Research Paper

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Studies on Growth Effects of Catechin on Probiotic Bacteria

Engineering

KEYWORDS : green tea, catechin, probiotic bacteria

Kavitha kottol	Research Scolar, Faculty of Engineering, Avinashilingam University, Tamilnadu
Sudha.P	Associate Professor, Faculty of Engineering, AvinashilingamUniversity, Tamilnadu
A.Reni	Associate Professor, Faculty of Engineering, Avinashilingam University, Tamilnadu

ABSTRACT

Tea is rich in polyphenols and other phenolics that have been widely reported to have beneficial health effects. However, dietary polyphenols are not completely absorbed from the gastrointestinal tract and are metabolized by the gut micro flora so that they and their metabolites may accumulate to exert physiological effects. This study is focussed on the effect of tea catechins in probiotics bacterium Lactobacillus acidophilus culture. The green tea catechin is extracted and isolated by using solvent extraction method, the percentage of concentration of catechin was optimized as 1% because maximum growth of probiotic bacterium is observed in that concentration and the bacterial concentration was optimized as 9.0-10.0 log 10 cfu/ml based on IDF standards.

INTRODUCTION

Tea (*Camellia sinensis*), is one of the most widely consumed beverage and its medicinal properties are well known. It is estimated that about 2.5 million tons of tea leaves are produced each year throughout the world, with 20 % produced as green tea, which is mainly consumed in Asia, some parts of North Africa, the United States, and Europe. The association between tea consumption, especially green tea, and human health has long been appreciated (Weisburger, 2000). Green tea contains polyphenols, which include flavanols, flavandiols, flavonoids, and phenolic acids; which compounds may account for up to 30 % of their dry weight. Most of the green tea polyphenols are flavonols, commonly known as catechins. Products derived from green tea are mainly extracts of green tea in liquid or powder form that vary in the proportion of polyphenols (45-90 %) and caffeine content (0.4-10 %). Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Fuller, 2002).The present study is focussed on addition of catechin and probiotic bacteria for contributing better intestional health for consumers.

REVIEW OF LITERATURE

Previously, Hansel et al. (1992) described that tea contains 9-13 % epigallocatechin gallate, 3-6% epicatechin gallate and epigallocatechin, 1-3 % epicatechin and the overall catechin content is 10mg/1g of green tea leaves. Extraction process is the key to the withdrawal of active components of green tea (Danrong *et al.,* 2009). Methodology and efficiency of catechins extraction is critical while studying their functionality behavior (Yoshida *et al.,* 1999) as extraction conditions like solvent, temperature, time, pH and ratio of solvent to material affect catechins quantity and quality (Perva-Uzunalic *et al.,* 2006). The method must facilitate complete extraction of the compounds of interest, evading their chemical modification (Zuo *et al.,* 2002). Among popular solvents, water, aqueous mixtures of ethanol, methanol and acetone are frequently used to extract plant bioactive molecules (Lee and Ong, 2000; Sun and Ho, 2005). Increasing extraction time is positively correlated to polyphenols extraction (Druzynska *et al.,* 2007) conversely, prolonged extraction procedure with higher temperatures results in degradation of catechins. Temperature is a critical factor, if catechins are epimerized during extraction process; the resultant extract would not reflect the actual health claims (Yao *et al.,* 2004).

The word 'probiotic', derived from the Greek language, means 'for life'. Probiotics benefit the health of consumers by maintaining, or improving their intestinal microbial balance. Salminen *et al.* (1998) reported that the use of probiotic bacterial cultures stimulate the growth of preferred microorganisms, crowds out potentially harmful bacteria, and reinforces the body's natural defence mechanisms. The most commonly used bacteria for commercial probiotic applications are species of *Lactobacillus* and *Bifidobacterium*. These are traditionally added to fermented milk and other dairy products; however, beverages such as fruit juices may also represent an ideal delivery medium for these health-promoting cultures (Sheehan *et al.,* 2007)

MATERIALS AND METHODS

EXTRACTION OF CATECHIN:

Catechins rich fraction from green tea was separated using methanol (70 %) for 2 hours and constant temperature of 60-70 °C .The methanolic extract is sthen subjected to partition by chloroform and ethyl acetate to obtain their respective catechins rich fractions followed by rotary evaporation and vacuum drying (Row and Jin, 2006). The systematic scheme for catechins extraction is depicted in Figure 1. During extraction solvent was recovered through rotary evaporation.

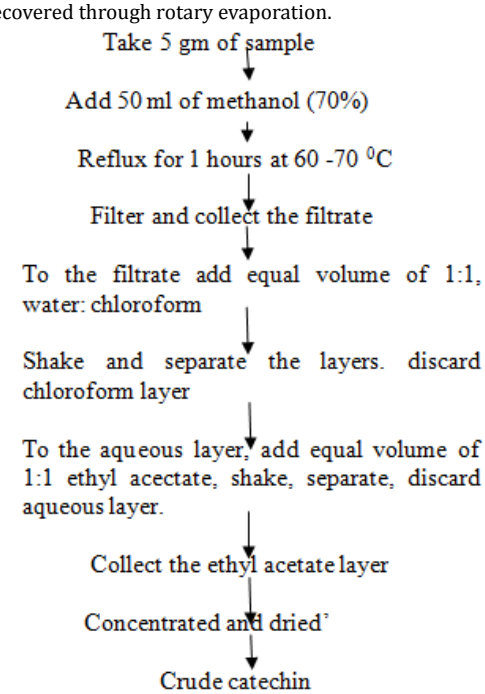

Figure 1. Extraction of Catechin

PREPARATION OF PROBIOTIC BACTERIA

Probiotic bacterium (*Lactobacillus acidophilus* -015) is used for this study and was obtained from NDRI Karnal, India.The probiotic culture was grown at 30 ºC for 24 hours in de Man Rogosa Sharpe (MRS) broth (dextrose 20.0 g/L; meat peptone 10.0 g/L; beef extract 10.0 g/L; yeast extract 5.0 g/L; sodium acetate 5.0 g/L; disodium phosphate 2.0 g/L; ammonium citrate 2.0 g/L; between 801.0 g/L; magnesium sulfate 0.1 g/L, manganese sul-

fate 0.05 g/L). Probiotic organisms were harvested by centrifuging (Remi C24) at 6000 rpm *at* 4°C for 20 min and the cell pellet was suspended in saline solution The plates were incubated as above and the bacterial counts were recorded (Puupponen *et al*., 2001).

GROWTH EFFECTS OF CATECHIN ON PROBIOTIC BACTERIA

Growth experiments with the probiotic bacterium were carried out at 37 °C by plate count method. Fifty microlitres of each bacterial suspension was inoculated into 5ml of MRS broth supplemented with different percentages of crude catechin and standard after which bacterial cell growth was monitored every 48 hours at 37 °C. The samples were diluted in peptone saline and the proper dilutions were plated. The plates were incubated as above and the bacterial counts were recorded.

ANTI-MICROBIAL ACTIVITY OF CRUDE CATECHIN:

The antimicrobial activity of catechin is determined by the following procedure. 10 ml of overnight cultures of *Salmonella Enteritis*, E.*coli* and *L.acidophius* was grown using BHI broth (*Salmonella Enteritis*, E.*coli*) and MRS broth (*L.acidophius).* The absorbance value is adjusted to 0.5 at 580 nm by adding medium. Then 0.1 ml of the inoculum is added to 10 ml of the media containing 1% catechin. Then the antimicrobial activity was observed by recording the absorbance value for 24 hours, 32 hours at 580nm (Haq *et al.,* 2011).

RESULTS AND DISCUSSION

The probiotic culture was grown at 30 ºC for 24 hours in de Man Rogosa Sharpe (MRS) broth and harvested by centrifuging at 6000 rpm *at* 4°C for 20 min and the cell pellet was suspended in saline solution followed by microencapsulation (Yoon *et al*., 2006). The probiotic bacterial count used in the study is optimized as 9.0-10.0 log10 cfu/ml (Shah and Ravula, 2000). International Dairy Federation (IDF) requires 10^7 cfu to 10^9 cfu to of *L. acidophilus* in products such as acidophilus milk at the time of sale. The dose needed for probiotics varies greatly depending on the strain and product. Although many over-the-counter products deliver in the range of 1–10 billion cfu/dose, some products have been shown to be efficacious at lower levels, while some require substantially more. The catechin optimization was done by incorporating the optimized bacterial count in different percentages of catechin ranging from 1% to 5 % and results shows that catechin stimulates the growth of bacterium but the bacterial growth is inversely proportional to the concentration of catechin as shown in figure 2.The growth varies from 7.2x 10^9 to 4.7 x 10^6cfu/ml. The researchers report that the growth of the probiotic Bifidobacterium and Lactobacillus strains were less affected by the tea compounds, and the level of inhibition varies depending on the bacterial species, concentration and chemical structure of the compound (Lee *et al.,* 2006).

The probiotic bacterium was incorporated in crude catechin and standard catechin at different concentrations ranging from 1% to 5% and the results (Figure 3) shows that the growth of probiotics was higher in crude catechin than in the standard catechin because the crude catechin extract contains carbohydrates and other growth promoters than the standard pure catechin. The results clearly indicate that the growth of bacteria is affected by concentration of catechin because in both cases the bacterial count decreased when concentration of catechin increased. It was observed that, 1% catechin has promoted the growth of bacteria to 7.8x10^9 cfu/ml than all other concentrations. Hence, 1% catechin concentration is found to be optimal.

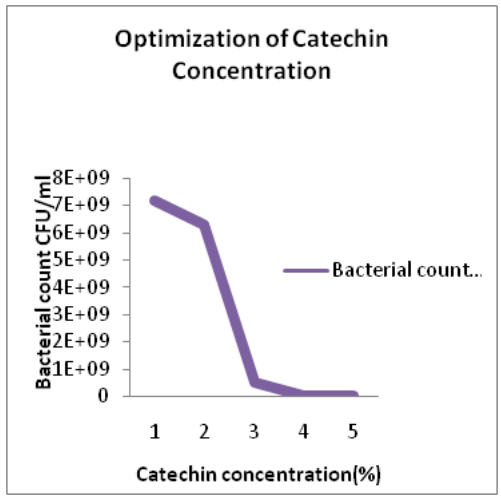

Figure 2. Optimization of the Concentration of Catechin

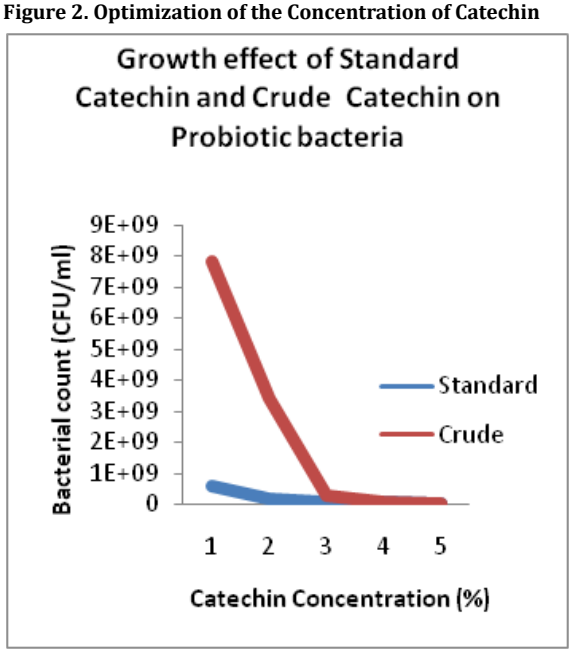

Figure 3. Study on growth effects of Standard Catechin and Crude Catechin on Probiotic bacteria.

ANTI-MICROBIAL ACTIVITY OF CRUDE CATECHIN:

The antimicrobial activity of catechin was determined by taking 10 ml of overnight cultures of *Salmonella Enteritis*, E.*coli* and *L.acidophius* grown using BHI broth (Salmonella Enteritis, E.*coli*) and MRS broth (*L.acidophius*). 0.1 ml of the inoculum is added in the media containing 1% catechin was evaluated for its antimicrobial activity and it was observed that the growth of pathogens was inhibited at 24 hours and 32 hours. The absorbance value indicated increase in the growth of bacterium. The result (Figure 4) showed that the catechin inhibited the growth of *Salmonella Enteritis*, E.*coli* and enhances the growth of lactobacillus bacterium. Hui *et al.,* (2006) has reported that the growth of certain pathogenic bacteria such as *Clostridium perfringens, Clostridium difficile* and *Salmonella Enteritis* was significantly repressed by tea phenolics and their derivatives, while commensal anaerobes like *Clostridium* spp., *Bifidobacterium* spp. and probiotics such as *Lactobacillus* sp. were less severely affected. This indicates that tea phenolics exert significant effects on the intestinal environment by modulation of the intestinal bacterial population, probably by acting as metabolic probiotics.

CONCLUSION

The results conclude that bacterial growth increases in the pres-

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ence of crude catechin than pure catechin because of the presence of the carbohydrates. The maximum growth of 7.2x10 ^{9} was observed at 1% catechin. The prebiotic property of catechin is proved by the antimicrobial assay which concludes that catechin inhibits the growth of pathogens *Salmonella Enteritis*, *E.coli* and promotes the growth of *lactobacillus acidophilus*. Thus the combination of catechin and probiotics form a symbiotic effect.

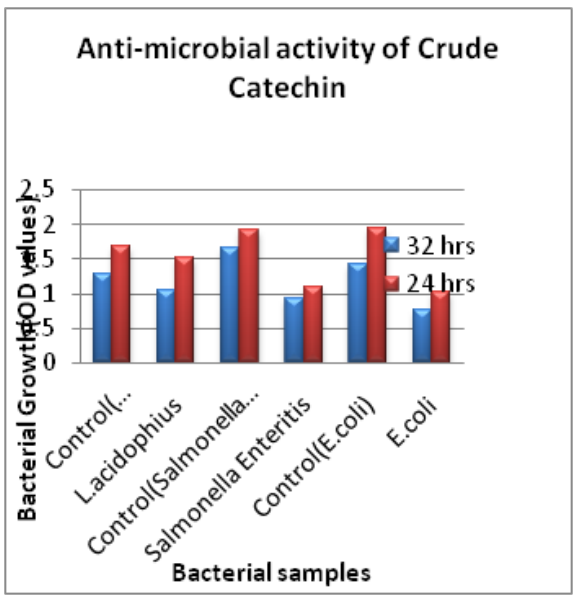

Figure 4. Anti-microbial activity of Crude Catechin:

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