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The study of prebiotic potential of peanuts and pistachios: The stimulatory effect on *Lactobacillus* growth

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Abstract
Gastrointestinal tract performs many functions in the body such as digestion, absorption, assimilation of the food; protection against pathogens and boosts immune system. Diseases of gastrointestinal tract have been majorly associated with improper dietary habit and lifestyle. Beneficial microorganisms (probiotics) in the gut play important nutritional and physiological roles. These microorganisms are fed on prebiotics. Prebiotics are supplements or foods that contain food ingredients that selectively stimulate the growth of probiotics. Peanuts and pistachios are widely consumed all over the India and have a great nutrient profile. They are rich in protein, fatty acids, phytochemicals, fibre and antioxidants.
In this present investigation prebiotic potential of peanuts and pistachios was estimated by studying the growth of *Lactobacillus acidpphilus* is presence and absence of peanuts and pistachios. Both quantitative and quantitative study showed the stimulation of lactobacillus in presence of different concentration of peanuts and pistachios. Phytochemical analysis (Qualitative and quantitative) revealed that was pistachio and peanuts rich in of polyphenols, flavanoids and proanthocynidins (Oligomeric polyphenol). As polyphenols and their metabolites posses strong prebiotic potential, it can be concluded that rich source of wide range of polyphenol are the most probable cause of their prebiotic potential of peanuts and pistachios.

Keywords: peanuts, pistachios, prebiotics and lactobascillus

Introduction
The human gastrointestinal (GI) tract consists of a dynamic and a complex population of microorganisms. These microorganisms exert a significant influence on the host during disease and homeostasis. Microorganisms are established during infancy; where multiple factors contribute to the establishment of the gut. There are many factors which governs the shaping of gut microbiota among which diet is considered as one of the main drivers which aids in development of microbiota in gut across the life time. Intestinal bacteria comprises of beneficial bacteria and pathogenic bacteria. The beneficial bacteria in the gut play a crucial role in maintaining immune and metabolic homeostasis and protects against pathogens. Dysbiosis, altered gut bacterial composition is associated with the pathogenesis of inflammatory diseases and infection. (Thursby and Juge, 2017) [1]
Probiotics are defined as 'live microorganisms which when administered in adequate amount confer health benefits to the host' (FAO/WHO, 2002). Alternatively, probiotics have been defined as live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance. Probiotics are used to improve the health of both animals as well as humans through the modulation of the intestinal microbiota. Currently several well characterised strains of Lactobacilli and Bifidobacteria are available for the use of humans to reduce the risk of several gastrointestinal infections or to treat such infections. Consumption of probiotics leads to some of the beneficial effects which include improvement of intestinal health by the regulation of microbiota and development and stimulation of the immune system, enhancing and synthesizing the bioavailability of nutrients, reducing the symptoms of lactose intolerance and reducing the risk of certain other diseases [2].
Nuts have been a part of human diet for a very long time. Nuts a concentrated food and stored well. Recent studies suggest that some early civilizations relied on nuts as a staple food before cereal grains. Nuts are highly valued for their attractive look and delicate taste. For a very long time vegetarians have valued nuts as a source of protein, whereas nuts have more than protein to offer. It has been suggested that eight different constituents might contribute to the nutritional benefits of nuts, namely linolenic acid, folic acid, arginine, fibre, vitamin E, potassium, copper and magnesium. Now the plant sterols and phenolic compounds have been added to this list. It has been proven to reduce hypertension, inflammation and interventional

studies show that nut intake have beneficial effects on oxidative stress, vascular reactivity, visceral adiposity and metabolic syndrome. (Ros, 2010) [3].

Among all the nuts Almonds, pistachios, walnuts, cashew nuts and peanuts are most popularly consumed nuts in India. Almond skins have a number of nutritional benefits mainly based on the presence of polyphenols and high dietary fibre content. In a study done, both natural and blanched almond skins showed significant increase in the population of *Bifidobacteria* and *Eubacterium rectale* group, resulting in prebiotic index that compared with the commercial prebiotic fructo- oligosaccharides at 24 hours incubation. (Mandalari, *et al*. 2010) [4].

The modification of microbiota to a 'beneficial' one is a promising approach for improving intestinal health as well as overall health. Phytochemicals and fibres that reach the colon such as those present in various nuts may provide substrates for the maintenance of healthy and diverse microbiota. The effects of increased consumption of nuts which are rich in phytonutrients as well as fibre, on human gut microbiota composition have not been investigated to date (except almonds). (Ukhanova, 2014) [5].

Materials & Methods

Collection of sample

The samples (Pistachios and peanuts) were bought from the local market of Kolkata, West Bengal; India. The samples were first sun dried to remove the moisture content and then finely ground to powder using a grinder. The powder was collected in an air tight container and stored in a cool, dry place.

Culturing of *Lactobacillus acidophilus* (NCDC 295)

Procedure: In a laminar air flow a stab culture was taken and was dug with the help of an inoculating loop or sterilized needle. The solid mass was broken and was transferred into a conical flask consisting of MRS Broth. The solution was mixed thoroughly and was kept in an incubator at 37 degree Celsius for 24 hours. This culture was maintained in a usable form by giving re-cultures regularly to avoid death of the micro-organism.

Preparation of MRS broth: Peptone (10 g), beef extract (5 g), yeast extract (5 g), dextrose (20 g), di potassium hydrogen phosphate (2 g), di ammonium hydrogen citrate (2 g), sodium acetate (5 g), magnesium sulphate (0.1 g), manganese sulphate (0.05 g), tween 80 (1 g), distilled water (1000 ml) were added in a conical flask. It was then mixed well with a pH of 6.5 and cotton plugged. The flask was then kept in an auto clave at 15 lbs. pressure (121°C) for 15 minutes.

Examination of lactobacillus growth in the presence and absence of peanuts and pistachioas

a) Qualitative study

For each samples (peanuts & pistachios) three petriplates were used, of which one was used as 'Control' plate, while the rest were used as 'Test' plates containing different amount of powered sample for example 0.5 gm and 1gm respectively along with MRS agar. The petri plates were prepared by pour plating and were incubated at 37ºC. Microbial growths were observed after 24 hours

b) Quantitative study: (Determination of growth curve of *lactobacillus acidophilus*)

MRS broth was poured into four clean 500 ml clear glass Nephelo culture flask. They were labelled as follows: 1) Blank 2) control 3) pistachio 4) peanuts. Two small porous bags (sterilized) were filled with 1 gram of ground pistachios and peanuts respectively and put it into Nephelo culture flasks filled with MRS broth named as pistachio, peanut. Equal amount of culture was added into the flasks except the blank. All the flasks were mixed carefully and were cotton plugged and kept it under incubator at 37 degree Celsius. Flasks were taken out at different time intervals to measure optical density. This was continued up to 20 hours [6].

Phytochemical analysis

Sample preparation

1 gram of each grounded sample i.e., pistachio and peanut was taken in 10 ml of ethanol was mixed thoroughly and was kept 48 hours. After that it was filtered for further analysis [7].

Qualitative method

1. Test for alkaloids

In a test tube sample was dissolved in hydrochloric acid (dilute) and was filtered. To the filtrate Wagner's reagent (2 gm iodine and 6 gm potassium iodide in 100 ml water) was added. Formation of reddish/ brown precipitate indicated the presence of alkaloids.

2. Test for anthraquinones

2 ml of chloroform was added to 0.2 gm of the sample; the mixture was shaken thoroughly for 5 minutes and filtered. To this 10 % ammonia solution was added and mixed well with filtrate obtained. Formation of bright pink colouration in the aqueous layer of the mixture indicates the presence of anthroquinines

3. Test for flavonoids

Few drops of 10% sodium hydroxide solution were added to 0.5 gm of sample. Formation of an intense colour which turns colourless on the addition of dilute acid indicates the presence of flavonoids.

4. Test for phenols

3- 4 drops of 10% ferric chloride solution was added to 0.5 gm of the sample in a test tube. Bluish black colour shows the presence of phenols.

5. Test for saponins

2 grams of sample was taken in a test tube and was boiled in 20 ml distilled water for 5 minutes and was filtered. Then 5 ml distilled water was mixed with 10 ml filtrate in a graduated cylinder, shaken vigorously and left to stand for 15 minutes for persistent frothing. Thereafter, 3 – 4 drops of olive oil was mixed with the froth and shaken. If there is an emulsion layer formed then it shows the presence of saponins.

6. Test for tannins

1 % gelatin solution containing 10 % sodium chloride was added to the sample (0.5 gram). Formation of white precipitate indicated the presence of tannins.

7. Test for phytosterols

Little amount of sample was mixed with chloroform and was filtered. The filtrate obtained was then treated with few drops of acetic anhydride. It was boiled and cooled. Concentrated sulphuric acid was poured in a keeping the test tube in a slanting position. Formation of brown ring at the junction suggests the presence of phytosterols.

Quantitative method

a) Determination of total phenolic content

The total phenolic content was determined spectrophotometrically with Folin Ciocalteau's phenol reagent. To prepare a calibration curve 0, 1, 2, 3, 5, 6, 7, 8, 9 and 10 ml of the Gallic acid solution was added into 100 ml volumetric flasks and then diluted with water. 0.5 ml of extract solution was mixed with Folin Ciocalteau's phenol reagent (10% v/v dilution in distilled water). Thereafter, 4 mL of anhydrous sodium carbonate (75%) was added, producing a blue colored solution. The resulting mixtures were vortexed and incubated at 40 degree Celsius for 30 minutes. The absorbance was then measured at 765 nm using UV-spectrophotometer using gallic acid as standard and against a blank. Total phenolic content was then expressed as mg/g gallic acid equivalent using calibration curve [7].

b) Determination of flavnoid content

0.5 mL of the extract was mixed with 0.5 mL of 2% aluminum chloride (prepared in ethanol).The mixture was incubated for 1 h at room temperature, after which the absorbance was read at 420 nm. Development of yellow color indicated the presence of flavonoid. Total flavonoid content was calculated as mg/g of quercetin equivalent using the calibration curve [7].

c) Determination of proanthocyanidins content

To prepare a calibration curve 0.1, 0.2, 0.3 0.4 and 0.5 ml of catechin was added in test tubes and then diluted with ethanol. To 0.5 ml of the sample solution 3 ml of 4 % vanillin-methanol solution and 1.5 ml of hydrochloric acid was added and vortexed. The mixtures were thoroughly mixed and were allowed to stand for 15 minutes at room temperature. The absorbance was then measured at 500 nm. Total proanthocynidin content was calculated as mg/g of catechin equivalent using the calibration curve.

The results of all microbial and chemical analysis were expressed as mean ± standard deviation of triplicate analyses [8].

Result & Discussion

Assess the growth of *Lactobacillus acidophilus* in presence and absence of peanuts and pistachios

Qualitative study

After 24 hours observation was done and it was found that the growth of *Lactobacillus acidophilus* is much higher in petri plate containing 0.5 and 1gm grounded sample (peanuts & pistachio) when compared to the control plate, whereas blank had no culture hence no growth was seen (Table :1).

Table 1: Effect of Peanuts and pistachios on the Lactobacillus acidophilus growth (Qualitative study)

Sample	Control	0.5 gm	1gm
Peanuts	+	++	+++
Pistachios	+	++	+++
Almond	+	++	+++

+: Low growth, ++: moderate growth, +++: high growth

Determination of growth curve of *Lactobacillus acidophilus* in the presence and absence of peanuts and pistachios

The flasks were taken out at regular time interval and its absorbance was measured at 680 nm in a colorimeter upto20 hours. A graph was plotted with absorbance against time (Fig 1).

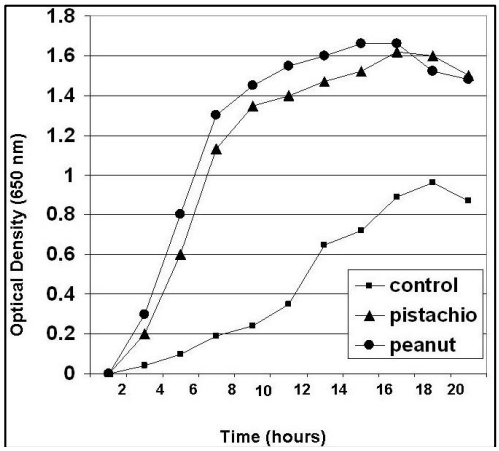

Fig 1: Effect of peanuts and pistachios on *Lactobacillus acidophilus* growth curve.

According to the above observation, the growth curve shows a clear picture that peanuts and pistachios show stimulatory effect on the growth of L. acidophilus compared to control. Microorganisms utilize the substrates provided by the growth medium to grow and reproduce. Nuts are a good source of a wide range of essential nutrients including B vitamins, vitamin E and minerals like calcium, iron, zinc, magnesium, potassium, selenium, manganese and copper. They are also abundant in phytochemicals like flavonoids, phenols, and plant sterols [3].

Dietary polyphenols contribute to the maintenance of gastrointestinal health by interacting with epithelial cells and largely by modulating the gut microbiota composition. Polyphenols may act as promoting factors of growth, proliferation, or survival for probiotics mainly *Lactobacillus* strains and thus exerting prebiotic actions and inhibiting the proliferation of pathogenic bacteria such as *Salmonella* and *Helicobacter pylori*. (Hervert-Hernandez and Goni, 2011) [9].

Finely ground almonds showed a significant increase in the population of *Bifidobacteria* and *Eubacterium rectale*, resulting in a higher prebiotic index than that was found for the commercial prebiotic products at 24 hours of incubation. There was no significant increase in the proportions of gut bacteria in response to defatted ground almonds. Therefore, the addition of finely ground almonds altered the composition of gut bacteria by stimulating the growth of *Bifidobacteria* and *Eubacterium rectale*. (Mandalari, *et al*. 2008) [10].

Almond skins have a number of nutritional benefits mainly based on the presence of polyphenols and high dietary fibre content. In a study done, both natural and blanched almond skins showed significant increase in the population of *Bifidobacteria* and *Eubacterium rectale* group, resulting in prebiotic index that compared with the commercial prebiotic fructo- oligosaccharides at 24 hours incubation. (Mandalari, *et al*. 2010) [4].

The skin covering the nut kernels are particularly rich in polyphenols and fibre that may help to explain the prebiotic effect metabolised by gut microbiota, altering the microbiome and changing microbiota profile. Polymerized polyphenols present in nuts are mainly proanthocynidins which could be a substrate for gut bacteria. (Lamuel-Raventos and Onge, 2017) [11].

Phytochemical analysis

Qualitative phytochemical analysis

Peanuta and pistachios were dissolved in organic solvent (ethanol) for 48 hours and different qualitative tests were

performed. The phytochemical analysis performed for alkaloids, anthroquinones, flavonoids, saponins, tannins and phytosterols. Results showed that peanuts and pistachios are rich in phenols and contains lesser amount of flavanoids and phytosterols (Table: 2).

Table 2: Results obtained from the qualitative analysis of phytochemicals in different nut samples.

Sample	Alkaloids	Anthro-quinones	Flavonoids	Phenols	Saponins	Tannins	Phytosterols
Almond	-	-	+	++	-	-	+
Pistachio	-	-	+	++	-	-	+
Walnut	-	-	+	++	-	-	+
Peanut	-	-	+	++	-	-	+

Note: Strongly present: ++; mildly present: +; absent: -

Quantitative analysis of the phytochemical content of peanuts and piatachios

Since qualitative analysis of peanuts and pistachios showed that it is very rich in phenol, and many experimental evidences showed that polyphenols have prebiotic potential [12-15], quantitative analysis were performed for phenols, flavanoids (Subgroup of polyphenol) and proanthocynidins (Oligomeric polyphenol) (Table 3).

Table 3: Quantitative analysis phenol, flavanoid and proanthocyanidine of peanuts and pistachios

Compound analysed	Pistachios	Peanuts
Phenol (mg/gm)	14.89 ± 2.34	12.82 ± 2.73
Flavanoids (mg/gm)	3.15 ± 0.75	0.83 ± 0.45
Proanthocyanidine (mg/gm)	2.07 ± 0.69	2.37 ± 0. 75

A human intervention study indicated that consumption of red wine polyphenols significantly increased the number of *Enterococcus, Prevotella, Bacteroides, Bifidobacterium, Bacteroides uniformis, Eggerthella lenta, and Blautia coccoides-E. rectale* group while the quantity of *Lactobacillus spp*. was unaltered [12]. On the other hand, when bacteria were cultured with various tea phenolics, the growth of pathogenic bacteria such as *Clostridium perfringens, Clostridium difficile and Bacteroides spp.* was significantly repressed, while commensal anaerobes like *Bifidobacterium and Lactobacillus* were affected less [13]. Vendrame *et al*. found a significant increase in the amount of *Bifidobacterium* after the consumption of a wild blueberry drink, suggesting an important role of the polyphenol present in wild blueberries on the intestinal microbiota composition modulation [14]. Cueva *et al*. analyzed the potential of flavan-3-ols from grape seed to influence the growth of intestinal bacterial groups using in vitro fermentation models. They found that the flavan-3-ol profile of a particular food source could affect the microbiota composition (Promoting the growth of *Lactobacillus/enterococcus* and decreasing *the C. histolyticum* Group) and its catabolic activity, inducing changes that could in turn affect the bioavailability and potential bioactivity of these compounds [15].

Overall observations indicate that peanuts and pistachios have stimulatory effect on lactobacillus growth (prebiotic potential) and rich source of wide range of polyphenol may be the most probable cause of their prebiotic potential.

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