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FORMULATION AND EVALUATION OF PROBIOTIC CHOCOLATE FROM AEGLE MARMELOS

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ABSTRACT
A functional food is a food that has been added with new ingredients or the present ingredients have be processed in a manner that it provides additional health benefits than the conventional form. Aeglemarmelos, commonly called as Aeglemarmelos 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. Most of the beneficial properties of Aeglemarmelos are derived from the pulp of the fruit.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 Aeglemarmelos and probiotics, a product is formulated that contains both Aeglemarmelos fruit extracts and probiotics, which forms a functional food and when consumed will provide the benefits of both. a probiotic chocolate per day is more than enough to ensure the optimal activity on the intestinal flora in parallel to its higher survival rates of chocolate –embedded probiotics in the human body.

KEYWORDS: Aeglemarmelos, beads, Lactobacillus sporogene, probiotic chocolate.

1 INTRODUCTION
Aeglemarmelos belonging to family Rutaceae, is commonly known as Aeglemarmelos in indigenous systems of medicine and has been regarded to possess various medicinal properties. The Aeglemarmelos is one of the sacred trees of the Hindus. Leaves are offered in prayers to Shiva and Parvathi since ancient times. Aeglemarmelos is a deciduous sacred tree, associated with Gods having useful medicinal properties, especially as a cooling agent. This tree is popular in Shiva and Vishnu temples and it can be grown in every house. Its leaves are trifoliate symbolizing the Thrimurthies-Brahma, Vishnu, Shiva, with spear shap leaflets resembling Thrisoolam the weapon of Lord Shiva. Many legends, stories and myths are associated with this tree. The leaflets are given to devotees as prasadam in Shiva temples and as Tulasi in Vishnu temples. In India flowering occurs in April and May soon after the new leaves appear and the fruit ripens in 10 to 11 months from bloom March to June The Aeglemarmelos tree has its origin from Eastern Ghats and Central India.[1]

As mentioned earlier, probiotics can be made available in the form of food, drug or dietary supplements. The concept of probiotics chocolate is not totally new. Many companies have claimed to produce probiotics chocolate with all the goodness of chocolate and benefits probiotics. But development of a product with fruit extract that is added with probiotics relatively new. Adding a layer of chocolate to the thickened fruit extract ensures its protection from spoilage creating organism since chocolate is not affected by spoilage creating microbes. Also the chocolate adds to the organoleptic property of the product.

1.1 Probiotic products
Due to the numerous positive effectsof probiotics on human and animal health, a number of probiotic products are now available in market. Probiotic products can either be available a dairy based products or non-dairy based.

Dairy based probiotic products: Dairy based probiotic products includes those that are
Developed by fermenting milk with probiotics. These products include -

- Yoghurt (spoonable, drinkable and shots)
- Dahi, kefir
- Cheese (long storage)

- **Non-dairy based probiotic products:** Nondairy based probiotic products include those

That is derived from sources other than milk. They include

- Fruit and berry juices (non fermented)
- Fermented vegetable juices (tomato, carrot juice)
- Yoghurt (oat yoghurt)
- Probiotic olives
- Probiotic salami
- Probiotic bread (*Lactobacillus* used in traditional sour dough bread)[2]

1.2 Functional foods

Due to the increase in population around the globe, the demand for food and its availability to satisfy the needs of human is increasing day by day. The United Nations Food and Agriculture Organization estimated that nearly 870 million people of the 7.1 billion people around the world are suffering from chronic undernourishment. Fifteen percent of these undernourished people are from developing countries In such a scenario where the availability of quality food's becoming scarce each day, the need for food with improvised qualities seems to be a solution. 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 Due to developments in food industries, many traditional food items are now being modified include beneficial components that will provide advantages beyond simplenourishment. The alteration of traditional foods also makes it easily acceptable by people thanintroduction of a new product.[3]

2 DRUG PROFILE

Aegel Marmelos

Systemic classification

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Sub-class: Rosidae
Order: Sapnidales
Family: Rutaceae
Genus: Aegle
Species: marmelos

2.1 AegelMarmelos tree

AegelMarmelos tree is one of the most used medicinal trees that are found in India. Apart from being used for its medicinal properties, it also holds religious values for Hindus who consider this tree as sacred. AegelMarmelos belongs to the family Rutaceae, the family to which citrus fruit belongs. Fig. 1 shows the photograph of aAegelMarmelos tree. The tree is slow growing and may reach a height of 40-50 feet. Tree has a short trunk and the bark is thick, soft and flaking. When wounded, the branches give out a clear gummy sap which hangs down like strands but eventually solidifies. The tree 6 bears alternate leaves that are in 2's and 3's. The branches sometime bear spines and the fruit is often drooping. The inflorescence of contains a bunch of fragrant flowers that vary from 4 to 7 in number [4]

Figure 1: Aegel Marmelos Tree.

2.2 Aegel Marmelos fruit

Aegel Marmelos plant is mostly prized for its fruit. The fruit is a pyriform, may vary from oval to round, and size varies from 10-20 cm in diameter. The fruit has a hard, woody exocarp and inside it is a thick, fleshy and aromatic, slightly sweet pulp. The color of the pulp may vary from bright orange to sunset yellow and the pulp contains seeds that are present in grooves and is surrounded by thick, clear mucilage. Both ripe and unripe fruits are used for their medicinal values. A number of phytochemicals are present in AegelMarmelos that makes in useful in many ailments.

2.3 Chemical constituents of AegelMarmelos fruit

Aegel Marmelos gets its medicinal values on basis of the various chemicals present in it like alkaloids, coumarins, polysaccharides, essential oils etc.

- Coumarins: The coumarins present in AegelMarmelos fruit includesmarmelosin, marmesin, imperatorin, marmin, alloimperatorin, methyl ether, xanthotoxol, scoparone, scopoletin, umbeliferone, marmelide and marmenol.
- Alkaloids: Aegelin, aegelinine, fragine, o-methylhalforodinine, o-isopentanylhalfordinol, N-2-[4-(3',3'-dimethylallyloxy)phenyl]ethyl cinnamide, o-(3,3-dimethylallyl) halofodinol, Ethyl cinnamide[5]
- Phenylpropenoids: Hydroxylcoumarins, phenylpropenes, lignans
- Polysaccharides: Galactose,arabinose,uronic acid, L-rhamanose
- Seed oils: Palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid

Tannis: Highest percentage of tannins recorded in AegelMarmelos fruit is 9% and was recorded

- Carotenoids: Imparts color to the fruit pulp

- Minor constituents: Ascorbic acid, sitosterol, crude fibers, α-amyrin, crude proteins [6]
- Proximate constituents: Table 1 shows the proximate constituents of Green and Ripe AegelMarmelos[7]

Table 1: Proximate analysis of Green and Ripe AegelMarmelos powder (%).

Powder	Moisture	Ash	Vitamin C	Protein	Fat	Carbohydrate
Green AegelMarmelos	2.64	0.64	56.99	1.75	0.67	93.73
Ripe AegelMarmelos	4.04	1.10	57.09	3.75	1.24	90.33

Table 2: Mineral Content of Green and Ripe Aegel Marmelos Powder (ppm).

Powder	Na	K	Ca	Mg	Zn	Cu	Fe
Green AegelMarmelos	55.6	1356	78.9	142	0.66	0.67	19.3
Ripe AegelMarmelos	119	4821	92.9	259	1.69	1.34	16.22

2.4 Therapeutic value of AegelMarmelos

1. Diarrhea and dysentery: In case of chronic diarrhea and dysentery without fever, half ripe or unripe fruit acts as a remedy. Half ripe fruit is considered best for the purpose but fully ripe fruits or even fruit powder has shown effective results. When the fruit is still unripe, it is cut, dried and ground into powder. The unripe fruit can also be consumed by baking and then consumption with brown sugar or jiggery. After use of fruit, the amount of blood passed in the fecal matter reduces and the fecal matter gets a more solid form.[8]

2. Antiulcer activity: Gastrouduoenal ulcer is a general disorder of the gastrointestinal tract. Many plants have shown effect in reducing ulcer like neem and turmeric. AegelMarmelos is known to show gastro protective activity. Unripe AegelMarmelos fruit extract serves the purpose. When used in rats, it produces a noteworthy inhibition of absolute ethanol induced gastric mucosal damage. This activity is shown due to the presence of a particular compound in the fruit, called, Luvangetin. Gastric ulcer is usually mediated by progress of oxidative stress. This compound, luvangetin might act by inhibition of oxidative stress producing compounds in the gastrointestinal tract thus preventing ulcer formation.[9]

3. Antidiabetic activity: Diabetes has become a common disease around the world. When the body cannot produce ample of insulin, the blood glucose level increases.Antidiabetics aim at reducing the blood glucose level by inducing the production of a higher amount of insulin. AegelMarmelos extract, when administered at a dose of 250 mg/kg of body weight, shows better result than glycenamide (antidiabetic drug). This antidiabetic effect may be due to the coumarins present in the fruit which induce the beta cells of islet of Langerhans to produce insulin. Aqueous extract of AegelMarmelos seeds reduces blood glucose level in case of severe diabetic patients

4. Antihyperlipidemic Activity: Increase in the concentration of cholesterol, triglycerides and fatty acids in blood causes arthrosclerosis and thickening of walls of arteries which may eventually cause ischemic heart disease, coronary heart disease, myocardial infarction and cerebro vascular accidents. Although a number of drugs are available to reduce the lipid concentration of blood, but their use is limited due to significant side effects. Oral administration of aqueous extract of AegelMarmelos fruits and seeds separately at a dose of 250 mg/Kg of body weight to diabetes induced rats has shown significant decrease in the blood lipid level. The effect may be due to fat mobilization from deposits which is caused due to hydrolysis of triglycerides. The extract also increases glucose utilization.[20][15][16]

5. Antioxidant Activity: 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. AegelMarmelos fruit has proven to show antioxidant activity. On administration of AegelMarmelos 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. Use of above mentioned dose of AegelMarmelos fruit extract shows better results than glibenclamide (36µg/kg). The antioxidant activity may be due topresence of flavonoids, alkaloids, sterols, tannins, phlobatannins and flavonoid glycosides[14]

6. Anticancer Activity: Cancer is one of the most dangerous diseases mainly because there is no complete treatment for it. Also the treatments that are available for cuing the symptoms have lots of side effects, and are also not cost effective. Hence search is going on to make available treatments of natural origin that will be cost effective and will show minimal side effects. AegelMarmelos 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[10]

7. Antibacterial Activity: AegelMarmelos 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 Xanthomonasvesicatoria.Methanol extracts of AegelMarmelos 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 aeuriginosa, Bacillus subtilis*and *Neisseria gonorrhoea.*[11]

8. Antifungal Activity: Extract of seeds of AegelMarmelos is effective against fungus such as Trichophytonrubrum, T, terrestrese, Epidermophytonfloccosum, Aspergillusniger, A. flavus and Aspergillusfumigatus[12]

9. Constipation: Ripe fruit has been considered as the best of all known laxatives. In case of constipation, administration of ripe fruits cleans and tones up the intestines. Its regular use for 2-3 months has been effective in removal of even old and accumulated fecal matter from bowels. For best results, the pulp of ripe fruit is crushed and made into a sherbet. Seeds are removed for reducing the bitterness and sugar and/or milk can be added to make it more palatable[13]

10. Radio Protective Effect: Today cancer treatment includes radiation therapy that often results in many side effects and toxicity. Radiation causes a slow alteration in genome base pair, my cause esophagitis in lung cancer, acute microsites and pharyngitis in case of neck and lung cancer. Due to many such profound effects of radiation therapy, its use is highly restricted unless utterly necessary. Hydroalcholic extract of AegelMarmelos fruits have been studied for their radio protective effects. Its effects have been proven in mice that were exposed to varying degrees of gamma radiation. Use of 20 mg/kg of bodyweight of hydroalcholic extract of AegelMarmelos fruit for 5 consecutive days before irradiation of gamma rays was seen to provide maximum protection. This action of AegelMarmelos may be due to lipid peroxidation along with elevation in GSH concentration in liver, kidneys, stomach and intestines of mice[14]

2.5 Products from Aegel Marmelos fruits

AegelMarmelos fruit is available in many forms. These include raw and ripe whole fruits, fruit powder, fruit extract, fruit extract powder, AegelMarmelos seeds, dried unripe fruit slices, ripe fruit drink, AegelMarmelos fruit tea (either entirely made of AegelMarmelos or a blend of AegelMarmelos fruit and other ingredients such as ginger or lemon or others), AegelMarmelos fruit juice, fruit juice jelly and jam.

3 INFORMATION REALATED TO PROBIOTICS

Eli Metchnikoff, who won Nobel Prize, defined "Probiotics" as live microbes which when administered in adequate amounts, confer a health benefit. The term "probiotics" is derived from two Greek words, "pro" meaning "for" and "biotics" meaning "life". Though probiotics is a fancy term now a days, but humans have been using probiotics unknowingly since ages. For example, it is a well-known fact that consumption of yoghurt or dahi provides a positive impact on the digestive system.

3.1 Mode of action of probiotics

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. Some mode of action of probiotics is listed below.

1. Prevention of growth of pathogenic bacteria: The normal microflora present in the intestine helps to keep an individual healthy by prevention of growth of pathogenic bacteria. But in case of some intestinal disorder, or after administration of high antibiotics, the normal microflora of intestines decreases in number. On administration of probiotics, these organisms go and colonize on the intestinal walls. Pathogenic microbes also need to colonize on the intestinal walls in order show their effect. Since they colonize on the surface of intestines, they compete with pathogenic microbes for adhesion. Along with this, they also help in restoration of normal microflora of the host [15]

Fig. 2 shows the effect of probiotics on the intestinal cells that prevent growth of pathogenic bacteria. This action might also be due to production of acids (like lactic acid by *Lactobacillus* spp.) that creates acidic environment and prevents growth of harmful microbes.

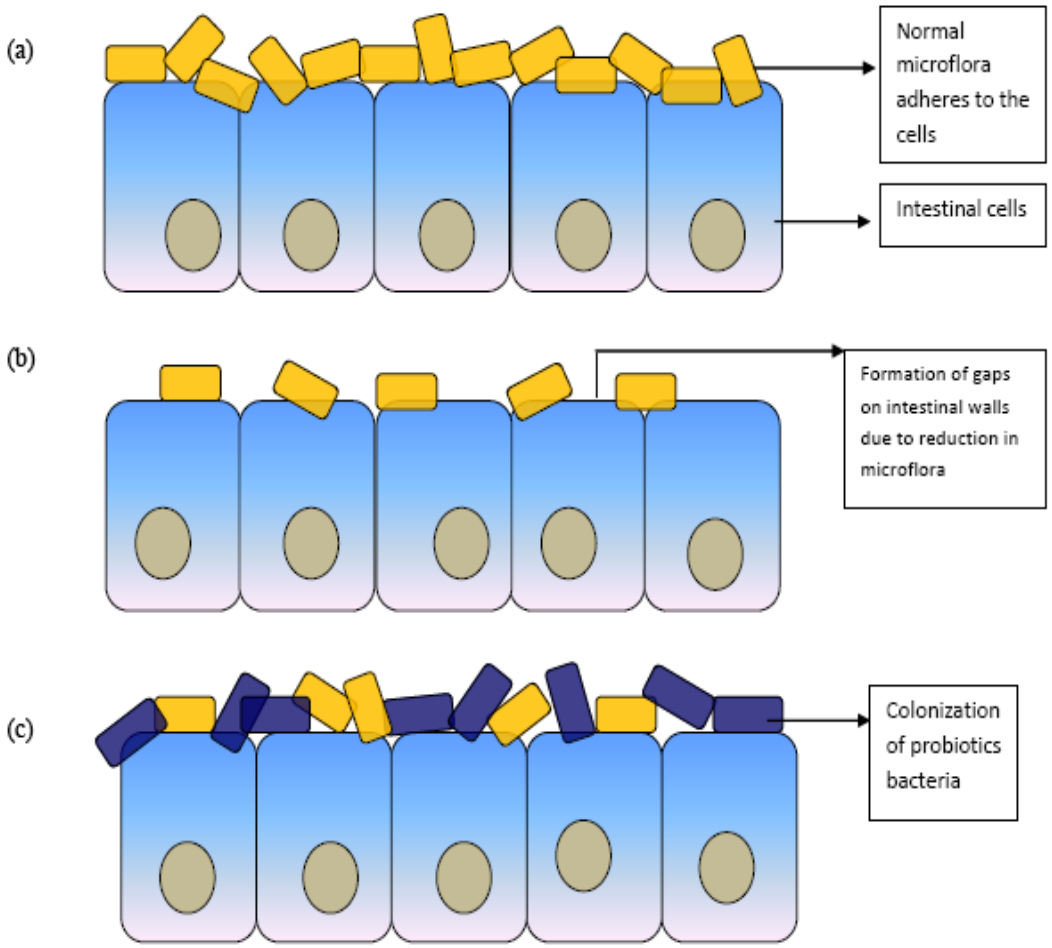

Figure 2: Role of probiotics bacteria in inhibition of growth of pathogenic bacteria.

(a) Normal microflora (yellow) covers the surface of intestinal cells. (b) After gastrointestinal problem or administration of antibiotics, there is reduction in number of normal microflora and the intestinal walls have space for growth of pathogenic microbes (c) consumption of probiotics(blue) causes their colonization on intestinal walls leaving minimal space for pathogenic microbes.

2. Immunologic effect: The intestine is lined by lymphoid tissue. Allergy is initiated by interaction of food components with the intestinal mucosa, which identifies allergens and initiates an immunologic response 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.

3. Alteration of colonic motility: Gastrointestinal problems such as constipation are directly related to movement of intestinal digest. If the movement of digested matter is not proper in the intestines, it leads to intestinal discomfort and constipation. Use of probiotics provides relief in such conditions, which might be due to stimulation of fermentation in large intestine[16]

3.2 Encapsulation of bacteria

Probiotics can only exert their function after they reach the intestines. But before reaching the intestines, they have to pass through a variety of harsh environment starting from the moth itself. The effect of probiotics depends on the number of viable cells that reach the intestines. First they have to tolerate enzymes present in the saliva. After reaching the stomach, probiotics are subjected to an acidic pH of 2-3. Next, the transit of probiotics from acidic to alkaline pH also decreases their viability[17]

Hence to ensure that a good amount of probiotic cells reach the intestine, it is necessary to protect the cells from the harsh environment. This can be achieved by a number of methods. Encapsulation of cells in a hydrocolloid matrix is one such method. Suspension of cells in the matrix ensures its protection from enzymes, acidic and alkaline pH. The use of biopolymers 19 such as alginate, k-carrageenan, xanthan gum, and gellan gum to trap cells is a common technique to protect cells. The presence of a barrier between the cells and the in vivo conditions ensures their survival[18]

Lactobacillus sporogenes

The probiotic organism used in this project was *Lactobacillus sporogenes*, available by the commercial brand name of "SPORLAC"

Systemic classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillicales
Genus: Bacillus

Species*: coagulans Bacillus coagulans*is usually marketed by the name of *L. Sporogenes*because of its spore forming ability. It is Gram Positive, spore forming and lactic acid producing bacillus which was first discovered in 1933. L. sporogenes has got the GRAS (Generally Recognized As Safe) status and is used as a probiotic. The band name for *L. sporogenes*is "sporlac", which is available in powder and tablet form. This probiotic is orally administered which passes into the stomach in spore form. Upon reaching the duodenum it becomes active and starts to multiply. *L. sporogenes*is not a permanent resident of the gut. It is removed slowly through fecal matter, after 7 days of dosage [19]

4 AIMS AND OBJECTIVES

The aim of this experiment was to make a probiotic chocolate with the goodness of Aegel Marmelos pulp and probiotics.

The objectives were

1. To prepare water extract of AegelMarmelos
2. Isolation, screening and encapsulation of probiotic bacteria
3. Preparation of probiotic chocolate

5 MATERIALS AND METHODS

5.1 Collection of aegelMarmelos

Aeglemarmelos was collected.The fruits were handpicked from the tree when they were ripe.

5.2 Preparation of aegelMarmelos water extract

For the preparation of water extract of AegelMarmelos, semi ripe/ripe fruit was 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^{o}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.

5.3 Isolation, Screeningand Encapsulationof Probiotic

Isolation of probiotic bacteria

The probiotic sample was dispensed in MRS broth and incubated at 37^{o}C for 16 hours. A loop full of this culture was plated on MRSA (de Man Rogosa Sharpe Agar) by spread plate method at concentration of 10-3. Incubation of plates was done at 37^{o}C for 48 hours.

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 on the five samples and their results were obtained.

pH tolerance test

The cultures were inoculated in MRS broth and incubated at 37^{o}C for overnight. From this fresh culture, serial dilution was made in PBS up to 10-3. One ml of this sample was inoculated in MRS broth at pH 6.8, pH 2 and pH 3. At the end of 1 hour, 2 hours and 3 hours 100 µl of this culture was inoculated on plates containing MRSA (HIMEDIATM)(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.) by spread plate method. The plates were incubated at 37^{o}C for 48 hours. The growth obtained in plates were compared to the control (pH 6.8) to deduce the results[20]

Bile tolerance test

MRSA 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^{o}C for 24 hours. Formation of halo zone at the end of incubation confirmed bile tolerance of the organism[21]

5.4 Encapsulation of probiotic bacteria

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^{o}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 capsules/ 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^{o}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[22]

5.5 preparation of probiotic chocolate

Probiotic chocolate was prepared using dried AegelMarmelos extract, probiotic capsules/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: AegelMarmelos extract was added to deionized water or milk at the concentration of 0.05 g/ml and heated at 100oC. 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 24 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^{o}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^{o}C for 15 minutes to harden the chocolate. Upon hardening, 2 g of thickened AegelMarmelos extract was added to each mold and it was covered with melted chocolate on the top. The mold was allowed to freeze at -20^{o}C for 2 hours to set the mixture. As the mixture sets, it was carefully removed from the mold to obtain the product.

6 RESULTS AND DISCUSSION

6.1 preparation of water extract of bael

On mixing the pulp of bale with water, most of the seeds were removed in order to reduce the bitterness of the extract. The mixture was homogenized by crushing the pulp and then drying it on a wide, flat and clean surface at 40^{o}C. Figure. 3 shows pulp in water solution of bale and dried bale water extract.

(a) Solution of bael pulp in water **(b) dried bael water extract**

Figure 3: Shows Pulp in Water Solution of Bale and Dried Bale Water extract.

6.2 Screening and encapsulation of probiotic bacteria Lactobacillus

Sporogenes showed resistance to stress conditions of pH and bile as foundgut. Hence it was used in the development of the product

pH tolerance of probiotic bacteria

Table 3: shows the pH tolerance test (*Lactobacillus sporogenes*).

pH	Number of colonies	
	Hour 1	Hour 2
6.8	12	12
2.0	2	4
1.0	1	2

Preparation of Probiotic Capsules/Beads

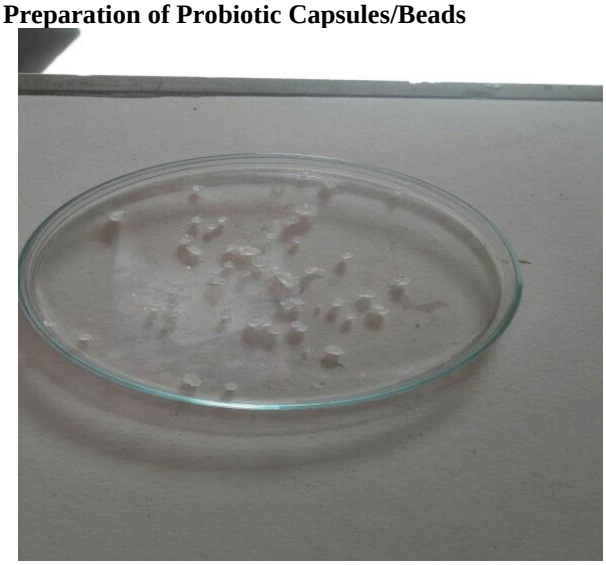

Figure 4: Prepared Lactobacillus sporogen beads.

6.3 Preparation of Probiotic Chocolate

a) Filling of mold with thickened bael extract **b) Milk based probiotic chocolate**

Figure 5: Preparation of probiotic chocolate from bael water extract.

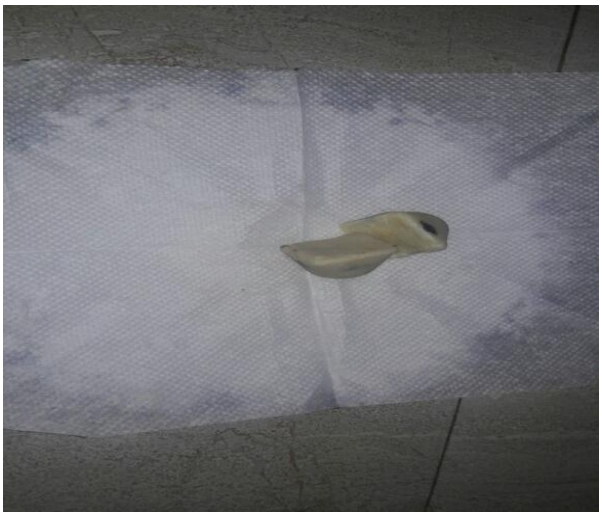

Figure 6: Preparation of probiotic chocolate (a) Filling of mold with thickened bael extract (milk based (b) Milk based probiotic chocolate. (c) Thickened baelextract (water based).

CONCLUSION

The probiotic chocolate that was formulated using bael extract, probiotic beads and chocolate functional food since it has the beneficial effects of bael, probiotic and chocolate. The idea combining all these ingredients and making a product with the goodness of each ofingredients 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 flavor of the product.

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