FUNCTIONALLY MODIFIED MILK USING PROBIOTICS AND PLANT DERIVATIVES

A PROJECT REPORT

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BONAFIDE CERTIFICATE

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ABSTRACT

Plants have many bioactive compounds which posses certain properties like antioxidant, antidiabetic and antibacterial activities. This project aims to enhance the properties of milk by adding bioactive compounds from plant materials and probiotics. The plant materials selected for the study were *Vitis vinifera* (seeds), *Psidium guajava* (leaves), *Trigonella foenum graecum* (seeds). The plant constituents were extracted using ethanol, methanol and hot water. The above extracts were subjected to quantitative assay for α- amylase inhibition, invitro antioxidant assays and antibacterial activity test. The extracts added milk samples were subjected to the above tests. The antibacterial activity was measured against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* for probiotics and plant extracts added milk . The results were analyzed and the extract and probiotics added milk samples were observed to possess significant alpha amylase inhibiting, antioxidant and antibacterial potential. An organoleptic test was performed for milk sample in which extract and probiotics were added in the ratio 1:1:100. The TLC analysis revealed the presence of rutin and ferulic acid related compounds.

Keywords: Antioxidant, α -amylase inhibitor, Antibacterial,TLC

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µg	Microgram
µl	Microliter
DPPH	1.1-diphenyl-2- picryl hydrazyl.
g	Gram
IDDM	Insulin Dependent.
mg	Milligram
min	Minute
ml	Milliliter
M	Molarity
NIDDM	Non Insulin Dependent Diabetes Mellitus.
PPHG	Post- Prandial Hyperglycemia
ROS	Reactive Oxygen Species
NaOH	Sodium Hydroxide
TLC	Thin Layer Chromatography

INTRODUCTION

1. INTRODUCTION

1.1 Milk:

India is the largest producer of milk in the world, and the Indian dairy industry is witnessing rapid changes. In search of better returns, the industry is widening its focus to include traditional milk products, and these are emerging as new profit centres for the commercial sector. Ethnic dairy products account for 90% of all dairy products consumed. The exact components of raw milk vary by species, but it contains significant amounts of saturated fat, protein and calcium as well as vitamin C. Cow's milk has a pH ranging from 6.4 to 6.8, making it slightly acidic.

The constituents of milk that are most important in food preparation are enzymes, vitamins, pigments, salts, sugar, fat, and proteins. The enzymes of cow's milk are proteinases, lactase, diastase, lipase, catalase and peroxidase. The appearance of milk is white. This is due to light rays reflected by the colloidally dispersed constituents of the milk, the calcium caseinate, and calcium phosphate.

Milk contains two classes of yellow or orange pigments. The water-soluble pigment, which imparts a yellow color with a green fluorescence to the whey of milk, was formerly called lactochrome. A name recently suggested for this pigment is lactoflavin. A fat-soluble pigment, carotene, found in the fat gives the milk a more or less yellow tinge, which is more pronounced as the fat particles become more concentrated and form cream. The chief pigment of butter fat is the carotene, but little xanthophyll being found. The depth of color depends upon the amount of pigment present. The color of carotene in solution varies from yellow to orange and to a deep red-orange as the concentration increases.

Milk contains salts of potassium, sodium, magnesium, calcium, phosphates, chlorides, and citrates. Traces of sulfates and carbonates are found. Iron is present in small amount. Iodides are also found in small amounts, the amount being greater in some localities than in others. Calcium and magnesium are in combination with the casein to form calcium and magnesium caseinates. To enhance the milk with

antioxidant,antimicrobial and amylase inhibitory properties ,probiotics and derivatives like fenugreek ,grapes and guava are to be added.

1.2 Probiotics:

Probiotics is defined as microbial food supplements with beneficial effects on the consumers. The gut flora can be broadly divided into 3 main groups: *Bifidobacteria* (probiotic beneficial 'good' bacteria), bacteroides species (pathogenic micro organisms) and prebiotics (non digestible food ingredients that encourage the growth of *Bifidobacteria*). Most probiotics fall into the group of organisms known as lactic acid producing bacteria and are normally consumed in the form of yoghurt and other fermented foods. The most commonly used species of lactic acid bacteria in probiotic preparations include *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* species.

The term probiotic was derived from the Greek, meaning "for life." The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have stated that there is adequate scientific evidence to indicate that there is potential for probiotic foods to provide health benefits and that specific strains are safe for human use. An expert panel commissioned by FAO and WHO defined probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host."

1.3 *Trigonella foenum graecum* (FENUGREEK):

Fenugreek is used both as a herb (the leaves) and as a spice (the seed, often called *methi*). The plant is cultivated worldwide as a semi-arid crop. Fenugreek may have potent antiviral properties, having relieved common cold symptoms in a group of volunteers. Fenugreek seeds are a rich source of the polysaccharide galactomannan. They are also a source of saponins such as diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens.The soluble fibre derived from fenugreek seeds has been identified chemically as galactomannans just like the other soluble fibre of guar seeds,psyllium husk etc(Song B.K et al.,1989) Other bioactive constituents of fenugreek include mucilage, volatile oils, and alkaloids such as choline and trigonelline. Fenugreek is frequently used in the production of flavoring for artificial maple syrups. The taste of

toasted fenugreek, like cumin, is additionally based on substituted pyrazines. By itself, fenugreek has a bitter taste. In India and China it has also been used to treat arthritis, asthma, bronchitis, improve digestion, maintain a healthy metabolism, increase libido and male potency, cure skin problems (wounds, rashes and boils), treat sore throat, and cure acid reflux.

Fenugreek also has a long history of use for the treatment of reproductive disorders, to induce labor, to treat hormonal disorders, to help with breast enlargement, and to reduce menstrual pain. Studies have shown that participants with type 2 diabetes had significantly lower blood sugar levels after eating fenugreek. Fenugreek seeds contain a lot of mucilage, which helps sooth gastrointestinal inflammation by coating the lining of the stomach and intestine.

1.4 *Psidium guajava*(GUAVA):

Guava belongs to the genus *Psidium* (meaning "pomegranate" in Latin) which contains about 100 species of tropical shrubs and small trees.Guava (*Psidium guajava*) is widely cultivated and its fruit is popular in Asia. Guava leaves have a high content of antioxidants. Antioxidants can help prevent and repair cell damage from free radicals (oxidants), thus slowing the aging process and reducing ones risk of diseases linked to aging (such as heart disease, cancer, Alzheimer's disease, mascular degeneration, cataracts, and osteoarthritis). Guava leaf extracts can inhibit diarrhea caused by bacteria, including *Salmonella spp., Staphylococcus aureus,* and *Escherichia coli.* It has been effective in stopping diarrhea in individuals with cholera. A decoction of guava leaves can increase the secretion of digestive enzymes and also help halt vomiting. It is believed that guava leaves can benefit weight loss by preventing starch from converting into sugar.The antioxidant ,free radical scavenging effects and protection from UBV-induced oxidation of aqueous extracts from guava leaf were also stronger than that of water soluble extracts of some neutraceutical herbs (Chen et al.,2007). Guava leaves have been used as a folk remedy to treat diabetes for many years. It reduces glucose levels in the blood without effecting levels of insulin.It also elieves arthritis pain when made into a paste and applied over the affected area.It speeds up the healing process and relieving pain in wounds and sores. It relieves toothaches and soothes sore throat.

1.5 *Vitis vinifera* (GRAPE):

Grape is a non-climacteric fruit that grows on the perennial and deciduous woody vines of the genus Vitis. Grape phytochemicals such as resveratrol (a polyphenol antioxidant), have been positively linked to inhibiting any cancer, heart disease, degenerative nerve disease, viral infections and mechanisms of Alzheimer's disease. Grape seed extracts are industrial derivatives from whole grape seeds that have a great concentration of vitamin E, flavonoids, linoleic acid, and OPCs. Typically, the commercial opportunity of extracting grape seed constituents has been for chemicals known as polyphenols, including oligomeric proanthocyanidins recognized as antioxidants. Grape seed extract may have other possible anti-disease properties, such as wound healing in which grape seed proanthocyanidins induced vascular endothelial growth factor and accelerated healing of injured skin in mice The phenolics in seeds may inhibit oral sugar metabolism and retard growth of certain bacteria causing dental caries thereby preventing tooth decay. In osteoporosis, the grape seed extracts enhanced bone density and strength in experimental animals. Grape seed proanthocyanidins decreases tumor numbers and reduces the malignancy of papillomas thereby acting against skin cancer.

The dietary proanthocyanidins may protect against carcinogenesis and provide supplementation for sunscreen protectionthus protecting the skin from ultraviolet damage.There is good evidence that grape seed extract can help treat chronic venous insufficiency and edema.

1.6 DIABETES MELLITUS AND ALPHA AMYLASE INHIBITORS:

Diabetes mellitus is one of the oldest diseases known to mankind and yet with the tremendous scientific advances witnessed, medical science cannot claim that it knows all that needs to be known about this disease, including its management. This is the main reason for the persistent interest all over the world to explore alternative remedies from the so-called "alternative systems" of medicine.

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to

due course turns into a syndrome called diabetes mellitus. Diabetes can cause many complications. Acute complications (hypoglycemia, ketoacidosis) may occur if the disease is not adequately controlled. Serious long-term complications include cardiovascular disease, chronic renal failure, retinal damage, nerve and microvascular damage, which may cause erectile dysfunction (impotence) and poor healing. Poor healing of wounds, particularly of the feet, can lead to gangrene which can require amputation. Adequate treatment of diabetes, as well as increased emphasis on blood pressure control and lifestyle factors (such as smoking and keeping a healthy body weight), may improve the risk profile of most aforementioned complications.

The enzyme α-amylase (EC 3.2.1.1) catalyzes the hydrolysis of the (1-4) - α-D-glycosidic linkages of starch, amylose, amylopectin, glycogen, and various maltodextrins. α-amylases are produced by a diverse variety of organisms: bacteria, fungi, plants, and animals. Two kinds of α-amylases are produced by many mammals, salivary α-amylase from the parotid gland and pancreatic α-amylase from the pancreas. The extracts from herbs are able to significantly inhibit the α-amylase enzyme and researchers are now trying to identify the specific active compounds which are responsible for inhibition. When the active component has been isolated and characterized the scientist believe it should be possible to evaluate whether the active compound is likely to have advantages in terms of efficacy or side effects over currently marketed anti-diabetic drugs that interfere with starch digestion. Recent advances in understanding the activity of α-amylase have led to the development of the anti-diabetic drugs.

LITERATURE REVIEW

2. LITERATURE REVIEW

Milk is an important source of all basic nutrients required for mammals including human beings. Milk from various mammals such as cow, buffalo, goat, sheep, camel, etc. is used for different nutritional purposes, e.g., feeding to young ones. Milk contains a number of nutraceutical agents such as conjugated linoleic acid (CLA), vitamins A, E and β-carotene, minerals like Ca, Cu, Mg and Na, lactalbumin, lactoferrin that have anticancer, antithrombogenic, antimicrobial, antioxidant, anticardio, antiviral, anti-inflammatory and other health benefits. The phytochemical of certain plants are known to have therapeutic properties. Since Indians have a great preference for milk and milk products, increasing the CLA, calcium, vitamins A, C and E, addition of probiotics and plant derived compounds (like Fenugreek) in milk may enhance it's qualities and will be of beneficial for human health and dairy industries.

2.1 Milk

Milk has long been and always be consumed as part of a healthy balanced diet. Nowadays the nutrient science moves beyond the study of essential nutrients and consumers turn to traditional and healthy dairy products (Michaelidou A.M.,2008). The increasing concern of consumers about their health and new lifestyles that are driving them away from healthy dietary habits has prompted the industry to become involved in the need for food products which contribute to the prevention of illness. The natural drinks (soy-based drinks or drinkable yogurts) that consumers consider healthy constitute one of the food industry sectors with highest growth worldwide (Rivas et al., 2007).Dairy may be ideal vehicle for creating value added products because it already contains many beneficial nutrients and excellent source of nine nutrients (calcium, potassium, phosphorous, proteins, vitamin B12, riboflavin, A and niacin). Milk is one of the most nutrient-dense foods, filled with a unique blend of carbohydrates, proteins, fats, vitamins and minerals. Milk is packed with essential nutrients and it has more nutrients than any other single food (Knowles et al., 2006). The probiotics and plant derivatives can be added to make the milk a functionally modified one.

2.2 Probiotics

Probiotics represents an expanding research area. A Medline search of the term probiotics illustrates the significant increase in research undertaken in this area during the past five years. Several potential mechanisms have been proposed for how lactobacilli reduce the duration of rotavirus diarrhea, but none have been proven and each theory has flaws. The first is competitive blockage of receptor sites (Bernet et al., 1994), in which lactobacilli bind to receptors, thereby preventing adhesion and invasion of the virus. This concept might be plausible if there was evidence for specific receptor competition. The second potential mechanism may be that the immune response is enhanced by lactobacilli, leading to the observed clinical effect. This is supported by the protective effect which local immunoglobulin A (IgA) antibodies appear to confer against rotavirus. A third mechanism could involve a signal(s) from lactobacilli to the host that down regulates the secretory and motility defenses designed to remove perceived noxious substances.

The ability of lactobacilli and bifidobacteria to modify the gut microbiota and reduce the risk of cancer is in part due to their ability to decrease β-glucuronidase and carcinogen levels (Hosoda et al., 1996). Studies with L. casei Shirota injected into mice showed a significant increase in natural killer cell activity from mesenteric node cells but not of Peyer's patch cells or spleen cells (Matsuzaki and Chin, 2000), supporting the concept that some probiotic strains can enhance the innate immune response. Other animal studies clearly indicate that probiotic strains can modify immune parameters. Legend has it that fermented milks were used to help the healing of wounds and to fight infection before antiseptics and antibiotics were available. Nevertheless, the application of viable lactic acid bacteria to an infected wound would represent a paradigm shift in current surgical practice. In a series of animal studies, *Lb.. fermentum* RC-14 and proteins produced by this organisms were shown to prevent severe *Staphyloccus aureus* surgical implant infection. Although this does not prove human efficacy, the concept illustrates a different approach to wound infection management.

2.3 *Trigonella foenum-graecum* (FENUGREEK)

Fenugreek seeds are a rich source of the polysaccharide galactomannan. Other bioactive constituents of fenugreek include volatile oils, and alkaloids such as choline and trigonelline. Several human intervention trials demonstrated that the antidiabetic effects of fenugreek seeds ameliorate most metabolic symptoms associated with type-1 and type-2 diabetes in both humans and relevant animal models by reducing serum glucose and improving glucose tolerance. Fenugreek is currently available commercially in encapsulated forms and is being prescribed as dietary supplements for the control of hypercholesterolemia and diabetes by practitioners of complementary and alternative medicine.

Fenugreek is noted for its several pharmacological properties especially its hypoglycaemic effects (Reena Randhir PhD and Kalidas Shetty 2007). In addition to these main components, some minor components like alkaloids (trigonolline, cholin, gentianine, carpaine etc), free unnatural amino acids (4- hydroxyisoleucine), and individual spirostanols and furastanols like diosgenin, gitogenin, yamogenin etc have also been identified, isolated and characterised as the principal components responsible for its varying biological effects (Trivedi PD et al 2007). Various solvent extraction techniques have been developed over the years to commercially extract the total fiber from fenugreek (Sharma R.D et al., 1990). The seeds have been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values (Srinivasan,K., 2006).

Fenugreek seeds are reported to have restorative and nutritive properties and are shown to stimulate digestive processes (Khosla et al.,1995). The seeds are rich in proteins and contain the unique amino acid 4-hydroxyisoleucine, which is one of the active ingredients for blood glucose control by inducing insulin release in both rats and humans (Broca C et al., 1999). Type 2 diabetes is increasing globally with ensuing concern about the cost of management and control. Many oral hypoglycaemic agents, such as biguanides and sulfonylurea are available along with insulin for its treatment but these synthetic agents can produce serious side effects including abnormal colon function. The use of synthetic α-glucosidases inhibitors such as acarbose, cause adverse side effects such as abdominal distention due to the excessive inhibition of pancreatic

enzymes, resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Gallagher AM et al., 2003).Hence, research on the development and utilization of antidiabetic plants with mild inhibition of pancreatic enzymes is beneficial. These plants contain phenolic substances and proteins, which interact with digestive enzymes thereby modulating their activity (Payan F., 2004). The health beneficial effects of fenugreek are attributed to its chemical composition (3–5% moisture, 25–30% protein, 20–30% galactomannan, 20–25% insoluble fiber, 7–9% lipids, 5–7% saponings and 3–4% ash) that include mucilaginous fiber, lysine-rich protein, free amino acids, saponins, flavonoids, and volatile oils (Raju and Bird, 2006).

2.4 *Vitis vinifera* (Grapes):

Grapes (*Vitis vinifera.*) belong to the world's largest fruit crops with a global production of around 69 million tons in 2006(FAOSTAT, 2007). The skins and seeds of grapes are known to be rich sources of phenolic compounds, both flavonoids and non-flavonoids. Although grape skins are confirmed as rich sources of phenolic compounds and recent results indicate higher antimicrobial potential of natural extracts than shown by selected antioxidants alone against several microorganisms (Serra et al., 2008) reports for antimicrobial activity of grape skin extracts are scarce. The seeds constitute a considerable proportion of the pomace, amounting to 38-52% on a dry matter basis. Their oil is rich in unsaturated fatty acids, in particular linolinic acid (Schieber et al., 2002).

Apart from being a rich source of high-value fatty oil, grape seeds have also been appreciated because of their content of phenolic compounds such as gallic acid, catechin and epicatechin and a wide variety of procyanidins. The latter are also reffered to as condensed tannins. Grape seed extracts and procyanidins have been a matter of intense investigations with respect to their potentially beneficial effects on human health. Recent reports indicate a wide range of biological activities. E.g. antioxidant properties and radioprotective effects (Castillo et al., 2000), prevention of cataract (Yamakoshi et al., 2002), antihyperglycemic effects (Pinent et al., 2004), modulation of the expression of antioxidant enzyme systems (Puiggros et al., 2005), improvement of insulin sensitivity and prevention of hypertriglyceridemia (Al-Awwadi et al., 2005), inhibition of aromatase and suppression of aromatase expressioin (Kijima et al., 2006), inhibition of protein

kinase activity of the epidermal growth factor receptor, protective effects against oxidative damage in mouse brain cells (Guo et al., 2007), and anti-inflammatory effects (Terra et al., 2007).

2.5 *Psidium guajava* (Guava Leaves)

Guavas, particularly the leaves have been a subject for diverse research in chemical identity of their constituents, pharmacological properties and history in folk medicine. Most research has been restricted to the Apple Guava (*P. guajava*) however, and any additional beneficial properties of other species remain essentially unstudied. From preliminary medical research in laboratory models, extracts from Apple Guava leaves or bark are implicated in therapeutic mechanisms against cancer, bacterial infections, inflammation and pain. Essential oils from guava leaves have shown strong anti-cancer activity in vitro (Manosroi et al., 2006) Guava leaves are used in folk medicine as a remedy for diarrhea and, as well as the bark, for their supposed antimicrobial properties and as an astringent. Guava leaves or bark are used in traditional treatments against diabetes (Ojewole J.A.O 2005).

2.6 Antimicrobial activity

The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds 1996; Lis-Balchin & Deans 1997). While some of the oils used on the basis of their reputed antimicrobial properties have well documented *in vitro* activity, there are few published data for many others (Morris *et al.* 1979 ; Ross *et al.* 1980 ; Yousef & Tawil 1980; Deans & Ritchie 1987; Hili *et al.* 1997). Some studies have concentrated exclusively on one oil or one micro-organism. While these data are useful, the reports are not directly comparable due to methodological differences such as choice of plant extract(s), test micro-organism(s) and antimicrobial test method (Janssen *et al.* 1987).Most of the foodborne bacterial pathogens examined were sensitive to extracts from plants such as cinnamon, clove, garlic, mustard, onion and oregano. Phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons have been recognized as major antimicrobial components in spices. (Erdogan ceylan et al., 2007)

The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms. The results obtained with *Pseudomonas aeruginosa* was particularly interesting, since it was inhibited by clove, jambolan, pomegranate and thyme extracts (Nascimento Gislene G. F et al.,) Grape seed extracts were tested for antibacterial activity by pour plate method against *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*The methanol, acetone and N, N-dimethylformamide (DMF) fractions of leaves of *Psidium guajava L.* were evaluated for antibacterial and antifungal activity. The antibacterial activity was more pronounced against gram-positive bacterial and fungal strains. Moderate activity was shown against the gram-negative bacterial strains studied (Rathish Nair et al.,2007)

The antibacterial activity of guava (*Psidium guajava*) extracts against 21 strains of foodborne pathogens was determined. No significant effects of temperature and pH were found on guava and neem extracts against cocktails of *L. monocytogenes* and *S. aureus*. Study suggest that guava and neem extracts possess compounds containing antibacterial properties that can potentially be useful to control foodborne pathogens and spoilage organisms (M.D. Mahfuzul Hoque et al.,2007).

2.7 Antioxidants:

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases , through xanthine oxidase activity , atmospheric pollutants and from transitional metal catalysts , drugs and xenobiotics. In addition, chemical metabolization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting can result in increased radical activity and damage(Makari et al.,2008). Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long term complication

of diabetes (Saha et al.,2008). They also have a significant role in causation of disease like cirrhosis and cardiovascular disease (Hertog MGL and Feskens EJMS,1993).

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, parkinson's disease, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metaboloites with antioxidant and antiradical properties (Augustin et al., 2005). Therefore dietary antioxidants are necessary to lessen the overall effect of antioxidant stress. Plants are potent biochemical factories and have been compounds of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e any part of the plant may contain active compounds (Hennebelle et al.,2006). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant.

The medicinal actions of the plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink M, 1999).Antioxidant based drugs formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, alzheimer's disease, and cancer have appeared during the last 3 decades (Zetola et al., 2002). This has attracted a great deal of research interest in natural antioxidants. Subsequently , a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oil seeds, beans, fruits, and vegetables have increased . Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger and several Chinese medicinal plants extract. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compound found in natural foods , vitamins C and E, betacarotene, and tocopherol are known to posess antioxidant potential(Prior R.L.,2003).

2.8 Diabetes:

Diabetes mellitus is one of the oldest diseases known to mankind and yet with the tremendous scientific advances witnessed, medical sciences cannot claim that it knows all that needs to be known about this disease, including its management. Diabetes is defined as a state in which homeostatis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalance does not return to normal and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus.

There are two main categories of this disease. Type 1 diabetes mellitus also called as insulin-dependent diabetes mellitus (IDDM) and Type 2, the non-insulin dependent diabetes mellitus (NIDDM). Diabetes can cause many complications. Acute complications (hypoglycemia,ketoacidosis or nonketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long term complications include cardiovascular disease, chronic renal failure, retinal damage, nerve and microvascular damage, which may cause erectile dysfunction and poor healing.

2.9 α-amylase inhibitors:

The extracts from herbs are able to significantly inhibit the α-amylase enzyme and researchers are now trying to identify the specific active compounds which are responsible for inhibition. This kind of inhibition shows beneficial effects on glycemic index control in diabetic patients. The favoured hypothesis about physiological roles of the enzyme inhibitors in seeds is that they act as storage or reserve proteins as regulators of endogenous enzyme or as defensive agents against the attacks of the animal predators and insect or microbial pests (Heidari et al.,2005). Certain inhibitors are reported to be antinutritional factors (Lalit Sexena et al., 2007). α-amylase and its inhibitors are drug design targets for the development of compounds for treatment of diabetes, obesity and hyperglycemia. These inhibitors show remarkable structural variety leading to different modes of inhibition (Xiaoyan HAO et al., 2009).

OBJECTIVES

3. OBJECTIVE

- To determine the antimicrobial, antioxidant and Alpha amylase inhibiting property of the selected plant samples (*Vitis vinifera seeds, Pisidium guajava leaves.Trigonella foenum-graecum*).
  the extracts using thin layer chromatography (TLC).

MATERIALS AND METHODS

4.1 Extraction of compounds from plant samples:

4.1.1 Materials:

- Dried samples of *Psidium guajava*(Guava) leaves, *Trigonella foenum graecum* (Fenugreek) seeds and *Vitis vinifera* (Grape) seeds.

4.1.2 Equipments:

- Shaker
- Incubator
- Microwave oven
- Vortex mixer
- TLC applicator

4.1.3 Sample collection:

Fresh guava *(Psidium guajava)* leaves were collected in and around Saravanampatti, Coimbatore. Grape (*Vitis vinifera*) and fenugreek *(Trigonella foenum graecum)* seeds were bought from the market.

4 .1.4 Organic solvent Extraction procedure:

- The samples such as *Psidium guajava* (Guava) leaves, *Trigonella foenum graecum* (Fenugreek) seeds and *Vitis vinifera* (Grape) seeds were initially washed and dried in incubator at 50^0c until dry.
- The dried samples were powdered using mixer grinder until fine powder was obtained.
- For methanolic and ethanolic extracts, 1g of the powdered samples were dissolved in 30ml of the solvents and incubated in shaker at 150rpm overnight.
- After the overnight extraction, the methanolic and ethanolic extracts were filtered and the filtrate is dried in incubator at 45-49^0c until dry.
- Then the dried extracts were taken out of the incubator and scrapped to get the residue, which was weighed and dissolved in water for further use.

4.1.5 Hot water extraction procedure:

50ml of distilled water is placed in water bath and was allowed to reach a temperature of 80^0c. Then 1g of powdered sample was dissolved in 50ml of hot water with continuous stirring for 15 min. The extracts were filtered wherever needed or else the unfiltered extract is directly used. If filtered, the extracts were allowed to dry and the dried extracts were scrapped out and used. For antimicrobial sensitivity test 1g extract was dissolved in 10 ml of hot water

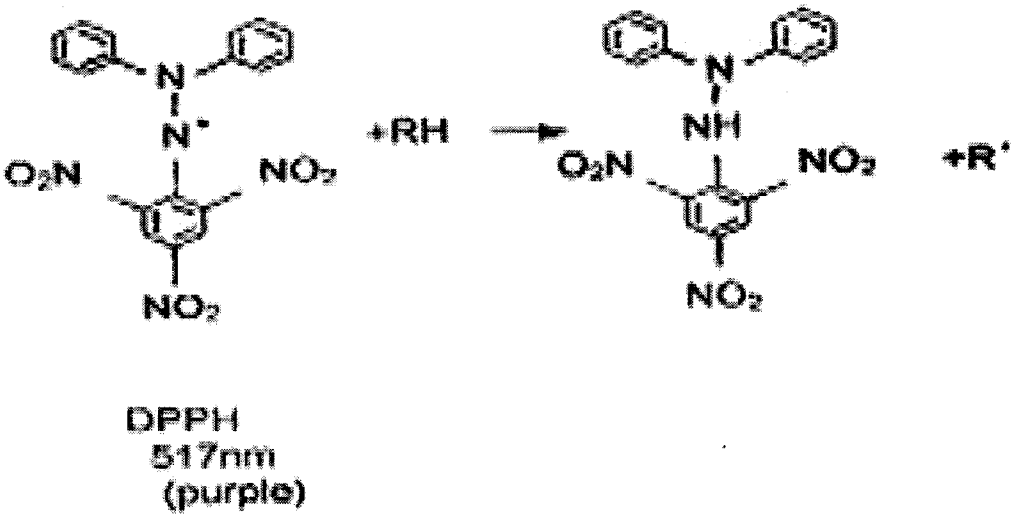
4.1.6 Milk sample preparation:

The raw milk is diluted 100 times with distilled water to carry out DPPH assay. The extracts and milk were taken in the ratio of 1:100 for DPPH assay. For antimicrobial assay raw milk is being directly used and the ratios chosen were 80:20, 60:40, 50:50 of milk and extracts. The milk contains 1 sachet of probiotics.

4.2. DPPH (1,1-Diphenyl-2-picryl-hydrazil) Radical Scavenging Assay

4.2.1 Principle:

DPPH reacts with absolute ethanol to yield a purple color radical (DPPH). 1, 1-diphenyl-2-picryl-hydrazil (DPPH·) using the method of Blois (1958)The presence of antioxidants which included polyphenolics and flavonoids in the sample will scavenge the formed DPPH radical and there by a decreased color will be observed which is spectrophotometrically measured at 517nm.



4.2.2 Sample preparation:

- About 50 mg of the dried extracts was weighed in a 50 ml standard flask and made up to the mark with distilled water (1ml=1mg). From this 1:10 dilution was performed i.e., 1:10, 2:10 up to 5:10 the extract corresponded to 100µg to 500µg was used for the experimental analysis.
- Raw milk was diluted hundred times with distilled water (1:100). This 100ml of diluted milk was taken and 1ml of the extract was added.

4.2.3 Procedure:

- 0.5 ml of DPPH solutions was taken in three test tubes. To this 2ml of the extract (100-500µg/ml), 2ml of diluted raw milk and 2ml of milk and extract mixture was added.

- The reaction mixture was vortexed for 10 s and allowed to stand at room temperature for 30 minutes. The absorbance was recorded at 517 nm by using (Beckman DU-530) UV-Vis spectrophotometer and compared with 75% methanol which acted as control solution.

- The percentage of DPPH scavenging activity was expressed in percentage [(Control-Test)/Control] ×100.

4.3 Antimicrobial assay:

1. For the Methanolic, ethanolic and hot water extracts of Vitis ***vinifera*** *seeds,* ***Psidium guajava*** leaves and ***Trigonella foenum graecum*** seeds.
2. For the mixture of milk and the three extracts of ***Vitis vinifera*** seeds, ***Psidium guajava*** leaves and ***Trigonella foenum graecum*** seeds.
3. For the mixture of milk and probiotics.

4.3.1 Principle:

Bauer-Kirby (Bauer et al., 1966) is done either by disc diffusion method or by well diffusion method. In disc diffusion method, known quantities of bacteria are grown on agar plates in the presence of thin discs containing relevant antibiotics. In well diffusion method, wells of required capacity are bore on the surface of the agar and then the antibiotic solutions are added to the wells. The antibiotics will start to diffuse into the medium. If the bacteria are susceptible to a particular antibiotic, a clear zone will be formed around the discs/ wells where bacteria are not capable of growing (called a zone of inhibition). The zone diameter (zone of inhibition) is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs. The size of the zone of inhibition is determined by the type of medium used, the solubility and rate of diffusion of the antibiotic, the amount of inoculum, as well as the effect of the antibiotic.

4.3.2 Preparation of inoculum:

The mother cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of culture from the mother culture to conical flasks containing nutrient broth. The cultures were incubated in shaker overnight at 37°C. The optical density for the overnight grown cultures corresponds to a CFU of 2.0×10^6 CFU/ml. Subcultures were prepared by streaking a loopful of culture from the Active cultures to test tubes containing nutrient agar slant. The subcultures were stored for future use.

4.3.3 Extracts Preparation:

About 100mg of the dried extract (*Vitis vinifera* seeds, *Psidium guajava* leaves and *Trigonella foenum-graecum* seeds) was dissolved in 1ml of distilled water. The hot water extracts were added as such without drying.

4.3.4 Mixture of Milk and extracts:

About 100mg of the dried extract (*Vitis vinifera* seeds, *Psidium guajava* leaves and *Trigonella foenum-graecum* seeds) was dissolved in 1ml of distilled water. The hot water extracts were added as such without drying. The milk and extracts were mixed in the ratio of 80:20, 60:40, 50:50.

4.3.5 Mixture of milk and probiotics:

1 sachet of commercially available probiotics is added to 100ml of milk.

4.3.6 Procedure:

Muller Hinton agar was poured into the sterile petri plates and allowed to solidify. A loopful of cultures (*Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa*) were streaked on the agar surface. The streaking was done by rotating the plate to 90^0 until we obtain a uniform distribution of the culture and then wells of 9mm diameter were bored on the agar.

4.3.6.1 Extract:

Extracts of volume 20,40,60,80 and 100µl corresponding to the concentration of 2,4,6,8 and 10mg were added to the well. In control well the antibiotic solution for the particular strain was added.

4.3.6.2 Mixture of milk and probiotics:

Milk and probiotic mixture of volume 100µl was added to the well. In control well the antibiotic solution for the particular strain was added

4.3.6.3 Mixture of milk+ probiotics and extracts

Milk and extract mixtures of volume 100µl for each ratio (80:20, 60:40, 50:50.) were added to the well. In control well the antibiotic solution for the particular strain was added.

The plates were incubated at 37^0c overnight. The zones of inhibition were measured.

4.4 α-Amylase inhibitory assay

4.4.1 Principle:

The method of Bernfield (1995) was adopted with some modifications. This method is used to test the presence of free carbonyl group (C=O) which was present in the reducing sugars. This involves the oxidation of the aldehyde functional group: for example aldehyde group in glucose or maltose. Simultaneously, 3, 5-dinitrosalicylic acid (DNS) was reduced to 3-amino, 5-nitrosalicylic acid under alkaline conditions.

Oxidation

- **Aldehyde group [-Cho] → Carboxyl group [-COOH]**

Reduction

- **3,5-dinitrosalicylic acid → 3-amino, 5-nitrosalicylic acid**

Because dissolved oxygen can interfere with glucose oxidation, sulfite, which was not necessary for the color reaction, was added in the reagent to absorb the dissolved oxygen. The above reaction scheme shows that one mole of sugar will react with one mole of 3,5-dinitrosalicylic acid. However, it was suspected that there were many side reactions, and the actual stoichiometry of the reaction was more complicated than that previously described. The type of side reaction depends on the exact nature of the reducing sugars. Different reducing sugars generally yield different color intensities; thus, it was necessary to calibrate for each sugar. In addition to the oxidation of the carbonyl groups in the sugar, other side reaction such as the decomposition of sugar also competes for the availability of 3, 5-dinitrosalicylic acid. As a consequence, carboxymethyl cellulose can affect the calibration curve by enhancing the intensity of the developed color. One can determine the background absorption on the original cellulose substrate solution by adding cellulose, immediately stopping the reaction and measuring the absorbance, i.e. following exactly the same procedures for the actual samples. When the

effects of extraneous compounds were not known , one can effectively include a so-called internal standard by first fully developing the color for the unknown sample; then , a known amount of sugar was added to this sample. The increase in the absorbance upon the second color development was equivalent to the incremental amount of sugar added.

4.4.2 Reagents:

- 1% starch solution (freshly prerpared)
- **Dinitrosalicylic acid reagent (DNS reagent):** 1g of dinitrosalicylic acid was dissolved in 100ml of 1% NaOH. Then 30g crystalline phenol and sodium sulphite was added.

4.4.3 Procedure for testing α-Amylase activity:

1. To 100 µl of amylase, 100 µl of extract and 200 µl of water was added.
2. Then about 200 µl of 20mM phosphate buffer was added and kept for 20 minutes at room temperature.
3. After 20 minutes, 200 µl of 1% starch solution was added and kept for 15 minutes at room temperature.
4. To this 1 ml of DNS reagent was added and mixed well. It is then incubated at 100°C for 10 minutes.
5. The absorbance was recorded at 540 nm using a spectrophotometer and the percentage inhibition of α-amylase was calculated.

Inhibition % = {(control - test) / (control)}x100

The inhibition of α-amylase was tested with varying concentration of plant extracts.

4.5 Seperation of compounds by Thin Layer Chromatography (TLC)

4.5.1 Priniciple:.

Thin Layer Chromatography (TLC) (Roger et al., 1987) is a solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase). Solids most commonly used in chromatography are silica gel (SiO_2 x H_2O) and alumina (Al_2O_3 x H_2O). Both of these adsorbents are polar. Silica is acidic. Alumina is available in neutral, basic, or acidic forms. Thin Layer Chromatography (TLC) is a sensitive, fast, simple and inexpensive analytical technique. It is a micro technique; as little as 10^{-9}g of material can be detected, although the sample size is from 1-100x10^{-6} g. TLC involves spotting the sample to be analyzed near one end of a sheet of glass or plastic that is coated with a thin layer of an adsorbent. The sheet, which can be the size of a microscope slide, is placed on end in a covered jar containing a shallow layer of solvent. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent the stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed onto the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate.

The TLC analysis was performed for the hot water extracts of *Vitis vinifera*, *Psidium guajava* and *Trigonella foenum graecum* to confirm the presence of rutin and ferulic acid related compounds.

4.5.2 Materials:

- Silica gel,G grade with 13% gypsum.
- TLC plate (20×20 cm)
- TLC developing tank.
- Mobile phase
    - Flavonoids- Ethyl acetate: Ethanol: Water(5:1:1)
    - Phenolic acid-Ethyl acetate: Toluene: Acetic acid(50:40:20)

- Spraying agent
    - Flavonoids-Ammonia
    - Phenolic acid-10% Ferric chloride

4.5.3 Procedure for preparation of TLC plates:

1. Clean 20×20 cm glass plates were taken. Before using it was rinsed well with detergent and then with water. After that the plates were dried and wiped well with tissue paper that was soaked in benzene.
2. For one plate about 20g of silica gel G was weighed and transferred to a wide-mouth conical flask.
3. To the beaker about 30-35 ml water was added (quantity of water may vary with different batches of silica gel) and was shaken thoroughly for 30 sec to get a uniform slurry.
4. The silica gel was uniformly applied over the plates by means of a spreader whose thickness was already adjusted to 0.25 or 0.5 mm.
5. The plates were coated with the silica gel were dried in air at room temperature .After that, plates were activated by keeping it in the oven at 110^0C for 2 hrs.
6. Without disturbing the silica gel layer, the standard compound and test samples of 20µl were spotted with the help of a capillary tube or a microlitre syringe.
7. About 120 ml of the solvent mixture of the corresponding ratio was added to the TLC tank. In order to saturate the chamber a filter paper was placed over the inner sides of the tank.
8. The TLC plate was placed inside the chamber and tightly covered by means of the lid.
9. When the solvent system reaches the top of the plate, it was removed and air dried.
10. Finally the plate was sprayed uniformly with the respective spraying reagent and the spots were noted.

4.6 Functionaly modified milk preparation and organoleptic test:

4.6.1 Preparation of value added milk samples:

Milk and dairy products tailored to meet specific nutritional requirements to become more attractive and valuable to major groups of consumers. It can be done by several ways. The plant sources such as *Vitis vinifera* seeds, *Psidium guajava* leaves and *Trigonella foenum graecum* seeds were added individually to the milk in the ratio 1:100. Organoleptic means relating to perception by a sensory organ. It involves the use of sensory organs for testing of any food items. Eg., Red chillies, wine , pepper, etc. Value added milk was prepared using guava leaves, grape seeds and fenugreek seeds extract and an organoleptic evaluation of the same was conducted by 20 volunteers on 05/4/2011. The value added milk was evaluated with senses of sight, smell, taste, consumer acceptability rather than by a scientific or chemical evaluation.

Table 4.6.2 Score card for organoleptic evaluation of Functionally modified milk samples.

CRITERIA	STANDARD SCORE	EVALUATION			
		SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
1.**COLOUR AND APPEARANCE**					
Good/ Acceptable	3				
Fair/ Moderately acceptable	2				
Poor/ Not acceptable	1				
2. **TASTE**					
Good/ Acceptable	3				
Fair/ Moderately acceptable	2				
Poor/ Not acceptable	1				
3.**FLAVOUR**					
Good/ Acceptable	3				

Fair/ Moderately acceptable	2				
Poor/ Not acceptable	1				
4.**SMELL**					
Good/ Acceptable	3				
Fair/ Moderately acceptable	2				
Poor/ Not acceptable	1				

SAMPLE 1-Raw milk
SAMPLE 2- Raw milk+ probiotics+*Vitis vinifera* seeds
SAMPLE 3- Raw milk+ probiotics+*Psidium guajava* leaves.
SAMPLE 4- Raw milk+ probiotics+*Trigonella foenum graecum* seeds.

RESULTS AND DISCUSSIONS

5. RESULTS AND DISCUSSION

5.1 DPPH (1,1-diphenyl-2-picrylhydrayl) Scavenging Activity: (Giuseppe Ruberto et .al)

The stable DPPH radical model is a widely used, relatively quick method for the evaluation of free radical scavenging activity. The effect of antioxidants on DPPH radical scavenging activity is thought to be due to their hydrogen donating ability. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidant activity.

Figure 5.1.1 DPPH scavenging activity of Rutin standard

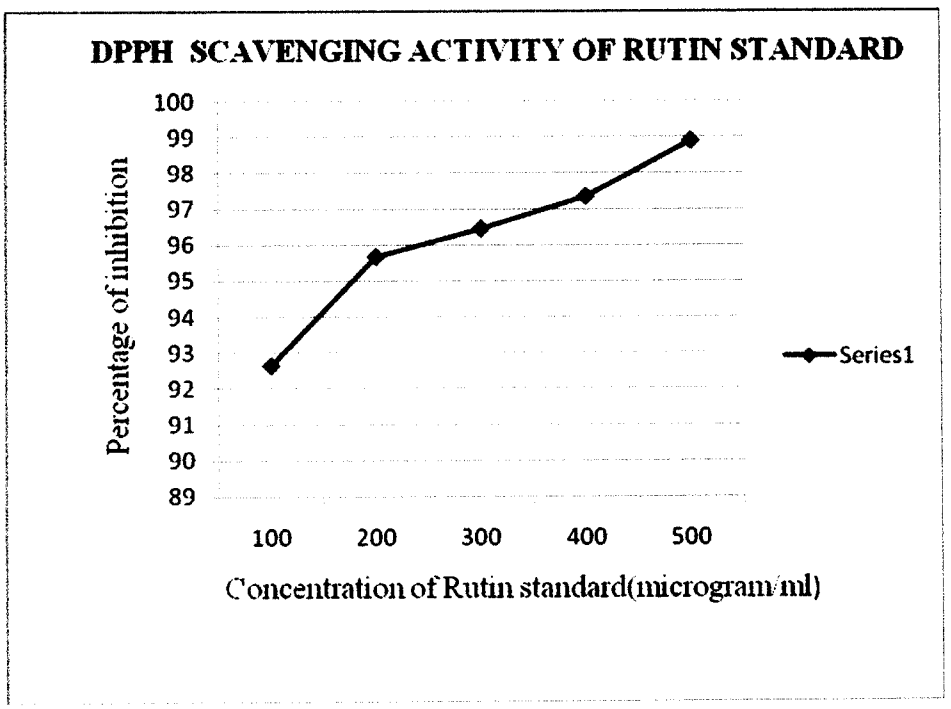
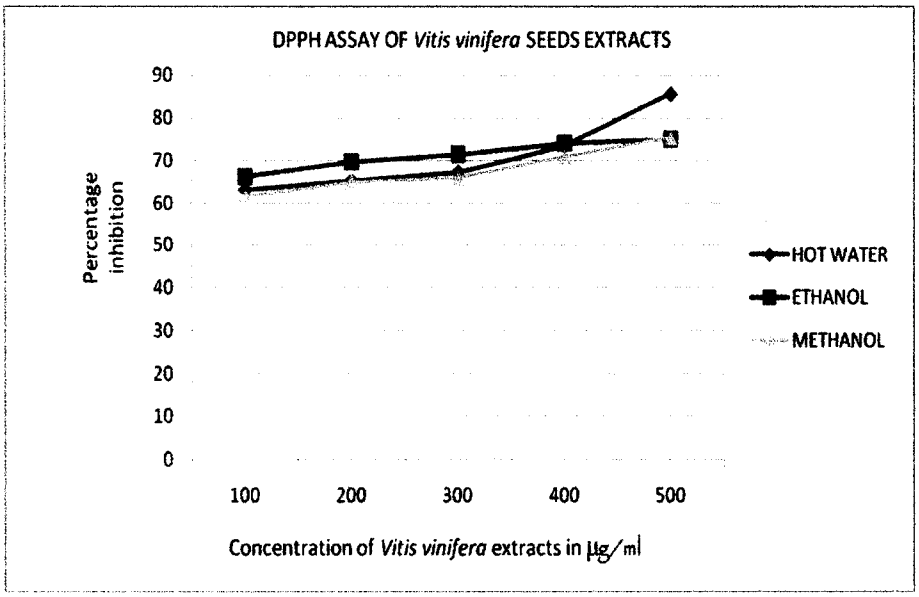


Figure 5.1.2 DPPH scavenging activity of *Vitis vinifera* seeds



From the results obtained, it was seen that the scavenging power of the extract was found to be in the order of hot water>methanol>ethanol.

Figure 5.1.3 DPPH scavenging activity of *Psidium guajava* leaves

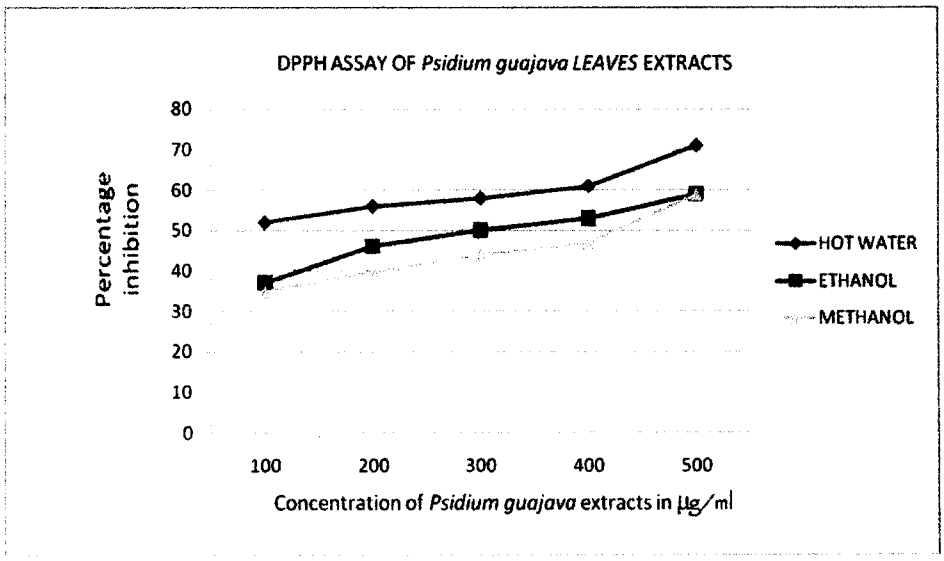
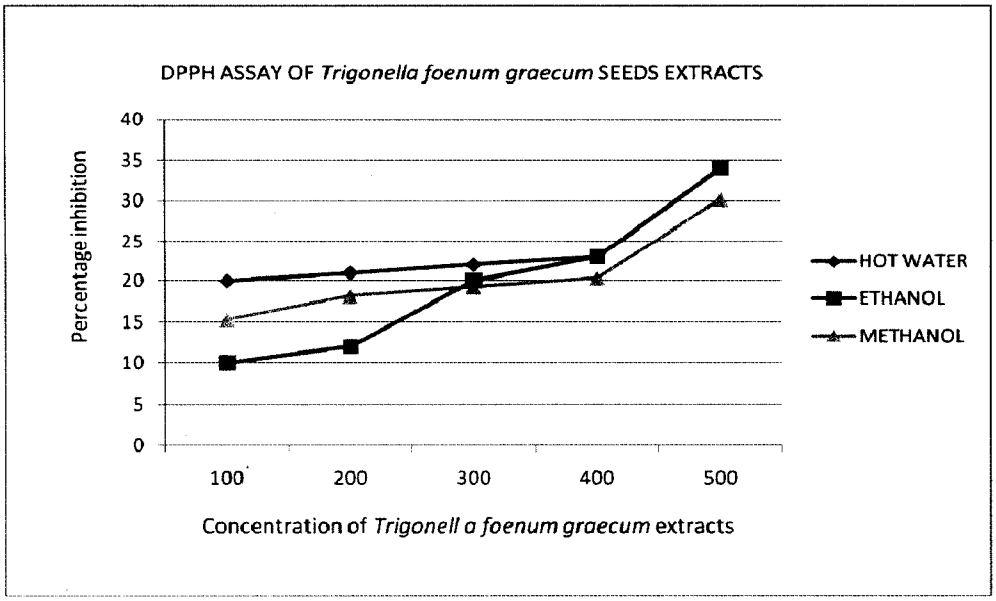


Figure 5.1.4 DPPH scavenging activity of *Trigonella foenum graecum*



The scavenging power of the extracts was found to be in the order of hot water and ethanol> methanol.
The free radical scavenging property of milk alone was found to be 30.8%
When the extracts were added to milk and tested for their DPPH scavenging activity, the following results were obtained.

Table 5.1.4 DPPH scavenging activity of milk+extracts

	Hot water extract+milk			Methanol extract+milk			Ethanol extract+milk		
	Guava	Grape	Fenugreek	Guava	Grape	Fenugreek	Guava	Grape	Fe
Scavenging activity(%)	44.16 ±0.76	42.83 ±0.28	41.13 ± 0.61	35.6 ±0.66	33.26 ±0.47	31.52 ±0.64	37.46 ±0.83	36.13 ±0.32	3 ±

It can be concluded that the DPPH scavenging activity was found to be significant for guava and grape when compared to fenugreek.

5.2 Antimicrobial sensitivity test :(P.Anurada et. Al)

The antimicrobial assay was performed for the methanolic, ethanolic and hot water extracts of *Vitis vinifera* seeds, *Psidium guajava* leaves and *Trigonella foenum graecum* seeds. The following tables and diagram shows the effect of the extracts on four bacterial strains(*Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa*) and also the effect of raw milk, mixture of raw milk and probiotics and mixture of raw milk,probiotics and extracts(60:40) against the four strains.

Table 5.2.1.1 Antibacterial activity of *Vitis vinifera* methanolic extract on test organisms

Organism	**Type of organism**	**Concentration of methanolic extract(in mg) Zone of inhibition(in mm)**			
		4	**6**	**8**	**10**
Bacillus subtilis	Gram positive	4	6	8	10
Staphylococcus aureus	Gram positive	4	6	9	10
Klebsiella pneumoniae	Gram positive	4	7	10	11
Pseudomonas aeruginosa	Gram negative	5	7	8	11

Table 5.2.1.2 Antibacterial activity of *Vitis vinifera* ethanolic extract on test organisms

Organism	Type of organism	Concentration of ethanolic extract(in mg) Zone of inhibition(in mm)			
		4	6	8	10
Bacillus subtilis	Gram positive	3	4	6	7
Staphylococcus aureus	Gram positive	2	5	7	8
Klebsiella pneumoniae	Gram positive	3	5	6	8
Pseudomonas aeruginosa	Gram negative	4	7	9	10

Table 5.2.1.3 Antibacterial activity of *Psidium guajava hot water* extract on test organisms

Organism	Type of organism	Concentration of hot water extract(in mg) Zone of inhibition(in mm)			
		4	6	8	10
Bacillus subtilis	Gram positive	6	7	9	10
Staphylococcus aureus	Gram positive	4	6	7	9
Klebsiella pneumoniae	Gram positive	6	8	10	11
Pseudomonas aeruginosa	Gram negative	5	7	8	10

Table 5.2.1.4 Antibacterial activity of *Psidium guajava methanolic* extract on test organisms

Organism	Type of organism	Concentration of Methanolic extract(in mg) Zone of inhibition(in mm)			
		4	**6**	**8**	**10**
Bacillus subtilis	Gram positive	7	8	10	11
Staphylococcus aureus	Gram positive	5	7	8	10
Klebsiella pneumoniae	Gram positive	6	8	10	11
Pseudomonas aeruginosa	Gram negative	4	6	8	10

Table 5.2.1.5 Antimicrobial activity of raw milk+probiotics and extracts:

SAMPLES	ORGANISMS			
	Bacillus subtilis	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudor aerugin*
Raw milk	8	6	9	9
Raw milk+probiotics	10	9	10	1
Raw milk+probiotics:Methanolic grape extract(60:40)	12	-	14	1
Raw milk+probiotics:Ethanolic grape extract(60:40)	12	-	15	1
Raw milk+probiotics:Hot water grape extract(60:40)	10	-	12	1
Raw milk+probiotics:Hot water guava extract(60:40)	-	-	15	1
Raw milk+probiotics:Methanolic guava extract(60:40)	-	-	13	1
Raw milk+probiotics:Ethanolic guava extract(60:40)	-	-	11	1

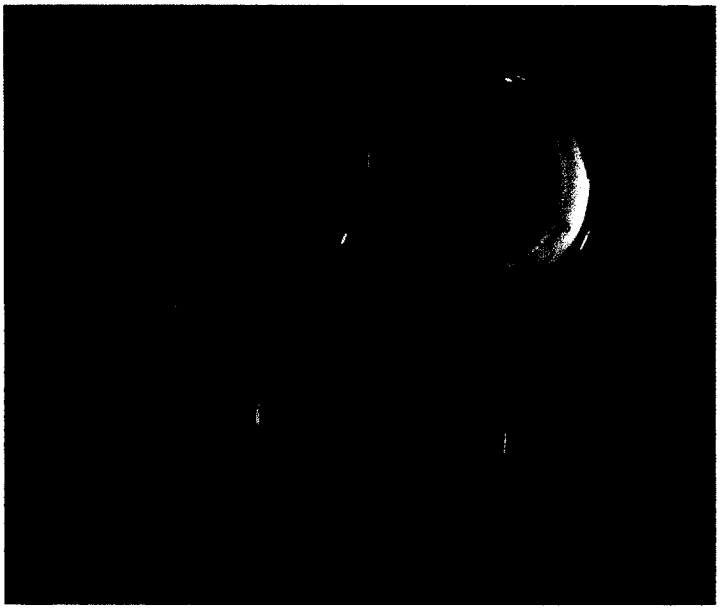


Figure 5.2.2.2 Antimicrobial **activity of *Vitis vinifera* seeds ethanolic extract**

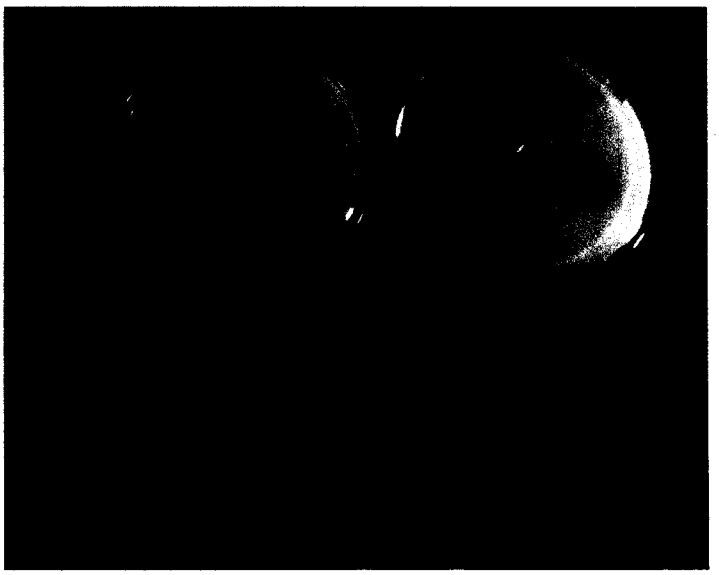


Figure 5.2.2.3 Antimicrobial activity of *Psidium guajava* leaves hot water extract

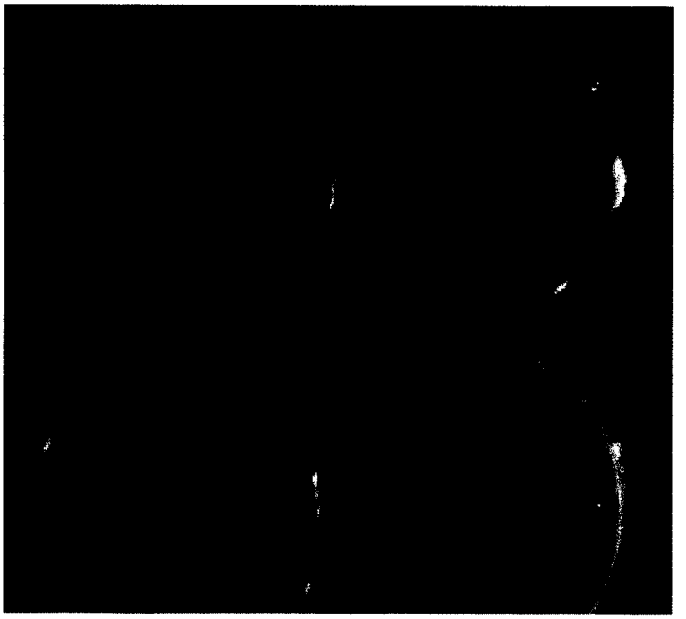


Figure5.2.2.4 Antimicrobial activity of *Psidium guajava* leaves methanolic extract

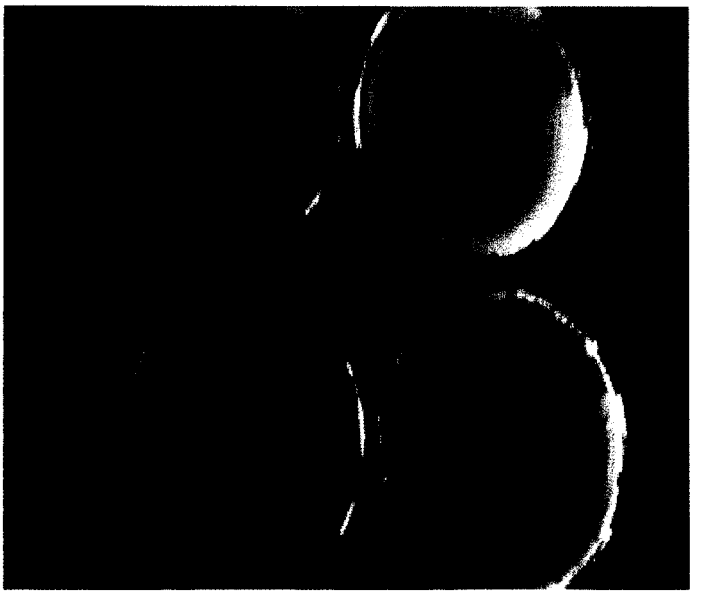
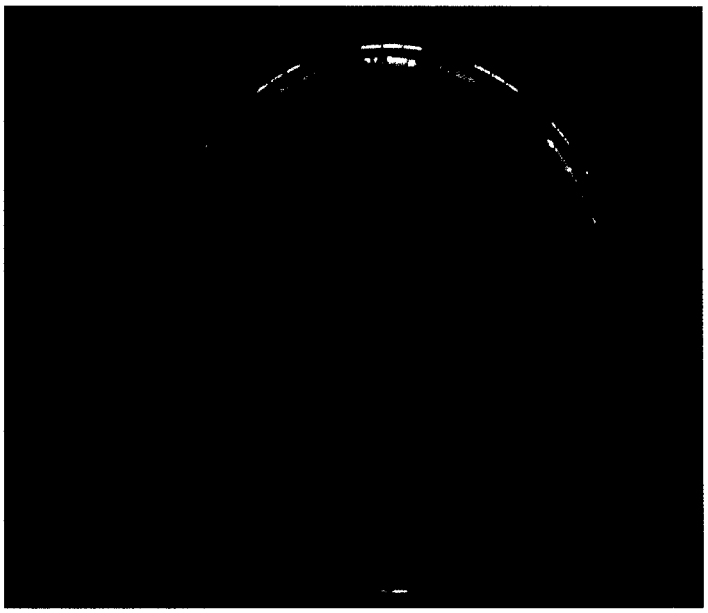
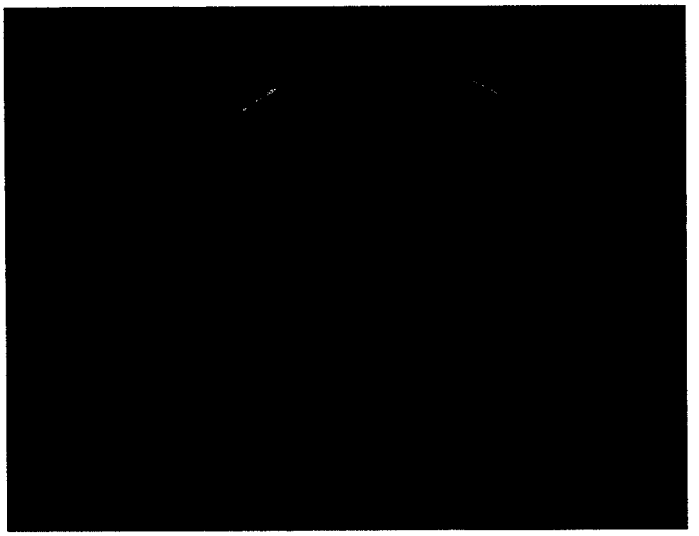


Figure 5.2.2.5 Antimicrobial activity of milk+probiotics

Figure 5.2.2.6Antimicrobial activity of milk+*Vitis vinifera* methanolic extract against *Bacillus subtilis*





Grape and guava extracts showed a significant zone of inhibition at different concentrations. The addition of individual extracts to the probiotics added milk showed significant zone of inhibition against *Bacillus subtilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Fenugreek extracts showed a negligible zone of inhibition against the four strains.

Trigonella foenum graecum seeds were checked for α amylase inhibitory activity by DNS (3,5 Di Nitro Salicylic acid) method using 1% starch as substrate. The enzyme used was porcine pancreatic α amylase (PPA).

Figure 5.3.1 α amylase inhibitory activity for Hot water extracts of *Vitis vinifera* seeds, *Psidium guajava* leaves and *Trigonella foenum graecum* seeds

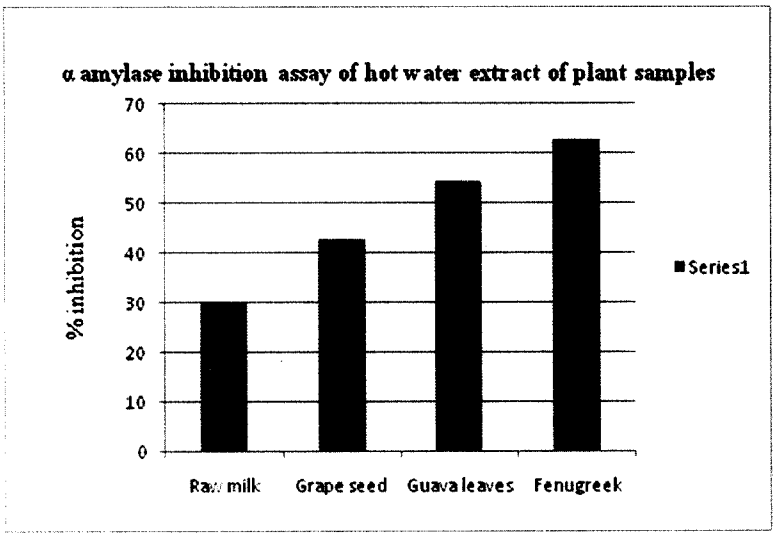
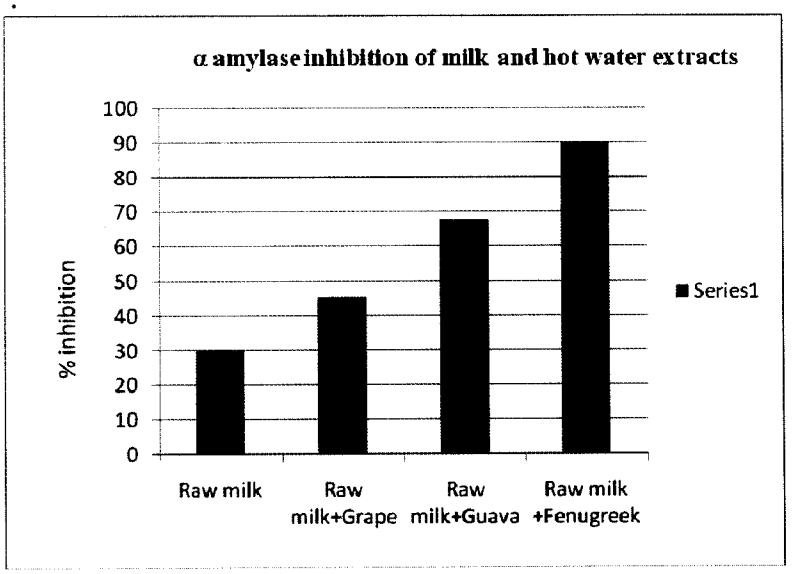


Figure 5.3.2 α amylase inhibitory activity of milk samples

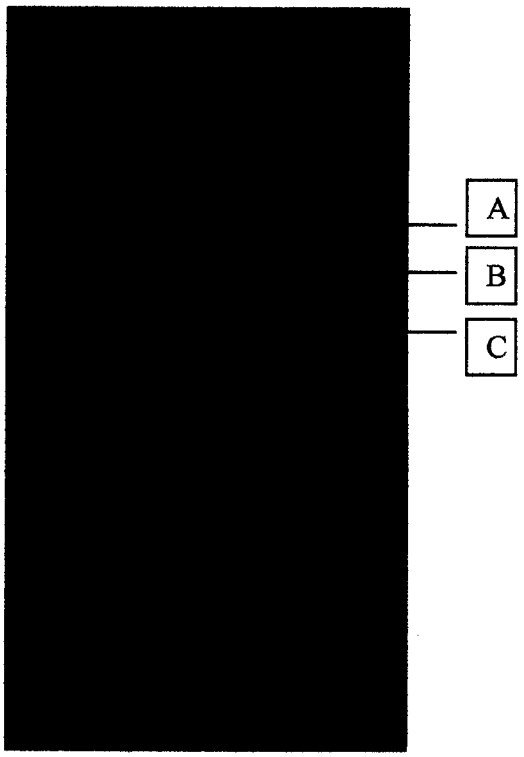


The results of the assay implied that the hot water extracts of the plant samples showed the maximum α amylase inhibition of 42.8%,54.4% and 62.9% and the mixture of milk and hot water extracts showed the α amylase inhibition of 45.6%,67.8% and 89.9%. The results indicated that the α amylase inhibitory activity of value added milk sample showed the maximum percentage of inhibition than the hot water extracts of plant samples.

5.4 TLC Results: (Roger et al.,1987)

TLC was carried out for plant samples to find out the presence of rutin and ferulic acid related compound.

Figure 5.4.1 Rutin analysis of *Vitis vinifera* and *Psidium guajava*

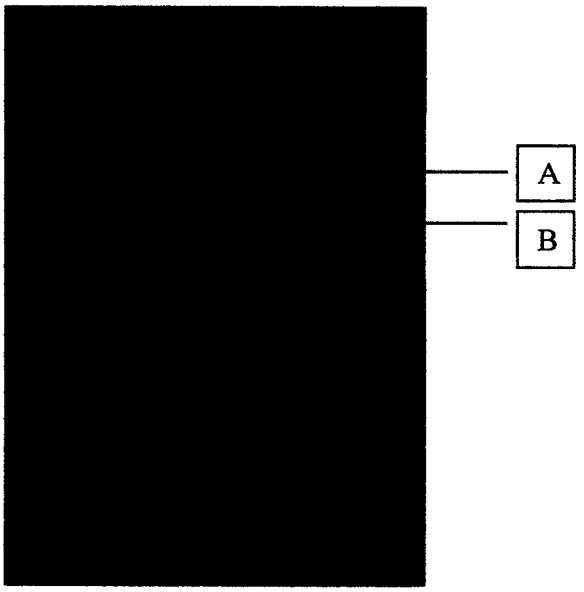


A- Rutin standard

B- Guava

C- Grape

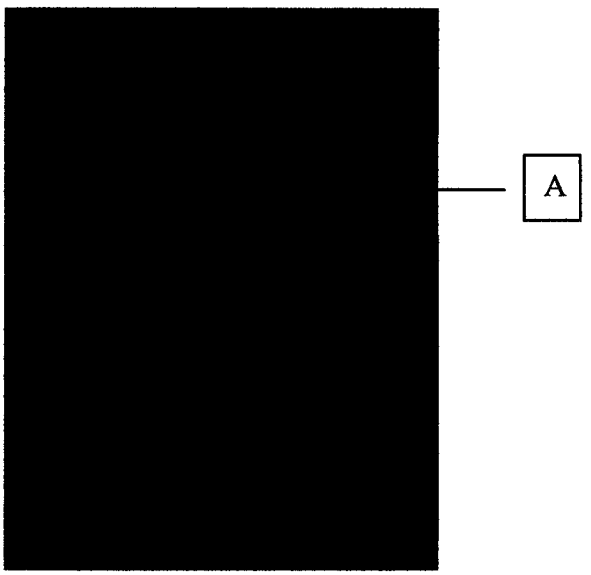
Figure 5.4.2 Ferulic acid analysis of *Vitis vinifera*



A- Ferulic acid

B- Grape

Figure 5.4.3 Ferulic acid analysis of *Psidium guajava*



A-Guava

5.5 Results of Organoleptic evaluation of Functionally modified milk:

The results of organoleptic evaluation of functionally modified milk are shown in the table.

Table 5.5.1 Organoleptic Evaluation of Functionally modified milk:

SAMPLE	DETAILS	AVERAGE SCORE			
		COLOUR AND APPEARANCE	TASTE	FLAVOUR	SMELL
1	Raw milk	3	3	3	3
2	Rawmilk+ probiotics+*Vitis vinifera* seeds	3	2.7	2.8	2.6
3	Rawmilk+ probiotics+*Psidium guajava* leaves.	3	2.9	2.7	2.55
4	Rawmilk+ probiotics+ *Trigonella foenum graecum* seeds.	3	2.6	2.5	2.35

CONCLUSION

6. CONCLUSION

Antimicrobial, antioxidant and alpha amylase inhibiting properties of plant samples *Vitis vinifera* (seeds), *Psidium guajava* (leaves), *Trigonella foenum graecum* (seeds) extracted with ethanol, methanol and hot water was analysed. Antimicrobial activity against Bacillus *subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* was performed and it was found that *Vitis vinifera* and *Psidium guajava* added to the milk containing probiotics showed a good antimicrobial activity. The antioxidant property of the plant extracts added to the milk were found significant and the α amylase inhibitory property of the fenugreek extracts was found significant. These results suggest that plant compounds from good grade sources offers an attractive health-giving dairy products towards diabetes and also the scavenging of free radicals can have potential for prevention of deadly diseases.

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