Inhibitory Effects of *Lactobacillus acidophilus* and *Lactobacillus casei* Isolated from “kunun zaki” (A Nigerian Fermented Beverage) against *Helicobacter pylori*  


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The attempt to use probiotic lactic acid bacteria from a popular fermented beverage in Nigeria (kunun zaki) is a quest to find an ideal cure for *Helicobacter pylori*-induced gastritis, gastric malignancies and peptic ulcer. The lactic acid bacteria counts of the samples of the beverage were determined and the organisms were identified using standard bacteriological methods and tested against *Helicobacter pylori*. The results showed the mean Lactic acid bacterial count to be 4.5x 10^8 cfu/ml while the microbial flora in the beverage were *Lactobacillus acidophilus* (50%), *Lactobacillus casei* (20%), *Lactobacillus plantarum* (10%), *Bacillus cereus* (10%) and *Saccharomyces cerevisiae* (10%) with their respective frequencies of occurrences in parentheses. The inhibitory effect of *Lactobacillus acidophilus* with the mean zone of inhibition of 51.25mm was found to be more effective against *Helicobacter pylori* strain J99, while *Lactobacillus casei* with the zone of inhibition of 39.50mm was more effective against strain P12. The synergistic effect of the two lactobacilli combined in equal proportion against both strain J99 and strain P12 with the mean zones of inhibition of 80.00mm and 77.75mm respectively for the *H. pylori* strains were significantly higher than those of the individual lactobacilli used. It could be concluded from this study that the two *Lactobacillus* sp from ‘kununzaki’ demonstrated strong inhibitory effects against *Helicobacter pylori* and further studies are recommended to validate if they could serve as probiotic alternatives to the treatment of *H. pylori*- induced peptic ulcers.  

Key words: lactic acid bacteria, kununzaki, *Helicobacter pylori*, Inhibitory effects  

INTRODUCTION  

According to the Food and Agricultural Organization of the World Health Organization, probiotics are live microorganisms which when administered in adequate amounts may confer a health benefit on the host (FAO/WHO, 2001). A probiotic can also be defined as a living microbial species that on administration, may have a positive effect on pathogenic microorganisms and improve the health conditions of the consumer (Myllyluoma et al., 2008). Probiotics originally, are used to improve the health of both men and animals through the consumption of fermented foods that contain probiotic microorganisms like the *Lactobacillus sp* and through modulation of intestinal microbiota. Presently, several of well-characterized strains of *Lactobacilli* and *Bifidobacteria* are available for human use in reducing the risk of gastrointestinal infections or treat such infections (Salminen et al., 1996). Some of the beneficial effects of probiotic consumption include improvement intestinal health by the regulation of microbiota, stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance and reducing the risks of certain other diseases (Yadav et al., 2008).  

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Also, as highlighted by (Toma et al., 2006), some of the health benefits of probiotic microorganisms include, resistance to enteric pathogens, aid in digestion of lactic acid, immune system modulation, anti-colon cancer effect, decreases detoxification and allergy, anti-extensive effective, eradication of infections caused by Helicobacter pylori, neutralization of dietary carcinogens and enhancement of nutrient value amongst others. At present, the most studied probiotics are lactic acid-producing bacteria, particularly Lactobacillus sp, examples include Lactobacillus casei, L. fermentum, L. acidophilus, L. plantarum, L. reuteri, L. bulgaricus, L. rhamnosus, L. lactis, L. brevis, L. delbreucki, L. gresius and a host of others. Lactic acid bacteria are fermentative bacteria that share common metabolic and physiological characteristics, gram positive, acid tolerant, nonsporulating, either rod or coccus shaped, anaerobic and nonrespiring. They are catalase and oxidase negative but are capable of fermenting several sugars such as glucose, fructose, sucrose, manitol, xylose and raffinose. Lactic acid bacteria constitute a diverse group of microorganisms associated with plants, meat, fermented drinks or foods and dairy products. They are mostly used in the manufacture of acidophilus milk, yogurt, buttermilk and cheeses (Cooney, 1997).

Kununzaki is a fermented food drinks that is locally prepared and consumed in Northern Nigeria. It is made of sorghum grains and has great nutritional value and health benefits because it contains useful probiotic microorganisms (Egbere et al., 2017). Many probiotic bacteria especially Lactobacillus sp has been isolated from ‘kunun zaki’ by few researchers (Egbere et al., 2017).

Recent works have been linked to probiotic bacteria with inhibitory effects against peptic ulcer. It is now established that the indigenous microbial communities have beneficial properties to the host. However, it is not precisely known which species of microorganisms play the principal role in these beneficial properties (Toma et al., 2006).

Helicobacter pylori is a gram negative, oxidase-, urease-, catalase positive, spiral and microaerophilic bacteria and a highly prevalent pathogen associated with chronic gastritis, gastric malignancies and peptic ulcer (Myllyouma et al., 2008). Helicobacter pylori infection rates vary with age, ethnicity, socioeconomic status, sanitary environments and lifestyle (Myllyouma et al., 2008). The organism is flagellated which enables it to colonize the gastric epithelium. It produces enzyme urease which hydrolyzes urea into carbon dioxide and ammonia and elevates pH in the surroundings of the bacteria thereby causing chronic infection due to complex balance between host factors and virulence bacterial factors. Studies by previous researchers, such as (Egbere et al., 2009; Egbere et al., 2007; Kutshik et al., 2012) have established that Lactobacillus casei and Lactobacillus acidophilus are frequently occurring in ‘kunun zaki’ sold in Nigerian markets. However, their inhibitory action against infectious agents has not been fully established. This present research seeks to determine the inhibitory effect of the two probiotic bacteria (Lactobacillus casei and Lactobacillus acidophilus) isolated from “kunun zaki” against Helicobacter pylori.

MATERIALS AND METHODS

General Asepsis

All methods including media preparation, sample handling and sample collection were carried out under aseptic conditions and careful handling. The workbench was made aseptic by cleaning with sterilizing reagents, flaring the wire loop, innoculating needle and bottle corks. Glass wares and materials used were also sterilized using the hot air oven to obtain absolute sterility.

Collection of Samples

A total of ten Kununzaki samples used for this research were collected from different refreshment spots at the University of Jos main campus Bauchi Road Jos, Plateau State. The samples were kept at room temperature for a day and were labeled properly from A-J to avoid mix up, this was to allow Lactobacillus acidophilus and Lactobacillus casei to proliferate and grow in the samples. Clinical strains of Helicobacter pylori P12 and J99 were obtained from the Nigerian Institute of Medical Research Apapa, Lagos State, Nigeria.

Media Preparation

All media including nutrient agar, de’Man Rogosa Sharpe (MRS) agar, GC agar and peptone water were prepared according to the manufacturer’s instructions.

SAMPLE PROCESSING

Serial Dilution

Kunun zaki samples were serially diluted up to the 10^-7 dilution power according to methods described by (Cheesbrough, 2000).

Inoculation of samples

Two dilution factors (10^-5 and 10^-7) obtained from samples A-J were inoculated on twenty Petri dishes containing de’ Man Rogosa Sharpe agar (ten for each dilution factor) using pour plate method by (Cheesbrough, 2000). The petri dishes were incubated at 37°C for 48 hours under anaerobic conditions using anaerobic jar. Distinct colonies were observed, counted, mean taken, expressed as cfu/ml and the total plate count and morphological features were recorded.
Microscopy

The Microscopic examination was done according to (Cheesbrough, 2000). A colony was picked from Petri dish using a sterilized wire loop and with sterile distilled water. The colony was then emulsified on sterile grease free slide to make a smear. This was allowed to air dry and then heat fixed by passing the slide over a flame from Bunsen burner. This was repeated for all the other colonies obtained. The smears were stained using gram staining techniques. The stained slides were then viewed under the microscope at the magnification of x100 oil emulsion and microscopic features were recorded accordingly.

Confirmatory Test for Helicobacter pylori strains

This test was done in order to confirm if the clinical isolates obtained from Nigerian Institute of Medical Research (NIMR) Apapa, Lagos State, Nigeria were truly Helicobacter pylori. Clinical isolates of Helicobacter pylori (strains P12 and J99) were grown on GC agar and viewed under the microscope at the magnification of x100 oil emersion for confirmation. The two clinical isolates collected from the National Institute of Medical Research were confirmed to be spiral, flagellated, gram negative, oxidase and urease positive.

Biochemical Test for Lactobacilli species Isolated from Kunun zaki Samples

The isolates obtained from kunun zaki were subjected to biochemical tests as described by (Cheesbrough, 2000) which comprises of the various tests needed to identify each of the bacterial isolates. The tests used included Catalase, Oxidase, and Triple sugar ion test (TSI) and sugar fermentation test.

Preservation of Isolates

Bacteria isolates which showed very close characteristics of Lactobacillus casei and Lactobacillus acidophilus were then inoculated into agar slants and preserved at 4°C for two days prior to experimentation.

Preparation of Bacterial Suspensions

Suspensions of isolates of Lactobacillus acidophilus and Lactobacillus casei were made using sterile peptone water and incubated at appropriate conditions for 48 hours before introduction into the agar wells. This was done by aseptically picking out five colonies of each pure culture of the lactobacilli into the peptone water and having the suspension thoroughly mixed for homogeneity. Also, suspension of amoxicillin was prepared in sterile distilled water with the concentration 125g/5ml (serving as a control) using sterile pasture pipettes, 0.05ml of each suspension were dispensed into agar wells as described according to methods of below.

Determination of Inhibitory Effects of L. acidophilus and L. casei

Well Diffusion Assay

After the foregoing processes, strains of Helicobacter pylori P12 and J99 were inoculated and incubated at appropriate conditions. A sterile swab stick was used to pick colonies of Helicobacter pylori P12 and circles were made round in four places on a Petri dish containing GC agar. In between the circles, a sterile pipette was used to bore wells and 0.05mls of suspension containing Lactobacillus acidophilus was then introduced into the well using sterile pasture pipettes. To the second Petri dish containing GC agar, 0.05mls of amoxicillin suspension was introduced to into four agar wells accordingly to serve as control.

This was repeated for Helicobacter pylori J99 using same Lactobacillus acidophilus and the plates were incubated at 37°C for 72 hours under a microaerophilic conditions after which the zones of inhibition were measured and recorded using the method and according to the standard described by Clinical and Laboratory Standard Institute (CLSI) formerly known as National Committee for Clinical Laboratory Standards (NCCLS). The same process was performed for Lactobacillus casei on separate Petri dishes and the results were recorded accordingly.

Statistical Analysis

Mean and Standard Deviation statistical tools were used to measure the central of tendency between the probiotic organism and amoxicillin control using Microsoft excel.

RESULTS

The results on lactic acid bacteria count of kunun zaki sampled within University of Jos main campus are shown in Table1. The results indicate that Lactic Acid Bacteria counts range from 1.4x10⁵cfu/ml to 9.0x10⁵cfu/ml with a mean count of 4.46x10⁵cfu/ml.

The colonial morphology and microscopic features of lactic acid bacteria are shown in Table 2. Four of the isolates occur in chains while six occurs singly, seven of the isolates were found to be thin rods, one as large rods, one as ovoid and the other as cocci.

Characteristics used in the identification of Lactobacillus casei include its ability to ferment all sugars except xylose, non-gas production during glucose fermentation and its non-motile behavior. Lactobacillus acidophilus was characterized by excess acid production with glucose. Lactobacillus plantarum was also isolated based on its biochemical features as shown in Table 3.
Table 4 depicts percentage frequency of occurrence of microorganisms present in the ‘Kunun zaki’ samples. From the table, it was observed that Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Saccharomyces cerevisiae and Bacillus cereus had 50%, 20%, 10%, 10% and 10% frequencies of occurrences respectively.

Tables 5 and 6 indicate the zones of inhibition of Lactobacillus casei and Lactobacillus acidophilus against Helicobacter pylori strain P12 and J99 respectively. The results were analysed according to standards by the National Committee for Clinical Laboratory Standards (NCCLS), 2013 publication. Mean values that is ≤13 are considered as resistant, mean values of 14-17 are considered as intermediate while mean values ≥18 are considered as susceptible or inhibitory.

Table 1: Colonial Morphology and Microscopy of Microbial Isolates Obtained from ‘Kununzaki’ Samples.

<table>
<thead>
<tr>
<th>Isolate Code</th>
<th>Size</th>
<th>Colour</th>
<th>Elevation</th>
<th>Margin</th>
<th>Gram reaction</th>
<th>Shape</th>
<th>Arrangement</th>
<th>Motility</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB1</td>
<td>M</td>
<td>White</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Thin Rods</td>
<td>Chains</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
<tr>
<td>LB2</td>
<td>L</td>
<td>White</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Ovoid</td>
<td>Chains</td>
<td>-</td>
<td>Bacillus sp</td>
</tr>
<tr>
<td>LB3</td>
<td>S</td>
<td>White</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Thin Rods</td>
<td>Single</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
<tr>
<td>LB4</td>
<td>S</td>
<td>Creamy</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Thin Rods</td>
<td>Chains</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
<tr>
<td>LB5</td>
<td>S</td>
<td>Creamy</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Thin Rods</td>
<td>Single</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
<tr>
<td>LB6</td>
<td>M</td>
<td>White</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Thin Rods</td>
<td>Single</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
<tr>
<td>LB7</td>
<td>L</td>
<td>Creamy</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Cocci</td>
<td>Single</td>
<td>-</td>
<td>Saccharomyces sp</td>
</tr>
<tr>
<td>LB8</td>
<td>L</td>
<td>Creamy</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Large Rods</td>
<td>Single</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
<tr>
<td>LB9</td>
<td>M</td>
<td>White</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Thin Rods</td>
<td>Single</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
<tr>
<td>LB10</td>
<td>S</td>
<td>White</td>
<td>Raised</td>
<td>Round</td>
<td>+</td>
<td>Thin Rods</td>
<td>Chains</td>
<td>-</td>
<td>Lactobacillus sp</td>
</tr>
</tbody>
</table>

L=large, M=medium and S=small, + = Gram positive
LB1-LB10= Bacterial Isolates

Table 2: Biochemical Characteristics of the Isolates Obtained from ‘Kununzaki’ Samples.

<table>
<thead>
<tr>
<th>Isolate Code</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Glucose</th>
<th>fructose</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Gas</th>
<th>Most probable Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>LB2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>LB3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>LB4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>LB5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>LB6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Lactobacillus casei</td>
</tr>
<tr>
<td>LB7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>LB8</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Lactobacillus plantarum</td>
</tr>
<tr>
<td>LB9</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>LB10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Lactobacillus casei</td>
</tr>
</tbody>
</table>

+ = Positive, - = Negative, LB1-LB10= Bacterial Isolates
Table 3: Inhibitory Effects of Two \textit{Lactobacilli} species Against \textit{Helicobacter pylori} Strain P12.

<table>
<thead>
<tr>
<th>S/No</th>
<th>Test Organisms</th>
<th>Zones of Inhibition (mm)</th>
<th>Mean zone of Inhibitions (mm)</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( W_1 )</td>
<td>( W_2 )</td>
<td>( W_3 )</td>
</tr>
<tr>
<td>1</td>
<td>\textit{Lactobacillus acidophilus} + \textit{Helicobacter pylori} P12</td>
<td>65</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>\textit{Lactobacillus casei} + \textit{Helicobacter pylori} P12</td>
<td>73</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>\textit{Lactobacillus acidophilus} + \textit{Lactobacillus casei} + \textit{Helicobacter pylori} P12</td>
<td>78</td>
<td>85</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>Control (amoxicillin) + \textit{Helicobacter pylori} P12</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\( W_1 \)-\( W_4 \)= Agar Diffusion Wells

Table 4: Inhibitory Effects of Two \textit{Lactobacilli} species Against \textit{Helicobacter pylori} strain J99.

<table>
<thead>
<tr>
<th>S/No</th>
<th>Test Organisms</th>
<th>Zones of Inhibition (mm)</th>
<th>Mean zone of Inhibitions (mm)</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( W_1 )</td>
<td>( W_2 )</td>
<td>( W_3 )</td>
</tr>
<tr>
<td>1</td>
<td>\textit{Lactobacillus acidophilus} + \textit{Helicobacter pylori} J99</td>
<td>70</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>\textit{Lactobacillus casei} + \textit{Helicobacter pylori} J99</td>
<td>75</td>
<td>25</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>\textit{Lactobacillus acidophilus} + \textit{Lactobacillus casei} + \textit{Helicobacter pylori} J99</td>
<td>80</td>
<td>85</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>Control (amoxicillin) + \textit{Helicobacter pylori} J99</td>
<td>33</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

\( W_1 \)-\( W_4 \)= agar diffusion wells

\textbf{DISCUSSION}

\textbf{Lactic Acid Bacteria Count}

The study has shown that the mean lactic acid bacteria load \((4.46\times10^8\text{cfu/ml})\) of ‘kunun zaki’ sampled within University of Jos premises is lower when compared with that of (Kutshik \textit{et al.}, 2012) which was \(4.0\times10^{10}\text{cfu/ml}\). This may be due to differences in environmental factors, weather, sampling area, personal hygiene of the ‘kunun zaki’ producer, water and materials used for production, number of days the ‘kunun zaki’ was kept for fermentation and period of research.

The number of lactic acid bacteria in kunun zaki in this study is comparable to the counts in yoghurt and juice milk samples analysed and reported by Elizaquível \textit{et al.}, 2014 and Guevarra and Barraquio, 2015 respectively. The counts of the lactic acid bacteria in kunun zaki qualifies it to be addressed as a probiotic product (if all other...
requirements are being met) considering the fact that standard of Codex Alimentarius Commission which stipulates that a probiotic must be sufficient in quantity of not less than 10^6 cfu/ml provided the organisms meet the other requirements of probiotic potentials like survival simulated conditions of acidity and alkalinity of the human gut, antibiotic sensitivity and antimicrobial properties amongst others (Codex, 2003)

However, the mean lactic acid bacteria load observed is capable of suppressing intestinal pathogens, thereby reducing gastrointestinal disturbances and preventing gastric malignancies (Patel et al., 2015). (Cats et al., 2003) in addition, the study has shown that 'kununzaki' is very rich in lactic acid bacteria as reflected by the eight species of lactobacilli (Figure 1) isolated from the samples hawked in the study area.

**Percentage Frequency of Occurrence of Bacteria in ‘Kununzaki.’**

The study on the frequency of occurrence (Figure 2) has equally showed that *Lactobacillus acidophilus* was the most predominant (50%). This may be due to tolerance to excess lactic acid bacteria present in the 'kununzaki' samples. This is followed by *Lactobacillus casei* (20%) and *Lactobacillus plantarum* (10%) with their respective frequency of occurrences in parentheses. This study agreed with work done by (Egbere et al., 2007) on the microorganisms associated with 'kununzaki' and that of (Kutshik et al., 2012).

Other microorganisms identified were *Bacillus sp* and *Saccharomyces sp*. This may be as a result of contamination due to prolonged storage and poor hygiene during production. The presence of *Bacillus sp* could cause food poisoning if the product is consumed in excess. The source of these organisms in kunun zaki has been linked to principally the raw materials (grains, sweet potato and spices) used in the production of the beverage.

**Inhibitory Effects of Lactobacilli on Helicobacter pylori.**

The study has shown that the two lactobacilli isolated from 'kunun zaki' (*Lactobacillus acidophilus* and *Lactobacillus casei*), demonstrated high inhibitory effects on the two strains of *Helicobacter pylori*, namely, strain P12 and J99 (Tables 3 and 4). The inhibitory effects of the two lactobacilli were far higher than the standards for amoxicillin and indeed higher than the minimal recommended zone of inhibition (≤13.00mm) considered as the minimal zone diameter of resistance by Clinical Laboratory Standard, 2013 publication. The zones of inhibition of the lactic acid bacteria against *Helicobacter pylori* strainP12 (33.75mm) and J99 (51.25mm) were three times higher than that of amoxicillin (12.50mm and 17.75mm) respectively.

Generally, *Lactobacillus acidophilus* was found to be more inhibitory to the two strains of *Helicobacter pylori*. The synergistic inhibitory effects which involved the combination of *Lactobacillus casei* and *Lactobacillus acidophilus* were far greater (80.00mm and 77.75mm) than the effects of the individual *Lactobacillus sp*. This may be due to possible secretion of inhibitory substances such as bacteriocins, lactic acid and lactoferrin that that are capable of causing a bactericidal or bacteriostatic effect on *Helicobacter pylori* (Sgouras et al., 2004).

*Helicobacter pylori* strain P12 showed resistance to amoxicillin while strain J99 is intermediate to amoxicillin. This may be due to the fact that P12 genome contains three plasticity zones on their cell surface, two of which encode type IV features of genomic islands; that also has more pathogenic surface proteins such as CagA, CagI, CagL and CagY than strain J99. This may actually be the reason for its high Pathogenicity and resistance of *H. pylori* to most antibiotics used against peptic ulcer. From the statistical analyses the inhibitory effects of the lactobacilli used against *H. pylori* were significantly high and were above the effects of test antibiotic used as control.

The study also showed that there is a large variation in the inhibitory effects of the lactic acid bacteria against J99 strain of *Helicobacter pylori* than strain P12 of *H. pylori*.

In comparison to work done by (Sgouras et al., 2004), antimicrobial activity was evident only when *Lactobacillus casei* strain Shirota cells were used. Their results suggest that, just the presence of living *Lactobacillus casei* strain Shirota is required for *Helicobacter pylori* inhibition.

**CONCLUSION**

At the end of this work, the numbers of Lactobacilli species present in the kununzaki samples were enumerated and they were isolated and characterized using appropriate bacteriological techniques. At the end of the in vitro test, it could be concluded that the two lactobacilli from kununzaki have demonstrated high in vitro inhibitory actions against *Helicobacter pylori* and so may be able to stabilize or restore the endogenous intestinal microflora judging from its in vitro action. This may result from a specific action against pathogenic organisms, such as competition for nutrients or adhesion sites on epithelial cells, as well as through the specific production and secretion of antibacterial substances. It could be concluded from this study that *Lactobacillus sp* from kununzaki demonstrated strong inhibitory effects against *Helicobacter pylori*, a major causative agent of peptic ulcer. Therefore, 'kununzaki' that is highly rich in viable *Lactobacillus casei* and *Lactobacillus acidophilus* are required for *Helicobacter pylori* growth inhibition in vitro. *Lactobacillus sp* have demonstrated a beneficial effect in various conditions associated with a disrupted gastrointestinal microenvironment, such as peptic ulcer, traveller's diarrhea and...
shigellosis, acute rotavirus diarrhoea and antibiotic-associated diarrhoea as demonstrated by (Sgouras et al., 2004; Egber et al., 2007; Kutshik et al., 2012) respectively.

Finally, viable Lactobacillus casei and Lactobacillus acidophilus, especially when isolated from ‘kunun zaki’, could be capable of inhibiting the in vitro growth of Helicobacter pylori. This effect may not be explained by lactic acid production, but by close contact of live Lactobacillus casei and Lactobacillus acidophilus cells with Helicobacter pylori in a specialized (GC agar) medium. The potential therapeutic effects of Probiotic should not be dismissed, particularly given the positive effects documented for the prevention and treatment of a wide range of human gastrointestinal diseases (Wang et al., 2016; Elmahmood and Doughari, 2007).

RECOMMENDATIONS

The consumption of such kunun zaki can possibly close the therapeutic gap in the eradication of peptic ulcer. Notwithstanding, lactic acid bacteria are generally harmless to man, but harmful to intestinal pathogens, therefore more studies on this special group of microorganisms will take the field of industrial, food and medical microbiology to a great height of disease eradication, healthy living, eradication of public health problems and disease prevention.

Due to increased awareness on probiotics, many proven and approved probiotic strains are available in the market as supplements in dehydrated form. But fermented food drinks such as ‘kunun zaki’ could serve as common carriers of probiotic microbes, especially Lactic acid bacteria (LAB) since they support growth of these organisms during fermentation (Elmahmood and Doughari, 2007; Ngene et al., 2019). This local beverage (kunun zaki) could be a possible recommendation for peptic ulcer patients when therapy fails, along with therapy or as preventive measures against peptic ulcers after further research on this aspect is validated.

Although, the effect of Lactobacillus casei and Lactobacillus acidophilus on Helicobacter pylori growth and activity in humans may be promising, additional larger scale studies on the in vivo inhibitory effect of the two lactobacilli would be necessary.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES


Inhibitory Effects of *Lactobacillus acidophilus* and *Lactobacillus casei* isolated from "kunun zaki" (a Nigerian Fermented Beverage) against *Helicobacter pylori*


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