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Fermentation of *Psidiumguajava* Juice by Using Probiotic Lactic acid Bacteria *Lactobacillus Plantarum*

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Abstract

The main objective of this study was to produce fermented probiotic drink with the help of *Lactobacillus plantarum*. The fermentation was carried at 37°C for 72 hr under aerobic condition. The parameters like pH, microbial load, antioxidant activity (DPPH), acidity & concentration of reducing sugar had been measured. The antibacterial against *E.coli*, *Staphylococcus* and *pseudomonas* was determined. Fermented juice was subjected cytotoxic activity against some cancer cell line & it showed activity. The presence of various types of organic acids was determined by HPLC analysis.

Keywords: Probiotics; *L. plantarum*; Guava juice; Aerobic condition; HPLC; Antioxidant

Introduction

Probiotics are food supplements containing beneficial bacteria or yeast. Generally probiotics contain Lactic Acid Bacteria. The main intention to prepare this probiotic drink is to assist in reviving the body's naturally own micro-flora within the digestive tract to reestablish themselves. Some of the medical importance of the probiotics are: prevention of colon cancer, reducing the cholesterol, reducing inflammation, improving assimilation & absorption & the most important is managing lactose tolerance .*Lactobacillus plantarum* which is a gram+ve, aerotolarent bacteria that generally produces D, L-isomers of lactic acid and the most diversified member of the genus Lactobacillus, found in many fermented products, plant matter & saliva. It is a very flexible & versatile species. *L.plantarum* quickly dominates the microbial population & starts producing Lactic Acid & Acetic Acid by the EM pathway just within 48 hr. It has a significant anti-oxidant activity &maintains the permeability in the intestine. The antimicrobial substances produced by *L.plantarum* help them to survive in humans.

Materials and Method

Microorganism culture

The organism of interest *L. plantarum* was provided by 'Microbiology & Molecular Biology Lab', VIT University, Vellore, TN, India. The supplied culture was inoculated by quadrant streaking on MRS agar plate. Plates were made & kept in 37°C for 24 hr in incubator. The very next day the obtained colonies were gram stained & observed under microscope (40 × &100 ×) [1]. The resultant colonies were transferred to MRS broth & kept for 24 hr at 37°C. Glycerol Stock was made from the above mentioned broth culture & kept at-20°C for further use.

Raw materials collection

Fresh guava fruits were collected and washed under water. Juice was prepared. The juice was pasteurized at 80°C for 5 mins [2].

Inoculum preparation

Fresh MRS broth was prepared & inoculated with the Glycerol Stock (containing *L. plantarum*) & kept at 37°C for 24 hr. For mass production, 150 ml of MRS broth was prepared and inoculated from the early prepared broth culture and incubated [3]. The broth was subjected to check for cell population under Spectrophotometer after 24 hr at 600 nm.

Cell extraction

The broth was centrifuged at 8000rpm in 4°C 10min [4]. Supernatant was discarded & pallet was washed with NaCl solution. The Sodium Phosphate buffer was prepared & added to the pallet to make it diluted.

Fermentation of the Guava juice: The extracted cells were introduced into the Pasteurized guava juice and kept for farther analysis [5].

Microbial load count: Microbial load was assayed from zeroth day sample to 3rd day sample on MRS agar plate. For each day's sample 3 plates (Replica1,Replica 2, &control) were maintained.

pH: pH was measured by pH meter from 0th day sample to 3rd day in every 24 hr interval by ELUTECH® pH meter.

Reducing sugar assay: To determine the percentage of reducing sugar present in the samples (0th day to 3rd day) DNSA method was used. 0.1 mg to 1 mg/ ml glucose solution was prepared .To 1 ml of the sample 3 ml of DNSA (3,5dinitrosalicylic acid) was added, heated for 20 min in water bath. The sample was diluted to 25 ml.O.D was measured at 550 nm.Standard curve with concentration of glucose on X-axis and O.D on Y-axis was plotted [6].

Acidity assay: Total acidity and volatile acidity of the samples was checked (0th day to 3rd day). 10 ml of distilled water and 5drops of 1% phenolphthalein solution was added to 10 ml of fermenting sample and the mixture was titrated to the first persistent pink color with 0.1N NaOH [7].

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Antioxidant activity, (DPPH radical scavenging assay)

1 ml of the Fermented Guava juice sample(supernatant) was diluted to 4 ml in methanol,0.6 ml of 1 mM DPPH solution was prepared and added with methanol, incubated at room temperature for 30 minutes, Absorbance was determined at 570 nm.percentage of free radical in sample was calculated by standard DPPH Assay formula [8].

Antimicrobial assay

Supernatant of the sample was prepared. The antimicrobial activity of the sample was determined against *Ecoli, Pseudomonas, S aureus* in Muller Hinton agar plate.

HPLC analysis

To determine the organic acids produced in the sample HPLC technique was accomplished.

Cytotoxic activity

Cell viability test: The viability of cells was assessed by MTT assay using MCF-7 breast cancer cell lines and Prostate cancer cell lines [9].

Reagents

- MTT (3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyl tetrazolium bromide)
- Dimethyl sulfoxide
- Phosphate buffered saline (PBS) (pH 7.4)

The cells were plated separately in 96 well plates at a concentration of 1 × 105 cells/well. After 24 hr, cells were washed twice with 100 µl of serum-free medium and starved for an hour at 37°C. After starvation, cells were treated with the test materialfor 24 hr. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/ ml) was added and incubated for 4 hr at 37°C in a CO_2 incubator [10].

The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by pipetting up and down. Spectrophotometrical absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graph pad prism5 software [11].

Result and Discussion

The production of Probiotic drink from Guava fruit using *Lactobacillus plantarum* was obtained after 72 hr of fermentation. MRS broth as the pre-culture medium had a pH of 5.6. The changing in pH level in the medium is one of the crucial probiotic characteristics for the survival in the food medium (Figure 1). The guava juice which was selected as the medium had a pH of 6.The microbial population in the medium at 48 hr showed many colonies (TMTC) (Figure 2) (Table 1). After 72 hr of fermentation the microbial population dropped as it was expected [12].

This decline in the pH implies that various organic acids were produced in the sample vigorously. The total acidity and volatile acidity of the sample during the course of fermentation increased tremendously from zeroth day to final day offermentation (Figure 3).

The data obtained from the experiment for DPPH scavenging activity showed a positive result (Figure 4). The antioxidant activities of the extract was assessed on the basis of the free radical scavenging

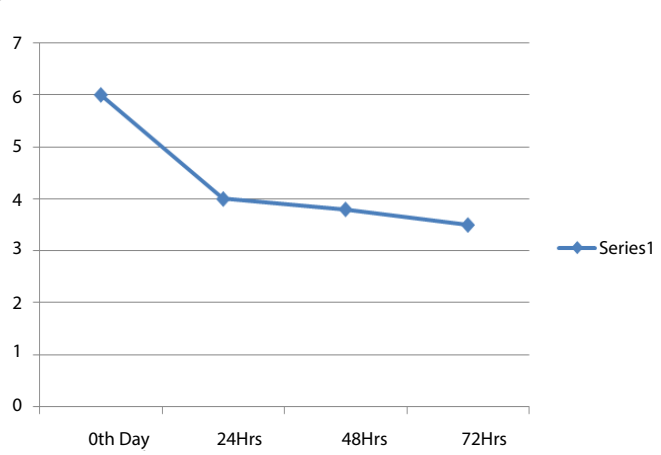

Figure 1: pH of the Sample.

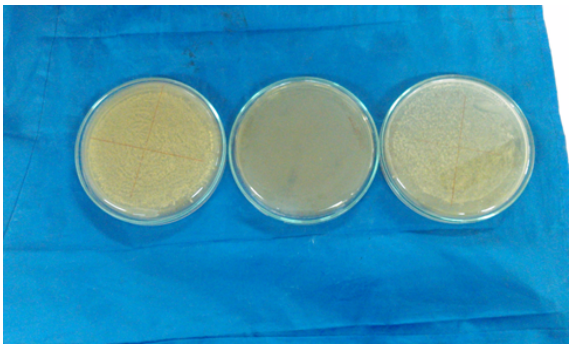

Figure 2: Microbial load.

0th Day	24Hrs	48Hrs	72Hrs
6	4	3.8	3.5

Table 1: Fermentation the Microbial Population.

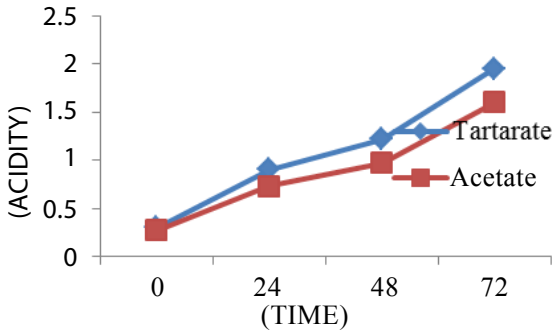

Figure 3: Graph showing the change in acidity.

effect of the stable 1,1-diphenyle-2-picrylhydrazyl(DPPH)-free radical activity [13] (Table 2).

The concentration of reducing sugar showed consistent increase in the value. The concentration of reducing sugar in zeroth day as well as in second day was 0.1, followed by increase in its concentration from 48-72 hr (Figure 5).

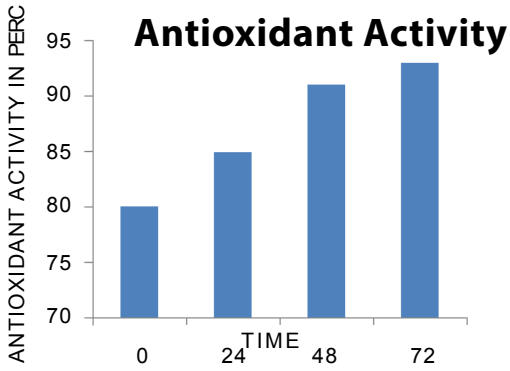

Figure 4: Histogram for Antioxidant activity.

0th Day	1st Day	2nd Day	3rd Day
0.1	0.1	0.16	0.22

Table 2: Increase in its concentration from 48-72hr.

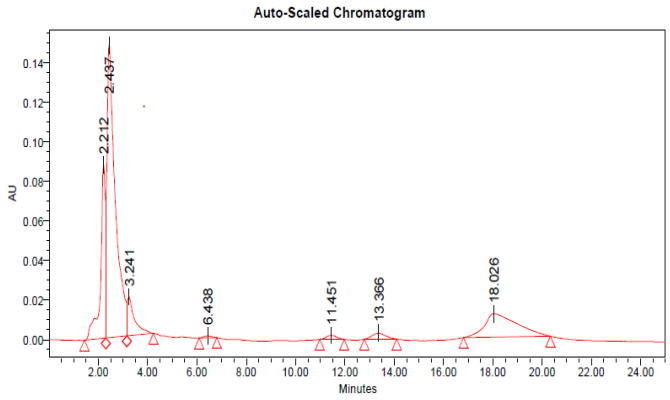

Figure 5: HPLC report of the sample.

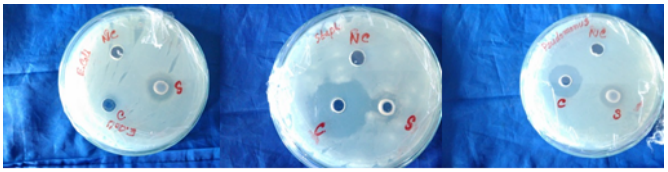

Figure 6: Antimicrobial activity.

By performing HPLC on the probiotic drink taking some organic acids as standard (ascorbic acid, acetic acid & lactic acid) to assess the presence of organic acid in the sample. By analyzing the peaks from the HPLC report of the sample it can be concluded that the sample contains acetic acid, lactic acid & ascorbic acid (Figure 5).

Antibacterial activity of the probiotic drink made from guava using *Lactobacillus plantarum* was seen in respect of zone of inhibition against *E coli, Pseudomonas and S aureus* (Figure 6). The maximum zone of inhibition was seen around both *Psudomonus & E.coli* (Table 3).

On Prostate cancer cell line this fermented product showed a satisfactory outcome. It was applied in amounts of 5 µl, 10 µl & 20 µl. It was about 15µl which showed better result against Prostate cancer cell line. 28% of Prostate Cancer cells were killed by 15 µl of the fermented product (Figure 7) [14].

The fermented product was demonstrated on Breast cancer (MCF 7) cell lines.12% of Cancer cells were killed by 15µl of the fermented product (Figures 8-10).

Conclusion

So, by the result we can draw the conclusion that the fermented juice is having satisfactory anti-cancer activity against the Prostate cancer than Breast cancer cells and confers health benefits.

	Optical density		% of cell viability	
5 µl	1.122	1.166	91.96721	95.57377
10 µl	1.095	1.11	89.7541	90.98361
20 µl	0.975	0.881	79.91803	72.21311

Table 3: Optical density and % of cell viability.

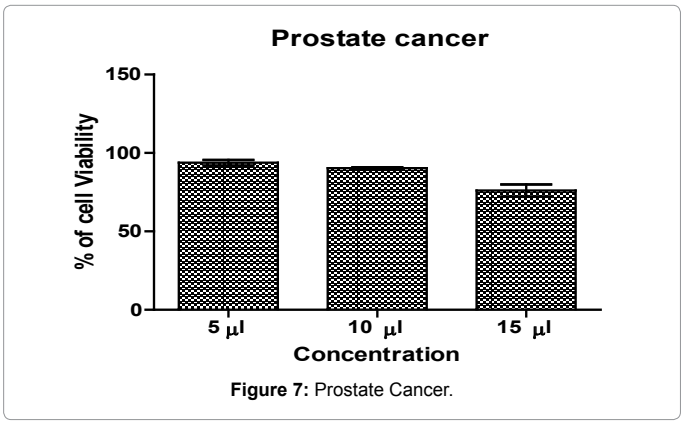

Figure 7: Prostate Cancer.

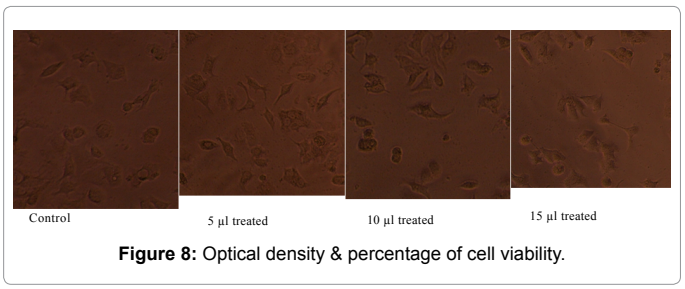

Figure 8: Optical density & percentage of cell viability.

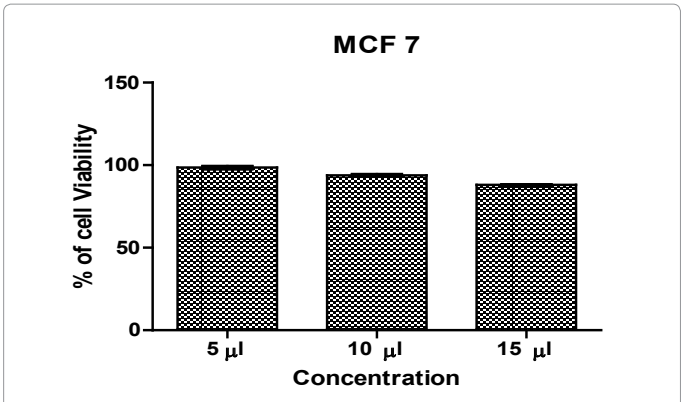

Figure 9: MCF 7 (Breast cancer).

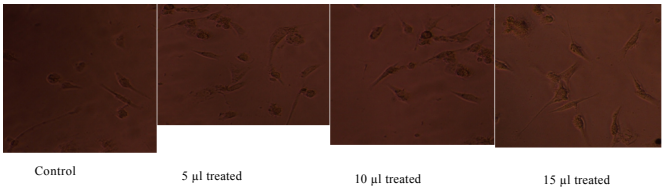

Figure 10: Optimal density and % of cell viability.

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