

Studies on Anti Diarrhoeal Activity of Synbiotic Plums Juice

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Abstract- This study evaluated that effect of prebiotic food containing oligosaccharide to enhance the growth and activity of probiotic strains. Plums juice probioticated using different strains of probiotics are *Lactobacillus kefiranofaciens*, *Candida kefir*, and *saccharomyces boluradii*. To select a suitable prebiotics like inulin for the development of synbiotic plums juice and for food preservation. Synbiotic plums juice tested for antibacterial activity against diarrhoea causing pathogen such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella paratyphi A*, *Shigella dysenteriae*, *Vibrio cholerae*. Analysis of identified compound from synbiotic plums juice using GC-MS.

Index Terms- Antibacterial activity, GC-MS analysis, Plums, Probiotics, Synbiotics

I. INTRODUCTION

Diarrhoea is a common symptom of intestinal disorders and it is a global threat to human health. It is a leading cause of morbidity and mortality, with over 1000 million episodes and over 4 million deaths annually in children under 5 years of age. Diarrhoeal infection is a second killer disease of children in the developing countries. Diarrhoea caused by *Escherichia coli* is common in India with occasional outbreaks (Kahali et al., 2004). Where as *Escherichia coli* (58.4%) *Salmonella* sp (20%) and *Shigella* sp (20%) were found to be extremely uncommon agents of childhood diarrhea making only 1.6 per cent of the positive culture in Yeman (Banajeh et al., 2001). Synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the friendly flora of the human intestine. Research and development of synbiotic products have been increasingly focusing on evidence of functional benefits including resistance to infection, antibacterial activity, and improved immune status (Gibson and Roberfroid, 1995). A synbiotic product should be considered a functional food rather than some obscure chemistry formulation. In the synbiotic present scenario, food is no longer consumed for satisfaction of hunger alone but for promoting nutrition and health. The concept of functional foods has gained universal acceptance as a preventive and therapeutic approach to combat many disease that decrease the work productivity due to poor health. The objectives of the study were to isolate and identify the beneficial bacteria [probiotics] from fermented milk sample such as yoghurt, kefir, butter, cheese, and koumiss. Effective probiotic organisms identified, and inoculated with plums juice and allowed for fermentation. Administration of prebiotics, the non-digestible

food ingredients that beneficially affect the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the column thus improving host health offers an attractive alternative. Among prebiotics, non-digestible carbohydrate like inulin and oligofructose have received much attention. Inulin consists of 2-60 fructose units linked by a β -(2-1) glycosidic linkage often with a terminal glucose unit. Many researches proved that consumption of prebiotics, such as inulin, could stimulate intestinal peristalsis by means of increasing fecal bulk and moisture (Gibson et al., 1995). The keeping above facts in view present investigation was undertaken to evaluate prebiotics strains for their compatibility with plums juice in the presence of inulin for synbiotic fruit juice preparation.

Plums have the natural nutritional components of 0.95 grams protein, 17 calories and 1.4 grams of dietary fiber and it also contain potassium, phosphorus, magnesium, calcium, iron, sodium, zinc, copper, manganese, selenium, vitamin C, niacin, vitamin B1, vitamin B2, vitamin B6, folate, pantothenic acid, vitamin A, K, E, and also contain some other vitamins in small amounts. In addition to that fermented juice with inulin might be a good source of probiotic and also nutritional components even after 2 weeks storage at 4°C so inulin act as food preservative.

II. MATERIALS AND METHODS

A. Preparation of synbiotic plums juice

Plums was purchased from a local market. Juice was prepared from homogenized skin less slices and was filtered it properly and 100ml of plums juice were inoculated with 2ml of MRS broth containing probiotic bacteria and yeast (*Lactobacillus kefiranofaciens* *Candida kefir*, and *saccharomyces boluradii*) they were allowed for fermentation (Yoonky et al., 2006) After fermentation juice were separated into two different container. One of that container inulin could be added and it was used for antibacterial analyses.

B. Test organisms

The bacterial test organisms were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella paratyphi A*, *Shigella dysenteriae*, *Vibrio cholerae* were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The organisms were maintained on agar slant and were subsequently subcultured into newly prepared nutrient agar media. All the chemicals and medium used in this study were supplied by Himedia Pvt. Ltd., Mumbai, India.

C. Preparation of Inoculum

Inoculum was prepared by adding one loopful of test pathogen in 50ml of BHI broth and then incubated at 37°C for 24hrs.

D. Isolation and Characterization of Probiotics

Fermented milk samples such as yoghurt, kefir, cheese, and koumiss were collected from market. The milk samples were enriched and inoculated into the MRS broth (Man rogosa sharpe) The enriched samples were incubated under static conditions for a week. Probiotic isolation was carried out by streaking the enriched milk sample on MRS agar media and incubated at 37°C. Isolated bacterial cultures were characterized using colony morphology, bio-chemical test and in selective medium, carbohydrate fermentation (Table 1).

E. Agar well diffusion assay

The antibacterial activity of synbiotic plums juice was evaluated by agar well diffusion method. (Chung et al., 1990) Muller Hinton agar medium was prepared and poured into the petriplates and allowed to solidify. Then it was inoculated with a swab of culture and spread through out the medium uniformly with a sterile cotton swab. Using sterile cork borer (10mm diameter) wells were made in the agar medium. The test compound was introduced into the separate well in a single plate.(fermented juice, fermented juice with inulin) All the plates were incubated at 37°C for 24h. The antagonistic test was performed intriplicate and their efficiency was determined by measuring the diameter of zone of inhibition around the well. In triplicate assay mean value was taken for analysis (Table 2).

F. GC-MS analysis

The volatile constituents from juice was analysed using GC-MS (GC Clarus 500 Perkin Elmer) with Elite-1 column and a mass detector, which was operated in EI mode at 70eV. Injector and detector temperatures were set at 250°C (Al-Delaimy and Ali, 1970). Plums juice (1µl) was injected and analysed with a column held initially at 110°C for 2min and then increased by 5°C per min up to 280°C. Helium was used as carrier gas (1ml/min). The relative amount of individual components of the total juice expressed as percentage peak area relative to total peak area. Quantitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds, or by retention indices (RI) and mass spectra.

III. RESULT AND DISCUSSION

As Table 1, shows isolation characteristics of probiotic bacteria and yeast. From these observation all the three strains characterized by morphology, biochemical test, different growth condition of pH, temperature and salt concentrations. Probiotics such as *Lactobacillus kefirifaciens*, *Candida kefir*, *saccharomyces boluradii* grown well temperature range of 20-30°C at 6.5 salt concentration and mainly characterized by 18

type of carbohydrate fermentation. In some cases week and negative sugar fermentation was observed.

From Table 2 it is very clear that both fermented plums juice and fermented plums juice with inulin showed growth inhibition activities of five diarrhoeal causing test pathogens. Fermented plums juice with inulin shows the higher antibacterial activity than the normal fermented plums juice (Fig 1).

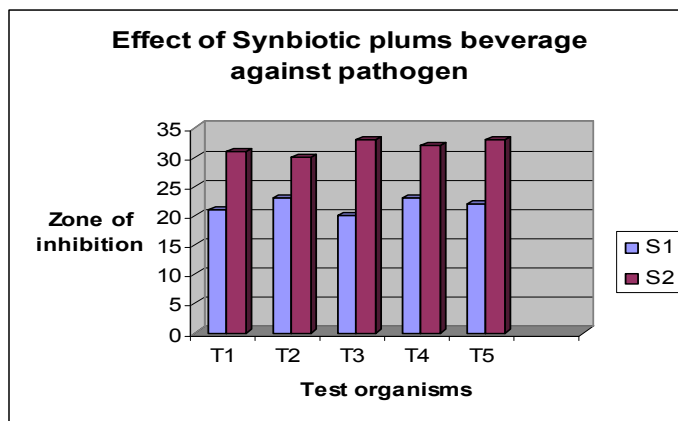


Figure 1: Effect of Synbiotic plums beverage against pathogen

S₁-Juice probicated with organism, S₂-Fermented beverage with Inulin

- T1 – *Staphylococcus aureus*
- T2 – *E.coli*
- T3 – *Salmonella Paratyphi A*
- T4 – *Shigella dysenteriae*
- T5 – *Vibrio Cholerae*

Similar type of work was to evaluated the influence of prebiotic additives on gluten-free breads, and to assess the effectiveness of selected prebiotics inulin (Grzelak, 2006). Since both fruit juice extract showed unique range of zone of inhibition. To further study the nature of components present in the fruit juice extract GC-MS was performed (Fig 2).

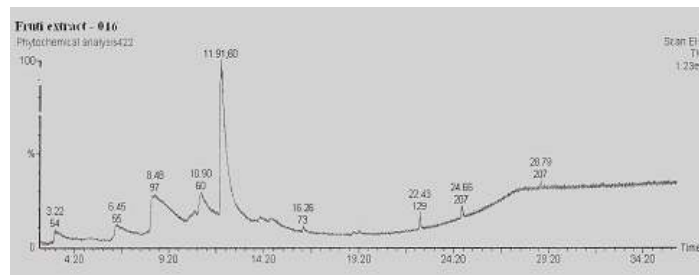


Figure 2: Gas Chromatography Mass Spectrum analysis of fermented plums juice

The major compounds of GC-MS analysis and their retention time were listed in Table 3, these include 1 H-Imidazole, 2,4 dimethyl (2.71%), 4 H-Pyran-4-one, 2,3, dihydro-3,5 dihydroxy-6-methyl (6.02%), 2- furancarboxaldehyde, 5-hydroxymethyl (

34.64%), 1,2,3,5- cyclohexaneterol (55.35%), Hexanedioic acid, bic 20ethylhexyl ester (0.64%), and 1,2- Benzenedicarboxylic acid, dissooctyl ester (0.65%). These observation when correlated with water soluble compounds common in most plant which may be responsible for its antibacterial activity (**Astal, 2004**).

Probiotic approach through plums juice increases residence bacteria which are beneficial to human health. The inhibitory action of probiotic bacteria and yeast is mainly due to accumulation of main primary metabolites such as lactic acid , acetic acid, ethanol and carbon dioxide. It is earlier reported as lactic acid bacteria were also able to control the growth of gram

negative pathogens including food borne pathogens by the production of organic acids and hydrogen peroxide (**Lu and Walker, 2001 and Ito et al., 2003**). Similar type of incidence have been reported as Production levels and the proportions among these compounds depend on the strain, medium compounds and physical parametrs (**Tannock, 2004**). The chromato graphic analysis of oils obtained from the juice of fatty acid, palmitic acid, stearic and the unsaturated acids, ollic besides metalinic acids (benzene dicarboxylic acid) fatty oils of fermented plums juice can be used as natural “anti bacterial potential activity” after further studies. It can be concluded that fatty oils of ‘fermented plums juice’ can be used for developing plant derived anti microbial drugs.

Table: 1

Isolation characteristics of Probiotics

Characteristics	<i>Lactobacillus kefiranofaciens</i>	<i>Candida kefir</i>	<i>Saccharomyces boluradii</i>
Cellwall	G+ve	Chitin mannose PPM, PLM	Chitin mannose PPM, PLM
Morphology	Rod	Yeast like pseudohyphae	Pseudohyphae
Motility	NM	-	-
Spore forming	NS		
Selectivemedium	MLR	YMA	SGA
Growth at 15 ⁰ C-20 ⁰ C 20 ⁰ C -30 ⁰ C 30 ⁰ C-40 ⁰ C 40 ⁰ C-50 ⁰ C	+	+	+
pH 3.5 4.5 6.5 8.5	+	+	+
Salt 6.5 10%	+	+	+

Carbohydrate fermentation			
Arabinose	+	+	+
Cellobiose	+	+	W
Esuculin		+	-
Fructose	+	+	+
Galactose	+	+	-
Gluconicacid	+	-	+
Lactorose	+	+	+
Maltose	+	-	+
Mannitol	+	-	+
Mannose	+	-	+
Mellibiose	+	+	+
Raffinose	-	-	+
Rhamnose	-	-	+
Ribose	+	-	+
Salicin	+	-	+
Sorbitol	+	-	-
Sucrose	+	-	+
Xylose	-	-	+

(++) - Luxurious growth
 (+) - Growth
 (W) - Weak Growth
 (-) - No growth

PPM – Phosphopetidomannan
 PLM – Phospholipomannam
 MLR – Modified Lactobacillus Agar medium
 TJA – Tomato Juice Agar medium
 LBB – Lacto bacillus bulgaricus agar medium
 YMA – Yeast morphology agar medium
 SGA – Sabrouds glucose medium
 NM – Nonmotile
 NS - Nospore

Table – 2
 Antibacterial activity of Plums juice

Pathogens	S₁	S₂
<i>Staphylococcus aureus</i>	+	++
<i>Escheriacoli</i>	+	++
<i>Salmonella paratyphi A</i>	+	++
<i>Shigella dysenteriae</i>	+	++
<i>Vibriocho lerae</i>	+	++

S₁ - Juice probicated with organism
 S₂ - Fermented beverage with Inulin

Table – 3

Major compounds identified from the fermented Plums juice

No.	Retention time (min)	Name of the Compound	Pear Area %
1	3.22	1H – Imidazole, 2,4 dimethyl	2.71
2	6.45	4H – Pyran-4-one, 2,3,dihydro-3.5-dihydroxy-6-methyl-	6.02
3	8.48	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	34.64
4	11.91	1,2,3,5-Cyclohexanetetrol	55.35
5	22.43	Hexanedioic acid, bic (20ethylhexyl) ester	0.64
6	24.66	1,2 – Benzenedicarboxylic acid, diisooctyl ester	0.65

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