods. Fermentation of fruits and vegetables is booming because of its proven health benefits for chronic diseases (Fessard et al., 2016). This mainly lactic fermentation, known as lactofermentation, is a process in which the sugars present in food are metabolised by lactic acid bacteria to produce organic acids, alcohol and/or carbon dioxide (Fessard et al., 2017). This process has been used for millennia and is thought to be beneficial in removing antinutritional compounds, increasing antioxidant and anti-inflammatory properties, and improving the digestibility of foods (Fessard et al., 2016). The bioactive compounds in the fermented product are enhanced at the end of the fermentation process. Lactic acid bacteria play a crucial role in food technology, particularly in food fermentation (Kandler & Weiss, 1986). They are known for their GRAS classification and are considered non-toxic by the WHO. These bacteria are probiotic and have the ability to produce molecules with antioxidant and anti-inflammatory properties. Because of their probiotic properties, it would be conceivable to use these bacteria to carry out a controlled fermentation of two fruits and vegetables known for their antihypertensive effects (*Garcinia kola* seeds and *Hydrocotyle bonariensis* leaves), with the aim of producing high quality foods capable of regulating intestinal health. In recent years, a significant link has been established between gut dysbiosis and the development of diabetes, hypertension, obesity and cardiovascular diseases. As a result, the synergistic effect of probiotics and prebiotics could lead to functional foods with a range of positive effects on the body's health (Zhuo et al., 2022). The aim of this study is to promote the consumption of two types of lacto-fermented plants for the prevention of arterial hypertension in Côte d'Ivoire.

2. Method

The biological material consisted of two fruits and vegetables (*Hydrocotyle bonariensis* leaves and *Garcinia kola* seeds) and strains of lactic acid bacteria. The fruits and vegetables were purchased at the Adjamé market (Abidjan, Côte d'Ivoire) and then transported to the National Floristic Centre of the Félix Houphouët-Boigny University (Abidjan) for identification using analytical flora. The lactic acid bacteria strains were isolated from cocoa fermented in Côte d'Ivoire.

2.1 Isolation and Identification of Lactic Acid Bacteria Strains

Lactic acid bacteria were isolated from cocoa fermented in Côte d'Ivoire. For this purpose, samples purchased at the Adjamé market were fermented at 30 °C for 72 h. Then 1 g of each sample was placed in a tube containing 9 mL of sterile peptone water (adjusted to pH 3 with HCl). The mixture was incubated at 30 °C for 2 h. Isolates were then plated on sterilised Man, Rogosa, Sharpe (MRS) agar supplemented with 0.1% nystatin and poured into Petri dishes. All plates were incubated at 30 °C for 48-72 hours. After incubation, colonies with the morphological characteristics of lactic acid bacteria were selected for Gram staining and oxidase and catalase tests to confirm that they belonged to the lactic acid bacterial groups. Isolates with the characteristics of lactic acid bacteria, *i.e.*, Gram positive, oxidase and catalase negative, were selected, purified and stored at -20 °C in MRS broth supplemented with 20% glycerol in Eppendorf tubes for phenotypic characterization.

2.2 Screening of Probiotic and Technological Properties of Isolated Strains

Resistance to acidity, bile salts, antibiotics, intestinal and non-intestinal pathogens are the parameters evaluated to highlight the probiotic properties of the strains (Hyronimus et al., 2000). Acid resistance was assessed by culturing lactic acid bacteria at 37 °C for 3 hours in MRS broth adjusted to pH 2 with 3 M hydrochloric acid, followed by neutralisation of acidity, adding 0.1 M phosphate buffer (pH = 6.2). A control was performed in normal MRS broth. Inoculation was then performed on MRS agar to determine survival. Resistance to bile salts was assessed in MRS broth supplemented with a pH indicator, bromocresol purple (BCP), and 0.3% of a mixture of bile salts (Sigma) (Pithva et al., 2014). Resistance of lactic acid bacterial isolates to bile salts is indicated by a change in colour of the BCP from purple to yellow (Pithva et al., 2014). Xylene is a hydrophobic substance used to assess the hydrophobicity of the surface of microbial cells by measuring their ability to adhere to this hydrocarbon (Rosenberg et al., 1980). Xylene is a hydrophobic substance used to assess the hydrophobicity of the surface of bacterial cells by measuring their ability to adhere to this hydrocarbon (Rosenberg et al., 1980). The bacterial pellet from one millilitre of overnight incubated culture was washed twice with PBS buffer (pH 7.4). The pellet obtained after washing was dissolved in 3 ml of the same buffer to measure the optical density (A₀) at 600 nm. An equivalent volume of xylene was then homogenised by vortexing for 5 minutes and

incubated for 1 hour at 37 °C. The bacterial adhesion capacity (BAC) was determined using the formula $BAC \% = [(A0 - A1)/A0] \times 100$ after reading the optical density of the organic material (A1) at 600 nm. The standardised diffusion technique of the AntibioGram Committee of the French Society of Microbiology was used to evaluate the antibiotic resistance of lactic acid bacterial isolates. 100 µl of a culture incubated for 24 hours at 37 °C were plated on MRS agar. Each antibiotic (chloramphenicol, penicillin, erythromycin, kanamycin, sulfa, cephalosporin, rifampicin, nalidixic acid and tygecycline) was then added and incubated at 37 °C for 24 hours. Pathogenic microorganisms such as *Staphylococcus*, *Pseudomonas*, *Escherichia coli*, *Salmonella*, *Klebsiella*, *Candida* and *Aspergillus* were used to evaluate the antimicrobial activity of lactic acid bacterial strains. Pre-cultures of lactic acid bacterial strains and pathogens were performed separately in MRS broth for 18 h at 37 °C. 100 µL of precultures of pathogenic strains were inoculated onto MRS agar and wells were made using a Pasteur pipette handle. Each well was inoculated with 50 µl of lactic acid bacteria preculture. The Petri dishes were incubated successively at 4 °C for 2 hours to ensure optimal diffusion of the substance and at 37 °C for 24 hours to assess the diameters of the inhibition zones (Tadesse et al., 2005). The production of exopolysaccharides was determined by inoculating the strains onto MRS agar supplemented with sucrose at 40 g/L (Abdellah et al., 2015). The production of amylase, cellulase, phytase and tannase by strains was assessed by replacing glucose in the MRS culture medium with soluble starch, carboxymethylcellulose, phytic acid and tannic acid, respectively (Krabi et al., 2015). The Petri dishes were incubated for 48 hours at 30 °C under anaerobic conditions. After incubation, Lugol's iodine solution was used to detect the production of pectinase, cellulase and phytase. In the case of tannase, the formation of a clear zone around the bacterial growth is an indicator of a positive result. The anti-inflammatory and antioxidant properties of the isolates were assessed using the supernatant obtained after 48 h of culture. The supernatant was sterilised using an MF-Millipore™ membrane filter (0.22 µm; Merck, Germany) after centrifugation of the culture at 6000 rpm/10 min. Antioxidant activity was assessed by mixing 1 ml of bacterial supernatant with 2 ml of 70% ethanol (Benhammou et al., 2007) and centrifuged at 6,000 rpm for 10 minutes. A mixture of 50 µl of the supernatant and 1950 µl of a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.6 mg/ml) was then prepared. The blank consisted of 50 µl of 70% ethanol and 1950 µl of DPPH. After 30 min incubation in the dark, the absorbance was measured at a wavelength of 517 nm. The percentage of discolouration induced by DPPH was calculated using the formula $I (\%) = ((ODc - ODex)/ODc) \times 100$. The anti-inflammatory activity of strains was determined using the bovine serum albumin inhibition test (Anoop et al., 2015). In this method, a volume of 0.45 ml of 5% aqueous bovine serum albumin solution and 0.05 ml of bacterial culture supernatant or reference drug (diclofenac) or pure water (control tube) were mixed. The pH of the solution was adjusted to 6.3 with 1 N hydrochloric acid. The tubes were incubated at 37 °C for 20 minutes and then heated to 57 °C for 3 minutes and then cooled. Before measuring the absorbance at 416 nm, 2.5 ml of phosphate buffer, previously prepared at pH 6.3, was introduced into the tubes. The following formula was used to calculate the inhibition of protein denaturation content: $\% \text{ inhibition} = 100 - [(OD \text{ sample} - OD \text{ control})/OD \text{ control}] \times 100$.

2.3 Molecular Identification of Isolated Bacteria

The method used is colony PCR, which involves making a cell extract from a clean colony and 300 µL of water DNase free in an Eppendorf tube (Gupta, 2019). Hypervariable regions of the 16S RNA gene, about 500 base pairs long, were amplified by PCR. The primers used are F27 (5'-AGAGTTTGATCCTGGCTCAG-3') and R520 (5'-ACCGCGGCTGGC-3'). The reaction mixture consisted of 15 µL sterile water, 25 µL 2X Phusion Master Mix, 2.5 µL F primer, 2.5 µL R primer, and 5 µL cell extract. The amplification was carried out at Université Paul Sabatier (Toulouse, France) according to the programme, consisting of an initial denaturation at 95 °C for 4 minutes, followed by 35 reaction cycles including a denaturation phase at 95 °C for 1 minute, a starter hybridisation phase at 54 °C for 1 minute, an extension phase at 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes. The amplicons were visualised by UV transillumination after 1% (w/v) agarose gel electrophoresis at 70 V for 2 hours in a Tris-Acetate-EDTA 1 buffer, followed by GelRed staining. PCR products were purified using a commercial kit (OMEGA bio-tek E.Z.N.A. Gel Extraction Kit). Sequencing was performed by MWG Eurofins using the F27 primer. The BLAST from NCBI database site (blast.ncbi.nlm.nih.gov/) was used to find the closest sequences relative to the amplified 16S RNA genes to identify the species of LAB.

2.4 Lacto-fermentation Process

Fruit and vegetables delivered to the laboratory were inspected for defects or imperfections, then treated with 70% bleach and carefully rinsed with tap water. The materials were then ground in a porcelain mortar and mixed with distilled water at a 1:2 (w/v) ratio. The resulting ground material was inoculated with 10⁷ cells of selected probiotic lactic acid bacterial strain. The strain was pre-cultured for 24 hours at 30 °C in MRS culture medium derived from pure colonies. A 50 µL aliquot of the pre-culture was then mixed with 50 µL of methylene blue and

placed on a Thomas cell counting slide to quantify viable cells. The sample was examined under a light microscope and the cell count was performed immediately at 40× magnification, taking into account the quadrants of the Thomas cell. The concentration of bacterial cells (NC, cells/ml) was determined using the equation $NC = n \times 4 \times 10^6 \times fd$, n is the average number of cells and fd is the dilution factor. Subsequently, 30 g of each type of plant material was infected with a load of 10^7 cells. Fermentation was carried out for 48 hours at 30 °C. After an initial evaluation, strain C26 was selected for the fermentation of *Hydrocotyle bonariensis* leaves and *Garcinia kola* seeds. After, 30 g of each fermented product was subjected to lyophilization in 250 ml containers using a Biobase lyophilizer set at -45 °C and 1 Pa for 48 hours, after an initial freezing period at -60 °C for 8 hours. The dried product was then ground in a blender to preserve it for biochemical and nutritional analysis.

2.5 Determination of Phytochemical Compounds, Total Sugars, Antioxidant and Anti-inflammatory Activity

The polyphenol content was determined by the Folin-Ciocalteu method (Singleton, 1999). The method of (Meda et al., 2005) was employed to determine the flavonoid content. Tannins were determined by the method of (Broadhurst & Jones 1978). According to (Benhammou et al., 2007), DPPH (diphenyl picrylhydrazyl) was used as a stable free radical to measure free radical scavenging activity. In this experiment, antioxidants converted the purple DPPH to a yellow molecule, with the intensity of the colour being inversely related to the ability of the antioxidant to donate protons (Benhammou et al., 2007). The bovine serum albumin (BSA) protein denaturation inhibition assay examined the anti-inflammatory efficacy of the samples using the method of (Anoop et al., 2015). The sulphuric phenol technique of (Dubois et al., 1956) was used to measure total sugars.

2.6 Determination of Anti-nutritional Compounds

Phytates were determined using Wade's reagent (Latta & Eskin, 1980). One gram of dried sample was mixed with 20 ml of 0.65N HCl, stirred for 12 hours. The mixture was centrifuged for 40 minutes at 12,000 rpm. Five (5) ml of the supernatant was added to 3 ml of Wade's reagent. The mix was allowed to stand for 15 minutes before the optical density was measured at 490 nm on a spectrometer, using the control as a reference. Sodium phytate at 10 µg/ml was used as a standard. The method used to measure oxalates in the samples was that of (Day & Underwood, 1991). One gram of sample powder was homogenised in 15 ml of 3M H₂SO₄ with stirring for one hour. The mixture was filtered with Whatman paper. The filtrate was titrated with 0.05M KMnO₄ until a stable pink colour was obtained.

2.7 Statistical Analysis

The properties of neglected wild edible fruits and vegetables were assessed before and after lactofermentation. The experiments were conducted in triplicate and provided as mean±standard deviation. XLSTAT version 25.0 was used to perform one-way ANOVA to discover significant mean differences. Duncan's test was performed at a significance level of $\alpha = 0.05$.

3. Results and Discussion

3.1 Biochemical Identification and Screening of Strains Isolates for Functional Properties

The results indicate that 34 isolates with lactic acid bacterial morphological characteristics, in particular a cocci or bacillus morphology, positive to Gram staining and negative to catalase and oxidase tests, were isolated. The isolates were screened for lactic acid bacteria starters by assessing their resistance to acidity and bile salts, as well as their antioxidant and anti-inflammatory activities (Table 1). Screening of isolates as potential starters has highlighted important characteristics for their use as probiotics or starter cultures. Resistance to acidity and bile salts is an important, as these conditions simulate the environment of the gastrointestinal tract (Tamang et al., 2016). Among the 34 isolates, only seven showed good tolerance to acidity, with survival rates ranging from 36.36% to 88.89% at pH 2. This variability suggests that they possess more effective resistance mechanisms, such as regulation of intracellular pH or modification of their cell wall (Ren et al., 2018). Qualitative analysis revealed the ability of the isolates to synthesise exopolysaccharides (Figure 1) and to resist bile salts. The resistance to 0.3% bile salts observed in these isolates is an essential criterion for their probiotic potential. Indeed, the survival in this environment is necessary for the bacteria to colonise the gut and exert beneficial effects (Begley et al., 2005). Of these seven isolates, only two showed antioxidant and anti-inflammatory activities, with values ranging from 27.50±0.00% to 28.73±1.45% and from 27.5±0.35% to 67.15±0.02%, respectively. These isolates were C6 and C26. These isolates were selected for further testing (Table 2). The production of secondary metabolites, such as exopolysaccharides, is a crucial criterion for their antioxidant activities (Wang et al., 2017). These metabolites can help neutralise free radicals and reduce oxidative stress in the body (Zommiti et al., 2018). With regard to anti-inflammatory activity, several scientific experiments indicate that certain strains, in particular *Lactobacillus* and *Bifidobacterium*, possess anti-inflammatory properties by modulating cytokine production and

reducing the expression of inflammatory mediators (Klemashevich et al., 2014). One of the major mechanisms involved is the ability of these strains to interact with immune cells and influence the inflammatory response through the gut-immune system axis (Tsai et al., 2012).

Table 1. Health characteristics of isolated strains

Strains	Resistance to 0.3% bile salts	Exopolysaccharides synthesis	Survival rate (%) to pH 2	Activity antioxidant (%)	Anti-inflammatory activity (%)
C6	+	+++	75.47±0.55 ^b	28.73±1.45 ^a	27.5±0.35 ^b
C18	+++	+++	61.11±2.06 ^c	0±0	0±0
C23	+++	+++	88.89±2.55 ^a	0±0	0±0
C24	+	+++	76.54±5 ^b	0±0	0±0
C26	+++	+++	64.39±1.06 ^c	27.57±0.12 ^a	67.15±0.02 ^a
C30	++	+++	74.87±1.87 ^b	0±0	0±0
C31	+	+++	36.36±2.06 ^d	0±0	0±0

Note. Values with similar superscript alphabet letter along the column are significantly different at $P \leq 0.05$; +: Low; ++: Medium; +++: High.

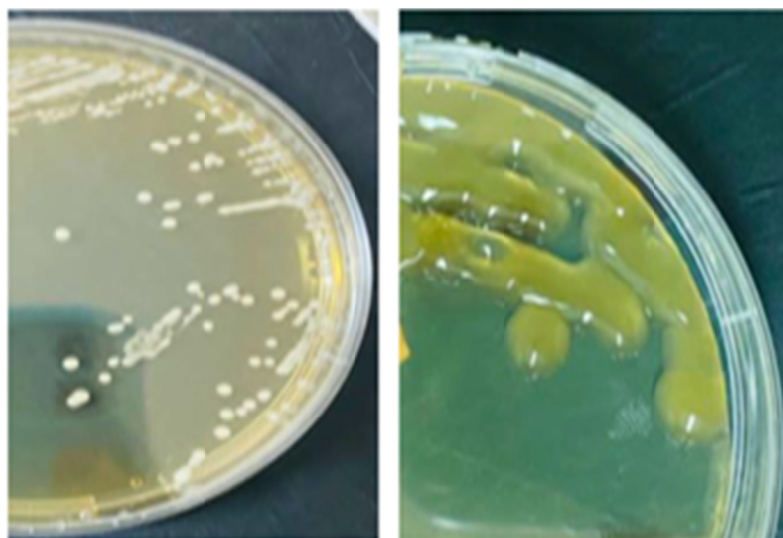


Figure 1. Exopolysaccharide production, appearance of isolates on MRS (left) and (right) on MRS-saccharose agar with exopolysaccharide synthesis (sticky and shiny colony forms)

3.2 Techno-functional Properties and Identification of Selected Isolates

Ribosomal RNA sequencing analysis of the two strains showing antioxidant and anti-inflammatory activities revealed that strains C6 and C26 were identified as *Lactiplantibacillus plantarum* and *Weissella paramesenteroides*, respectively, with similarities of 99% and 98% (Table 2). The enzyme profile analysis showed that two isolates had a similar enzyme profile with regard to the production of tannase, cellulase and phytase. Both strains showed a significant capacity to degrade cellulose and phytic acid. The production of tannase is an important feature that reduces antinutritional effects and improves nutrient bioavailability (Belur et al., 2011). In addition, tannin degradation is thought to be associated with probiotic benefits, by allowing better uptake of polyphenols by the host (Osawa et al., 2000). The ability of the strains to metabolize cellulose gives them an ecological advantage, particularly in the fermentation of fiber-rich materials such as agro-industrial by-products (Abdel-Rahman et al., 2011). This activity could be useful in digesting dietary fiber and improving intestinal health, by contributing to the bioavailability of nutrients trapped in the cellulose matrix of plants (Behera et al., 2018). The phytase activity of both isolates is a particularly sought-after criterion in probiotic and fermentative strains. Phytase degrades phytic acid, an antinutrient present in cereals and legumes, releasing essential minerals such as calcium, iron and zinc (Greiner & Konietzny, 2006). This property could enhance the

nutritional value of fermented foods and play a beneficial role in preventing micronutrient deficiencies. One notable finding was that only *Weissella paramesenteroides* C26 showed strong amylolytic activity, which enables it to degrade starch. This ability allows *Weissella paramesenteroides* C26 to hydrolyse starch into fermentable sugars, favouring its adaptation to environments rich in complex carbohydrates (Falasconi et al., 2020). Amylolysis is an important trait for starter cultures used in the manufacture of traditional fermented products, such as fermented cereal-based beverages (e.g., mahewu and pozol) or sourdough breads (Salas-Millian & Aguayo, 2024). Both strains have the ability to metabolise all sugars (glucose, galactose, sorbitol, threose, xylose) used in the fermentation profile. The raffinose is only degraded by strain C26. The ability of both strains to metabolise a variety of sugars, including hexoses (glucose, galactose), pentoses (xylose, threose) and polyols (sorbitol), gives them an ecological advantage, especially in the fermentation of foods rich in complex carbohydrates (Linares et al., 2016). The strain *Weissella paramesenteroides* C26 is able to metabolise raffinose, suggesting that it possesses enzymes such as α -galactosidase. This enzyme is essential for the metabolism of indigestible oligosaccharides found in legumes and certain cereals, which could give this strain probiotic interest by reducing colonic fermentation and digestive discomfort associated with these sugars (Coda et al., 2012). Both strains have a heterofermentative phenotype. A heterofermentative strain confers technological advantages, as acetic acid and ethanol production contribute to the preservation of fermented foods by increasing their microbiological stability (Gänzle, 2015). In addition, gas production promotes texture and volume development in fermented products such as sourdough bread (Wang & Wang, 2024). They were resistant to concentrations of 2 and 4% NaCl. However, strain C6 was sensitive in the presence of 6.5% NaCl, unlike strain C26 which was resistant at this concentration. The increased salt resistance of strain C26 suggests that it may be better adapted to saline environments and fermentations requiring high NaCl concentrations, which is important for the preservation of fermented foods (Hurtado et al., 2012). In terms of inhibition of pathogenic strains, strain C26 showed a more effective profile than strain C6. In fact, the inhibition diameters recorded for strain C26 ranged from 6.67 ± 0.58 mm to 18.33 ± 1.15 mm, while those for strain C6 ranged from 5.67 ± 0.58 mm to 14.33 ± 0.58 mm. The ability of strain to inhibit pathogens is often due to the production of bacteriocins, hydrogen peroxide and organic acids that lower the pH and inhibit bacterial growth (Settanni et al., 2008). This antimicrobial activity is particularly useful in agri-food and probiotic applications, as it enhances the microbiological safety of food and the protection of the gut microbiota against pathogenic infections (Stephen et al., 2017). In the antibiotic susceptibility test, strains C6 and C26 showed different susceptibilities to the antibiotics tested. Strain C6 was resistant to sulphonamide, nalidixic acid, rifampicin and cephalotin. Strain C26 was resistant to penicillin G and nalidixic acid. The antibiotic susceptibility shows that these strains could be compatible with probiotic applications, as they do not show widespread resistance that could be a problem in clinical or food use (Campedelli et al., 2019; Sharma et al., 2014). In the light of the analyses carried out, the C26 strain was selected for further work.

Table 2. Techno-functional properties and identification of strains

Strains		C6	C26
Fermentative type		Hétérofermentative	Hétérofermentative
Fermentative profile	Glucose	+	+
	Galactose	+	+
	Raffinose	-	+
	Sorbitol	+	+
	Threhalose	+	+
	Xylose	+	+
	2	+	+
NaCl concentration (% w/v)	4	+	+
	6,5	-	+
Titratable acidity (%)		1.72±0.09 ^a	1.86±0.063 ^a
Antagonist test (Inhibition diameter in mm)	<i>Aspergillus</i>	10.67±0.58 ^b	16.33±0.58 ^a
	<i>Staphylococcus</i>	9.67±0.58 ^b	15±1 ^a
	<i>E. coli</i>	5.67±0.58 ^a	6.67±0.58 ^a
	<i>Candida</i>	14.33±0.58 ^a	12.67±1.15 ^a
	<i>Klebsiella</i>	5.67±0.58 ^b	18.33±1.15 ^a
	<i>Salmonella</i>	5.67±0.58 ^b	12.33±0.58 ^a
	<i>Pseudomonas</i>	8.67±0.58 ^b	16.67±0.58 ^a
Enzymatic synthesis (diameter in mm)	Amylases	0.6/M	1.9/H
	Cellulase	1.6/H	3.0/H
	Phytase	1.5/H	2.0/H
	Tannase	0.6/M	0.6/M
Antibiotic inhibition diameters in mm	Chloramphénicol	16/S	24/S
	Pénicilline G	13/I	09/R
	Erythromycine	20/S	20/S
	Kanamycine	12/I	16/S
	Sulfamide	06/R	22/S
	Tigécycline	22/S	20/S
	Acide nalidixique	06/R	06/R
	Rifampicine	06/R	13/I
	Cephalotine	06/R	20/S
Sequence similarity isolated strains and catalogue species	Species	<i>Lactiplantibacillus plantarum</i>	<i>Weissella paramesenteroides</i>
	Max score	854	667
	Query cover (%)	99	98
	Per. Iden (%)	100	100
	Accession number	CP101640.1	MH899266.1

Note. Values are means standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are significantly different at $P \leq 0.05$; (+) for tolerance and (-) for no tolerance; S: Susceptible to antibiotic; I: Intermediate (medium resistance to antibiotic); R: Resistant to antibiotic; M: Medium enzyme producer; H: High enzyme producer.

3.3 Nutritive and Anti-nutritive Properties of Two Plants Fermented by a *Weissella paramesenteroides* Strain C26

The nutritional and anti-nutritional data of *Hydrocotyle bonariensis* leaves and *Garcinia kola* seeds after fermentation by strain C26, *Weissella paramesenteroides*, are given in Table 3. The results show a decrease in the concentration of reducing sugars after fermentation in *Garcinia kola*, from 0.22 ± 0.01 to 0.08 ± 0 mg/ml. After fermentation, an increase in the amount of reducing sugars was observed in *Hydrocotyle bonariensis* leaves, from 0 ± 0 to 0.03 ± 0.01 mg/mL. The reduction in the concentration of reducing sugars in *Garcinia kola* shows that lactic acid bacteria consume the reducing sugars to produce energy via lactic acid fermentation, leading to

the production of lactic acid and other secondary metabolites (Mugula et al., 2003). This reduction may be beneficial in the context of a low-glycemic index diet, as the reduction in simple sugars could reduce blood glucose levels after food ingestion (Anumudu et al., 2024). In contrast to *Garcinia kola*, reducing sugars increase in fermented *Hydrocotyle bonariensis* leaves. This increase could be related to the hydrolysis of complex polysaccharides present in the leaves under the action of enzymes produced by *Weissella paramesenteroides*. Indeed, scientific research has shown that certain lactic acid bacteria possess hydrolytic enzymes capable of breaking down polysaccharides into simpler sugars, making the latter more bioavailable (Wang et al., 2021). However, the concentration of total sugars in the two plants did not appear to vary at the end of fermentation. The fact that the total sugar concentration of both plants does not change significantly after fermentation corroborate with other studies on the fermentation of plant matrices by strains of this group, where the enzymatic action results in the conversion of sugar forms rather than total consumption (Verni et al., 2022). There was a significant increase in the concentrations of total polyphenols, flavonoids and tannins after fermentation of *Hydrocotyle bonariensis* leaves and *Garcinia kola* seeds. A significant reduction in oxalate levels was observed after fermentation of *Hydrocotyle bonariensis* leaves and *Garcinia kola* seeds, from 968 ± 88 to 557.33 ± 25.4 mg/mL and from 498.67 ± 23.2 to 234.67 ± 22.4 mg/mL, respectively. A decrease in phytate concentration was also observed in both matrices, although this decrease was not statistically significant. The increase in total polyphenols, flavonoids and tannins after fermentation could be due to the activity of microbial enzymes, such as tannases and cellulases, capable of hydrolyzing these complexes and releasing the polyphenols in their free, more bioavailable form (Filannino et al., 2015). Furthermore, increasing their concentration after fermentation could enhance the antioxidant potential of *Hydrocotyle bonariensis* and *Garcinia kola* extracts, making them more interesting for nutraceutical applications (Alvarez-Martinez et al., 2020). This result is in line with studies showing that fermentation improves the availability of polyphenols in several foods, such as cocoa, tea and cereal grains (Rollán et al., 2019).

Table 3. Nutritional properties of the two plants after controlled fermentation by *Weissella paramesenteroides* strain C26

Parameters	<i>Hydrocotyle bonariensis</i> leaves		<i>Garcinia kola</i> nuts	
	Unfermented	Fermented	Unfermented	Fermented
Phytates (mg/mL)	8.52 ± 1.23^a	7.12 ± 0^a	6.84 ± 0.31^a	6.66 ± 0.55^a
Oxalates (mg/mL)	968 ± 88^d	557.33 ± 25.4^c	498.67 ± 23.2^b	234.67 ± 22.4^a
Tannins (mg/mL)	0.47 ± 0.06^{ab}	1.19 ± 0.12^a	2.38 ± 0.12^{ab}	3.54 ± 0.01^a
Total polyphenols (mg/mL)	0.19 ± 0^{ab}	0.97 ± 0.05^a	0.33 ± 0.01^{ab}	0.87 ± 0^a
Flavonoides (mg Quercetin/mL)	0.003 ± 0^{ab}	0.007 ± 0^a	0.03 ± 0.001^{ab}	0.062 ± 0.001^a
Reducing sugars (mg/mL)	0 ± 0^{ab}	0.03 ± 0.01^a	0.22 ± 0.01^{ab}	0.08 ± 0^a
Total sugars (mg/mL)	0.04 ± 0^{ab}	0.05 ± 0^{ab}	0.38 ± 0.05^{ab}	0.38 ± 0.05^{ab}

Note. Values represent means and standard deviations of triplicate measurements. Values with the same superscript in a column are significantly different at $P \leq 0.05$.

3.4 Protein, Antioxidant and Anti-inflammatory Content of the Two Fermented Plants by a *Weissella paramesenteroides* Strain C26

Figure 2 shows the protein content, anti-inflammatory and antioxidant activities of both fermented and unfermented plants. All matrices show the presence of protein as well as anti-inflammatory and antioxidant activities. There is an increase in these compounds after fermentation compared to unfermented matrices. The protein concentrations of the fermented matrices increased significantly, from 6 ± 0.02 to 8.68 ± 0.02 g/100 g dry matter (DM) for *Hydrocotyle bonariensis* leaves and from 8.21 ± 0.01 to 11.12 ± 0.02 g/100 g DM for *Garcinia kola* seeds. The anti-inflammatory activities of the fermented matrices also increased to $72.96 \pm 0.89\%$ and $98.11 \pm 2.67\%$. With regard to antioxidant activities, an increase in content was also observed after fermentation of *Hydrocotyle bonariensis* leaves, from 60.74 ± 4.09 to $83.55 \pm 0.68\%$. However, the concentration seems to decrease after fermentation of *Garcinia kola* seeds, this decrease was not significant. The increase in anti-inflammatory activity after fermentation may be related to the conversion of certain phenolic compounds into more active forms, enhancing their anti-inflammatory role (Filannino et al., 2015). These observations are in line with studies on tea and soy fermentation, which show improved anti-inflammatory properties through the generation of active polyphenols and bioactive peptides (Abedin et al., 2024).

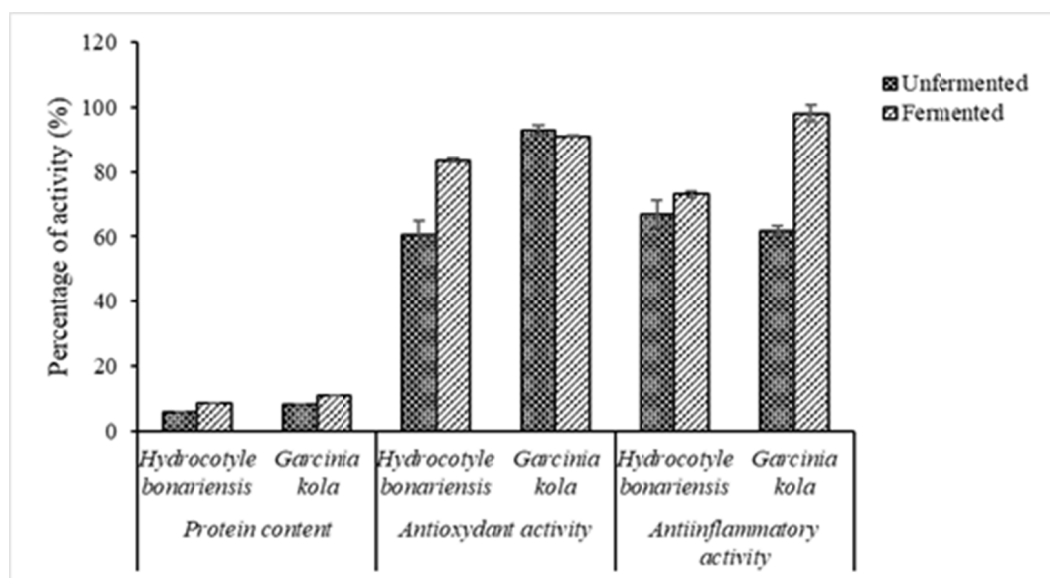


Figure 2. Protein content, anti-inflammatory and antioxidant activities of both fermented and unfermented plants

3.5 Vitamin and Mineral Composition of the Two Fermented Plants by a *Weissella paramesenteroides* Strain C26

After fermentation, the fermented products were found to have a higher concentration than the unfermented products (Table 4). Vitamin levels in *Hydrocotyle bonariensis* increased significantly after fermentation, with an increase to 1.17 ± 0.01 mg/100 g dry matter for vitamin A, 0.04 ± 0 mg/100 g dry matter for vitamin D and 2.08 ± 0.01 mg/100 g dry matter for vitamin E. Mineral concentrations measured in the same matrix increased significantly after fermentation. For *Garcinia kola* fruit, the chromium content increased as well as vitamin A and E after fermentation. The vitamin D content remained constant during the fermentation. Concerning the minerals (chromium, magnesium, zinc, sodium, potassium) there was an important increase in concentrations after fermentation (Table 4). The increase in vitamin A, D and E content in fermented matrices could be explained by several mechanisms, in particular the degradation of phytates and oxalates (Adebo et al., 2022). These anti-nutritional compound complex with minerals and vitamins, making them unavailable. In the course of this work, it was observed that fermentation of the matrices significantly reduced the levels of these anti-nutritional compounds. These result suggest that certain mineral and vitamins are initially complexed by these anti-nutritional compounds; thereby limiting their bioavailability. Lactofermentation would therefore have made these foods more nutritious. This technique could therefore be recommended for the treatment of neglected fruits and vegetables used in the prevention of a number.

Table 4. Vitamin and mineral content of fermented by *Weissella paramesenteroides* strain C26 and unfermented matrices.

Vitamin and mineral content (mg/100 g dry matter)	<i>Hydrocotyle bonariensis</i> leaves		<i>Garcinia kola</i> nuts	
	Unfermented	Fermented	Unfermented	Fermented
Chromium	0.07 ± 0.02^c	0.6 ± 0.01^b	0.6 ± 0.01^b	0.7 ± 0.01^a
Magnesium	190.67 ± 1.56^b	194.4 ± 1.11^a	10.63 ± 0.02^d	20.4 ± 0.02^c
Zinc	0.09 ± 0.01^b	1.28 ± 0.01^a	0.03 ± 0^c	0.04 ± 0^c
Sodium	21.61 ± 0.02^b	23.72 ± 0.25^a	3.5 ± 0.01^d	4.36 ± 0.01^c
Potassium	19.63 ± 0.12^b	22.43 ± 0.12^a	2.46 ± 0.01^d	3 ± 0.02^c
Vitamin A	0.87 ± 0.24^b	1.17 ± 0.01^a	0.73 ± 0.12^b	0.83 ± 0.12^b
Vitamin D	0.03 ± 0^c	0.04 ± 0^b	0.06 ± 0^a	0.06 ± 0^a
Vitamin E	1.85 ± 0.01^b	2.08 ± 0.01^a	0.58 ± 0.01^d	0.75 ± 0.01^c

Note. The values are means standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are significantly different at $P \leq 0.05$.

4. Conclusion

This study highlights the nutritional and functional improvements in *H. bonariensis* and *G. kola* following controlled fermentation by *W. paramesenteroides*. The results show an increase in protein and essential minerals, as well as a reduction in anti-nutritional factors. Fermentation also led to significant improvements in antioxidant and anti-inflammatory properties. These results suggest that lactic fermentation could enhance the therapeutic potential of these neglected food plants. While these properties are associated with beneficial effects in the context of chronic diseases such as hypertension, further research is required to confirm whether they can contribute to the prevention or management of arterial hypertension.

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Authors Contributions

The concept was developed by H.D.O. and A.A.A. The methodology was designed by all authors. Data acquisition and analysis were carried out by H.D.O., K.E.N., S.G.S. and C.C. The manuscript was drafted by H.D.O. Critical revision of the manuscript was undertaken by S.N. and C.C. Funding was provided by H.D.O. and A.A.A., under the supervision of A.A.A., S.N. and C.C. Final approval was given by C.C.

Finding

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed Consent

Obtained.

Ethics Approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

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Data Sharing Statement

No additional data are available.

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