



Probiotic Chocolate Preparation Using *Aegle marmelos*

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ABSTRACT:

World population is increasing, the demand for food and its availability to satisfy the needs of human is increasing day by day. Functional food fits the picture since functional foods are the kind of food that provides benefits beyond basic nutrition. Examples of functional foods include fortified foods, dairy supplements and whole grains. In the present study, probiotics chocolate with bael water extract was prepared. The bael plant contains number of phytochemicals that makes in useful in many ailments. Bael gets its medicinal values on basis of the various chemicals present in it like alkaloids, polysaccharides, essential oils etc. The probiotics bacteria were encapsulated with bael water to get effective results.

Probiotic bacteria *Lactobacillus sporogenes* showed resistance to stress conditions of pH and bile found in the gut. Hence it was used in the development of the product. By combining all these ingredients to get product with all beneficial properties, these ingredients give the synergistic effect in the functional food. Coating the product with chocolate also provides protection of the inner contents of the product and even increases the anti-inflammatory effect of chocolate. Recent research has found that regular moderate consumption of dark chocolate confers benefits even on the young and healthy.

KEYWORDS: Probiotic, Bael extract, Chocolate, *Lactobacillus sporogenes*.

INTRODUCTION

Probiotics refers to live microbes, which when consumed in adequate amounts confers some health benefits. A number of beneficial effects have been attributed to the use of probiotics. The term "probiotics" is derived from two Greek words, "pro" means "for" and "bios" means "life". Probiotics refer to live microorganisms which when administered in adequate amounts, confer a health benefit on its host. The term Probiotics was coined in 1965 by Lilly and Stillwell, who defined them as "microbially derived factors that stimulate growth of other organisms" (Guarner et al., 2008).

The action of probiotics is mainly due to its colonizing and pH alteration activities in the gut. The activity of probiotics also depends on the strain and amount of probiotics consumed. The immunologic benefits provided by probiotics such as prevention of allergies is due to activation of macrophages that increase the antigen presentation to B lymphocytes and increases secretion of Immunoglobulin A. Another reason may be that probiotics alter cytokine profiles and they induce hypersensitiveness to food allergens (Falk et al., 1998; Guarner et al., 2008). Ulcers that are induced by *Helicobacter pylori* are prevented by consumption of probiotics. This action is due to the colonization of probiotics on intestinal mucosa, thus unavailability of space for *H. pylori* to adhere. Our diet contains a number of complex and simple compounds. The simple compounds are easily broken down by the organism while the complex food components are difficult to assimilate. Like in case of

carbohydrate uptake, mammals lack the necessary machinery to degrade complex polysaccharides such as starch, glycans, xylans etc. these complex polysaccharides move to the large intestine that is colonized by normal microflora. Synergistic action of normal microflora and probiotics break these complex molecules into simpler ones that are then readily absorbed (Resta, 2009). Probiotics have the ability to bind aflatoxin to their surfaces and then degrade them (Gratz, 2007). Probiotics, especially Lactic Acid Bacteria have been reported to inhibit growth of molds and aflatoxin biosynthesis due to production of lactic acid and lactic acid metabolites.

Aegle marmelos, commonly called as bael is a plant of the Rutaceae family and is known since ancient times on account of its medicinal properties. The leaves, bark, fruits and flowers are edible and confers different health benefits. The fruits of the plant are widely consumed and its pulp has a sweet, aromatic flavor and imparts a characteristic taste when consumed. The parts of the plant are often found to be ingredients of many ayurvedic medicines and are also used locally in certain illness such as diarrhea, constipation, gastro intestinal disorders, irritable bowel syndrome etc. Probiotics on the other hand refers to live microbes, which when consumed in adequate amounts confers some health benefits. A number of beneficial effects have been attributed to the use of probiotics. In order to provide the advantageous effects of both bael and probiotics, a product is formulated that contains both bael fruit extracts and probiotics, which

forms a functional food and when consumed will provide the benefits of both.

Bael plant is very useful medicinal tree. Its each and every part of the plant is useful. Bael plant is mostly prized for its fruit. Bael gets its medicinal values on basis of the various chemicals present in it like alkaloids, coumarins, polysaccharides, essential oils etc. It contains Polysaccharides such as Galactose, arabinose, uronic acid, L-rhamnose. Tannins present in bael fruit was found to be highest of about 9% (Chakraborty et al., 2012) Carotenoids: Imparts color to the fruit pulp. Minor constituents: Ascorbic acid, sitosterol, crude fibers, α -amyrin, crude proteins (Farooq, 2005). The bael fruit also has therapeutic value which includes Antihyperlipidemic activity, Antiulcer activity, Antidiabetic activity of Aqueous extract of bael seeds reduces blood glucose level in case of severe diabetic patients (Kamalakkannan and Prince, 2003; Maity et al., 2009), Diarrhea and dysentery Antioxidant activity of bael Normal metabolic activities give rise to free radicals. These free radicals, mainly oxygen free radicals, referred as ROS (Reactive Oxygen Species) causes oxidative stress. ROS are harmful for the body as they damage macromolecules, DNA, proteins and lipids. Antioxidants are compounds that scavenge the free radicals and reduce oxidative stress. Bael fruit has proven to show antioxidant activity. On administration of Bael fruit extract of 250 mg/kg of body weight, the activity of ROS scavengers such as glutathione peroxidase, glutathione reductase, superoxide dismutase (SOD) and catalase is shown to increase considerably. Bael extract has been found to have antibacterial properties. Its extract is effective against a number of pathogenic species such as *E.coli*, *Pseudomonas salanacearum*, *Aeromonas spp.* and *Xanthomonas vesicatoria*. Methanol extracts of Bael fruit is also effective against multidrug resistant *Salmonella typhi*. Seed extracts are effective against *Salmonella typhi*, *Salmonella paratyphi*, *Proteus vulgaris*, *Streptococcus fecalis*, *Vibro cholera*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Neisseria gonorrhoea* (Rusia and Srivastava., 1988). Anticancer Bael extract has been found successful in inhibition of in vitro proliferation of human tumor cell lines including Lecukenic K562, T-Lymphoid Jurat, Beta-Lymphoid Raji, Erythro Leukemic HEL (Lampronti et al, 2003) Antifungal activity is found to be very effective against fungus such as *Trichophyton nubrum*, *T. terrestris*, *Epidermophyton floccosum*, *Aspergillus niger*, *A. flavus* and *Aspergillus fumigatus* (Pitre and Srivastava., 1987).

Cocoa is good for your heart because of fermentation by gut bacteria, creating anti-inflammatory compounds that improve blood vessel function. These new findings, about dark chocolate improves vascular function and cardiovascular health in general. Other recent research has found that regular moderate consumption of dark chocolate confers benefits even on the young and healthy. Cocoa phenolics are bioactive compounds possessing anti-oxidant, anti-radical, antiplatelet, anti-inflammatory, anti-carcinogenic and anti-cariogenic products and are also considered to be prebiotics (Redovnikovic et al.2009).

MATERIALS AND METHODS

1. COLLECTION OF BAEI:

Aegle marmelos were collected. These fruits were handpicked from the tree when they were ripe.

2. PREPARATION OF BAEI WATER EXTRACT:

For the preparation of water extract of bael, semi ripe/ripe fruit were used. The shell was broken and the pulp was mixed in water at the concentration of 0.1 mg/ml. To smoothen the solution the pulp was crushed and seeds were removed and it was strained through a mesh with pore size of 1 mm. The obtained solution was dried at 40°C for 48 hours. The product formed after drying was ground into a fine powder and kept in an air tight container for further use.

3. ISOLATION, SCREENING AND ENCAPSULATION OF PROBIOTIC BACTERIA:

Collection of probiotic sample The probiotic sample, Sporlac tablets, containing probiotic organism *Lactobacillus sporogenes* (Uni-Sankyo Ltd) was obtained from Apollo Pharmacy, Isolation of probiotic bacteria The probiotic sample was dispensed in MRS broth and incubated at 37°C for 16 hours. A loop full of this culture were plated on MRSA (de Man Rogosa Sharpe Agar) by spread plate method Incubation of plates was done at 37°C for 48 hours.

a) SCREENING OF PROBIOTIC BACTERIA:

For the screening of the probiotic, it was subjected to test whether they can tolerate the various stress factors in the gut. pH and bile tolerance tests were conducted and their results were obtained.

b) pH TOLERANCE TEST:

The cultures were inoculated in MRS broth and incubated at 37°C for overnight. From this fresh culture, serial dilution was made in PBS up to 10³. One ml of this sample was inoculated in MRS broth at, pH 3 and pH 2, pH 1. by spread plate method. The plates were incubated at 37°C for 48 hours. The growth

obtained in plates was observed (Sahadeva et al., 2011).

b) **BILE TOLERANCE TEST:**

MRSA Plate was prepared with 0.3 % bile salt (HiMedia). A loop full of this sample inoculated at the centre of the plate and the plates were incubated at 37°C for 24 hours. Halo zone formed around the growth at the end of incubation confirmed bile tolerance of the organism (Islam et al., 2012).

GROWTH, ISOLATION AND PREPARATION OF PROBIOTIC SAMPLE:

The probiotic sample was inoculated in 250 ml of MRS broth and incubated for 48 hours at 37°C. At the end of 48 hours, the bacterial cells were isolated by centrifugation at 7000 rpm for 15 minutes. The supernatant was discarded and pellet was taken. Sample was prepared by dilution of the bacterial cell pellet in 1 ml autoclaved water.

PREPARATION OF BEADS:

Three percent sodium alginate and 0.05 M calcium chloride solution was prepared using deionized water and both the solutions were autoclaved at 121°C (15 psi) for fifteen minutes. To the autoclaved sodium alginate solution, bacterial sample was added and the solution was homogenized using vortex. This solution was added drop wise using a syringe with needle diameter of 1 mm to the calcium chloride solution. The capsules/beads formed were allowed to harden for 10 minutes, washed in deionized water twice and spread on a petriplate to dry (Manasouripour et al., 2013)

PREPARATION OF PROBIOTIC CHOCOLATE:

Probiotic chocolate was prepared using dried bael extract, probiotic beads, sugar and chocolate. A thickening agent (corn starch) is used to thicken the sample. The prepared chocolate can either have a water base or a milk base.

PREPARATION OF THICKENED EXTRACT:

Bael extract was added to deionized water or milk at the concentration of 0.05 g/ml and heated at 100°C. Upon heating sugar at the concentration of 0.1 g/ml and corn starch at the concentration of 0.03 g/ml was added to the solution. The mixture was allowed to thicken and allowed to cool down. Probiotic beads were added and homogenized by mixing.

PREPARATION OF MOLD AND MAKING OF CHOCOLATE:

Milk chocolate (Cadbury) was melted at 50°C in water bath and 1 g of melted chocolate was used to line the walls of the mold of suitable shape. It was cooled at 4°C for 15 minutes to harden the chocolate. Upon hardening, 2 g of

thickened bael extract was added to each mold and it was covered with melted chocolate on the top. The mold was allowed to freeze at -20°C for 2 hours to set the mixture. As the mixture sets, it was carefully removed from the mold to obtain the product. Ice tray was used as mold.

RESULT AND DISCUSSION:

Bael water extract was prepared by removing the seeds in order to remove the bitterness of the extract. The mixture is homogenized by crushing the pulp and dried to get powder of bael fruit.

SCREENING AND ENCAPSULATION OF PROBIOTIC BACTERIA

Lactobacillus sporogenes showed resistance to stress conditions of pH and bile as found in the gut. Hence it was used in the development of the product. As the probiotics bacteria can able to survive in acidic pH.

BILE TOLERANCE TEST:

The bile tolerance test for *Lactobacillus sporogenes* confirms that the organism can able to survive in 0.3 % of bile salt concentration. *Lactobacillus sporogenes* shows the halo zone proves that the microbe can tolerate the high bile concentration.

Probiotic chocolate with bael water extract was prepared. Preparation of mold by lining with liquid chocolate. Filling of mold with thickened bael extracts (milk based). Milk based probiotic chocolate. Thickened bael extract (water based) the chocolate prepared were of two types i.e. milk based and water based. The difference between the two lies in taste and flavor. While the water based chocolate has a stronger aroma and a sharp taste of bael, the milk based chocolate has a milder flavor. Cocoa phenolics are bioactive compounds possessing anti-oxidant, anti-radical, antiplatelet, anti-inflammatory, anti-carcinogenic and anti-cariogenic products and are also considered to be probiotics. Redovnikovic et al., (2009). Dark chocolate was used due to the presence of flavonoids, which possess various therapeutic properties and a prebiotic effect enhancing the antibacterial activity of probiotic chocolate (Dakshinamoorthy et al., (2016).

Preparation of probiotic chocolate with bael water extract was carried out in order to provide the advantageous effects of both bael and probiotics the product was formulated that contains both bael fruit extracts and probiotics that forms a functional food and that when consumed will provide the benefits of both.

The results of the current study shows the similar results to that of Ms. Swapna –Sonali Panda. They carried out the similar

experiment. The pH tolerance test showed that the optimum growth was observed at pH 2 and pH 3, as well as bile tolerance test showed the

same results of halo zone formation. The organism can grow at lower pH (i.e. acidic pH) and can able to tolerate the bile salt concentration.

Table 1 Composition of Bile Salt media

Gms/Litre	Ingredients	Composition
1.0 %	peptone,	
0.8 %	egg extract,	
0.4 %	yeast extract,	
2.0 %	glucose,	
0.5 %	sodium acetate trihydrate,	
0.1 %	polysorbate 80 (also known as Tween 80),	
0.2 %	dipotassium hydrogen phosphate,	
0.2 %	triammonium citrate,	
0.02 %	magnesium sulfate heptahydrate,	
0.005 %	manganese sulfate tetrahydrate,	
1.0 %	agar,	
pH	adjusted to 6.2 at 25°C.)	



Figure 2. Bael Pulp



Figure 3 Bael water extract

pH TOLERANCE OF PROBIOTIC BACTERIA:

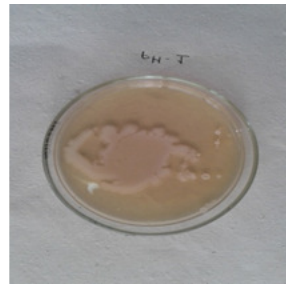


Figure 4: pH 1

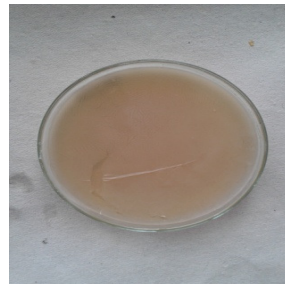


Figure 5: pH 2

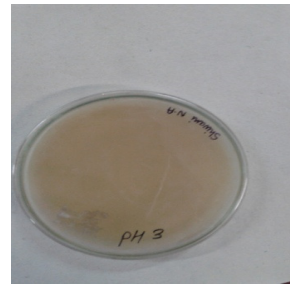


Figure 6: pH 3

BILE TOLERANCE TEST:



Figure 7 Bile tolerance

PREPARATION OF PROBIOTIC BEADS:



Figure 8 Beads

THE PREPARATION OF PROBIOTIC CHOCOLATE WITH BAEI WATER EXTRACT.

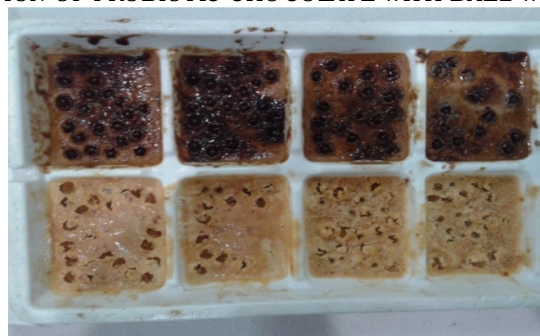


Figure 9 Probiotic chocolate

CONCLUSION :

In the present study the probiotic chocolate that was formulated using bael extract, probiotic beads and chocolate is a functional food since it has the beneficial effects of bael, probiotic and chocolate. Researchers are trying to the development of drugs using natural sources, development of modern drugs from *A. marmelos* can be emphasized for the control of various intestinal diseases. A systemic research and development work should be undertaken for the development of products for their better economic and therapeutic utilization. The aim of combining all these ingredients and making a product with the goodness of each of the ingredients gives the synergistic effect in the functional food. Coating the product with chocolate aims at protecting the inner contents from spoilage creating microbes and it also increases the flavor of the product. Chocolate contains some compounds such as antioxidant polyphenol compounds, such as catechin and a small amount of dietary fibre.

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