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Evaluation of Bio control Efficacy of Synbiotic Cherry (*Prunus avium. L.,)* Juice

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ABSTRACT

The aim of the research was to evaluate that the effect of symbiotic fermented cherry juice containing fructo oligosaccharide to enhance the growth and activity of probiotic strains include *Lactobacillus acidophilus* was tested for their antibiotic susceptibility, and tolerance to bile. Antifungal activity of symbiotic cherry juice could differ in their antagonistic activity against fungal disease which could be due to the metabolite secreted by the lactic acid bacteriocin specially type of organic acids and added fructo oligo saccharide as a probiotic and for food preservation synbiotic cherry juice was identified and their major compounds was detected using gas chromatography-mass spectrum (gc-ms).

1. INTRODUCTION

Probiotics are non pathogenic, technologically suitable for industrial process and produce antimicrobial substances including organic acids, hydrogen peroxide and bacteriocins(biologically active protein) (Dunne *et al.,*1999). Lactic acid bacteria make up extremely important group of probiotic dairy products. (Lourens-hattingh and Viijoen 2001). The recent trend in food technology is to combine probiotics with prebiotics products containing there both components are generally termed as "synbiotics" (Holzapfel and schilling 2002). Synbiotics have been reported to provide different health benefits such as antimicrobial, anticarcinogenic, immunomodulatory, antidiarrhoeal, antiallergenic, hypolipidaemic and hypoglycaemic activities. The synbiotic effects of fermented foods and drinks not only populate our intestines with beneficial bacteria, but also helps in improving, the survival. Implantation and growth of newly added micro flora strains.

In recent years, consumer demand for synbiotics products has increased and probiotics as well as prebiotics components are incorporated into drinks and marked as supplements in the form of capsules, tablets and freeze dired products. Beverages such as fruits or vegetables juice delivery medium for the supplementation of such as health promoting components.

2. MATERIALS AND METHODS

2.1. Sample enrichment:

Cherries were collected from market and were used for isolated probiotic bacteria and yeast. The cherry sample was inoculated and allowed to ferment at room temperature for a week spontaneously without any additives through the cherry juice endogenous of micro organisms. The enrichment process of the collected organisms inoculated same was carried out as follows low volume of inoculated cherry was added to 80ml MRS broth (man rogosa sharpa) in 150ml conical flask. The enriched sample was incubated under anaerobic conditions. The enrichment process was conducted in triplicate and repeated on weekly basis for one month period.

2.2. Probiotic isolation and characterization:

The isolation process was carried out by streaking the enriched samples on MRS agar media and incubated at 37c. The probiotic are characterized by using colony morphology,biochemical test and in selective medium carbohydrate fermentation (Table 1). Probiotic bacteria *Lactobacillus acidophilus* confirmed by using acid tolerance test, bile tolerance test and cell adhesion test, and antimicrobial activity.

2.3. Probiotication of synbiotic cherry juice:

The Probiotic bacteria *Lactobacillus acidophilus* was inoculated and fermented for 24 hours. After 24 hours the 1% probiotics fructo oligosaccharides were added into the fermented probiotic juice. probiotic cherry juice was fermented for 24 hours. After fermentation the synbiotic cherry juice was prepared.

2.4. Bile tolerance:

Probiotic strains were cultivated in MRS broth enriched with 2% of oxgall (dehydrated bile,becton and Dickinson) at 37 c for 24 hours. The growth was checked by spreading of 100 of culture of appropriate dilution on to MRS agar (oxoid) control cultures were without oxgall and cells counts were completed with those after 24 hours. Bacterial growth was expressed in colony forming units per milli liter (cfu/ml).

2.5. Detection of antagonistic activity of probiotics

2.5.1. Test organisms

The bacteria used as test organisms were *Aspergillus niger, candida albicans*, these were procured from MTCC (microbial type culture collection) IMTECH Chandigarh, India. The organisms were maintained on PDA and were subsequently subcultured into newly prepared agar media. All the chemicals and medium used in this study were supplied by Himedia Pvt.Ltd., Mumbai , India.

2.5.2. Agar well diffusion assay

The antifungal activity of synbiotic cherry 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 throughout the medium uniformly with a sterile cotton swab. Using sterile cork borer (10mm diameter) wells were made in the agar medium. The test compound (synbiotic cherry juice extract) was introduced into the separate well in a single plate. All the plates were incubated at 37 c for 24h. The antagonistic test was performed in triplicate 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).

2.5.3. GC-MS analysis

The volatile constituents from symbiotic cherry juice was analysed using GC-MS with Elite-1 column and a mass detector, which was operated in El mode at 70ev.injector and detector temperatures were set at 250 c Al-Delaimy and All, (1970). Synbiotic cherry juice (1ml) was injected and analysed with a column held initially at 110 c for 2min and then increased by 5 c per min up to 280c. Helium was used as carrier gas (1 ml/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 time and mass spectra with those of authentic reference compounds, or by retention indices (RI) and mass spectra. (Table 3).

TABLE-1
IDENTIFICATION OF PROBIOTIC ORGANISMS

S.NO	General characteristics	*Lactobacillus acidophilus*
1	Colony morphology	Brown colour colonies
2	Gram staining	+ve , rod
3	Motility	Non-motile
4	Catalase	-ve
5	Oxidase	-ve
6	Cellobiose	+ve
7	Lactose	+ve
8	Mannitol	-ve
9	Mannose	+ve
10	Galactose	+ve

TABLE – 2
INHIBITORY ACTIVITY OF SYNBIOTIC CHERRY JUICE AGAINST TEST PATHOGENS

S.No	ORGANISM	ZONE OF INHIBITION (in mm)			
		S1	S2	S3	ANTIBIOTICS
1.	*Aspergillus niger*	+	++	+++	Nystatin ++
2.	*Candida albicans*	+	++	+++	Candicidin ++

S1 - Nonfermented Cherry juice.

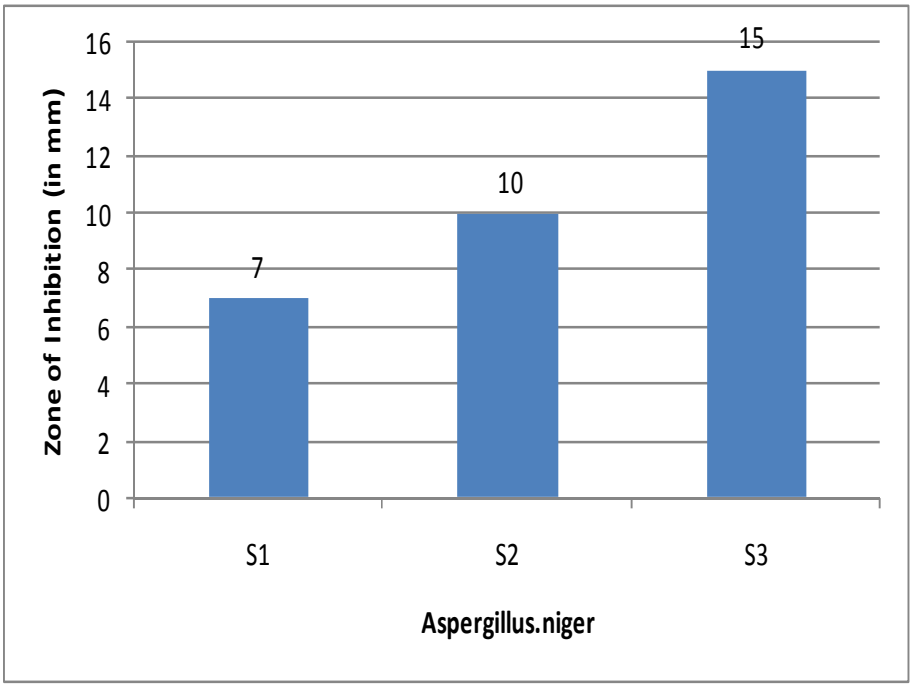
S2 - Fermented cherry juice.

S3 - Fermented cherry juice with fructo oligo saccharide.

TABLE- 3
COMPONENTS INDENTIFIED FROM THE SYNBIOTICS CHERRY JUICE

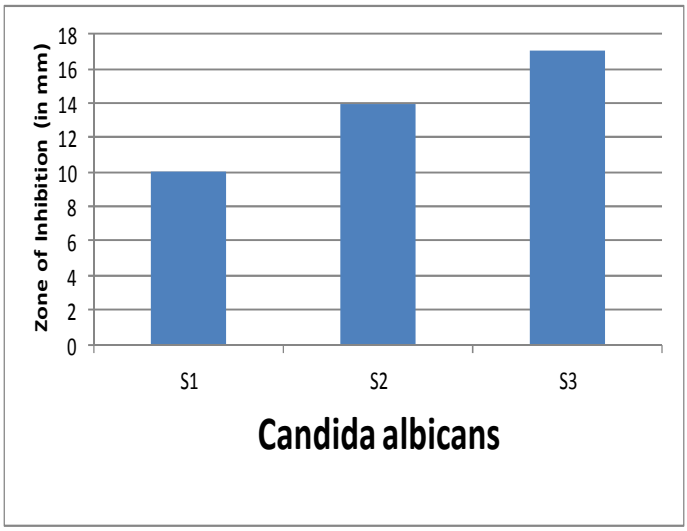
S.NO	RT	Name of the compound	Molecular Formula	Mw	Peak Area%	Biological Activity
1.	8.90	Cetene	$C_{16}H_{32}$	224	13.88	Antioxidants
2.	10.81	Pentaphen	$C_{11}H_{16}O$	164	13.52	Neuroleptic action
3.	11.12	1-Nonadecence	$C_{19}H_{38}$	226	18.86	Antibacterial
4.	13.43	1-Docosene	$C_{22}H_{44}$	208	17.44	Antibacterial
5.	14.83	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	10.32	Antioxidants
6.	16.05	1-Tricosanol	$C_{23}H_{48}O$	340	12.10	Antifungal
7.	18.86	17-Pentatriacontene	$C_{35}H_{70}$	490	9.61	Antidiarrhoeal
8.	21.71	Docosanaic acid,1,2,3, -propanetriyl ester	$C_{69}H_{134}O_6$	1058	4.27	Antifungal

RT – Retention Time
MW- Molecular



S1 –Non Fermented Cherry Juice
S2 – Fermented Cherry Juice
S3- Fermented Cherry Juice with fructo oligo saccharide

FIGURE – 1
ANTAGONOSITIC ACTIVITY OF SYNBIOTIC CHERRY JUICE TEST PATHOGEN ON *ASPERGILLUS NIGER*



S1 –Non - Fermented Cherry Juice
S2 – Fermented Cherry Juice
S3- Fermented Cherry Juice with fructo oligo sacharide

FIGURE – 2
ANTAGONOSITIC ACTIVITY OF SYNBIOTIC CHERRY JUICE TEST PATHOGEN ON *CANDIDA ALBICANS*

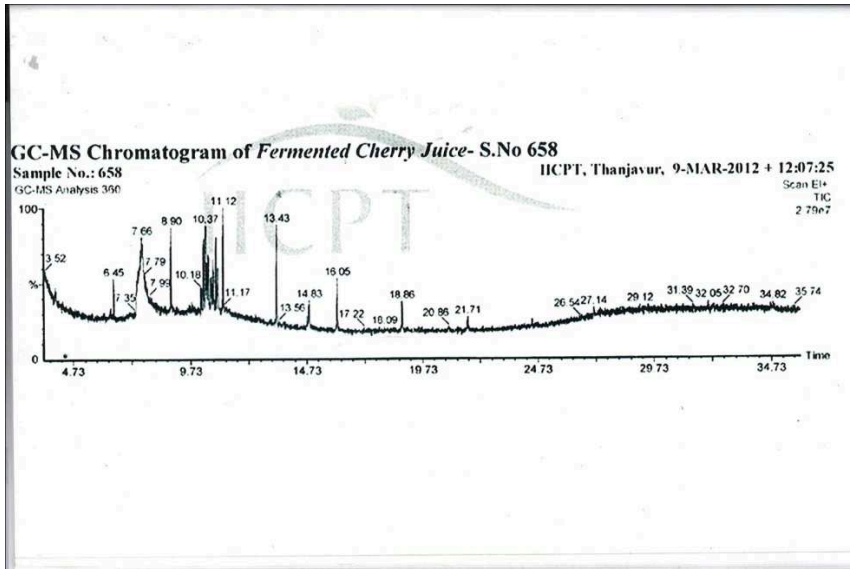


FIGURE--3
GAS CHROMATOGRAPHY-MASS SPECTRUM ANALYSIS OF SYNBIOTIC CHERRY JUICE

3. RESULT AND DISCUSSION

The synbiotic fermented cherry juice was most effective against the fungal pathogens such as *Aspergillus niger* and *Candida albicans.* In addition to that significant in antagonistic effect against the test pathogens between fermented and non fermented cherry juice fermented cherry juice (S2)and fermented cherry juice with fructo oligo saccharide (S3) was performed (Table 2; Fig 1 & 2) however among the three samples higher antagonistic activity was shown by fermented cherry juice with fructo oligo saccharide (S3) similar incidence was reported as the probiotic *Lactophillus acidophilus* with prebiotic,fructo oligo saccharide at different concentration (0.5%)results in good probiotic-prebiotic combination for the preparation of symbiotic cherry juice (Anju kurien *et al.,* 2005).

The reports of chromatograms and compounds from cherry juice given in (Table 3 & Fig 3) chromatographic analysis of compounds obtained from the milk beverage of fatty acid palmitic acid, stearic and the unsaturated acids,ollic besides metalinic acids, fatty oils of fermented cherry juice can be used as natural antifungal potential activity after further studies. Probiotic approach through cherry juice extract increases residence fungi 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 pathogens by the production of organic acids and hydrogen peroxide (Lu and Walker, 2001 and Ito et al., 2003).Similar types of incidence have been reported as production levels and the proportions among these compounds depend on the strain. Medium compounds and physical parameters (Tannock, 2004). The chromatographic analysis of compounds obtained from these synbiotic cherry juice extract can be used as natural "anti fungal activity "for developed plant derived anti microbial drugs,

4. CONCLUSION

The aim of the research was to evaluate that the effect of symbiotic fermented cherry juice containing fructo oligosaccharide to enhance the growth and activity of probiotic strains include *Lactobacillus acidophilus* was tested for their antibiotic susceptibility, and tolerance to bile. Antifungal activity of symbiotic cherry juice could differ in their antagonistic activity against fungal disease which could be due to the metabolite secreted by the lactic acid bacteriocin specially type of organic acids and added fructo oligo saccharide as a probiotic and for food preservation synbiotic cherry juice was identified and their major compounds was detected using gas chromatography-mass spectrum (gc-ms).

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