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# *GRANULES* OF UNISTRAIN *LACTOBACILLUS* AS NUTRACEUTICAL ANTIOXIDANT AGENT

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**ABSTRACT:** The present study was conducted with the aim to prepare probiotics *Lactobacillus acidophilus* (*L. acidophilus*) granules which are stable at room temperature. *Lactobacillus acidophilus* 2285 probiotics was obtained from the N.C.I.M (National Collection of Industrial Microorganism), Pune. The formation of the semi-solid mass occurred after the further incubation at 34 °C from range (33 °C to 37 °C) in an incubator kept for the less than 24 hour time duration. This mass was homogenized and converted into granule formulation. The viability of the granule formulation was achieved with a maximum viable cell count after 24 hours of incubation in de Man, Rogosa, and Sharpe (M.R.S) agar media. Spray dried and tray dried powder of the probiotics is used for granulation, these drying methods served as a cheap alternative to the expensive freeze-drying procedure. The selected strain of *L. acidophilus* NCIM 2285 assessed for antioxidant activity. The antioxidant activity of *L. acidophilus* was demonstrated by *in-vitro* test using 2, 2--diphenyl-1-picrylhydrazyl free radical scavenging assay. The results showed that intact cells and cell-free extract of two formulations exhibited obviously higher antioxidative activity in scavenging DPPH radical than standard *L. rhamnose GG*, which was shown to have an antioxidative activity used as a positive control.

**INTRODUCTION:** As microorganisms are the oldest sources of medicines and are no means of exhausted, search for new microbial products as drugs is still a productive approach, especially to serve as Probiotics. Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'.

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The concept of probiotics (which means 'for life') and their beneficial effects were initially revealed by Nobel laureate, Elie Metchnikoff, who proposed that ingesting microorganisms could have substantial health benefits for humans. The numerous species of microorganisms in the adult human gut are known as the microbiota [1].

The microbiota of a newborn develops rapidly after the birth. It is initially dependent mainly on the mother's microbiota, mode of delivery, birth environment, and rare genetic factors. The maternal vaginal and intestinal flora constitutes the source of bacteria, which colonizes the intestine of the newborn. [12] Imbalance of intestinal microflora results in Poor nutritional response reduced the efficacy of medications, physiological dysfunction,

physical discomfort, accelerated aging, cancer, deficient immune response and susceptibility to infection.

Their beneficial effects include reinforcement of the natural defense mechanisms and protection against gastrointestinal disorders. [2] The potential benefits of lactic acid bacteria for human health include improvement of lactose intolerance, prevention of intestinal infection, reduction of serum cholesterol, stimulation of the immune system, anti-carcinogenic action, and anti-oxidative effects. Recent studies showed that LAB could be successfully used to manage diarrhea, food allergies and inflammatory bowel disease (IBD) [3].

Probiotics are available in foods and dietary supplements, produced in various Dosage forms including powders, granules, pastes, liquids, capsules, and tablets, etc. The dose of probiotics is usually given as the number of colonies forming units (CFU) [4]. The optimal dosage has not been established, but the results of clinical studies show that the minimum daily therapeutic dose should be $10^6$ to $10^{11}$ CFU/day [5]. Microorganisms have anti-oxidative systems to maintain free radical levels that are not toxic to the cells. Recently, there has been an interest in the antioxidant effects of microorganisms and their role in health and disease. Several investigations were conducted to study anti-oxidative properties of lactic acid bacteria. *Lactobacillus* with antioxidant activity may be used to help the human body by reducing oxidative damage. They are able to degrade the superoxide anion and hydrogen peroxide [6]. The metabolic antioxidant activities of Probiotics may be assigned to ROS scavenging, enzyme inhibition, chelate Ferrous, and reduction activity or inhibition of ascorbate autoxidation in the intestine by neutralizing free radicals.[12 - 14].

## MATERIAL AND METHODS:

**Procurement of *L. acidophilus*:** *Lactobacillus acidophilus* 2285 probiotics which was obtained from the N.C.I.M (National Collection of Industrial Microorganism), Pune; for long-term maintenance, this organism was stored as glass bead cultures in freezer at -20°C. Once this bead of a deep-frozen culture were transferred into de Man, Rogosa, Sharpe (MRS) broth and incubated overnight at 37°C for 24 hours; turbidity was observed indicating the colony growth. Later the 1ml of the broth were diluted with saline in a various ratio as 1:100, 1:1000, 1:10000 till $10^{12}$ times and were placed on MRS agar media; colony forming units (CFU) were obtained.

**pH Survival Studies [7]:** A single isolated colony was subcultured in MRS broth adjusted to different pH using NaOH (1.0 M) or HCI (1.0 M), pH values of 4.0, 5.0, 6.0, 7.0 and 8.0 were selected and incubated at 37°C for 24 hours to observe the ability of the growth of *L. acidophilus* under different pH values.

**Thriving off the *Lactobacillus acidophilus* in Natural Media [8]:** The milk of buffalo was pasteurized before use to nil the other microbes if present in the milk. The effects obtained in the case of the buffalos were based on the content difference in fat and nutrients of both animals. The microbes from early MRS media plates were transferred via nichrome lope in aseptic condition to the media of Milk of buffalos. Later these flasks of milk inoculated by microbes of *Lactobacilli* were kept in the incubator at 33 °C to 37 °C. The semi-solid beads formed were broken down and converted into a liquid state by homogenization.

**Thermal Method to Generate the Dried Particles [9]:** To maintain the viability and motility along with the stability of the microbial culture to the desired level it needed to be converted into the dried powder form. This can be achieved by converting into dried powder form by below given methods as:

**Tray Drying Method [10]:** The technique is quite simple requires merely the heating chamber. After adding 5% maltodextrin in aseptic condition, the broth was poured into the tray drier and dried at 40°C for 48 hours and collected in powder form. Silver foil was used for drying purpose, with evacuating the chamber initially at 100°C with concentrated alcohol.

**Spray Dry Method [11]:** The spray-drying process of *Lactobacillus acidophilus* done in the various media was undertaken in a laboratory scale spray dryer (JISL mini-spray dryer). The feed solution was pneumatically atomized into a vertical, concurrent drying chamber using a two-fluid nozzle at a constant flow rate ($5ml.min^{-1}$) to ($20ml.min^{-1}$).

The outlet temperature was adjusted from 100°C to 110°C by varying the air inlet temperature. The dried powder was collected in a single cyclone separator.

The excipients used for the spray dry were starch and maltodextrin solution. The viability at different combination was carried out for starch and maltodextrin solution as 1:1, 1:2, 2:1, *etc* to check for good results.

**FIG. 1: SPRAY DRYER**

**Formulating *L. acidophilus* as Granules:** Wet granulation is a size enlargement process in which a liquid is used to achieve agglomeration of solid particles in a formulation.

Microcrystalline cellulose, lactose monohydrate, and corn starch were first sieved through a 300 µm diameter sieve to break up lumps, povidone water solution was added as a binder. All these materials were blended by hand mix method for 15 min. Three different volumes of edible broth medium containing *L. acidophilus* NCIM 2285 and 2.0 % (m/V) skim milk were mixed with the powder mass for 10 min.

**Antioxidant Activity Screening [5]:** Preparation of intact cells and intracellular cell-free extracts:
Cells were harvested by centrifugation at 4°C for 30 min (3,000 rpm) after overnight incubation at 37°C and the pellet was washed twice with 20 mM sodium phosphate buffer (SPB, pH 7.4), then re-suspended in SPB. Washed cell suspension was disrupted with an ultrasonic cell disrupter (4°C) and filtration. Cell debris was removed by centrifugation (5,000 rpm) for 10 min and adjusted to 1 mg $ml^{-1}$. For the preparation of intact cells, cells were washed twice with SPB and re-suspended in SPB. The total cell number was adjusted to $10^9$CFU $ml^{-1}$

**DPPH Free Radical Scavenging Assay[15]:** 0.8 ml of intact cells or intracellular cell-free extract and 1 ml of freshly prepared DPPH (1,1-Diphenyl-2-Picrylhydrazyl radical) solution (0.2 mM in methanol) were mixed and allowed to react for 30 min. Blank samples contained either PBS. The scavenged DPPH was then monitored by measuring the decrease in absorbance at 517 nm. *L. rhamnosus GG* (standard) which was shown to have an antioxidative activity was used as a positive control. All determinations were performed in triplicate. The decrease in the absorbance indicated the antioxidant activity means the lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The scavenging ability was defined as follows.

$$\% \text{ Scavenging Activity: } 1 - \frac{\text{Absorbance sample}}{\text{Absorbance blank}} \times 100$$

## RESULT AND DISCUSSION:

**Plating Method:** The transfer of the microbial inoculums was carried out in aseptic condition in MRS media by serial dilution and kept for the incubation in incubator at 33 °C to 37 °C. It was found that the growth rate and CFU count was optimal at 34 °C; when kept for periods of 24 hours from the MRS broth to MRS agar media.

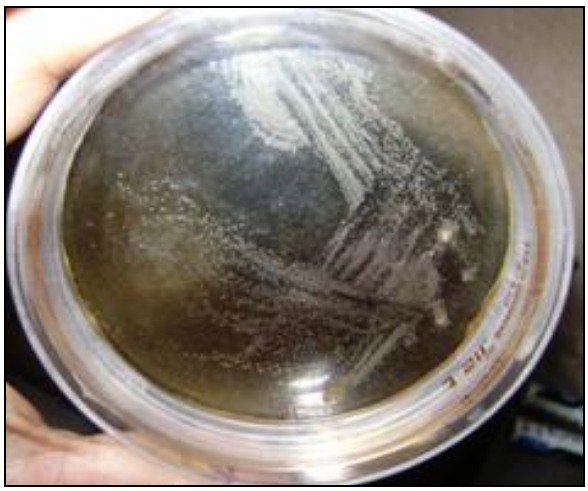

**FIG. 2: PLATE OF *L. ACIDOPHILUS* ON MRS MEDIA WITH DILUTION**

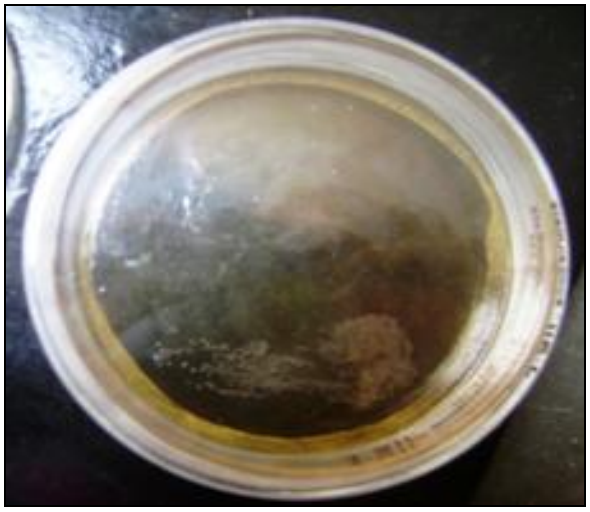

**FIG. 3: PLATE OF *L. ACIDOPHILUS* ON MRS MEDIA WITHOUT DILUTION**

**pH Survival Studies:** The effect of pH on growth of microbes was as follows:

**TABLE 1: THE EFFECT OF pH ON GROWTH OF MICROBES**

| S.no. | pH | Observation |
|---|---|---|
| 1 | 3 | Clear |
| 2 | 4 | Slight turbid |
| 3 | 5 | Very turbid |
| 4 | 6 | Very turbid |
| 5 | 7 | Turbid |
| 6 | 8 | Clear |
| 7 | 9 | Clear |

The study revealed that *Lactobacillus acidophilus* preferred to grow in medium with pH between 4 and 7.

**Tray Drying Method:** This method yield solid mass with the low percentage of moisture. The tray drying was carried out by taking the combination of the Maltodextrin and starch in different ratios and the cell count obtained by serial dilution were as follows.

**TABLE 2: CFU COUNT FOR DIFFERENT CONCENTRATION OF MALTODEXTRIN: STARCH IN MILK**

| Maltodextrin: Starch ratio | Microbial count as $10^9$ CFU/gm | | |
|---|---|---|---|
| 1:1 | 21 | 22 | 24 |
| 1:2 | 25 | 27 | 29 |
| 2:1 | 38 | 34 | 39 |

The results were optimal for the milk as by spray dry. The 2:1 ratio of (Maltodextrin and starch) gave good cell count as 38 X $10^8$ CFU/gm (average).

**Spray Drying Method:** The spray drying was carried out by taking the combination of the starch and maltodextrin in different ratios and the cell count obtained by serial dilution were as follows for different the milk.

**TABLE 3: CFU COUNT FOR DIFFERENT CONCENTRATION OF MALTODEXTRIN: STARCH IN MILK**

| Maltodextrin: Starch ratio | Microbial count as $10^8$ CFU/gm | | |
|---|---|---|---|
| 1:1 | 12 | 12 | 12 |
| 1:2 | 15 | 14 | 14 |
| 2:1 | 35 | 34 | 34 |

The results were optimal for the milk as by spray dry. The 2:1 ratio of (Maltodextrin and starch) gave good cell count as 35 X $10^8$ CFU/gm (average).

**DPPH Free Radical Scavenging Ability:** The DPPH radical scavenging method is widely used to evaluate antioxidant activities, because of its simplicity, rapidity, sensitivity, and reproducibility compared with other methods. The principle of the assay is based on the reduction of a methanolic DPPH solution in the presence of a hydrogen-donating antioxidant, leading to the formation of non-radical form DPPH-H. The antioxidant is able to reduce the stable radical DPPH from purple to yellow - colored diphenylpicrylhydrazine. Formulation 1 and 2 were tested for their antioxidative capacity in both intact cells and intracellular cell-free extracts in this study. *L. rhamnosus GG* (Std.), which was shown to have an antioxidative activity in the previous reports, was used as a positive control.

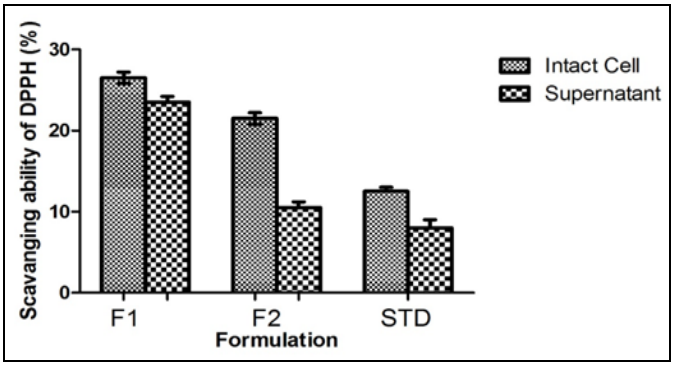


**FIG. 4: DPPH FREE RADICAL SCAVENGING ABILITY (n=3)**

As shown in the **Fig. 5**, the scavenging DPPH rate of intact cells was higher than that of cell-free extracts.

The intact cells of formulation 1 and 2 exhibited the scavenging DPPH radical by 27.50 and 23.99%, respectively. The results also showed that intact cells and cell-free extract of two formulations exhibited obviously higher antioxidative activity in scavenging DPPH radical than the standard used.

**SUMMARY AND CONCLUSION:** *L. acidophilus* was obtained from the N.C.I.M (National Collection of Industrial Microorganisms), Pune. It was obtained in form of bead, once this bead of a deep-frozen culture was transferred into de Man, Rogosa, Sharpe (MRS) broth and incubated overnight at 37 °C for 24 hours; turbidity was observed indicating the colony growth. Latter sub-culturing was carried out by serial dilution in the natural media like milk of buffalos.

It resulted in the formation of the semi-solid mass after the further incubation at 34 °C from range (33°C to 37 °C) in an incubator kept for the less than 24 hours' time duration.

To process it further these semi-solid beads were broken down-converted into the liquid state by homogenization. To maintain the viability and motility along with the stability of the microbial culture to the desired level it was needed to be converted into the dried powder form. This was achieved by converting into dried powder form by the spray dry and tray dry technique. The latter technique with proper excipients in granule formulation gave more positive results. The spray-drying process of Lactobacillus acidophilus was done in the various media in a laboratory scale spray dryer (JISL mini-spray dryer).

*In-vitro* antioxidant studies carried out by DPPH free radical scavenging ability method using Lactobacillus acidophilus powder and standard as *L. rhamnosus GG*; in which formulation shown higher antioxidative activity in scavenging DPPH radical than the standard used.

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**CONFLICT OF INTEREST:** The authors have no conflict of interests to declare regarding the publication of this paper.

## REFERENCES:

1. Barbosa J, Borges S and Teixeira P: *Pediococcus acidilactici* as a potential probiotic to be used in food industry. International Journal of Food Science & Technology 2015, 50: 1151–1157.
2. Maciel, G, Chaves, K, Grosso C and Gigante M: Microencapsulation of Lactobacillus acidophilus La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. Journal of Dairy Science. 2014; 97: 1991–1998.
3. Tripathi M and Giri S: Probiotic functional foods: Survival of probiotics during processing and storage. Journal of Functional Foods, 2014, 9: 225–241.
4. Yu J and Kim H: Oxidative stress and cytokines in the pathogenesis of pancreatic cancer. Journal of Cancer Prevention. 2014; 19: 97–102.
5. Asikin Y, Takahashi M, Mishima T, Mizu M, Takara K and Wada K: Antioxidant activity of sugarcane molasses against 2,2 ′-azobis(2-amidinopropane) dihydrochloride-induced peroxyl radicals. Food Chemistry. 2013; 141: 466–472.
6. Mustapha N, Bouhlel I, Chaabane F, Bzeouich IM, Ghedira K and Hennebelle T *et al.*: Aqueous extract of Crataegus azarolus protects against DNA damage in human lymphoblast Cell K562 and enhances antioxidant activity. Applied Biochemistry and Biotechnology. 2014; 172: 2266–2275.
7. Huang H, Sun Y, Lou S, Li H and Ye X: *In vitro* digestion combined with cellular assay to determine the antioxidant activity in Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) fruits: a comparison with traditional methods. Food chemistry. 2014; 146: 363–370.
8. Mali I, Wang H, Grant W, Feldman M and Forstner M: Modeling Commercial Freshwater Turtle Production on US Farms for Pet and Meat Markets. PLoS ONE. 2015; 10.
9. Preidis G and Versalovic J: Targeting the human microbiome with antibiotics probiotics, and prebiotics: gastroenterology enters the metagenomics era. Gastroenterology. 2009; 136: 2015-2031.
10. Montalban-Arques A, De Schryver P, Bossier P, Gorkiewicz G, Mulero V and Gatlin DM: Selective Manipulation of the Gut Microbiota Improves Immune Status in Vertebrates. Frontiers in Immunology. 2015; 6: 512.
11. Gong S, Wang F, Shi H, Zhou P, Ge Y and Hua L: Highly pathogenic Salmonella Pomona was first isolated from the exotic red-eared slider (*Trachemys scripta* elegans) in the wild in China: Implications for public health. Science of the Total Environment. 2014; 468: 28–30.
12. Bernstein C: Inflammatory bowel disease: a global perspective. World Gastroenterology Organisation Global Guidelines. 2009, 1-24.
13. Patil A, Shinde S, Kakade P and D'souza J: Lactobacillus Model Moiety a New Era Dosage Form as Nutraceuticals and Therapeutic Mediator, Biotechnology and Bioforensics, Springer Singapore 2015; 11-21.
14. Patil A and D'souza J: *Aloe vera* phytochemical constituents and medicinal properties: review, World Journal of Pharmaceutical Research 2016; 4(5): 709-728.

15. Patil A and D'souza J: Antioxidant Study and Phenolic Content of *Caralluma Fimbriata* Herb, World Journal of Pharmaceutical Research 2015; 3(7): 565-575.

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