



## The effects of *Lactobacillus acidophilus* (Probiotics) and other fungicidal agents against vagina *Candida albicans*

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### Abstract

*Candida albicans* is a diploid fungus that grows both as yeast and as filamentous cells and is the causal agent of opportunistic oral and genital infections in humans. Probiotics are viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance. The Interrelation of *Lactobacillus acidophilus* probiotics on *Candida albicans* was investigated using standard microbiological procedures. Tablets of *Lactobacillus acidophilus* probiotics procured from the United States of America carefully manufactured by NATURE'S BOUNTY, showed oval, white to yellowish colour, with smooth border, non-bright and convex colonies on MRS agar. Under the microscope, the colonies showed Gram positive cocci arranged with single, or pair or pair to chains, non-spore forming cells. Two hundred and fifty isolates (100%) of *Candida albicans* were susceptible (sensitive) to Nystatin and Fluconazole. The results of susceptibility of *C. albicans* isolates showed that 240 isolates (96%) were sensitive to the suspension of *L. acidophilus* probiotic with varying zones of inhibition at different concentrations. Comparatively, the commercial antifungal drugs are better than the suspension from the probiotic, *Lactobacillus acidophilus*. These results showed the high activity of Lactobacilli to inhibit the *Candida* growth.

**Keywords:** *Lactobacillus acidophilus*, *Candida albicans*, antifungal properties

### Introduction

*Candida albicans* is a virulent strain of yeast which is naturally present in everybody (Oscar *et al.*, 2000), often within areas of mucous membrane such as the inside of the mouth, on moist skin, vagina, intestines, lungs, on or under the fingers and toenails (Vazques and Sobel, 2000). Some common conditions that *Candida* overgrowth is responsible for include thrush, vaginal yeast infections and even diaper rash (Ping, 2002). Candidiasis or Yeast Hypersensitivity occurs when the yeast overgrowth becomes a systemic problem (Debbie *et al.*, 2004). When the immune system is suppressed, the yeast can multiply rapidly, penetrate the intestinal lining and move into the bloodstream. They are present as part of the normal

flora (in moist places like the intestinal system, mouth, etc) in the human body.

Hospital acquired fungal infections cause serious morbidity and mortality and it is clear that fungal diseases have emerged as important public health problems (Chen *et al.*, 2006). *Candida* constitutes 80% of hospital-acquired fungal infections. In recent years, there has been a significant increase in non-albicans *Candida* species, especially in blood cultures, *C. albicans* and non-albicans species pose a high risk of infection in the blood circulation system of children (Levi *et al.*, 1998). Yeast population is controlled by probiotics or "friendly" bacteria (Cheryl *et al.*, 2001; Boonnaert and Rouxhet, 2000).

The term Probiotics literally means "for life", is used to describe organisms that are used medicinally including bacteria such as *Lactobacillus* which are naturally present in small intestine and vagina and have been shown to inhabit unhealthy organisms and play an important role in maintaining human health (Rolfe, 2002). The primary role of *Lactobacillus* is to reinforce protective mucosal surfaces and prevent the entrance and attachment of harmful microorganisms and allergens (Wee *et al.*, 2006). Strains of probiotics, like *Lactobacillus acidophilus* are of high value for protection against pathogenic yeast infections of the vagina, intestinal and oral cavity, and can be described as one of the body's primary defense mechanisms against *Candida* (Mercenier *et al.*, 2003).

Typically, probiotics are consumed as part of cultured foods such as acidophilus milk, yoghurt (Simin and Woel-Kyu, 2000). The topical application of such yoghurt products has been reported to control yeast and bacterial infections, and the ingestion of these preparations has been recommended to reduce the symptoms of sore mouth caused by *Candida* infections (Boonnaert and Rouxhet, 2000). Research has shown that *L. acidophilus* was the most popular species of probiotic bacteria that produces substances that slow or prevent the growth of *Candida* (Yang, 2000). Several reports have shown that Lactobacilli produce metabolites that inhibit various bacteria (Mikolajcik and Hamaden, 1975, Shahani *et al.*, 1976; Shahani *et al.*, 1977), but few investigators have included fungi in their studies. Guillot, (1958) reported that an inhibitor produced in casein broth by *L. acidophilus* inhibited *C. albicans*. *Lactobacillus acidophilus* has been reported to have a therapeutic effect in women with vaginal infections caused by *Candida*. It has been reported that Lactic acid bacteria (LAB) can produce antimicrobial substances with the capacity to inhibit the growth of pathogenic and spoilage microorganisms. Organic acids, hydrogen peroxide, diacetyl and bacteriocins are included among these compounds. Recent years have witnessed an increase in the studies exploring the antifungal effects of *Lactobacillus* strains. These studies have primarily focused on protection against urogenital candidiasis and prevention of fungal contamination of food (Barousse *et al.*, 2004).

This study reports on the inhibitory effects of *Lactobacillus acidophilus* and commercial fungicidal agents against *C. albicans* *in vitro*.

## Materials and Methods

### Sample Collection and Treatment

Two hundred and fifty (250) vaginal swabs were collected from women diagnosed with Recurrent Vaginal Candidiasis (RVC) by Specialist Physicians of obstetrics and Gynecology Departments of Federal Medical Centre, Life Spring Specialist Hospital and Ikenegbu Hospital, all in Owerri Municipal Council of Imo State, during the period of March to July 2014. The common criteria of patients included in this study were complaint of Recurrent Vaginal Candidiasis cases. Vaginal swabs were collected aseptically from pregnant and non pregnant women of age range 17-50 years old and some were pregnant. The swabs were swirled inside test tubes containing normal saline (0.9%) and transported immediately to laboratory (Emmons *et al.*, 1974). These swabs were cultured on different media for isolation and identification of *Candida albicans*.

### Yeast isolates

All vaginal swabs were cultured at 37°C for 72hrs on Christensen's Urea Agar (CUA) as primary media for isolation of yeast colony from pathogenic samples (Faro, 1996); Sabouraud's Dextrose Agar (SDA) for isolation and diagnosis of yeast samples (plates) (Ferrer, 2000).

Emmon's Sabouraud's Dextrose Agar (ESDA) for diagnosis of *Candida* species from positive *Candida* cultures (Ferrer, 2000).

Complete diagnosis of positive *Candida* cultures as *C. albicans* were carried out by the methods of Fidel and Sobel (1996); Forbes *et al.* (2002) which included; macroscopic appearance (colony morphology) of *Candida* colonies on CUA, SDA, and ESDA medium for shape, size, colour and odour of colonies; microscopic appearance (Gram stain method) to study the hyphae and buds. Chlamydospore formations, germ tube formation, growth on 37°C, were also done (Gould and Bowi, 1982; Gunston and Fairbrother, 1975). The yeast isolate were also screened against some sugars such as galactose, glucose, trehalose, maltose, lactose and sucrose (Cheesbrough, 2000).

### **Lactobacillus acidophilus Probiotics**

Tablets of *Lactobacillus acidophilus* probiotics Manufactured by NATURE'S BOUNTY, INC. Bohemia, NY, 11716 U.S.A. ©2013 Nature's Bounty Inc were procured from the United States of America.

Confirmation tests were done (Holt *et al.*, 1994) such as; macroscopic appearance of cultured colonies on MRS agar for its shape, color and viscosity of colonies; microscopic appearances of colonies after Gram stain (shape of cells, sporulation and capsule formation); biochemical tests (John *et al.*, 2008) ; growth on MRS agar at 45°C, production of ammonia from arginine, and production of acid and curd on litmus Milk agar and Carbohydrates fermentation including galactose, glucose, lactose, trehalose, mannitol, xylose, sucrose, fructose, mannose, arabinose and raffinose (Kaewsrirachan *et al.*, 2006).

### **Preparation of *Lactobacillus acidophilus* for antifungal assay**

*L. acidophilus* suspension was prepared by dissolving 1g of the *L. acidophilus* probiotics tablet in 4ml of deionized water which contain  $1 \times 10^8$  cells/ml (Kandler *et al.*, 1986). It was then standardized to  $1.5 \times 10^8$  cells using 0.5 McFarland turbidity standards. It was further diluted serially to different concentrations of  $250 \text{gml}^{-1}$ ,  $125 \text{gml}^{-1}$ ,  $62.5 \text{gml}^{-1}$ ,  $31.25 \text{gml}^{-1}$  and  $15.625 \text{gml}^{-1}$ .

Stock solution of antifungal Nystatin and Fluconazol were done with concentration of  $1 \times 10^4 \mu\text{g/ml}$  of solution to be used in susceptibility test according to Klebanoff and Coombs (1991), whereas filter paper discs were done from *L. acidophilus* bacterial suspension and solution of the two antifungal agents (Lanchares and Hernandez, 2000).

### **Preparation of fungal inocula**

*Candida albicans* suspension was prepared by using 1 ml of yeast inocula that contain  $1 \times 10^5$  cells/ml calculated by hemacytometer (Klebanoff and Coombs, 1991). Fungal spores on SDA slant were taken with sterile wire loop and introduced into sterile test tubes containing potato broth, and standardized to turbidity equivalent to  $1.5 \times 10^8$  cells by McFarland standard (Chessbrough, 2000)

### **Susceptibility test**

Susceptibility to *L. acidophilus* and two listed antifungal agents were determined against *C. albicans* isolates by means of an agar well diffusion assay. MRS broth was inoculated with 1ml each of the different concentrations of *L. acidophilus* probiotics and incubated at 37°C for 24hrs. After incubation, the cultures were centrifuged at 6000 rpm for 15 min (Al-Kafaji, 1992), and filtered through millipore filter unit ( $0.22 \mu\text{m}$ ). Saboruad agar plates were inoculated with 0.1ml of *C. albicans* by a spreader then, 5mm well was made with the cork borer. The wells were filled with different concentrations of *L. acidophilus* supernatant, and then incubated at 37°C for 24 hours. Inhibition zone around the well were measured in (mm) and compared with that of control which contained MRS broth only.

### **Minimum Inhibitory Concentration (MIC) ASSAY**

The Minimum Inhibitory Concentration assay technique was used to determine the lowest concentration of a particular antibiotic needed to kill an organism. To evaluate MIC and MFC, the procedure was done according to Atlas *et al.* (1995).

Serial dilutions of the *L. acidophilus* probiotics (representing different concentrations of the probiotics,  $250 \text{mgml}^{-1}$ ,  $125 \text{mgml}^{-1}$ ,  $62.5 \text{mgml}^{-1}$ ,  $31.25 \text{mgml}^{-1}$  and  $15.625 \text{mgml}^{-1}$ ) were added to a growth medium (MRS broth) in separate test tubes. These tubes were then inoculated with an equal volume of standardized *C. albicans*. The tubes are allowed to incubate overnight. Broth tubes that appear turbid are indicative of fungal growth while tubes that remain clear indicate no growth. The turbidity of the broth tubes was determined with a Uniscope spectrophotometer at 340nm.

### **Statistical analysis**

Analysis of the data obtained from these variations was performed by measuring degree of response of *Candida albicans* isolates and other antifungal agents to different concentrations of *Lactobacillus acidophilus* probiotics and means value according to Ringdahl (2000).

## Results

The characteristics and identities of the *Candida albicans* and *L. acidophilus* are shown in Tables 1 and 2. *C. albicans* on the culture media were white creamy, smooth, homogenous colonies as microscopic appearance, while microscopic appearance showed Gram positive staining cocci, budded cells with pseudohyphae. Other features of diagnosis of *C. albicans* isolates are listed in Table 1 which showed the characteristic reactions of physiological tests, sugar fermentation tests and sugar assimilation. All the two hundred and fifty (250) vaginal swabs collected from women diagnosed of Candidiasis were confirmed

positive yeast cultures. All results of the AP1-*Candida* technique were confirmed.

Results of confirmation of *L. acidophilus* probiotics showed oval, white to yellowish colour, with smooth border, non-bright and convex colonies. Under the microscope, the colonies were Gram positive cocci arranged with single, or pair to chains, non-spore forming cells. Also, the colonies were negative to oxidase, gelatinase, catalase; grow on MRS at 45°C and produce acid and curd on litmus milk agar. Results of carbohydrate fermentation showed positive reaction (Table 2).

**Table 1: Results of Characteristic Test of *C. albicans***

| Physiological test       | Reaction | Sugar Ferm. | Reaction | Sugar Assim. | Reaction |
|--------------------------|----------|-------------|----------|--------------|----------|
| Chlamydospores formation | +        | Galactose   | +        | Sucrose      | +        |
|                          |          | Glucose     | +        | Maltose      | +        |
| Germ tube formation      | +        | Trehalose   | ±        | Lactose      | ±        |
| Growth on 37°C           | +        | Maltose     | +        | Melibiose    | -        |
| Capsule Formation        | -        | Lactose     | +        | Xylose       | +        |
| Urease production        | -        | Sucrose     | -        | Trehalose    | +        |
|                          |          |             |          | Galactose    | +        |
|                          |          |             |          | Inositol     | -        |
|                          |          |             |          | Cellobiose   | -        |

**Table 2: Results of Biochemical and Carbohydrates Fermentation of *L. acidophilus***

| Biochemical tests     | Reaction | Carbohydrate ferm. | Reaction |
|-----------------------|----------|--------------------|----------|
| Oxidase               | -        | Galactose          | +        |
| Gelatinase            | -        | Glucose            | +        |
| Catalase              | -        | Lactose            | +        |
| Growth at 45°C        | -        | Trehalose          | +        |
| Production of Ammonia | -        | Manitol            | +        |
| Production of acid    | -        | Zylol              | +        |
|                       |          | Sucrose            | +        |
|                       |          | Fructose           | +        |
|                       |          | Manose             | +        |
|                       |          | Arbinose           | +        |
|                       |          | Rafiuose           | +        |

### Susceptibility Test

The comparative *in-vitro* susceptibility of *C. albicans* isolates to the activity of *L. acidophilus* suspension and two selective effective antifungal agents (Nystatin and Fluconazole) are shown in Table 3. Two hundred and fifty isolates (100%) of *Candida* were susceptible (sensitive) to Nystatin and Fluconazole with varying zones of inhibition. This high activity to inhibit the growth of all isolates comes from their polyenes

compounds that integrate directly to sterol and ergosterol which are found in the plasmic membrane of *Candida* cells to form pores that lead to gradual changing in the cell permeability and death of these cells (Ringdahl, 2000). The results of susceptibility of *C. albicans* isolates showed that 240 isolates (96%) were sensitive to the suspension of *L. acidophilus* probiotic with the zones of inhibition varying with different concentrations.

**Table 3. Susceptible of *C. albicans* isolates to *L. acidophilus* Probiotics**

| C.<br><i>albicans</i><br>isolates | Negative<br>Control | Positive<br>Control I    | Positive<br>Control II | <i>L. acidophilus</i> Zones of inhibition (mm) |          |           |            |             |
|-----------------------------------|---------------------|--------------------------|------------------------|------------------------------------------------|----------|-----------|------------|-------------|
|                                   | Water               | Fluconazole<br>(50mg/ml) | Nystatin<br>(25mg/ml)  | 250mg/ml                                       | 125mg/ml | 62.5mg/ml | 31.25mg/ml | 15.625mg/ml |
| <b>Week 1</b>                     |                     |                          |                        |                                                |          |           |            |             |
| Ca1                               | 0                   | 11                       |                        | 10                                             | 8        | 4         | 8          | 8           |
| Ca2                               | 0                   | 7                        |                        | 1                                              | 1        | 1         | 1          | 1           |
| Ca3                               | 0                   | 8                        |                        | 4                                              |          | 2         | 2          | 1           |
| Ca4                               | 0                   | 14                       |                        | 4                                              | 4        | 2         | 2          | 1           |
| Ca5                               | 0                   | 6                        |                        | 8                                              | 6        | 6         | 12         | 4           |
| Ca6                               | 0                   |                          | 5                      | 10                                             | 8        | 2         | 1          | 2           |
| Ca7                               | 0                   |                          | 2                      | 10                                             | 8        | 2         | 1          | 2           |
| Ca8                               | 0                   |                          | 6                      | 12                                             | 4        | 4         | 2          | 1           |
| Ca9                               | 0                   |                          | 1                      | 4                                              | 4        | 1         | 1          | 1           |
| Ca10                              | 0                   |                          | 4                      | 2                                              | 12       | 4         | 2          | 10          |
| Ca11                              | 0                   | 8                        |                        | 6                                              | 2        | 1         | 1          | 1           |
| Ca12                              | 0                   | 14                       |                        | 3                                              | 1        | 1         | 0          | 0           |
| Ca13                              | 0                   | 24                       |                        | 6                                              | 6        | 1         | 0          | 0           |
| Ca14                              | 0                   | 24                       |                        | 3                                              | 1        | 1         | 0          | 0           |
| Ca15                              | 0                   | 15                       |                        | 2                                              | 1        | 3         | 3          | 2           |
| Ca16                              | 0                   |                          | 13                     | 2                                              | 2        | 4         | 2          | 1           |
| Ca17                              | 0                   |                          | 12                     | 8                                              | 2        | 2         | 3          | 1           |
| Ca18                              | 0                   |                          | 12                     | 4                                              | 6        | 4         | 4          | 2           |
| Ca19                              | 0                   |                          | 13                     | 4                                              | 4        | 3         | 4          | 3           |
| Ca20                              | 0                   |                          | 4                      | 4                                              | 2        | 5         | 1          | 2           |
| Ca21                              | 0                   | 6                        |                        | 8                                              | 4        | 5         | 7          | 7           |
| Ca22                              | 0                   | 8                        |                        | 2                                              | 2        | 1         | 3          | 2           |
| Ca23                              | 0                   | 4                        |                        | 10                                             | 8        | 5         | 5          | 2           |
| Ca24                              | 0                   | 12                       |                        | 6                                              | 5        | 6         | 7          | 4           |
| Ca25                              | 0                   | 2                        |                        | 4                                              | 6        | 5         | 3          | 2           |
| Ca26                              | 0                   |                          | 2                      | 4                                              | 4        | 10        | 5          | 2           |
| Ca27                              | 0                   |                          | 3                      | 4                                              | 4        | 12        | 2          | 2           |
| Ca28                              | 0                   |                          | 2                      | 2                                              | 2        | 3         | 4          | 2           |
| Ca29                              | 0                   |                          | 5                      | 8                                              | 2        | 8         | 8          | 2           |
| Ca30                              | 0                   |                          | 6                      | 4                                              | 4        | 6         | 3          | 3           |
| Ca31                              | 0                   | 18                       |                        | 2                                              | 2        | 1         | 1          | 5           |
| Ca32                              | 0                   | 20                       |                        | 1                                              | 1        | 0         | 0          | 0           |
| Ca33                              | 0                   | 28                       |                        | 0                                              | 0        | 0         | 0          | 0           |
| Ca34                              | 0                   | 11                       |                        | 2                                              | 6        | 6         | 4          | 8           |

|               |   |    |    |    |    |    |    |    |
|---------------|---|----|----|----|----|----|----|----|
| Ca35          | 0 | 12 |    | 0  | 0  | 0  | 2  | 0  |
| Ca36          | 0 |    | 15 | 4  | 3  | 2  | 1  | 1  |
| Ca37          | 0 |    | 16 | 1  | 0  | 0  | 0  | 0  |
| Ca38          | 0 |    | 13 | 4  | 3  | 2  | 0  | 0  |
| Ca39          | 0 |    | 13 | 0  | 0  | 0  | 1  | 0  |
| Ca40          | 0 |    | 12 | 1  | 1  | 1  | 1  | 1  |
| Ca41          | 0 | 12 |    | 4  | 6  | 4  | 6  | 4  |
| Ca42          | 0 | 21 |    | 6  | 1  | 0  | 0  | 3  |
| Ca43          | 0 | 13 |    | 5  | 2  | 2  | 0  | 2  |
| Ca44          | 0 | 22 |    | 1  | 1  | 2  | 0  | 0  |
| Ca45          | 0 | 18 |    | 6  | 2  | 1  | 0  | 0  |
| Ca46          | 0 |    | 11 | 0  | 0  | 1  | 0  | 0  |
| Ca47          | 0 |    | 15 | 8  | 2  | 2  | 0  | 0  |
| Ca48          | 0 |    | 12 | 0  | 0  | 6  | 0  | 2  |
| Ca49          | 0 |    | 13 | 1  | 1  | 6  | 2  | 2  |
| Ca50          | 0 |    | 18 | 0  | 0  | 0  | 0  | 0  |
| <b>Week 2</b> |   |    |    |    |    |    |    |    |
| Ca51          | 0 | 11 |    | 10 | 8  | 4  | 8  | 8  |
| Ca52          | 0 | 7  |    | 1  | 1  | 1  | 1  | 1  |
| Ca53          | 0 | 8  |    | 4  |    | 2  | 2  | 1  |
| Ca54          | 0 | 14 |    | 4  | 4  | 2  | 2  | 1  |
| Ca55          | 0 | 6  |    | 8  | 6  | 6  | 12 | 4  |
| Ca56          | 0 |    | 5  | 10 | 8  | 2  | 1  | 2  |
| Ca57          | 0 |    | 2  | 10 | 8  | 2  | 1  | 2  |
| Ca58          | 0 |    | 6  | 12 | 4  | 4  | 2  | 1  |
| Ca59          | 0 |    | 1  | 4  | 4  | 1  | 1  | 1  |
| Ca60          | 0 |    | 4  | 2  | 12 | 4  | 2  | 10 |
| Ca61          | 0 | 8  |    | 6  | 2  | 1  | 1  | 1  |
| Ca62          | 0 | 14 |    | 3  | 1  | 1  | 0  | 0  |
| Ca63          | 0 | 24 |    | 6  | 6  | 1  | 0  | 0  |
| Ca64          | 0 | 24 |    | 3  | 1  | 1  | 0  | 0  |
| Ca65          | 0 | 15 |    | 2  | 1  | 3  | 3  | 2  |
| Ca66          | 0 |    | 13 | 2  | 2  | 4  | 2  | 1  |
| Ca67          | 0 |    | 12 | 8  | 2  | 2  | 3  | 1  |
| Ca68          | 0 |    | 12 | 4  | 6  | 4  | 4  | 2  |
| Ca69          | 0 |    | 13 | 4  | 4  | 3  | 4  | 3  |
| Ca70          | 0 |    | 4  | 4  | 2  | 5  | 1  | 2  |
| Ca71          | 0 | 6  |    | 8  | 4  | 5  | 7  | 7  |
| Ca72          | 0 | 8  |    | 2  | 2  | 1  | 3  | 2  |
| Ca73          | 0 | 4  |    | 10 | 8  | 5  | 5  | 2  |
| Ca74          | 0 | 12 |    | 6  | 5  | 6  | 7  | 4  |
| Ca75          | 0 | 2  |    | 4  | 6  | 5  | 3  | 2  |
| Ca76          | 0 |    | 2  | 4  | 4  | 10 | 5  | 2  |
| Ca77          | 0 |    | 3  | 4  | 4  | 12 | 2  | 2  |
| Ca78          | 0 |    | 2  | 2  | 2  | 3  | 4  | 2  |
| Ca79          | 0 |    | 5  | 8  | 2  | 8  | 8  | 2  |
| Ca80          | 0 |    | 6  | 4  | 4  | 6  | 3  | 3  |
| Ca81          | 0 | 18 |    | 2  | 2  | 1  | 1  | 5  |
| Ca82          | 0 | 20 |    | 1  | 1  | 0  | 0  | 0  |
| Ca83          | 0 | 28 |    | 0  | 0  | 0  | 0  | 0  |
| Ca84          | 0 | 11 |    | 2  | 6  | 6  | 4  | 8  |
| Ca85          | 0 | 12 |    | 0  | 0  | 0  | 2  | 0  |

|               |   |    |    |    |    |    |    |    |
|---------------|---|----|----|----|----|----|----|----|
| Ca86          | 0 |    | 15 | 4  | 3  | 2  | 1  | 1  |
| Ca87          | 0 |    | 16 | 1  | 0  | 0  | 0  | 0  |
| Ca88          | 0 |    | 13 | 4  | 3  | 2  | 0  | 0  |
| Ca89          | 0 |    | 13 | 0  | 0  | 0  | 1  | 0  |
| Ca90          | 0 |    | 12 | 1  | 1  | 1  | 1  | 1  |
| Ca91          | 0 | 12 |    | 4  | 6  | 4  | 6  | 4  |
| Ca92          | 0 | 21 |    | 6  | 1  | 0  | 0  | 3  |
| Ca93          | 0 | 13 |    | 5  | 2  | 2  | 0  | 2  |
| Ca94          | 0 | 22 |    | 1  | 1  | 2  | 0  | 0  |
| Ca95          | 0 | 18 |    | 6  | 2  | 1  | 0  | 0  |
| Ca96          | 0 |    | 11 | 0  | 0  | 1  | 0  | 0  |
| Ca97          | 0 |    | 15 | 8  | 2  | 2  | 0  | 0  |
| Ca98          | 0 |    | 12 | 0  | 0  | 6  | 0  | 2  |
| Ca99          | 0 |    | 13 | 1  | 1  | 6  | 2  | 2  |
| Ca100         | 0 |    | 18 | 0  | 0  | 0  | 0  | 0  |
| <b>Week 3</b> |   |    |    |    |    |    |    |    |
| Ca101         | 0 | 11 |    | 10 | 8  | 4  | 8  | 8  |
| Ca102         | 0 | 7  |    | 1  | 1  | 1  | 1  | 1  |
| Ca103         | 0 | 8  |    | 4  |    | 2  | 2  | 1  |
| Ca104         | 0 | 14 |    | 4  | 4  | 2  | 2  | 1  |
| Ca105         | 0 | 6  |    | 8  | 6  | 6  | 12 | 4  |
| Ca106         | 0 |    | 5  | 10 | 8  | 2  | 1  | 2  |
| Ca107         | 0 |    | 2  | 10 | 8  | 2  | 1  | 2  |
| Ca108         | 0 |    | 6  | 12 | 4  | 4  | 2  | 1  |
| Ca109         | 0 |    | 1  | 4  | 4  | 1  | 1  | 1  |
| Ca110         | 0 |    | 4  | 2  | 12 | 4  | 2  | 10 |
| Ca111         | 0 | 8  |    | 6  | 2  | 1  | 1  | 1  |
| Ca112         | 0 | 14 |    | 3  | 1  | 1  | 0  | 0  |
| Ca113         | 0 | 24 |    | 6  | 6  | 1  | 0  | 0  |
| Ca114         | 0 | 24 |    | 3  | 1  | 1  | 0  | 0  |
| Ca115         | 0 | 15 |    | 2  | 1  | 3  | 3  | 2  |
| Ca116         | 0 |    | 13 | 2  | 2  | 4  | 2  | 1  |
| Ca117         | 0 |    | 12 | 8  | 2  | 2  | 3  | 1  |
| Ca118         | 0 |    | 12 | 4  | 6  | 4  | 4  | 2  |
| Ca119         | 0 |    | 13 | 4  | 4  | 3  | 4  | 3  |
| Ca120         | 0 |    | 4  | 4  | 2  | 5  | 1  | 2  |
| Ca121         | 0 | 6  |    | 8  | 4  | 5  | 7  | 7  |
| Ca122         | 0 | 8  |    | 2  | 2  | 1  | 3  | 2  |
| Ca123         | 0 | 4  |    | 10 | 8  | 5  | 5  | 2  |
| Ca124         | 0 | 12 |    | 6  | 5  | 6  | 7  | 4  |
| Ca125         | 0 | 2  |    | 4  | 6  | 5  | 3  | 2  |
| Ca126         | 0 |    | 2  | 4  | 4  | 10 | 5  | 2  |
| Ca127         | 0 |    | 3  | 4  | 4  | 12 | 2  | 2  |
| Ca128         | 0 |    | 2  | 2  | 2  | 3  | 4  | 2  |
| Ca129         | 0 |    | 5  | 8  | 2  | 8  | 8  | 2  |
| Ca130         | 0 |    | 6  | 4  | 4  | 6  | 3  | 3  |
| Ca131         | 0 | 18 |    | 2  | 2  | 1  | 1  | 5  |
| Ca132         | 0 | 20 |    | 1  | 1  | 0  | 0  | 0  |
| Ca133         | 0 | 28 |    | 0  | 0  | 0  | 0  | 0  |
| Ca134         | 0 | 11 |    | 2  | 6  | 6  | 4  | 8  |
| Ca135         | 0 | 12 |    | 0  | 0  | 0  | 2  | 0  |
| Ca136         | 0 |    | 15 | 4  | 3  | 2  | 1  | 1  |

|               |   |    |    |    |    |    |    |    |
|---------------|---|----|----|----|----|----|----|----|
| Ca137         | 0 |    | 16 | 1  | 0  | 0  | 0  | 0  |
| Ca138         | 0 |    | 13 | 4  | 3  | 2  | 0  | 0  |
| Ca139         | 0 |    | 13 | 0  | 0  | 0  | 1  | 0  |
| Ca140         | 0 |    | 12 | 1  | 1  | 1  | 1  | 1  |
| Ca141         | 0 | 12 |    | 4  | 6  | 4  | 6  | 4  |
| Ca142         | 0 | 21 |    | 6  | 1  | 0  | 0  | 3  |
| Ca143         | 0 | 13 |    | 5  | 2  | 2  | 0  | 2  |
| Ca144         | 0 | 22 |    | 1  | 1  | 2  | 0  | 0  |
| Ca145         | 0 | 18 |    | 6  | 2  | 1  | 0  | 0  |
| Ca146         | 0 |    | 11 | 0  | 0  | 1  | 0  | 0  |
| Ca147         | 0 |    | 15 | 8  | 2  | 2  | 0  | 0  |
| Ca148         | 0 |    | 12 | 0  | 0  | 6  | 0  | 2  |
| Ca149         | 0 |    | 13 | 1  | 1  | 6  | 2  | 2  |
| Ca150         | 0 |    | 18 | 0  | 0  | 0  | 0  | 0  |
| <b>Week 4</b> |   |    |    |    |    |    |    |    |
| Ca151         | 0 | 11 |    | 10 | 8  | 4  | 8  | 8  |
| Ca152         | 0 | 7  |    | 1  | 1  | 1  | 1  | 1  |
| Ca153         | 0 | 8  |    | 4  |    | 2  | 2  | 1  |
| Ca154         | 0 | 14 |    | 4  | 4  | 2  | 2  | 1  |
| Ca155         | 0 | 6  |    | 8  | 6  | 6  | 12 | 4  |
| Ca156         | 0 |    | 5  | 10 | 8  | 2  | 1  | 2  |
| Ca157         | 0 |    | 2  | 10 | 8  | 2  | 1  | 2  |
| Ca158         | 0 |    | 6  | 12 | 4  | 4  | 2  | 1  |
| Ca159         | 0 |    | 1  | 4  | 4  | 1  | 1  | 1  |
| Ca160         | 0 |    | 4  | 2  | 12 | 4  | 2  | 10 |
| Ca161         | 0 | 8  |    | 6  | 2  | 1  | 1  | 1  |
| Ca162         | 0 | 14 |    | 3  | 1  | 1  | 0  | 0  |
| Ca163         | 0 | 24 |    | 6  | 6  | 1  | 0  | 0  |
| Ca164         | 0 | 24 |    | 3  | 1  | 1  | 0  | 0  |
| Ca165         | 0 | 15 |    | 2  | 1  | 3  | 3  | 2  |
| Ca166         | 0 |    | 13 | 2  | 2  | 4  | 2  | 1  |
| Ca167         | 0 |    | 12 | 8  | 2  | 2  | 3  | 1  |
| Ca168         | 0 |    | 12 | 4  | 6  | 4  | 4  | 2  |
| Ca169         | 0 |    | 13 | 4  | 4  | 3  | 4  | 3  |
| Ca170         | 0 |    | 4  | 4  | 2  | 5  | 1  | 2  |
| Ca171         | 0 | 6  |    | 8  | 4  | 5  | 7  | 7  |
| Ca172         | 0 | 8  |    | 2  | 2  | 1  | 3  | 2  |
| Ca173         | 0 | 4  |    | 10 | 8  | 5  | 5  | 2  |
| Ca174         | 0 | 12 |    | 6  | 5  | 6  | 7  | 4  |
| Ca175         | 0 | 2  |    | 4  | 6  | 5  | 3  | 2  |
| Ca176         | 0 |    | 2  | 4  | 4  | 10 | 5  | 2  |
| Ca177         | 0 |    | 3  | 4  | 4  | 12 | 2  | 2  |
| Ca178         | 0 |    | 2  | 2  | 2  | 3  | 4  | 2  |
| Ca179         | 0 |    | 5  | 8  | 2  | 8  | 8  | 2  |
| Ca180         | 0 |    | 6  | 4  | 4  | 6  | 3  | 3  |
| Ca181         | 0 | 18 |    | 2  | 2  | 1  | 1  | 5  |
| Ca182         | 0 | 20 |    | 1  | 1  | 0  | 0  | 0  |
| Ca183         | 0 | 28 |    | 0  | 0  | 0  | 0  | 0  |
| Ca184         | 0 | 11 |    | 2  | 6  | 6  | 4  | 8  |
| Ca185         | 0 | 12 |    | 0  | 0  | 0  | 2  | 0  |
| Ca186         | 0 |    | 15 | 4  | 3  | 2  | 1  | 1  |
| Ca187         | 0 |    | 16 | 1  | 0  | 0  | 0  | 0  |

|               |   |    |    |    |    |    |    |    |
|---------------|---|----|----|----|----|----|----|----|
| Ca188         | 0 |    | 13 | 4  | 3  | 2  | 0  | 0  |
| Ca189         | 0 |    | 13 | 0  | 0  | 0  | 1  | 0  |
| Ca190         | 0 |    | 12 | 1  | 1  | 1  | 1  | 1  |
| Ca191         | 0 | 12 |    | 4  | 6  | 4  | 6  | 4  |
| Ca192         | 0 | 21 |    | 6  | 1  | 0  | 0  | 3  |
| Ca193         | 0 | 13 |    | 5  | 2  | 2  | 0  | 2  |
| Ca194         | 0 | 22 |    | 1  | 1  | 2  | 0  | 0  |
| Ca195         | 0 | 18 |    | 6  | 2  | 1  | 0  | 0  |
| Ca196         | 0 |    | 11 | 0  | 0  | 1  | 0  | 0  |
| Ca197         | 0 |    | 15 | 8  | 2  | 2  | 0  | 0  |
| Ca198         | 0 |    | 12 | 0  | 0  | 6  | 0  | 2  |
| Ca199         | 0 |    | 13 | 1  | 1  | 6  | 2  | 2  |
| Ca200         | 0 |    | 18 | 0  | 0  | 0  | 0  | 0  |
| <b>Week 5</b> |   |    |    |    |    |    |    |    |
| Ca201         | 0 | 11 |    | 10 | 8  | 4  | 8  | 8  |
| Ca202         | 0 | 7  |    | 1  | 1  | 1  | 1  | 1  |
| Ca203         | 0 | 8  |    | 4  |    | 2  | 2  | 1  |
| Ca204         | 0 | 14 |    | 4  | 4  | 2  | 2  | 1  |
| Ca205         | 0 | 6  |    | 8  | 6  | 6  | 12 | 4  |
| Ca206         | 0 |    | 5  | 10 | 8  | 2  | 1  | 2  |
| Ca207         | 0 |    | 2  | 10 | 8  | 2  | 1  | 2  |
| Ca208         | 0 |    | 6  | 12 | 4  | 4  | 2  | 1  |
| Ca209         | 0 |    | 1  | 4  | 4  | 1  | 1  | 1  |
| Ca210         | 0 |    | 4  | 2  | 12 | 4  | 2  | 10 |
| Ca211         | 0 | 8  |    | 6  | 2  | 1  | 1  | 1  |
| Ca212         | 0 | 14 |    | 3  | 1  | 1  | 0  | 0  |
| Ca213         | 0 | 24 |    | 6  | 6  | 1  | 0  | 0  |
| Ca214         | 0 | 24 |    | 3  | 1  | 1  | 0  | 0  |
| Ca215         | 0 | 15 |    | 2  | 1  | 3  | 3  | 2  |
| Ca216         | 0 |    | 13 | 2  | 2  | 4  | 2  | 1  |
| Ca217         | 0 |    | 12 | 8  | 2  | 2  | 3  | 1  |
| Ca218         | 0 |    | 12 | 4  | 6  | 4  | 4  | 2  |
| Ca219         | 0 |    | 13 | 4  | 4  | 3  | 4  | 3  |
| Ca220         | 0 |    | 4  | 4  | 2  | 5  | 1  | 2  |
| Ca221         | 0 | 6  |    | 8  | 4  | 5  | 7  | 7  |
| Ca222         | 0 | 8  |    | 2  | 2  | 1  | 3  | 2  |
| Ca223         | 0 | 4  |    | 10 | 8  | 5  | 5  | 2  |
| Ca224         | 0 | 12 |    | 6  | 5  | 6  | 7  | 4  |
| Ca225         | 0 | 2  |    | 4  | 6  | 5  | 3  | 2  |
| Ca226         | 0 |    | 2  | 4  | 4  | 10 | 5  | 2  |
| Ca227         | 0 |    | 3  | 4  | 4  | 12 | 2  | 2  |
| Ca228         | 0 |    | 2  | 2  | 2  | 3  | 4  | 2  |
| Ca229         | 0 |    | 5  | 8  | 2  | 8  | 8  | 2  |
| Ca230         | 0 |    | 6  | 4  | 4  | 6  | 3  | 3  |
| Ca231         | 0 | 18 |    | 2  | 2  | 1  | 1  | 5  |
| Ca232         | 0 | 20 |    | 1  | 1  | 0  | 0  | 0  |
| Ca233         | 0 | 28 |    | 0  | 0  | 0  | 0  | 0  |
| Ca234         | 0 | 11 |    | 2  | 6  | 6  | 4  | 8  |
| Ca235         | 0 | 12 |    | 0  | 0  | 0  | 2  | 0  |
| Ca236         | 0 |    | 15 | 4  | 3  | 2  | 1  | 1  |
| Ca237         | 0 |    | 16 | 1  | 0  | 0  | 0  | 0  |
| Ca238         | 0 |    | 13 | 4  | 3  | 2  | 0  | 0  |

|       |   |    |   |   |   |   |   |
|-------|---|----|---|---|---|---|---|
| Ca239 | 0 | 13 | 0 | 0 | 0 | 1 | 0 |
| Ca240 | 0 | 12 | 1 | 1 | 1 | 1 | 1 |
| Ca241 | 0 | 12 | 4 | 6 | 4 | 6 | 4 |
| Ca242 | 0 | 21 | 6 | 1 | 0 | 0 | 3 |
| Ca243 | 0 | 13 | 5 | 2 | 2 | 0 | 2 |
| Ca244 | 0 | 22 | 1 | 1 | 2 | 0 | 0 |
| Ca245 | 0 | 18 | 6 | 2 | 1 | 0 | 0 |
| Ca246 | 0 | 11 | 0 | 0 | 1 | 0 | 0 |
| Ca247 | 0 | 15 | 8 | 2 | 2 | 0 | 0 |
| Ca248 | 0 | 12 | 0 | 0 | 6 | 0 | 2 |
| Ca249 | 0 | 13 | 1 | 1 | 6 | 2 | 2 |
| Ca250 | 0 | 18 | 0 | 0 | 0 | 0 | 0 |

**MIC and MFC of *L. acidophilus* against *C. albicans***

The result revealed that concentrations 62.5mg/ml, 125mg/ml and 250mg/ml had little effect on *C. albicans* isolates when clear growth was noticed,

while concentration 15.625mg/ml and 31.25mg/ml led to minimized and sharp decrease in growth (MIC) of *C. albicans*, the concentration 15.625mg/ml inhibit (MFC) of *C. albicans* growth completely (Table 4).

**Table 4. Minimum Inhibitory Concentration (MIC) *L. acidophilus* Probiotics against *C. albicans* at 520nm Spectrophotometric reading**

| <i>C. albicans</i><br>isolates | Concentration of <i>L. acidophilus</i> Probiotics (mg/ml) |         |        |        |        |
|--------------------------------|-----------------------------------------------------------|---------|--------|--------|--------|
|                                | 250.000                                                   | 125.000 | 62.500 | 31.250 | 15.625 |
| <b>Week 1</b>                  |                                                           |         |        |        |        |
| Ca1                            | 1.490                                                     | 1.747   | 1.514  | 1.486  | 1.434  |
| Ca2                            | 2.490                                                     | 2.747   | 2.514  | 2.486  | 2.434  |
| Ca3                            | 1.607                                                     | 1.507   | 1.490  | 1.377  | 1.427  |
| Ca4                            | 1.624                                                     | 1.677   | 1.453  | 1.475  | 1.434  |
| Ca5                            | 0.947                                                     | 0.818   | 0.674  | 0.497  | 0.271  |
| Ca6                            | 1.138                                                     | 0.760   | 0.570  | 0.578  | 0.381  |
| Ca7                            | 1.016                                                     | 0.686   | 0.533  | 0.334  | 0.423  |
| Ca8                            | 0.857                                                     | 0.785   | 0.543  | 0.278  | 0.413  |
| Ca9                            | 0.997                                                     | 0.626   | 0.603  | 0.546  | 0.603  |
| Ca10                           | 0.924                                                     | 0.951   | 0.801  | 0.621  | 0.721  |
| Ca11                           | 0.363                                                     | 1.031   | 0.368  | 1.450  | 0.194  |
| Ca12                           | 2.339                                                     | 2.127   | 2.189  | 1.386  | 1.198  |
| Ca13                           | 2.388                                                     | 2.157   | 1.573  | 1.594  | 1.286  |
| Ca14                           | 2.260                                                     | 2.093   | 1.701  | 1.341  | 1.457  |
| Ca15                           | 2.481                                                     | 1.860   | 1.826  | 1.490  | 1.261  |
| Ca16                           | 2.496                                                     | 1.921   | 1.505  | 1.525  | 1.263  |
| Ca17                           | 2.782                                                     | 2.025   | 1.661  | 1.360  | 1.106  |
| Ca18                           | 2.381                                                     | 2.104   | 1.854  | 1.781  | 1.217  |
| Ca19                           | 2.709                                                     | 2.081   | 1.676  | 1.870  | 1.448  |
| Ca20                           | 2.871                                                     | 2.436   | 1.253  | 1.221  | 1.105  |
| Ca21                           | 1.169                                                     | 0.641   | 0.574  | 0.459  | 0.320  |
| Ca22                           | 1.414                                                     | 1.004   | 0.784  | 0.290  | 0.196  |

|               |       |       |       |       |       |
|---------------|-------|-------|-------|-------|-------|
| Ca23          | 2.751 | 2.612 | 2.169 | 1.770 | 1.431 |
| Ca24          | 1.874 | 3.100 | 2.369 | 1.649 | 1.735 |
| Ca25          | 2.351 | 2.189 | 1.743 | 1.690 | 1.618 |
| Ca26          | 2.153 | 1.906 | 1.674 | 1.492 | 1.575 |
| Ca27          | 2.110 | 2.265 | 2.168 | 2.816 | 1.830 |
| Ca28          | 2.458 | 2.322 | 2.127 | 1.834 | 1.636 |
| Ca29          | 2.038 | 2.176 | 1.900 | 1.731 | 1.740 |
| Ca30          | 1.740 | 1.767 | 1.720 | 1.658 | 1.652 |
| Ca31          | 2.623 | 1.906 | 1.627 | 1.373 | 1.249 |
| Ca32          | 2.646 | 2.052 | 1.759 | 1.417 | 1.122 |
| Ca33          | 2.456 | 1.507 | 1.676 | 1.584 | 1.486 |
| Ca34          | 2.722 | 2.083 | 1.275 | 1.226 | 1.188 |
| Ca35          | 2.010 | 1.717 | 1.664 | 1.571 | 1.371 |
| Ca36          | 2.218 | 1.400 | 1.338 | 1.336 | 1.168 |
| Ca37          | 0.427 | 0.303 | 0.185 | 0.140 | 0.157 |
| Ca38          | 1.340 | 1.108 | 1.130 | 1.138 | 1.233 |
| Ca39          | 0.399 | 0.268 | 0.277 | 0.509 | 0.191 |
| Ca40          | 1.174 | 1.061 | 1.081 | 1.080 | 1.231 |
| Ca41          | 0.873 | 0.342 | 0.302 | 0.331 | 0.236 |
| Ca42          | 1.523 | 1.246 | 1.209 | 1.256 | 1.173 |
| Ca43          | 1.643 | 0.403 | 0.212 | 0.252 | 0.245 |
| Ca44          | 1.466 | 1.270 | 1.334 | 1.130 | 1.284 |
| Ca45          | 0.460 | 0.320 | 0.337 | 0.304 | 0.261 |
| Ca46          | 0.796 | 0.312 | 0.235 | 0.235 | 0.273 |
| Ca47          | 1.363 | 1.221 | 1.153 | 1.184 | 1.097 |
| Ca48          | 1.224 | 1.109 | 1.100 | 1.016 | 1.017 |
| Ca49          | 1.354 | 1.265 | 1.135 | 1.178 | 1.081 |
| Ca50          | 0.184 | 1.634 | 0.412 | 0.341 | 0.669 |
| <b>Week 2</b> |       |       |       |       |       |
| Ca51          | 1.490 | 1.747 | 1.514 | 1.486 | 1.434 |
| Ca52          | 1.725 | 1.625 | 1.494 | 1.468 | 1.444 |
| Ca53          | 1.607 | 1.507 | 1.490 | 1.377 | 1.427 |
| Ca54          | 1.624 | 1.677 | 1.453 | 1.475 | 1.434 |
| Ca55          | 0.947 | 0.818 | 0.674 | 0.497 | 0.271 |
| Ca56          | 1.138 | 0.760 | 0.570 | 0.578 | 0.381 |
| Ca57          | 1.016 | 0.686 | 0.533 | 0.334 | 0.423 |
| Ca58          | 0.857 | 0.785 | 0.543 | 0.278 | 0.413 |
| Ca59          | 0.997 | 0.626 | 0.603 | 0.546 | 0.603 |
| Ca60          | 0.924 | 0.951 | 0.801 | 0.621 | 0.721 |
| Ca61          | 0.363 | 1.031 | 0.368 | 1.450 | 0.194 |
| Ca62          | 2.339 | 2.127 | 2.189 | 1.386 | 1.198 |
| Ca63          | 2.388 | 2.157 | 1.573 | 1.594 | 1.286 |
| Ca64          | 2.260 | 2.093 | 1.701 | 1.341 | 1.457 |
| Ca65          | 2.481 | 1.860 | 1.826 | 1.490 | 1.261 |
| Ca66          | 2.496 | 1.921 | 1.505 | 1.525 | 1.263 |

|               |       |       |       |       |       |
|---------------|-------|-------|-------|-------|-------|
| Ca67          | 2.782 | 2.025 | 1.661 | 1.360 | 1.106 |
| Ca68          | 2.381 | 2.104 | 1.854 | 1.781 | 1.217 |
| Ca69          | 2.709 | 2.081 | 1.676 | 1.870 | 1.448 |
| Ca70          | 2.871 | 2.436 | 1.253 | 1.221 | 1.105 |
| Ca71          | 1.169 | 0.641 | 0.574 | 0.459 | 0.320 |
| Ca72          | 1.414 | 1.004 | 0.784 | 0.290 | 0.196 |
| Ca73          | 2.751 | 2.612 | 2.169 | 1.770 | 1.431 |
| Ca74          | 1.874 | 3.100 | 2.369 | 1.649 | 1.735 |
| Ca75          | 2.351 | 2.189 | 1.743 | 1.690 | 1.618 |
| Ca76          | 2.153 | 1.906 | 1.674 | 1.492 | 1.575 |
| Ca77          | 2.110 | 2.265 | 2.168 | 2.816 | 1.830 |
| Ca78          | 2.458 | 2.322 | 2.127 | 1.834 | 1.636 |
| Ca79          | 2.038 | 2.176 | 1.900 | 1.731 | 1.740 |
| Ca80          | 1.740 | 1.767 | 1.720 | 1.658 | 1.652 |
| Ca81          | 2.623 | 1.906 | 1.627 | 1.373 | 1.249 |
| Ca82          | 2.646 | 2.052 | 1.759 | 1.417 | 1.122 |
| Ca83          | 2.456 | 1.507 | 1.676 | 1.584 | 1.486 |
| Ca84          | 2.722 | 2.083 | 1.275 | 1.226 | 1.188 |
| Ca85          | 2.010 | 1.717 | 1.664 | 1.571 | 1.371 |
| Ca86          | 2.218 | 1.400 | 1.338 | 1.336 | 1.168 |
| Ca87          | 0.427 | 0.303 | 0.185 | 0.140 | 0.157 |
| Ca88          | 1.340 | 1.108 | 1.130 | 1.138 | 1.233 |
| Ca89          | 0.399 | 0.268 | 0.277 | 0.509 | 0.191 |
| Ca90          | 1.174 | 1.061 | 1.081 | 1.080 | 1.231 |
| Ca91          | 0.873 | 0.342 | 0.302 | 0.331 | 0.236 |
| Ca92          | 1.523 | 1.246 | 1.209 | 1.256 | 1.173 |
| Ca93          | 1.643 | 0.403 | 0.212 | 0.252 | 0.245 |
| Ca94          | 1.466 | 1.270 | 1.334 | 1.130 | 1.284 |
| Ca95          | 0.460 | 0.320 | 0.337 | 0.304 | 0.261 |
| Ca96          | 0.796 | 0.312 | 0.235 | 0.235 | 0.273 |
| Ca97          | 1.363 | 1.221 | 1.153 | 1.184 | 1.097 |
| Ca98          | 1.224 | 1.109 | 1.100 | 1.016 | 1.017 |
| Ca99          | 1.354 | 1.265 | 1.135 | 1.178 | 1.081 |
| Ca100         | 0.184 | 1.634 | 0.412 | 0.341 | 0.669 |
| <b>Week 3</b> |       |       |       |       |       |
| Ca101         | 1.490 | 1.747 | 1.514 | 1.486 | 1.434 |
| Ca102         | 1.725 | 1.625 | 1.494 | 1.468 | 1.444 |
| Ca103         | 1.607 | 1.507 | 1.490 | 1.377 | 1.427 |
| Ca104         | 1.624 | 1.677 | 1.453 | 1.475 | 1.434 |
| Ca105         | 0.947 | 0.818 | 0.674 | 0.497 | 0.271 |
| Ca106         | 1.138 | 0.760 | 0.570 | 0.578 | 0.381 |
| Ca107         | 1.016 | 0.686 | 0.533 | 0.334 | 0.423 |
| Ca108         | 0.857 | 0.785 | 0.543 | 0.278 | 0.413 |
| Ca109         | 0.997 | 0.626 | 0.603 | 0.546 | 0.603 |
| Ca110         | 0.924 | 0.951 | 0.801 | 0.621 | 0.721 |

|               |       |       |       |       |       |
|---------------|-------|-------|-------|-------|-------|
| Ca111         | 0.363 | 1.031 | 0.368 | 1.450 | 0.194 |
| Ca112         | 2.339 | 2.127 | 2.189 | 1.386 | 1.198 |
| Ca113         | 2.388 | 2.157 | 1.573 | 1.594 | 1.286 |
| Ca114         | 2.260 | 2.093 | 1.701 | 1.341 | 1.457 |
| Ca115         | 2.481 | 1.860 | 1.826 | 1.490 | 1.261 |
| Ca116         | 2.496 | 1.921 | 1.505 | 1.525 | 1.263 |
| Ca117         | 2.782 | 2.025 | 1.661 | 1.360 | 1.106 |
| Ca118         | 2.381 | 2.104 | 1.854 | 1.781 | 1.217 |
| Ca119         | 2.709 | 2.081 | 1.676 | 1.870 | 1.448 |
| Ca120         | 2.871 | 2.436 | 1.253 | 1.221 | 1.105 |
| Ca121         | 1.169 | 0.641 | 0.574 | 0.459 | 0.320 |
| Ca122         | 1.414 | 1.004 | 0.784 | 0.290 | 0.196 |
| Ca123         | 2.751 | 2.612 | 2.169 | 1.770 | 1.431 |
| Ca124         | 1.874 | 3.100 | 2.369 | 1.649 | 1.735 |
| Ca125         | 2.351 | 2.189 | 1.743 | 1.690 | 1.618 |
| Ca126         | 2.153 | 1.906 | 1.674 | 1.492 | 1.575 |
| Ca127         | 2.110 | 2.265 | 2.168 | 2.816 | 1.830 |
| Ca128         | 2.458 | 2.322 | 2.127 | 1.834 | 1.636 |
| Ca129         | 2.038 | 2.176 | 1.900 | 1.731 | 1.740 |
| Ca130         | 1.740 | 1.767 | 1.720 | 1.658 | 1.652 |
| Ca131         | 2.623 | 1.906 | 1.627 | 1.373 | 1.249 |
| Ca132         | 2.646 | 2.052 | 1.759 | 1.417 | 1.122 |
| Ca133         | 2.456 | 1.507 | 1.676 | 1.584 | 1.486 |
| Ca134         | 2.722 | 2.083 | 1.275 | 1.226 | 1.188 |
| Ca135         | 2.010 | 1.717 | 1.664 | 1.571 | 1.371 |
| Ca136         | 2.218 | 1.400 | 1.338 | 1.336 | 1.168 |
| Ca137         | 0.427 | 0.303 | 0.185 | 0.140 | 0.157 |
| Ca138         | 1.340 | 1.108 | 1.130 | 1.138 | 1.233 |
| Ca139         | 0.399 | 0.268 | 0.277 | 0.509 | 0.191 |
| Ca140         | 1.174 | 1.061 | 1.081 | 1.080 | 1.231 |
| Ca141         | 0.873 | 0.342 | 0.302 | 0.331 | 0.236 |
| Ca142         | 1.523 | 1.246 | 1.209 | 1.256 | 1.173 |
| Ca143         | 1.643 | 0.403 | 0.212 | 0.252 | 0.245 |
| Ca144         | 1.466 | 1.270 | 1.334 | 1.130 | 1.284 |
| Ca145         | 0.460 | 0.320 | 0.337 | 0.304 | 0.261 |
| Ca146         | 0.796 | 0.312 | 0.235 | 0.235 | 0.273 |
| Ca147         | 1.363 | 1.221 | 1.153 | 1.184 | 1.097 |
| Ca148         | 1.224 | 1.109 | 1.100 | 1.016 | 1.017 |
| Ca149         | 1.354 | 1.265 | 1.135 | 1.178 | 1.081 |
| Ca150         | 0.184 | 1.634 | 0.412 | 0.341 | 0.669 |
| <b>Week 4</b> |       |       |       |       |       |
| Ca151         | 1.490 | 1.747 | 1.514 | 1.486 | 1.434 |
| Ca152         | 1.725 | 1.625 | 1.494 | 1.468 | 1.444 |
| Ca153         | 1.607 | 1.507 | 1.490 | 1.377 | 1.427 |
| Ca154         | 1.624 | 1.677 | 1.453 | 1.475 | 1.434 |

|       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|
| Ca155 | 0.947 | 0.818 | 0.674 | 0.497 | 0.271 |
| Ca156 | 1.138 | 0.760 | 0.570 | 0.578 | 0.381 |
| Ca157 | 1.016 | 0.686 | 0.533 | 0.334 | 0.423 |
| Ca158 | 0.857 | 0.785 | 0.543 | 0.278 | 0.413 |
| Ca159 | 0.997 | 0.626 | 0.603 | 0.546 | 0.603 |
| Ca160 | 0.924 | 0.951 | 0.801 | 0.621 | 0.721 |
| Ca161 | 0.363 | 1.031 | 0.368 | 1.450 | 0.194 |
| Ca162 | 2.339 | 2.127 | 2.189 | 1.386 | 1.198 |
| Ca163 | 2.388 | 2.157 | 1.573 | 1.594 | 1.286 |
| Ca164 | 2.260 | 2.093 | 1.701 | 1.341 | 1.457 |
| Ca165 | 2.481 | 1.860 | 1.826 | 1.490 | 1.261 |
| Ca166 | 2.496 | 1.921 | 1.505 | 1.525 | 1.263 |
| Ca167 | 2.782 | 2.025 | 1.661 | 1.360 | 1.106 |
| Ca168 | 2.381 | 2.104 | 1.854 | 1.781 | 1.217 |
| Ca169 | 2.709 | 2.081 | 1.676 | 1.870 | 1.448 |
| Ca170 | 2.871 | 2.436 | 1.253 | 1.221 | 1.105 |
| Ca171 | 1.169 | 0.641 | 0.574 | 0.459 | 0.320 |
| Ca172 | 1.414 | 1.004 | 0.784 | 0.290 | 0.196 |
| Ca173 | 2.751 | 2.612 | 2.169 | 1.770 | 1.431 |
| Ca174 | 1.874 | 3.100 | 2.369 | 1.649 | 1.735 |
| Ca175 | 2.351 | 2.189 | 1.743 | 1.690 | 1.618 |
| Ca176 | 2.153 | 1.906 | 1.674 | 1.492 | 1.575 |
| Ca177 | 2.110 | 2.265 | 2.168 | 2.816 | 1.830 |
| Ca178 | 2.458 | 2.322 | 2.127 | 1.834 | 1.636 |
| Ca179 | 2.038 | 2.176 | 1.900 | 1.731 | 1.740 |
| Ca180 | 1.740 | 1.767 | 1.720 | 1.658 | 1.652 |
| Ca181 | 2.623 | 1.906 | 1.627 | 1.373 | 1.249 |
| Ca182 | 2.646 | 2.052 | 1.759 | 1.417 | 1.122 |
| Ca183 | 2.456 | 1.507 | 1.676 | 1.584 | 1.486 |
| Ca184 | 2.722 | 2.083 | 1.275 | 1.226 | 1.188 |
| Ca185 | 2.010 | 1.717 | 1.664 | 1.571 | 1.371 |
| Ca186 | 2.218 | 1.400 | 1.338 | 1.336 | 1.168 |
| Ca187 | 0.427 | 0.303 | 0.185 | 0.140 | 0.157 |
| Ca188 | 1.340 | 1.108 | 1.130 | 1.138 | 1.233 |
| Ca189 | 0.399 | 0.268 | 0.277 | 0.509 | 0.191 |
| Ca190 | 1.174 | 1.061 | 1.081 | 1.080 | 1.231 |
| Ca191 | 0.873 | 0.342 | 0.302 | 0.331 | 0.236 |
| Ca192 | 1.523 | 1.246 | 1.209 | 1.256 | 1.173 |
| Ca193 | 1.643 | 0.403 | 0.212 | 0.252 | 0.245 |
| Ca194 | 1.466 | 1.270 | 1.334 | 1.130 | 1.284 |
| Ca195 | 0.460 | 0.320 | 0.337 | 0.304 | 0.261 |
| Ca196 | 0.796 | 0.312 | 0.235 | 0.235 | 0.273 |
| Ca197 | 1.363 | 1.221 | 1.153 | 1.184 | 1.097 |
| Ca198 | 1.224 | 1.109 | 1.100 | 1.016 | 1.017 |
| Ca199 | 1.354 | 1.265 | 1.135 | 1.178 | 1.081 |

|               |       |       |       |       |       |
|---------------|-------|-------|-------|-------|-------|
| <i>Ca200</i>  | 0.184 | 1.634 | 0.412 | 0.341 | 0.669 |
| <b>Week 5</b> |       |       |       |       |       |
| <i>Ca201</i>  | 1.490 | 1.747 | 1.514 | 1.486 | 1.434 |
| <i>Ca202</i>  | 1.725 | 1.625 | 1.494 | 1.468 | 1.444 |
| <i>Ca203</i>  | 1.607 | 1.507 | 1.490 | 1.377 | 1.427 |
| <i>Ca204</i>  | 1.624 | 1.677 | 1.453 | 1.475 | 1.434 |
| <i>Ca205</i>  | 0.947 | 0.818 | 0.674 | 0.497 | 0.271 |
| <i>Ca206</i>  | 1.138 | 0.760 | 0.570 | 0.578 | 0.381 |
| <i>Ca207</i>  | 1.016 | 0.686 | 0.533 | 0.334 | 0.423 |
| <i>Ca208</i>  | 0.857 | 0.785 | 0.543 | 0.278 | 0.413 |
| <i>Ca209</i>  | 0.997 | 0.626 | 0.603 | 0.546 | 0.603 |
| <i>Ca210</i>  | 0.924 | 0.951 | 0.801 | 0.621 | 0.721 |
| <i>Ca211</i>  | 0.363 | 1.031 | 0.368 | 1.450 | 0.194 |
| <i>Ca212</i>  | 2.339 | 2.127 | 2.189 | 1.386 | 1.198 |
| <i>Ca213</i>  | 2.388 | 2.157 | 1.573 | 1.594 | 1.286 |
| <i>Ca214</i>  | 2.260 | 2.093 | 1.701 | 1.341 | 1.457 |
| <i>Ca215</i>  | 2.481 | 1.860 | 1.826 | 1.490 | 1.261 |
| <i>Ca216</i>  | 2.496 | 1.921 | 1.505 | 1.525 | 1.263 |
| <i>Ca217</i>  | 2.782 | 2.025 | 1.661 | 1.360 | 1.106 |
| <i>Ca218</i>  | 2.381 | 2.104 | 1.854 | 1.781 | 1.217 |
| <i>Ca219</i>  | 2.709 | 2.081 | 1.676 | 1.870 | 1.448 |
| <i>Ca220</i>  | 2.871 | 2.436 | 1.253 | 1.221 | 1.105 |
| <i>Ca221</i>  | 1.169 | 0.641 | 0.574 | 0.459 | 0.320 |
| <i>Ca222</i>  | 1.414 | 1.004 | 0.784 | 0.290 | 0.196 |
| <i>Ca223</i>  | 2.751 | 2.612 | 2.169 | 1.770 | 1.431 |
| <i>Ca224</i>  | 1.874 | 3.100 | 2.369 | 1.649 | 1.735 |
| <i>Ca225</i>  | 2.351 | 2.189 | 1.743 | 1.690 | 1.618 |
| <i>Ca226</i>  | 2.153 | 1.906 | 1.674 | 1.492 | 1.575 |
| <i>Ca227</i>  | 2.110 | 2.265 | 2.168 | 2.816 | 1.830 |
| <i>Ca228</i>  | 2.458 | 2.322 | 2.127 | 1.834 | 1.636 |
| <i>Ca229</i>  | 2.038 | 2.176 | 1.900 | 1.731 | 1.740 |
| <i>Ca230</i>  | 1.740 | 1.767 | 1.720 | 1.658 | 1.652 |
| <i>Ca231</i>  | 2.623 | 1.906 | 1.627 | 1.373 | 1.249 |
| <i>Ca232</i>  | 2.646 | 2.052 | 1.759 | 1.417 | 1.122 |
| <i>Ca233</i>  | 2.456 | 1.507 | 1.676 | 1.584 | 1.486 |
| <i>Ca234</i>  | 2.722 | 2.083 | 1.275 | 1.226 | 1.188 |
| <i>Ca235</i>  | 2.010 | 1.717 | 1.664 | 1.571 | 1.371 |
| <i>Ca236</i>  | 2.218 | 1.400 | 1.338 | 1.336 | 1.168 |
| <i>Ca237</i>  | 0.427 | 0.303 | 0.185 | 0.140 | 0.157 |
| <i>Ca238</i>  | 1.340 | 1.108 | 1.130 | 1.138 | 1.233 |
| <i>Ca239</i>  | 0.399 | 0.268 | 0.277 | 0.509 | 0.191 |
| <i>Ca240</i>  | 1.174 | 1.061 | 1.081 | 1.080 | 1.231 |
| <i>Ca241</i>  | 0.873 | 0.342 | 0.302 | 0.331 | 0.236 |
| <i>Ca242</i>  | 1.523 | 1.246 | 1.209 | 1.256 | 1.173 |

|       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|
| Ca243 | 1.643 | 0.403 | 0.212 | 0.252 |       |
| Ca244 | 1.466 | 1.270 | 1.334 | 1.130 | 1.284 |
| Ca245 | 0.460 | 0.320 | 0.337 | 0.304 | 0.261 |
| Ca246 | 0.796 | 0.312 | 0.235 | 0.235 | 0.273 |
| Ca247 | 1.363 | 1.221 | 1.153 | 1.184 | 1.097 |
| Ca248 | 1.224 | 1.109 | 1.100 | 1.016 | 1.017 |
| Ca249 | 1.354 | 1.265 | 1.135 | 1.178 | 1.081 |
| Ca250 | 0.184 | 1.634 | 0.412 | 0.341 | 0.669 |

Table 5 shows the minimum fungicidal concentration of *L. acidophilus* probiotics that can kill *Candida*. Analysis of variance (ANOVA), shown in Table 6 indicates that there is significant difference in the minimum inhibition zone of *L. acidophilus* probiotics concentrations on *C. albicans* isolates. This is because the F.Ratio (42.8166) is greater than the Critical value (<0.0001). The one way analysis of variance shown in Fig 1 illustrates the means diamond plot of the

minimum inhibition zone for each concentration of the probiotic, with the X axis scaled proportional to the sample size of each concentration. The boxplots which compare the different diameters on a numeric response shows that the minimum inhibition diameter clearly differ with variability highest in 125mg/ml and 250mg/ml probiotics and the least in 31.25mg/ml and 15.625mg/ml probiotics.

**Table 5. Results showing the minimum fungicidal concentration (MFC) of *Lactobacillus acidophilus* probiotics**

| <i>C. albicans</i> isolates | Concentrations of <i>L. acidophilus</i> Probiotics (mg/ml) | Result             |
|-----------------------------|------------------------------------------------------------|--------------------|
| Ca1                         | 15.625                                                     | Very scanty growth |
| ca2                         | 15.625                                                     | No growth          |
| ca3                         | 31.25                                                      | No growth          |
| ca4                         | 15.625                                                     | No growth          |
| Ca5                         | 15.625                                                     | Very scanty growth |
| Ca6                         | 15.625                                                     | No growth          |
| Ca7                         | 31.25                                                      | No growth          |
| Ca8                         | 31.25                                                      | No growth          |
| Ca9                         | 31.25                                                      | Very scanty growth |
| Ca10                        | 31.25                                                      | No growth          |
| Ca11                        | 15.625                                                     | Very scanty growth |
| Ca12                        | 15.625                                                     | No growth          |
| Ca13                        | 15.625                                                     | No growth          |
| Ca14                        | 31.25                                                      | No growth          |
| Ca15                        | 15.625                                                     | Very scanty growth |
| Ca16                        | 15.625                                                     | No growth          |
| Ca17                        | 15.625                                                     | Very scanty growth |
| Ca18                        | 15.625                                                     | Very scanty growth |
| Ca19                        | 15.625                                                     | Very scanty growth |
| Ca20                        | 15.625                                                     | Very scanty growth |
| Ca21                        | 15.625                                                     | Very scanty growth |
| Ca22                        | 15.625                                                     | Very scanty growth |

|      |        |                    |
|------|--------|--------------------|
| Ca23 | 15.625 | No growth          |
| Ca24 | 31.25  | No growth          |
| Ca25 | 15.625 | No growth          |
| Ca26 | 31.25  | No growth          |
| Ca27 | 15.625 | Very scanty growth |
| Ca28 | 15.625 | Very scanty growth |
| Ca29 | 31.25  | No growth          |
| Ca30 | 15.625 | No growth          |
| Ca31 | 15.625 | Very scanty growth |
| Ca32 | 15.625 | Very scanty growth |
| Ca33 | 15.625 | Very scanty growth |
| Ca34 | 15.625 | Very scanty growth |
| Ca35 | 15.625 | Very scanty growth |
| Ca36 | 15.625 | Very scanty growth |
| Ca37 | 31.25  | No growth          |
| Ca38 | 125    | Scanty growth      |
| Ca39 | 15.625 | Scanty growth      |
| Ca40 | 125    | Very scanty growth |
| Ca41 | 15.625 | Scanty growth      |
| Ca42 | 15.625 | Normal growth      |
| Ca43 | 62.5   | No growth          |
| Ca44 | 31.25  | Scanty growth      |
| Ca45 | 15.625 | Scanty growth      |
| Ca46 | 31.25  | Scanty growth      |
| Ca47 | 15.625 | Scanty growth      |
| Ca48 | 31.25  | Normal growth      |
| Ca49 | 15.625 | Scanty growth      |
| Ca50 | 250    | Scanty growth      |
| Ca51 | 15.625 | Very scanty growth |
| Ca52 | 15.625 | No growth          |
| Ca53 | 31.25  | No growth          |
| Ca54 | 15.625 | No growth          |
| Ca55 | 15.625 | Very scanty growth |
| Ca56 | 15.625 | No growth          |
| Ca57 | 31.25  | No growth          |
| Ca58 | 31.25  | No growth          |
| Ca59 | 31.25  | Very scanty growth |
| Ca60 | 31.25  | No growth          |
| Ca61 | 15.625 | Very scanty growth |
| Ca62 | 15.625 | No growth          |
| Ca63 | 15.625 | No growth          |
| Ca64 | 31.25  | No growth          |
| Ca65 | 15.625 | Very scanty growth |

|       |        |                    |
|-------|--------|--------------------|
| Ca66  | 15.625 | No growth          |
| Ca67  | 15.625 | Very scanty growth |
| Ca68  | 15.625 | Very scanty growth |
| Ca69  | 15.625 | Very scanty growth |
| Ca70  | 15.625 | Very scanty growth |
| Ca71  | 15.625 | Very scanty growth |
| Ca72  | 15.625 | Very scanty growth |
| Ca73  | 15.625 | No growth          |
| Ca74  | 31.25  | No growth          |
| Ca75  | 15.625 | No growth          |
| Ca76  | 31.25  | No growth          |
| Ca77  | 15.625 | Very scanty growth |
| Ca78  | 15.625 | Very scanty growth |
| Ca79  | 31.25  | No growth          |
| Ca80  | 15.625 | No growth          |
| Ca81  | 15.625 | Very scanty growth |
| Ca82  | 15.625 | Very scanty growth |
| Ca83  | 15.625 | Very scanty growth |
| Ca84  | 15.625 | Very scanty growth |
| Ca85  | 15.625 | Very scanty growth |
| Ca86  | 15.625 | Very scanty growth |
| Ca87  | 31.25  | No growth          |
| Ca88  | 125    | Scanty growth      |
| Ca89  | 15.625 | Scanty growth      |
| Ca90  | 125    | Very scanty growth |
| Ca91  | 15.625 | Scanty growth      |
| Ca92  | 15.625 | Normal growth      |
| Ca93  | 62.5   | No growth          |
| Ca94  | 31.25  | Scanty growth      |
| Ca95  | 15.625 | Scanty growth      |
| Ca96  | 31.25  | Scanty growth      |
| Ca97  | 15.625 | Scanty growth      |
| Ca98  | 31.25  | Normal growth      |
| Ca99  | 15.625 | Scanty growth      |
| Ca100 | 250    | Scanty growth      |
| Ca101 | 15.625 | Very scanty growth |
| Ca102 | 15.625 | No growth          |
| Ca103 | 31.25  | No growth          |
| Ca104 | 15.625 | No growth          |
| Ca105 | 15.625 | Very scanty growth |
| Ca106 | 15.625 | No growth          |
| Ca107 | 31.25  | No growth          |
| Ca108 | 31.25  | No growth          |

|       |        |                    |
|-------|--------|--------------------|
| Ca109 | 31.25  | Very scanty growth |
| Ca110 | 31.25  | No growth          |
| Ca111 | 15.625 | Very scanty growth |
| Ca112 | 15.625 | No growth          |
| Ca113 | 15.625 | No growth          |
| Ca114 | 31.25  | No growth          |
| Ca115 | 15.625 | Very scanty growth |
| Ca116 | 15.625 | No growth          |
| Ca117 | 15.625 | Very scanty growth |
| Ca118 | 15.625 | Very scanty growth |
| Ca119 | 15.625 | Very scanty growth |
| Ca120 | 15.625 | Very scanty growth |
| Ca121 | 15.625 | Very scanty growth |
| Ca122 | 15.625 | Very scanty growth |
| Ca123 | 15.625 | No growth          |
| Ca124 | 31.25  | No growth          |
| Ca125 | 15.625 | No growth          |
| Ca126 | 31.25  | No growth          |
| Ca127 | 15.625 | Very scanty growth |
| Ca128 | 15.625 | Very scanty growth |
| Ca129 | 31.25  | No growth          |
| Ca130 | 15.625 | No growth          |
| Ca131 | 15.625 | Very scanty growth |
| Ca132 | 15.625 | Very scanty growth |
| Ca133 | 15.625 | Very scanty growth |
| Ca134 | 15.625 | Very scanty growth |
| Ca135 | 15.625 | Very scanty growth |
| Ca136 | 15.625 | Very scanty growth |
| Ca137 | 31.25  | No growth          |
| Ca138 | 125    | Scanty growth      |
| Ca139 | 15.625 | Scanty growth      |
| Ca140 | 125    | Very scanty growth |
| Ca141 | 15.625 | Scanty growth      |
| Ca142 | 15.625 | Normal growth      |
| Ca143 | 62.5   | No growth          |
| Ca144 | 31.25  | Scanty growth      |
| Ca145 | 15.625 | Scanty growth      |
| Ca146 | 31.25  | Scanty growth      |
| Ca147 | 15.625 | Scanty growth      |
| Ca148 | 31.25  | Normal growth      |
| Ca149 | 15.625 | Scanty growth      |
| Ca150 | 250    | Scanty growth      |
| Ca151 | 15.625 | Very scanty growth |

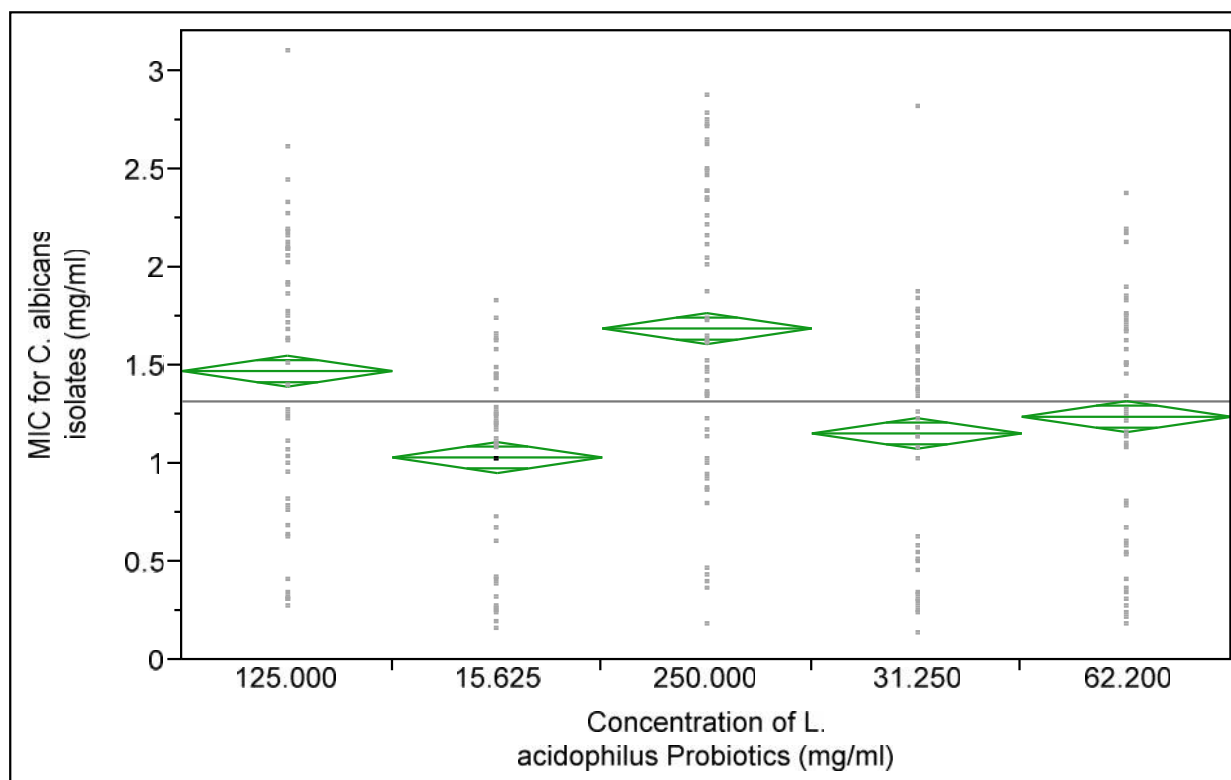
|       |        |                    |
|-------|--------|--------------------|
| Ca152 | 15.625 | No growth          |
| Ca153 | 31.25  | No growth          |
| Ca154 | 15.625 | No growth          |
| Ca155 | 15.625 | Very scanty growth |
| Ca156 | 15.625 | No growth          |
| Ca157 | 31.25  | No growth          |
| Ca158 | 31.25  | No growth          |
| Ca159 | 31.25  | Very scanty growth |
| Ca160 | 31.25  | No growth          |
| Ca161 | 15.625 | Very scanty growth |
| Ca162 | 15.625 | No growth          |
| Ca163 | 15.625 | No growth          |
| Ca164 | 31.25  | No growth          |
| Ca165 | 15.625 | Very scanty growth |
| Ca166 | 15.625 | No growth          |
| Ca167 | 15.625 | Very scanty growth |
| Ca168 | 15.625 | Very scanty growth |
| Ca169 | 15.625 | Very scanty growth |
| Ca170 | 15.625 | Very scanty growth |
| Ca171 | 15.625 | Very scanty growth |
| Ca172 | 15.625 | Very scanty growth |
| Ca173 | 15.625 | No growth          |
| Ca174 | 31.25  | No growth          |
| Ca175 | 15.625 | No growth          |
| Ca176 | 31.25  | No growth          |
| Ca177 | 15.625 | Very scanty growth |
| Ca178 | 15.625 | Very scanty growth |
| Ca179 | 31.25  | No growth          |
| Ca180 | 15.625 | No growth          |
| Ca181 | 15.625 | Very scanty growth |
| Ca182 | 15.625 | Very scanty growth |
| Ca183 | 15.625 | Very scanty growth |
| Ca184 | 15.625 | Very scanty growth |
| Ca185 | 15.625 | Very scanty growth |
| Ca186 | 15.625 | Very scanty growth |
| Ca187 | 31.25  | No growth          |
| Ca188 | 125    | Scanty growth      |
| Ca189 | 15.625 | Scanty growth      |
| Ca190 | 125    | Very scanty growth |
| Ca191 | 15.625 | Scanty growth      |
| Ca192 | 15.625 | Normal growth      |
| Ca193 | 62.5   | No growth          |
| Ca194 | 31.25  | Scanty growth      |

|       |        |                    |
|-------|--------|--------------------|
| Ca195 | 15.625 | Scanty growth      |
| Ca196 | 31.25  | Scanty growth      |
| Ca197 | 15.625 | Scanty growth      |
| Ca198 | 31.25  | Normal growth      |
| Ca199 | 15.625 | Scanty growth      |
| Ca200 | 250    | Scanty growth      |
| Ca201 | 15.625 | Very scanty growth |
| Ca202 | 15.625 | No growth          |
| Ca203 | 31.25  | No growth          |
| Ca204 | 15.625 | No growth          |
| Ca205 | 15.625 | Very scanty growth |
| Ca206 | 15.625 | No growth          |
| Ca207 | 31.25  | No growth          |
| Ca208 | 31.25  | No growth          |
| Ca209 | 31.25  | Very scanty growth |
| Ca210 | 31.25  | No growth          |
| Ca211 | 15.625 | Very scanty growth |
| Ca212 | 15.625 | No growth          |
| Ca213 | 15.625 | No growth          |
| Ca214 | 31.25  | No growth          |
| Ca215 | 15.625 | Very scanty growth |
| Ca216 | 15.625 | No growth          |
| Ca217 | 15.625 | Very scanty growth |
| Ca218 | 15.625 | Very scanty growth |
| Ca219 | 15.625 | Very scanty growth |
| Ca220 | 15.625 | Very scanty growth |
| Ca221 | 15.625 | Very scanty growth |
| Ca222 | 15.625 | Very scanty growth |
| Ca223 | 15.625 | No growth          |
| Ca224 | 31.25  | No growth          |
| Ca225 | 15.625 | No growth          |
| Ca226 | 31.25  | No growth          |
| Ca227 | 15.625 | Very scanty growth |
| Ca228 | 15.625 | Very scanty growth |
| Ca229 | 31.25  | No growth          |
| Ca230 | 15.625 | No growth          |
| Ca231 | 15.625 | Very scanty growth |
| Ca232 | 15.625 | Very scanty growth |
| Ca233 | 15.625 | Very scanty growth |
| Ca234 | 15.625 | Very scanty growth |
| Ca235 | 15.625 | Very scanty growth |
| Ca236 | 15.625 | Very scanty growth |
| Ca237 | 31.25  | No growth          |

|       |        |                    |
|-------|--------|--------------------|
| Ca238 | 125    | Scanty growth      |
| Ca239 | 15.625 | Scanty growth      |
| Ca240 | 125    | Very scanty growth |
| Ca241 | 15.625 | Scanty growth      |
| Ca242 | 15.625 | Normal growth      |
| Ca243 | 62.5   | No growth          |
| Ca244 | 31.25  | Scanty growth      |
| Ca245 | 15.625 | Scanty growth      |
| Ca246 | 31.25  | Scanty growth      |
| Ca247 | 15.625 | Scanty growth      |
| Ca248 | 31.25  | Normal growth      |
| Ca249 | 15.625 | Scanty growth      |
| Ca250 | 250    | Scanty growth      |

**Table 6: ANOVA Table**

| Source                                                    | Degree of Freedom | Sum of Squares | Mean Square | F Ratio | Prob > F |
|-----------------------------------------------------------|-------------------|----------------|-------------|---------|----------|
| Concentration of <i>L. acidophilus</i> Probiotics (mg/ml) | 4                 | 68.79274       | 17.19819    | 42.8166 | <.0001   |
| Error                                                     | 1245              | 500.0804       | 0.401671    |         |          |
| C. Total                                                  | 1249              | 568.8731       |             |         |          |



**Figure 1: One way ANOVA**

Table 7 shows the mean comparison student T-Test. From the table, positive values show pairs of mean that are significantly different. This means that for all positive values, the mean of the inhibition diameter are different. E.g. 125mg/ml vs 250mg/ml = 0.105348 (the

mean inhibition diameter at 125mg/ml concentration is significantly different from that of 250mg/ml), 31.25mg/ml vs 62.5mg/ml = -0.02557 (the mean inhibition diameter at 31.25mg/ml concentration is not significantly different from that of 62.5mg/ml).

**Table 7: Mean comparison for T-test (Students T)**

| T        |            | Alpha      |                |             |          |         |
|----------|------------|------------|----------------|-------------|----------|---------|
| 1.961871 |            | 0.05       |                |             |          |         |
| Level    | -<br>Level | Difference | Std<br>Err Dif | Lower<br>CL | Upper CL | p-Value |
| 250      | 15.625     | 0.65606    | 0.056687       | 0.544848    | 0.767272 | <.0001  |
| 250      | 31.25      | 0.53372    | 0.056687       | 0.422508    | 0.644932 | <.0001  |
| 250      | 62.2       | 0.44808    | 0.056687       | 0.336868    | 0.559292 | <.0001  |
| 125      | 15.625     | 0.4395     | 0.056687       | 0.328288    | 0.550712 | <.0001  |
| 125      | 31.25      | 0.31716    | 0.056687       | 0.205948    | 0.428372 | <.0001  |
| 125      | 62.2       | 0.23152    | 0.056687       | 0.120308    | 0.342732 | <.0001  |
| 250      | 125        | 0.21656    | 0.056687       | 0.105348    | 0.327772 | 0.0001  |
| 62.5     | 15.625     | 0.20798    | 0.056687       | 0.096768    | 0.319192 | 0.0003  |
| 31.25    | 15.625     | 0.12234    | 0.056687       | 0.011128    | 0.233552 | 0.0311  |
| 62.5     | 31.25      | 0.08564    | 0.056687       | -0.02557    | 0.196852 | 0.1311  |

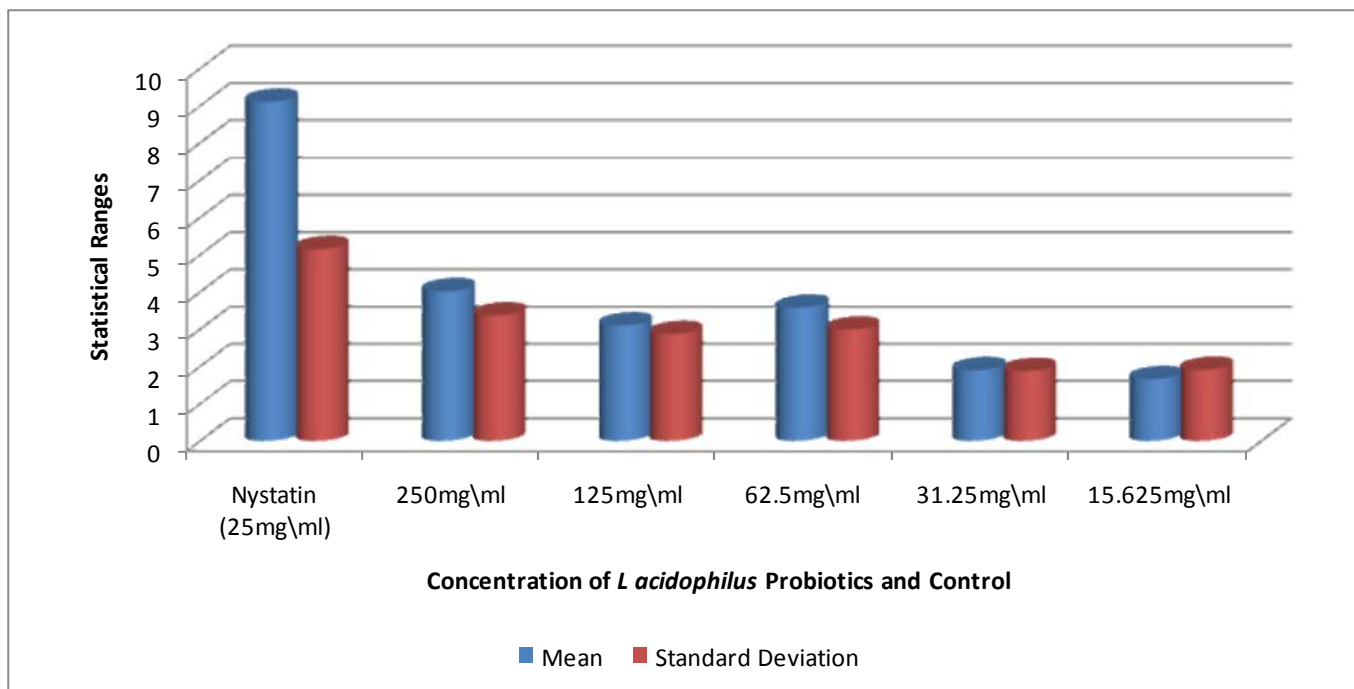
**Table 8** is the mean of distribution. From the Table, positive values show pairs of mean that are significantly different.

**Table 8: Mean comparison for T-test**

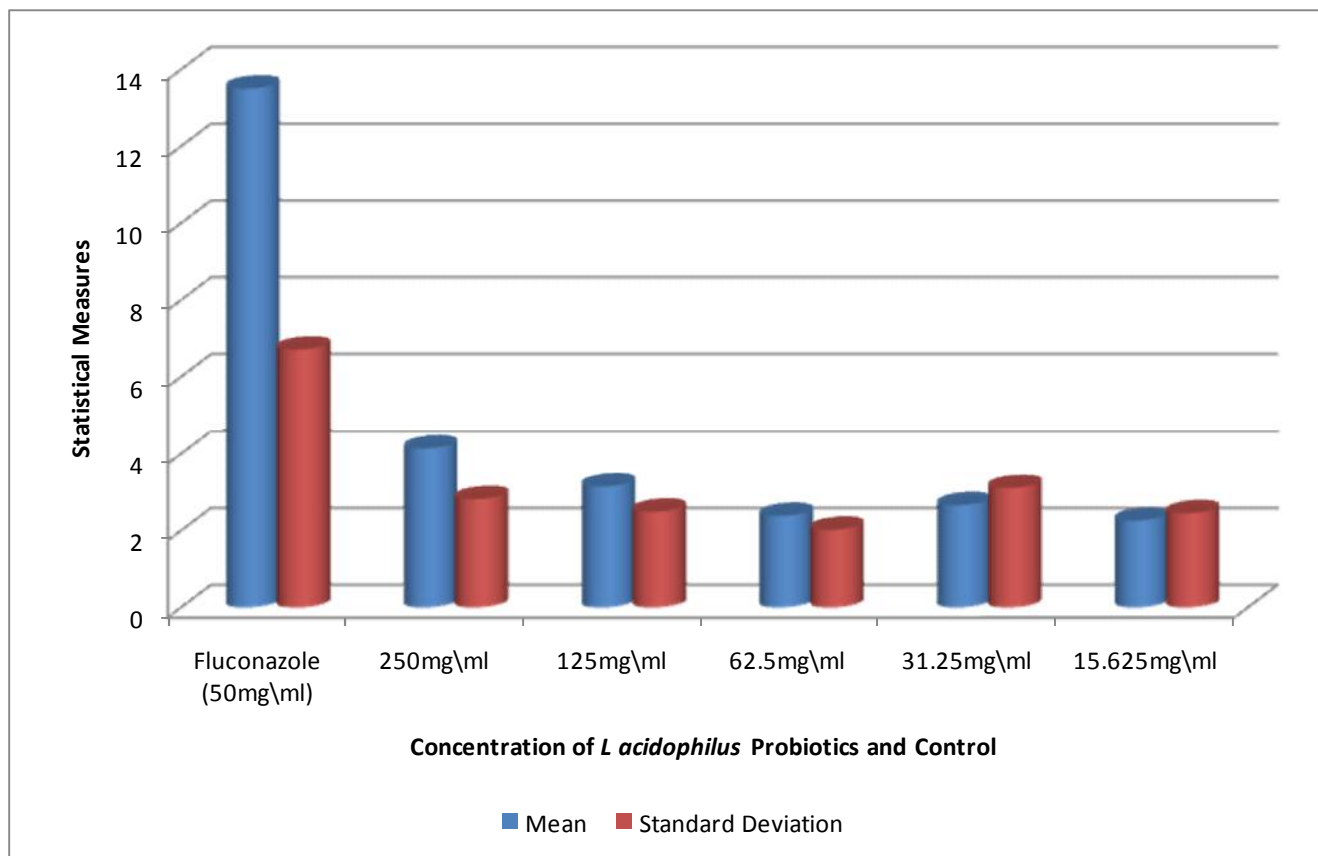
|             | 250mg/ml | 125mg/ml | 62.5mg/ml | 31.25 mg/ml | 15.625 mg/ml |
|-------------|----------|----------|-----------|-------------|--------------|
| 250 mg/ml   | -0.11121 | 0.105348 | 0.336868  | 0.422508    | 0.544848     |
| 125 mg/ml   | 0.105348 | -0.11121 | 0.120308  | 0.205948    | 0.328288     |
| 62.5 mg/ml  | 0.336868 | 0.120308 | -0.11121  | -0.02557    | 0.096768     |
| 31.25 mg/ml | 0.422508 | 0.205948 | -0.02557  | -0.11121    | 0.011128     |
| 15.625mg/ml | 0.544848 | 0.328288 | 0.096768  | 0.011128    | -0.11121     |

Figures 2 and 3 shows the descriptive statistics of nystatin and probiotics, and fluconazole and probiotics respectively. At concentrations of 25 mg/l

(nystatin) and 50 mg/ml (fluconazole), the inhibitions far exceeds the maximum concentration (250 mg/ml) of the probiotic



**Figure 2: Descriptive statistics of Nystatin and the Probiotics.**



**Figure 3: Descriptive statistics of Fluconazole and the Probiotics.**

## Discussion

*Lactobacillus* is a natural resistance factor against potential pathogenic microorganisms, by producing autogenic regulation factors e.g. organic acids, hydrogen peroxide and bacteriocins (Rastall and Maitin, 2002).

In recent years, the use of *Lactobacilli* as biotherapeutic agents has received wider attention and several studies provide evidence supporting the ability of *Lactobacilli* to prevent infection (Sullivan and Nord, 2002). Although many commercially available *Lactobacillus* products can be found in healthy food stores, their reliability is questionable and there is only little evidence proving their efficacy (Rastall and Maitin, 2002). Other drawbacks of the currently available products include poor product, viability, and possible contamination with other organisms (Sullivan and Nord, 2002).

Results of susceptibility shows that two hundred and fifty isolates (100%) of *Candida* were susceptible (sensitive) to Nystatin and Fluconazole (Table 3). This high activity to inhibit the growth of all isolates may comes from their polyenes compounds that integrates directly to sterol and ergosterol which are found in the plasmic membrane of *Candida* cells to form spores, that lead to gradual changes in the cell permeability and cell death (Ringdahl, 2000). These results were in agreement with the findings by Al-Shibly (2006). The results obtained from this study shows that *L. acidophilus* is sensitive to 96% of the *Candida albicans* (Table 3). The result also proved that the commercial antifungal agent were better with higher zones of inhibition (Tables 3), where as the antifungal activity of *Lactobacillus acidophilus* was better at the lower concentrations (Tables 4, 5).

Ostrzenski (1974) treated patients with vaginitis cases with a lyophilized culture of *L. acidophilus* suspended in cocoa butter, while Gunston and Fairbrother (1975) treated other patients with yoghurt. Each study gave inconclusive evidence that *Lactobacilli* were beneficial in treatment of vaginitis cases.

Statistical analysis indicates that there is significant difference in the minimum inhibition zone of *L. acidophilus* probiotics concentrations on *C. albicans* isolates (Table s 6, 7, 8, Fig 1). Figures 2 and 3 confirm the inhibitory activities of commercial antifungal against *Candida albicans* than the probiotics.

Many studies are certain that *Lactobacilli* are natural inhabitants of urogenital microflora and are believed to have a central role in the suppression of potential pathogens. *Lactobacilli* administered to the genital tract have a prominent role as a prophylactic aimed at improving the genital microflora defense against bacterial infection (Kaewsrirachan *et al.*, 2006). Also Wilson (2004) reported that *Lactobacilli* probiotic strains that can be used for the treatment of vaginal candidiasis should be able to produce metabolites that are fungistatic for *C. albicans*.

Data suggested that probiotics are beneficial against mucosal *Candida* infection (Mailander-Sanchez, 2012), and should be especially considered for women who suffer from more than three yeast infection per year (Falagas, 2006). Research shows that probiotics exert their beneficial action by suppressing the growth of *Candida albicans* and inhibiting *Candida's* ability to adhere to cell surface.

Thus, this study suggest that *L. acidophilus* bacteria could be useful agent for development of antifungal and antibacterial activity against vagina pathogens and may have great potential for use in external application and vaginal wash for preventing or reducing *Candida* growth or might also be useful for the treatment of Recurrent Vaginal Candidiasis.

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