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Original Research Article

Effect of Lactic Acid Bacteria on the Quality Attributes of Tea-Like Product from Blends of Drumstick *(Moringa oleifera)* Leaves and Ginger *(Zingiber officinale)*

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Abstract

Moringa oleifera leaves and Ginger crops still remain an underutilized commodity because of limited information on its wider use. *Moringa oleifera* is very rich in nutrition but has undesirable taste and flavor. However, addition of ginger takes the edge off the bitter spinachy taste of *Moringa oleifera* leaves. Lactic acid bacteria (LAB) are important microbes that have long been used in both traditional and modern industrial food fermentation. Lactic acid bacteria can be used for production of food due to their ability to coax microorganisms into producing In the process of food fermentation; microorganisms produce enzymes to break down complex compounds to simple bio-molecules for several biological activities such as proteinase, amylase, mannase, cellulase, and catalase. The aim of this research work is to determine the effect of Lactic acid bacteria (LAB) fermentation on the quality attributes of tea-like beverage produced from blends of *Moringa oleifera* leaves and Ginger*(Zingiber officinale).* The results showed that The phenotypic and biochemical characteristic of Blends of Drumstick leaves (*Moringa oleifera*) and Ginger (*Zingiber officinale*) reveals that the isolates were presumptively categorized to belong to the genus *Lactobacillus,* the isolates are gram positive, cocci, rod shaped, either, short rod or long rods, non-sporulating, ferments carbohydrate, non-motile and catalase negative. Technological properties of isolates are the properties that indicate the quality of the isolate to be used as a starter culture in the course of fermentation. A total of 35 colonies were isolated from the blend samples. Of them, 3 colonies were identified as *Lactobacillus* species, through observation of phenotypic characters, and identification, It was found that *L. plantarum* had tolerance under acid conditions, as its viable cell count remained at pH 2.0 and under 1.0 % bile salt condition, after 72 h incubation time. The proximate and energy content of fermented beverage produced from the blends shows that percent Dry matter, Moisture content, Crude protein, Ether extract , Crude fibre, Ash, carbohydrate and energy value were ranged from 10.00- 30.00, 4.0-5.0, 35.00 -71.00, 3.0-5.0, 11.00 - 18.00,3.0-10,7.0-15 and 74Kcal - 396Kcal respectively. The study concludes that The phenotypic and biochemical characteristic of blends of drum stick leaves (*Moringa oleifera*) and Ginger (*Zingiber officinale*) reveals that the isolates were to belong to the genus *Lactobacillus.* Also, Many of the LAB metabolites such as low molecular weight acids, alcohols, carbon dioxide, diacetyl and hydrogen peroxides have broad spectrum antimicrobial activity against other species of bacteria. The proximate and energy value of the beverage plays a crucial role in assessing its nutritional significance ($P<0.05$). Consequently, the fermentation of these blends with LAB species might provide a means of retaining more of the nutrients that are trapped in the matrix of the cells of these plant materials.

Keywords: *Moringa oleifera*, Ginger, lactic acid, Bacteria, fermentation.

1.0 INTRODUCTION

The world of tea and tea-like beverages offer consumers as much varieties and choices as wine or whisky (Mosher and Trantham, 2017). The production of this healthy beverage has evolved over centuries. In over 40 countries today, the art of tea and tea-like beverage manufacture brings a wealth of flavor and aroma to suit all occasion and all times (Zhang, 2021).Next to water, tea and tea-like beverages are the most common beverage of choice throughout the world, whether black, green or Oolong. Tea comes from a plant, called *Camellia sinensis*. Difference in color and

flavor depends on processing (Samanta, 2020).Tea leaves produce different components of rare and valuable substances such as caffeine, theobromine, theophylline, tannin (catechins), essential oils and different vitamins. The chemical composition of tea leaves has been demonstrated to have peculiar features that are responsible for important pharmacological and physiological properties (PreetiArya and Guarve, 2019). Tea is known as an excellent diaphoretic that stimulates metabolic processes in human body and plays a certain prophylactic role. Tea and tea-like beverage has proven to be one of the best drugs against mental fatigue due to the presence of tea alkaloids which includes Caffeine, Theobromine, Theophylline, they also contributes to cerebral vasodillation (Echeverri, Montes, Cabrera, Galán, and Prieto, 2010). Tea and Tea-like beverages produced from blends of *Moringa oleifera* leaves and ginger rhizomes appears to have potential health benefits, perhaps derived from its flavonoids. Flavonoids and other polyphenols, which are phytonutrients work as antioxidants that may help protect body cells from damage done by free radicals (Jamshidi-Kia *et al.*, 2020).

Generally, food and food substrates that undergo fermentation are invaded or overgrown by edible microorganisms whose enzymes, principally amylases, proteases and lipases; hydrolyze polysaccharides, proteins and lipids responsible to produce fermented food (Abormegah, 2019). Moreover, these enzymes also improve enjoyable flavors, aromas and textures which are more attractive to the human consumption (Maryam, Datsugwai, and Shehu, 2017).

Moringa oleifera is used as an alternative to imported food supplements to treat and combat malnutrition, especially among infants and nursing mothers, by virtue of its chemical constituents in developing nations (Jasper *et al*., 2017).*Moringa oleifera* has been found to be a good source of polyphones and antioxidants (Arise *et al*., 2010). Vanillin, omega fatty acids, and carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin are Phytochemicals that have been reported in the flowers, roots, fruits, and seeds of Moringa (Mumtaz, Kausar, Hassan, Javaid, & Malik, 2021). The leavesof *Moringa oleifera*, in particular, have been found to contain phenolics and flavonoids (Lin *et al*., 2018); these compounds, phenolics and flavonoids, have various biological activities, including antioxidant, anti-carcinogenic, immunomodulatory, antidia-betic, antiatherogenic, and heat to protective functions and the regulation of thyroid status (Parcheta *et al*., 2021). *Moringa oleifera* leaves contain trace elements that are essential to human health (Yabalak, 2018). These trace elements which includes, magnesium, iron, selenium, and zinc play an important role in metabolism. There has also been an increased interest in these elements together with reports relating trace element status and oxidative diseases (Gul *et al*., 2021). Therefore, it is very important to identify the mineral composition of *Moringa oleifera* leaves that are widely consumed by humans and animals.

Ginger has been used as a spice as well as medicine in India and China since ancient times. Ginger has been reported to be used to treat various types of other gastro-intestinal problems like morning sickness, colic, upset stomach, gas, bloating, heartburn, flatulence, diarrhea, loss of appetite, and dyspepsia (discomfort after eating) (Raut *et al.*, 2020). According to Indian Ayurvedic medicinal system, ginger is recommended to enhance the digestion of food (Ali *et al.*, 2008).

Besides these, ginger has been reported as a pain relief for arthritis, muscle soreness, chest pain, low back pain, stomach pain, and menstrual pain (Anh *et al*., 2020). Ginger can be used for treating upper respiratory tract infections, cough, and bronchitis. As an anti-inflammatory agent, it is recommended for joint problems (Timba *et al.*, 2019). Fresh juice of ginger has been shown to treat skin burns. Active component of ginger is used as a laxative and antacid medication. It is also used to warm the body for boosting the circulation and lowering high blood pressure. Because of its warming effect, ginger acts as antiviral for treatment of cold and flu (Qidwai *et al.*, 2003). Ginger is also used as a flavoring agent in foods and beverages and as a fragrance in soaps and cosmetics (Alam, 2013).

Lactic acid bacteria (LAB) are important microbes that have long been used in both traditional and modern industrial food fermentation (Adesulu-Dahunsi *et al*., 2020). There is addition of flavor by many food products fermented by LAB, such food products as yogurt, cheese, pickled cabbage and kefir milk are believed to convey health benefits (Hu *et al*., 2020). The health benefits which Lactic acid bacteria convey include stimulation of the human immune system and antimicrobial activity (Moradi *et al*., 2020). The addition of LAB to food products has increased in recent years because LAB constitute the biggest group of probiotic bacteria used and although they may be somewhat present in some foods, they may be externally added for a more consistent product. Many of the LAB metabolites such as low molecular weight lactic acids, alcohols, carbon dioxide, diacetyl and hydrogen peroxides have broad spectrum antimicrobial activity against other species of bacteria (Helander *et al*., 1997). This research focused on the Isolation and characterization of lactic acid bacteria (LAB) species from blends of *Moringa oleifera* leavesand *Ginger rhizome* (*Zingiber officinales)* with the application of isolates from the blend with the highest yield of lactic acid to various blends of *Moringa oleifera*leaves and *Ginger rhizome* (*Zingiber officinales).* These isolates will serve as the main ingredient that will modify the nutritional, prophylactic, diaphoretic, pharmacological and physiological properties of the product.In this

circumstance, the use of *Moringa oleifera* leaves and ginger rhizome for making tea-like beverage products containing LAB is expected to provide more beneficial effect.

2.0 MATERIALS AND METHODS

2.1.1 Materials:

The drum stick (*Moringa oleifera)* leaves and Ginger (*Zingiber officinale*) were obtained from kanti market Kazaure near the Hussaini Adamu Federal Polytechnic Kazaure, Jigawa state.

2.1.2 Formulationof blends for Isolation and Characterization of Lactic Acid Bacteria from blends of *Moringa oleifera* and Ginger

The Drum stick (*Moringa oleifera)* leaves were plucked from the stalk, weighed, washed, weighed again and wet milled using attrition mill. The slurry was divided into two portions. The ginger rhizome were selected, washed to remove adhering dirt, peeled carefully to avoid losing the essential oil which is situated close to the peel, weighed and wet milled using attrition mill to obtain a slurry. The slurry was portioned into two. Each portion from *moringa oleifera* and ginger rhizome were mixed to obtain blends proportions of 100:0(moringa/ginger), 90:10(moringa/ginger), 80:20(moringa/ginger), 70:30(moringa/ginger) 60:40(moringa/ginger) respectively. One part of the blends was subjected to fermentation for 5days to be able to obtain build up of lactic acid bacteria.

Table 1: Sample Formulation of Blends for Isolation and Characterization of LAB

S/N	Drumstick leaves(g) (*Moringa oleifera*)	Ginger(g) (*Zingiberofficinale*)	Blend ratio	Blend code
1	100	0	100:0	MG100
2	90	10	90:10	MG90
3	80	20	80:20	MG80
4	70	30	70:30	MG70
5	60	40	60:40	MG60
6	50	50	50:50	MG50

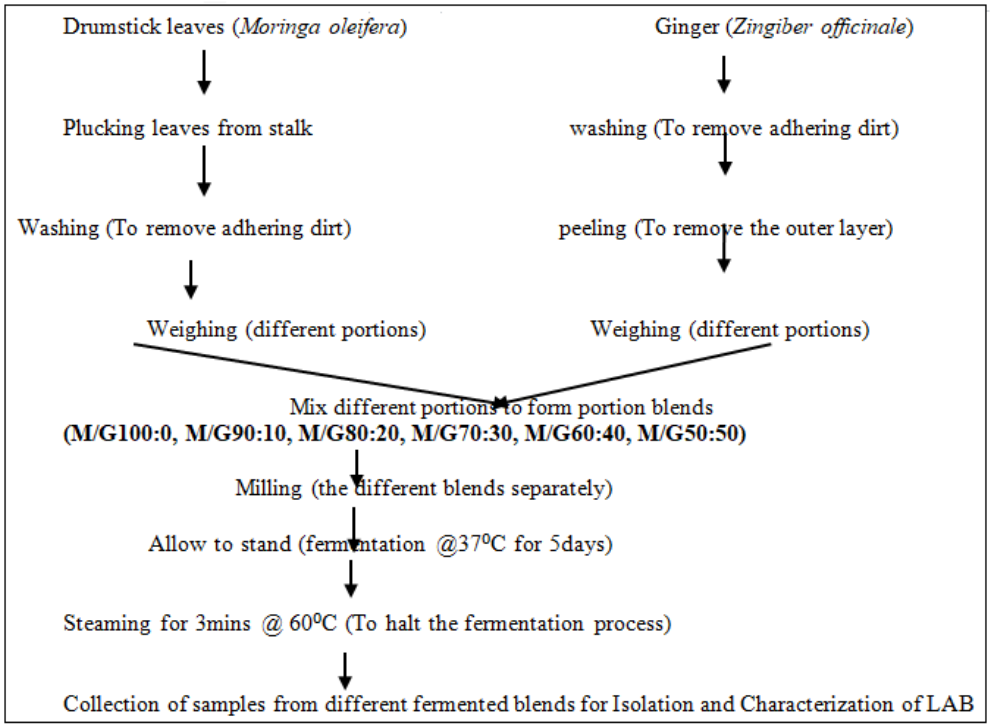

Fig 1: Flow Chart of Blends Formulation for Isolation and Characterization of LAB

2.1.3 Isolation and Characterization of Lactic Acid Bacteria from Blends of *Moringa oleifera* leaves Ginger

The bacterial isolates were characterized based on their cultural and biochemical properties which included production of coagulase, catalase, indole, urease, motility test, citrate utilization test, starch hydrolysis, Methyl Red-Voges Proskaeur (MR-VP), triple sugar iron test, utilization of sodium azide and various carbohydrates (glucose, lactose, maltose, fructose, mannitol, sucrose, and arabinose). The isolates were identified to the species level by comparing their characteristics with those of known Taxa, as described

by Buchanan and Gibbons (1974) in Bergey's Manual of Determinative Bacteriology (Krieg and Holt, 1974).

2.1.4 Serial Dilution

The samples were aseptically weighed and homogenized. From each sample, a 1:10 dilution was subsequently made using peptone water followed by making a 10 fold serial dilution. 0.1ml of each dilution which was sub-cultured in duplicates, into the M17, MRS Agar (Man, Rogosa and Sharpe agar. Merck, Germany) used for isolating lactic acid bacteria (Badis *et al*., 2004a; Guessas and Kihal, 2004).

The sub-cultures were identified according to their morphological, cultural, physiological and biochemical characteristics (Kandler and Weiss, 1986; Sharpe *et al*., 1979). The used test were Gram reaction; production of catalase, cytochrome oxidase and hydrogen peroxide; growth at 15ºC and 45ºC in 1week; acid production from carbohydrates (1%w/v) –L-arabinose, cellobiose, D- fructose, D-galactose, esculin, lactose, maltose, melabiose, mannitol, D-mannose, raffinose, rhamnose, D- ribose, salicin, sorbitol, sucrose, trehalose and D-xylose in MRS broth devoid of glucose and beef extract with chlorophenol red as indicator; production of acid and gas from 1% glucose (MRS broth without beef extract); methyl red and Voges-Proskauer test in MRVP medium; H&L test in O/F mdedium; production of ammonia from arginine; nitrate reduction in nitrate broth; Indole production in Tryptone broth and growth on ace.

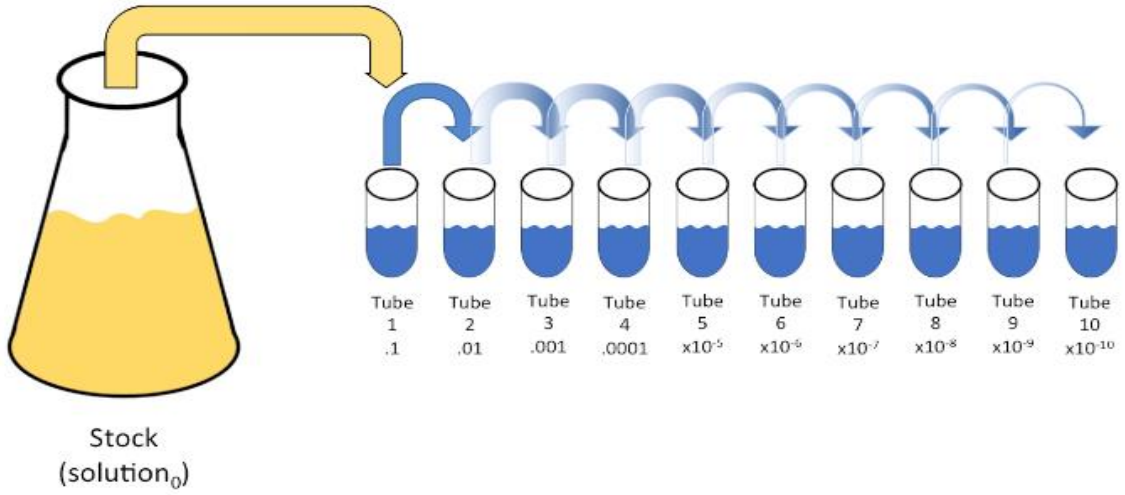

Figure 2: Serial Dilution

Source: JoVE Science Education Database. *Microbiology.* Serial Dilutions and Plating: Microbial Enumeration. JoVE, Cambridge, MA, (2020)

2.1.5 Culture Preservation

Cultures of LAB isolated were sub cultured into nutrient medium. The stock cultures were stored on agar slants at 4°C for subsequent use.

2.1.6. Morphological Characterization of Bacterial Isolates

Different tests were carried out on isolates, which include; Gram reaction, Spore staining, Coagulase, Motility, Oxidase, Indole production, Methyl red, Voges Proskauer, Sugar fermentation, Citrate utilization, Catalase test and test for H_2S gas production (Fawole and Oso, 2004).

2.1.7. Gram Staining Procedure

A thin smear of the bacteria cell was made on a clean glass slide and heat fixed by passing the slide over blue flame. The slide was flooded with a crystal violet (Primary stain)for 30-60 seconds and then washed under slow running clean tap water, a Lugol's iodine which act as a mordant was added for 30-60 seconds and then washed under slow running clean tap water, absolute alcohol was added for about 3 seconds to decolourize purple dye-iodine complexes from the bacteria, then washed under slow running clean tap water, safranin which is a secondary stain was added for 2 minutes, and then washed under slow running clean tap water and allowed to air dry. A drop of oil immersion was placed on the stained smear and examined under microscope using oil immersion objective (X 100) (Fawole and Oso, 2004).

2.1.8 Motility Test

Double strength of nutrient broth was prepared, and dispensed into test tube and corked, the test tubes were then sterilized at 121^{o}C for 15 minutes, after sterilization the broth was allowed to cool and the bacteria was inoculated into the broth and incubated at 37°C for 24hours. A clean glass cavity slide and cover slip were used in which Vaseline was applied to all edges of the cover slip; this allows the adhesion of the cover slip to the cavity slide. The culture broth inside each test tube was shaken and inoculating loop was used to transfer two loopful of the culture broth into the

cavity slide and covered with cover slip. The cavity slide was inverted quickly in order for the drop not to run off to one side and if it was examined under x40 objective microscope (Fawole and Oso, 2004).

2.1.9 Spore Staining Test

On a clean grease free slide, a smear of the isolate was made. It was later stained with 5% malachite green for ten minutes. The stain was washed off with distilled water, 0.5% aqueous safranin was used to counter stain for 15 minutes. This was washed off with distilled water and allowed to dry. Spore stained green while the body of bacterial stain red when viewed under the microscope (Fawole and Oso, 2004).

2.2 TECHNOLOGICAL PROPERTIES OF LAB ISOLATES (FOR SELECTION OF SUITABLE ISOLATES).

Technological properties of isolates are those properties that indicate the quality of the isolate to be used as a starter culture in the course of fermentation. These properties were investigated to find the suitable or appropriate starter culture for use in the fermentation of the tea-like beverage products from blends of drum stick leaves (*moringa oleifera)* and ginger (*Zingiber officinale).* The properties that were analyzed include:

2.3 Determination of Lactic acid, Hydrogen peroxide and Diacetyl production by LAB isolates.

For accurate measurements, the test organisms were grown in MRS broth: The broths were inoculated with 0.1 ml of a suspension of LAB specie and incubated. Incubation was for 72 h at 37°C. Cultures were centrifuged at 3000 rpm for 15min. Known volume of the supernatant fluid was used for all the titrations at specified time interval (Hujanen and Linko, 1996).

2.3.1. Quantitative Estimation of Lactic acid

The quantity of lactic acid produced by antimicrobial producing isolates at 24hrs, 48hrs and 72hrs was determined by transferring 25ml of broth cultures of test organisms into 100 ml flasks. The production of lactic acid was determined by titration with 0.25 mol/L NaOH and 1 ml of phenolphthalein indicator. The titratable acidity was calculated as lactic acid (% v/v) (Fortina *et al.*, 1990). Each millilitre of I N NaOH is equivalent to 90.08mg of lactic acid. The titratable acid was then calculated according to A.O.A.C. method (1995).

$$\text{Titratable acidity (\% Lactic acid)} = \frac{\text{ml NaOH x N NaOH x M.E. x 100}}{\text{Volume of sample used (ml)}} \quad \text{Equation1}$$

Where: ml NaOH= Volume of NaOH used; N NaOH= Normality of NaOH solution; M.E= Equivalence factor.

2.3.2 Determination of Diacetyl Formation

Diacetyl production was determined by transferring 25 ml of broth cultures of test organisms into 100 ml flasks. Seven and half millilitres of 1m Hydroxylamine solution was added to the flask and to a similar flask for residual titration. Both flasks were titrated with 0.IN HCL to a greenish yellow end point using bromophenol blue as indicator. The equivalence factor of HCL to diacetyl is 21.52 mg. The concentration of diacetyl produced was calculated according to the method of Food Chemicals Codex (1972).

$$AK = \frac{(b - s)\,(100E)}{W} \quad \text{Equation 2}$$

Ak = Percentage of diacetyl; b = No of ml of 0.IN HCl consumed in titration of sample; E = Equivalence factor; W = Volume of sample; S = No of ml of 0.IN HCl consumed in titration of sample.

2.3.3. Quantitative Estimation of Hydrogen Peroxide Formation

Hydrogen Peroxideproduction was determined by transferring 25ml of broth cultures of test organisms into 100 ml flasks. Twenty ml of dilute sulphuric acid was added to 25ml of the supernatant and titration was carried out with 0.1M potassium permanganate which is equivalent to 1.7mg of hydrogen peroxide. A decolourization of the sample was regarded as the end point (A.O.A.C., 1995).

$H_2O_2+2KMNO_4+3H_2S0_4 \rightarrow K_2SO_4+4H_2O+O_2$

$$H_2O_2 = \frac{\text{mlKMNO}_4 \times \text{NKMNO}_4 \times \text{M.E} \times 1OO}{\text{ml}H_2SO_4 \times \text{Vol of sample}} \quad \text{Equation 3}$$

ml $KMNO_4$= Volume of Sample used
$NKMNO_4$= Normality of $KMNO_4$
$mlKMNO_4$= Volume of H_2S0_4 used
M.E: Equivalent factor.

2.3.4 Acidification activity

MRS broth was inoculated with a 24h old culture of each isolate and incubated at 37 ºC for 72h. At intervals of 24, 48 and 72h, the culture was centrifuged at 3000 × g for 5min and the supernatant recovered was used for pH measurement using a pH meter. Change in pH was calculated as the change in pH from an initial 6.05 which was the pH of the MRS broth at the time of inoculation

2.4. Biochemical Characterization of Bacterial Isolates

2.4.1 Coagulase Test

A colony from the clinical organism was emulsified in normal saline on clean grease free slide and an equal volume of plasma were added and mixed

together aseptically. Clumps or precipitate in the mixture indicates a positive coagulase test, this shows that the organism produces coagulase enzyme while the absence of clumps gives a negative result (Fawole and Oso, 2004).

2.4.2 Catalase Test

A sterile wire loop was used to pick a colony of the organism of 24 hour old culture and placed in a drop of 3% hydrogen peroxide on a clean glass slide. Effervescence caused by the liberation of oxygen as a gas bubble indicates the production of catalase by the bacterium while a negative result indicates the absence of catalase (Fawole and Oso, 2004).

$$2H_2O_2\ (l) \longrightarrow 2H_2O\ (l) + O_2\ (g)$$

2.4.3 Oxidase Test

A piece of filter paper was placed in a sterile petri dish and 2-3 drops of freshly prepared oxidase reagent was added. Using a sterile glass rod, a colony of the test bacterium was picked and smeared on the filter paper and was observed for 10 seconds. The presence of blue-purple colour indicates a positive oxidase while no blue-purple color indicates anegative oxidase test (Fawole and Oso, 2001).

2.4.4 Urease Test

A little of the culture of the test bacteria was streaked over the surface of the agar slant of urease test medium with phenol red as indicator and incubated at 37°C for 7 days. A control of the basal medium containing no added urea was equally inoculated. A color change of the medium from yellow to pink or red was an indication of a positive result and no color change indicate a negative result (Fawole and Oso, 2001).

2.4.5 Indole Production

Sterile peptone water in tubes was inoculated with test isolate in a water bath for 48 hrs and 0.5ml of kovac's reagent was added to the culture and shook gently. A red color on the surface implied a positive reaction (Olutiola *et al*., 2000).

2.4.6 Citrate Test

Simmon's citrate agar was used for this test. The medium was prepared in bijou bottles and it was allowed to cool and the isolate was introduced and incubated, a change in the color of medium from green to blue was a positive test for citrate utilization.

2.4.7 Sugar Fermentation

Glucose, fructose, sucrose, lactose, maltose and mannitol were used for the fermentation test. 10ml of nutrient broth containing 1% of fermentable sugar was placed in a test tube. Phenol red was added as indicator and a Durham tube was inserted into each tube. All tubes were sterilized in an autoclave at 121^{o}C for 15 minutes. After cooling, tubes were inoculated and incubated at 30oC for 5-7 days and examined daily. Acid production was indicated by change in color from red to yellow. Gas produced accumulated in the Durham tube (Fawole and Oso, 2004).

2.4.8 Starch Hydrolysis

Nutrient agar plus 1% soluble starch was used for this test. The medium was sterilized and poured into sterile Petri dishes and allowed to solidity. Inoculation of test organism was done by streaking once across the plate and then incubated at 37^{o}C for 3-5 days. After 3 days of incubation, the plates were flooded with gram's iodine. Unhydrolized starch formed blue or blue black colour while area of hydrolysis appeared as brownish or clear zone. Reddish brown zones around the colony indicated partial hydrolysis of starch to dextran, which results from apha-amylase activity (Abiola and Oyetayo, 2016).

2.5 Characterization and Identification of Lactic Acid Bacterial Isolates

The bacterial isolates were characterized based on their cultural and biochemical properties which included production of coagulase, catalase, indole, urease, motility test, citrate utilization test, starch hydrolysis, Methyl Red-Voges Proskaeur (MR-VP), triple sugar iron test, utilization of sodium azide and various carbohydrates (glucose, lactose, maltose, fructose, mannitol, sucrose, and arabinose). The isolates were identified to the species level by comparing their characteristics with those of known taxa, as described by Buchanan and Gibbons (1974) in Bergey's Manual of Determinative Bacteriology (Krieg and Holt, 1974).

Dilutions of the resultant solution (10-fold to 10000-fold) were spread directly onto the surface of MRS agar plates containing 20g kg−1 NaCl. Plates were incubated under anaerobic conditions (Mitsubishi Anaero Pak System, Pack-Anaero; Mitsubishi Gas Chemicals, Tokyo, Japan) at 30°C for 3–5 days. Colonies of acid-producing bacteria, identified by a clear zone around each colony, were randomly selected from MRS plates and purified by replating on MRS agar plates. Colonies were re-selected and initially examined for Gram staining and the production of catalase. Only Gram-positive, catalase-negative strains were selected. The selected strains were stored at−80°C ina100gkg−1 skim milk broth.

2.6 Proximate Composition

Proximate compositions of the samples were determined using the AOAC methods (AOAC, 2005). The moisture content (MC) was determined by drying samples in an oven at 105°C for 24 hours to obtain %MC. Crude protein percentage was determined using the Kjeldahl method with the Kjeltec 8400 analyzer unit (FOSS, Sweden) and the percentage nitrogen (%N) obtained was used to calculate the percentage crude protein (% CP) using the relationship: % CP = % N X 6.25. Ether extract percentage was determined using

Soxhlet system HT-extraction technique of AOAC methods (AOAC, 2005). The percentage ash (%) was determined by incinerating the samples in a muffle furnace at 550°C for 4hrs. The ash was cooled in a desiccator and weighed. Crude fiber percentage (% CF) was determined by dilute acid and alkali hydrolysis of AOAC methods (AOAC, 2005). Carbohydrate was calculated by difference.

2.7. Energy Value:

The bomb calorimeter was used to calculate the energy value of the product.

2.8 Statistical Analysis

The data was statistically analyzed at P-value ($p<0.05$) significantly accepted and a comparison between the groups was performed using one-way analysis of variance (ANOVA) by Graphpad instat3 software (2000) version 3.05 by Graphpad Inc. The data are given as the mean ± standard deviation.

3.0 RESULTS AND DISCUSSION

3.1 Morphological andBiochemical Identification of Lactic acid Bacteria Isolates from Blends of Drumstick leaves (*Moringa oleifera*) and Ginger (*Zingiber officinale*).

The phenotypic and biochemical characteristic of Blends of Drumstick leaves (*Moringa oleifera*) and Ginger (*Zingiber officinale*) reveals that the isolates were presumptively categorized to belong to the genus *Lactobacillus*; this was done by particular reference to Bergey's Manual of systematic bacteriology (2000) according to their phenotypic profiles. The non-spore forming Gram-positive isolated bacterial strains showed rod shaped morphology under a microscope, and formed round milky colonies on Man, Rogosa and Sharpe (MRS) agar. The table shows that isolates are gram positive, they are cocci, rod shaped, either, short rod or long rods, non-sporulating, ferments carbohydrate, non-motile and catalase negative (Table 2).

Table 2: Morphological andBiochemical Identification of Lactic acid Bacteria Isolates from Blends of Drumstick leaves (*Moringa oleifera*) and Ginger (*Zingiber officinale*) Probable Isolates

Properties	***Lactococcus lactis***	***Lactobacillus plantarum***	***Lactobacillus acidophilus***
Gram Rxn	+	+	+
Cell Shape	Cocci	Short rods	Long rods
Arrangement of cells	Singly in pairs and chains	Singly in pairs and chains	Singly
Indole	-	-	-
Citrate	+	+	-
Spore	-	-	-
Motility	-	-	-
Glucose	+	+	+
Starch Hydrolysis	-	+	-
Mannitol	+	+	+
Lactose	-	+	+
Sucrose	+	-	+
Maltose	-	+	+
Catalase	-	-	-
Arabinose	-	+	+
Xylose	-	+	+
Urease	+	-	-
Oxidase	+	-	-
Coagulase	NT	NT	NT

KEY – Means absent, + means present; NT mean Not detected.

3.2 Technological Properties of Isolates from Blends of *Moringa oleifera* leaves and Ginger

Technological properties of isolates are the properties that indicate the quality of the isolate to be used as a starter culture in the course of fermentation. These properties were investigated to find the suitable or appropriate starter culture for use in the fermentation of the tea-like beverage products from blends of Drumstick leaves (*moringa oleifera)* and Ginger (*Zingiber officinale*). Many of the LAB metabolites such as low molecular weight acids, alcohols, carbon dioxide, diacetyl and hydrogen peroxides have broad spectrum antimicrobial activity against other species of bacteria (Helander *et al.*, 1997).

During fermentation process of *Moringa oleifera* leaves and ginger rhizome, 1.71 % to 1.99% lactic acid was found from LAB species in 10 gm leaves. This percentage of the lactic acid level was good. Which indicates that commercially higher amount of lactic acid can be produced from Moringa leaves and Ginger by using this easy and cheaper fermentation process. Lactic acid production increased with incubation time for all the isolates with the highest production of 1.71 % to 1.99(mg/L) being recorded for *Lactobacillus acidophilus Specie* and *Lactobacillus plantarum* respectively within 120 h of incubation. Reasonable quantity of lactic acid was produced by the isolates, and this was in agreement with the report of Pinthong *et al.*, (1980) who reported that lactic acid

bacteria could lead to products with sufficient acidity (low pH) for good keeping properties through production of organic acids.

Lactic acid is a major product of fermentation of carbohydrate by lactic acid bacteria, and this was evident in the isolates under investigation. This is an important technological property of LAB which may impart positively on the quality attributes of beverages especially shelf-stability, aroma and flavors characteristics.

LAB species gave the lowest hydrogen peroxide ranging between 0.006-0.012mg/L which is a good production against other species such as *proteus vulgaris* that produced hydrogen peroxide between the ranges of 0.062-0.071 which is the highest production so far. Hydrogen peroxide is associated with spoilage in a starter culture in food production. These differences may be attributed to the possible difference in lactose hydrolyzing/galactosidase enzymes activity in metabolic activity of strains and media used. Generally, estimation of hydrogen peroxide production by the isolate strains was observed to be very low when compared to lactic and acetic acids production.

The trend of acetic acid production was similar to that of lactic acid, with production observed of the two acids at 24 h of incubation. However, production of the acetic acid by the all strains of the isolates was lower than lactic acid throughout the incubation period.

Diacetyl was generally produced in minimal amounts and concentration was observed to reach a maximum concentration between 72 and 120 h by most of the strains. The highest concentration of 0.89, 0.94 and 0.94 mg/L was recorded for *Lactobacillus plantarum*, *Lactobacillu Fermentum* and *Lactobacillus specie* respectively. Reasonable quantity of diacaetyl was produced by the presumptive LAB isolates. Diacetyl has a strong, buttery flavor and is essential at low concentrations in many dairy products, and other functional food products. Furthermore, based on the performances of the presumptive *Lactobacillus* strains in terms of Diacaetyl production, it seems they could make a good bio-preservative culture. This is as a result of the strains' production of comparatively higher concentrations of the antimicrobial than the *Enterococci* strains. Production of relatively high Diacaetyl concentration has been observed to contribute significantly to exertion of antagonism by LAB species against most unwanted organisms (Jyoti *et al*., 2003).

The result of pH measurement in the growth medium indicated a reduction ranging between 4.4 and 3.2 by all the strains of LAB within 24 h; this could be a useful factor in exertion of antagonism against spoilage and pathogenic organisms that may be associated with food products.

Selection of a Starter Culture: A total of 35 colonies were isolated from the blend samples. Of them, 3 colonies were identified as *Lactobacillus* species, through observation of phenotypic characters, and identification. Further studies on acid tolerance, bile tolerance, and proteolytic activity were conducted, to select strains as starter culture in the *Moringa oleifera* and Ginger blends. It was found that *L. plantarum* had tolerance under acid conditions, as its viable cell count remained at pH 2.0 and under 1.0% bile salt condition, after 72 h incubation time. Other studies (Lee *et al*., 2011; Lim and Im, 2009) conducted on *L. plantarum* strain isolated from *Moringa oleifera* leaves and Ginger also indicated that *L. plantarum* strain had acid tolerance, compared with other species (Table 3).

Table 3: Technological Properties of Isolates from Blends of *Moringa oleifera* leaves and Ginger

Isolates	Fermentation period (hrs)	H_2O_2 mg/l	Lactic acid mg/l	Diacetyly mg/l	Acetic acid mg/l	$_pH$
Lactococccus lactis	24hrs	0.024	1.72	0.57	0.66	4.8
	72hrs	0.031	1.84	0.62	0.85	4.3
	120hrs	0.030	1.96	0.78	1.18	3.8
Lactobacillus acidophilus	24hrs	0.006	1.84	0.88	0.41	5.7
	72hrs	0.012	1.82	0.89	0.72	5.5
	120hrs	0.009	1.88	0.94	1.21	5.2
Escherichia coli	24hrs	0.004	0.38	0.43	0.52	4.0
	72hrs	0.031	0.52	0.49	0.68	3.7
	120hrs	0.060	1.04	0.54	1.06	3.3
Lactobacillus Fermentum	24hrs	0.064	1.71	0.69	0.48	4.2
	72hrs	0.071	1.96	0.82	0.76	4.0
	120hrs	0.062	1.98	0.94	1.14	3.8
Proteus vulgaris	24hrs	0.064	0.11	0.49	0.48	4.2
	72hrs	0.071	0.16	0.52	0.76	4.0
	120hrs	0.062	1.24	0.64	1.12	3.8
Lactobacillus plantarum	24hrs	0.006	1.82	0.86	0.62	4.4
	72hrs	0.062	1.98	0.88	0.90	4.0
	120hrs	0.064	1.99	0.89	1.26	4.0

3.3 Proximate and Energy Content of Fermented Beverage (*Moringa oleifera* leaves and Ginger) Samples (Powder)

The proximate and energy content of fermented beverage produced from the blends. The proximate and energy value of the beverage plays a crucial role in assessing its nutritional significance ($P<0.05$). Consequently, the fermentation of these blends with LAB species might provide a means of retaining more of the nutrients that are trapped in the matrix of the cells of these plant materials (Table 4).

Table 4: Proximate and Energy Content of Fermented Beverage (*Moringa oleifera* leaves and Ginger) Samples (Powder)

Samples	Dry matter %	Moisture content %	Crude protein %	Ether extract %	Crude fibre%	Ash %	Carbohydrate%	Energy value kcal
T1	20.00^{a}±2.35	5.00 ^{a}±0.25	35.00 ^{e}±6.23	5.00 ^{a}±0.51	12.00^{e}±6.23	10.00^{a}±2.59	15.00^{a}±0.89	74Kcal
T 2	30.00^{b}±2.35	5.00 ^{a}±0.25	40.00 ^{d}±6.20	5.00^{a}±0.51	13.00^{d}±6.20	9.00^{b}±2.56	15.00^{a}±0.89	108Kcal
T 3	30.00 ^{b}±2.35	5.00 ^{a}±0.25	45.00 ^{c}±6.53	5.00^{a}±0.51	14.00^{c}±6.53	8.00^{c}±2.51	15.00^{a}±0.89	162Kcal
T 4	22.00 ^{e}±2.31	5.00 ^{a}±0.25	47.00 ^{b}±6.71	5.00^{a}±0.51	15.00^{b}±6.71	7.00^{d}±2.30	15.00^{a}±0.89	197Kcal
T 5	26.00 ^{c}±2.31	5.00 ^{a}±0.25	51.00 ^{a}±6.32	4.00 ^{b}±0.52	16.00^{a}±6.32	6.00^{e}±2.40	13.00^{b}±0.59	207Kcal
T 6	26.00 ^{c}±2.31	5.00 ^{a}±0.25	59.00^{e}±5.81	4.00^{b}±0.52	17.00^{e}±5.81	5.00^{a}±0.55	13.00^{b}±0.5	238Kcal
T 7	26.00 ^{c}±2.31	5.00 ^{a}±0.25	60.00 ^{d}±5.80	4.00^{b}±0.52	18.00^{d}±5.80	4.00^{a}±0.57	13.00^{b}±0.59	257Kcal
T 8	26.00 ^{c}±2.31	5.00 ^{a}±0.25	65.00 ^{c}±5.82	4.00^{b}±0.52	16.00^{c}±5.82	4.00^{a}±0.58	13.00^{b}±0.59	279Kcal
T 9	25.00 ^{d}±2.35	5.00 ^{a}±0.25	70.00 ^{b}±5.82	3.00^{a}±0.00	13.00^{b}±5.82	3.00^{b}±0.56	12.70^{a}±2.07	290Kcal
T 10	2300 ^{e}±2.35	5.00 ^{a}±0.25	71.00 ^{a}±5.82	3.00^{a}±0.00	14.00^{a}±5.82	3.00^{b}±0.55	12.50^{a}±2.07	305Kcal
T 11	20.00 ^{e}±4.82	5.00 ^{a}±0.25	50.00^{a}±6.32	3.00^{a}±0.00	17.00^{a}±6.32	5.00^{b}±0.55	12.80^{a}±2.07	320Kcal
T 12	20.00 ^{e}±4.82	4.00 ^{b}±0.25	51.00^{a}±6.32	3.00^{a}±0.00	14.00^{a}±6.32	6.00^{b}±0.55	12.00^{a}±2.07	338Kcal
T 13	18.00 ^{f}±4.81	4.00 ^{b}±0.25	55.00^{a}±6.32	3.00^{a}±0.00	12.00^{a}±6.32	7.00^{b}±0.55	12.00^{a}±2.07	357Kcal
T 14	18.00 ^{f}±4.81	4.00 ^{b}±0.25	65.00 ^{c}±5.82	3.00^{a}±0.00	11.00^{c}±5.82	8.00^{b}±0.55	9.00^{d}±2.07	380Kcal
T 15	10.00 ^{g}±4.81	4.00 ^{b}±0.25	70.00 ^{b}±5.82	3.00^{a}±0.00	18.00^{b}±5.82	6.00^{b}±0.55	11.00^{b}±2.07	379Kcal
T 16	10.00 ^{c}±4.81	4.00 ^{b}±0.25	71.00 ^{a}±5.82	3.00^{a}±0.00	16.00^{a}±5.82	7.00^{b}±0.55	10.00^{c}±2.07	396Kcal

Values are means ± standard deviation of duplicate determinations

Means with different superscript letters in the same column are significantly different at ($P<0.05$).

T1 (100% *Moringa* leaves uninoculated); T2 (100%*Moringa* leaves+LL);
T3 (100%*Moringa*leaves+LP); T4 (100%*Moringa*leaves+LA);
T5 (90/10%*Moringa*/Ginger+LL); T6 (90/10%*Moringa*/Ginger+LP);
T7 (90/10%*Moringa*/Ginger+LA); T8 (80/20%*Moringa*/Ginger+LL);
T9 (80/20%*Moringa*/Ginger+LP); T10 (80/20%*Moringa*/Ginger+LA);
T11 (70/30%*Moringa*/Ginger+LL); T12 (70/30%*Moringa*/Ginger+LP);
T13 (70/30%*Moringa*/Ginger+LA); T14 (60/40%*Moringa*/Ginger+LL);
T15 (60/40%*Moringa*/Ginger+LP); T16 (60/40%*Moringa*/Ginger+LA);

LL: *Lactococccus lactis;* LP: *Lactobacilus plantarium;* LA: *Lactobacillus acidophilus.*

Dry matter (DM):

The proximate analysis revealed that the dry matter contents ranged between 10.00and 30.00, there was a significant difference in the beverage dry matter at ($P<0.05$) as a decrease in dry matter across the samples of the fermented beverage. The samples fermented with LAB 2 and LAB 1 gave better values for dry matter compared with other samples. This could be attributed to the action of LAB on the samples. The decrease in the dry matter content of the beverage samples inoculated with LAB isolates could be attributed to the metabolic activities of the fermenting cultures (Madigan *et al*., 2002).

Moisture content:

The proximate analysis revealed that the moisture content ranged between 4.00 and 5.00, there was a significant difference in the beverage moisture content at ($P<0.05$), there was a decrease in moisture content along the blend. The low moisture content observed in the samples could be attributed to the high activity of the LAB isolates inoculated and possibly due to some degree of evaporation at the temperature of incubation (37^{0}C).In addition, packaging of the fermented beverage samples with foil could have contributed to decrease in moisture content as evaporation was high and this is an indication that the samples could be stored favorably for a long period of time (Alinnor and Akalesi, 2011).

Crude protein:

Drumstick leaves *(Moringa oleifera)* is a good source of protein.Ginger supplementation increases with the decreases in the quantity of protein in the blends. Proximate and energy content revealed that the crude protein content ranged from 35.00 and 71.00, the blends fermented with LAB 2 gave a better protein yield compared to other blends. *Lactobacillus plantarum* used as ferment increases protein content, bioavailability of nutrients, improves shelf life of food (Djouldeet *al.*, 2003; Adetunde *et al.*, 2010; Mami *et al.*, 2010). They are excellent source of protein

(especially when dried), but poor in carbohydrates and fats, thus making them one of the best plant foods available in nature (Olusola 2006). *Lactobacillus plantarum* is found in the fermentation of corn pastes (Louembé *et al.*, 2003), Sauerkraut, Ogi and several other fermented food of vegetable origin (Yao *et al.*, 2009). In addition to its abilities to reduce anti-nutritional factor content in food such as phytates due to the production of phytase (Zamudio *et al.*, 2001), fibre by the β -galactosidase (Giraud, *et al*, 1993; Perumal *et al*, 2012), *Lactobacillus plantarum* used as ferment increases protein content, bioavailability of nutrients, improves shelf life of food (Dajanta *et al.*, 2003; Mami *et al.*, 2010) and increases the energy density of rich starch foods.

Ether extract:

The proximate and energy content revealed that the crude fat 3.00to 5.00. The content shows a decrease in fat content after inoculation with Lactic acid bacteria. There was a significant difference at ($P < 0.05$) for the fat content. This could be attributed to the actions of the Lactic acid bacteria as well as ginger supplementation. *Moringa oleifera* leaves are generally a poor source of fat. The decrease observed in fat content may also be attributed to the breakdown of fat into free fatty acids by the *Lactobacillus* spp., some of which might have been used in flavor and aroma generation; such may be due to reaction with other components of the mash to form esters which produced the characteristic aroma of the beverage. This agreed with the work of Ouoba *et al*., (2005), they reported on beneficial effects of lipase in the developments of characteristic flavors and aromas.

Crude Fibre:

The proximate and energy content revealed thatthe crude fibre ranged between 11.00 and 18.00.There was an increase in fibre content. This is as a result of the fact that *Moringa oliefera* and ginger are good sources of fibre. Fibre is an essential component of food as it aids in digestion and prevents constipation.Crude Fiber plays useful role in providing roughages that aid digestion. From the result obtained, the samples contain adequate fibre that will serve as a source of fibre in foods.

Ash Content:

The proximate analysis revealed thatthe Ash content ranged between 3.00 and 10.00. The ash content indicates that the blends of Drumstick (*Moringa oleifera)*leaves and Ginger are rich in mineral elements. The decrease in the ash content could be due to reduction of certain chemical components such as carbohydrate, moisture and fat as reported in this study (Adetunde *et al.*, 2010).

Carbohydrate Content:

The proximate and energy content revealed that the carbohydrate content ranged from 7.00 to 15.00.Carbohydrates are the principal sources of energy. There was a significant difference in the carbohydrate content at ($p<0.05$) in the mean carbohydrate values. There was a decrease in the carbohydrate content of beverage samples inoculated with LAB isolates. This could be attributed to the presence of LAB because Lactic acid bacteria have been reported as producers of certain enzymes such as amylase, galactanase, galactosidase, glucosidase and fructofuranosidase, which are involved in the degradation of carbohydrates. The decrease in the carbohydrate content of the samples could also be attributed to the breakdown of complex sugar to simple sugar by the Lactic acid bacteria inoculated in the samples.

Energy Value:

The proximate and energy content revealed that the energy value kcal ranged between74Kcal and 396Kcal.

Consequently, fermentation of this tea-like beverage product from blends of *Moringa oleifera* leaves and ginger rhizome with LAB species might provide a means of retaining more of the nutrients and increased energy value.

4. CONCLUSION

The phenotypic and biochemical characteristic of Blends of Drumstick leaves (*Moringa oleifera*) and Ginger (*Zingiber officinale*) reveals that the isolates were belong to the genus *Lactobacillus*; The *L. plantarum* strain isolated from *Moringa oleifera* leaves and Ginger also indicated that *L. plantarum* strain had acid tolerance.

The proximate and energy values confirmed that blends of *Moringa oleifera* leaves and ginger are an excellent food source, justifying its direct use in human nutrition or development of balanced diets for nutrition.

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6. ETHICAL APPROVAL

NO ethical approval is required in this study

7. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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