CHAPTER: 1
Review of literature
‘Let food be thy medicine and medicine be thy food’, this quote by Hippocrates, holds very true these days. There is growing interest in functional foods which can promote health beyond basic nutrition (Suvarna and Boby 2005). Microorganisms with health promoting attributes constitute an integral part of some of the functional foods. These microorganisms with health promoting activity or attribute are widely known as probiotics. The word “Probiotics” is derived from Greek and it means “prolife” or “for life”. This term was first coined by Lilly and Stillwell (Dunne et al. 1999) in the 1960’s and can be defined as “Live microorganism which when administered in adequate amounts confer a health benefit on the host” (Lee et al. 2008).

Role of microorganism in fermented foods or in its preparation has been known for centuries but scientific interest in this area was generated by pioneering work published by Ellie Metchinkoff in 1908. He suggested that Bulgarian peasants lived longer because of their yogurt consumption (Suvarna and Boby 2005). One independent study in 1930’s by Minoru Shirota, a Japanese physician, suggested that the right mix of bacteria in the gut could prevent disease (Brown and Valiere 2004). These and some other findings led to the increased commercial exploitation of probiotics. These probiotic properties are strain specific, many diverse probiotic strains have been released for commercial use and they constitute a substantial part of functional food market. According to one report, United States market potential for probiotic fortified functional food may be estimated to US $ 120 million per month (Senok et al. 2005).

Traditionally fermented milk and yoghurt has been prescribed by local healers for the treatment of diverse conditions like skin allergies and stomach upsets especially diarrhoea (Senok et al. 2005). During past decades there has been increased interest in the study of nutritional and therapeutic aspects of these products. Probiotic microorganisms are now included in wide range of consumer formulations including yoghurts, drinks, capsules and dietary supplements. As a result, probiotics based functional food have gained access to all markets across the globe. Independent research groups have suggested that lactic acid cultures and their fermented products
provide therapeutic and nutritional benefits to the consumers (Parvez et al. 2006). Potential beneficial effect of incorporation of viable lactic acid bacteria could also be due to the favourable alteration in Gastrointestinal (GI) microecology. Organisms used as probiotics majorly include the member of genera *Lactobacillus or Bifidobacterium, Escherichia coli, Bacillus subtilis, Saccharomyces boulardii* and *Enterococcus faecium* (Toole and Cooney 2008).

1.1 **Mechanisms of action of probiotics**

Understanding the molecular details of probiotic efficacy or mode of action has been investigated in number of *in vitro, in vivo* and clinical settings. Most of the probiotic strains exhibited common properties but there are differences in their mode of action. These facts signify that probiotic attributes are strain specific and each probiotic strain should be tested individually. The major mode of action includes:

1.1.1 **Altering the immune system**

Gut microbiota has direct contact with intestinal epithelial cells, which in turn has direct interface with immune system. At intestinal surface, microbes are recognised by receptors on the surface of epithelium, which is a prime activator of immunological response. Bacterial molecular structures like lipopolysaccharides (LPS), lipoteichoic acids and unmethylated CpG DNA motifs are recognised by pattern recognition receptors such as Toll-like receptors. Probiotic bacteria are also known to activate pro-inflammatory cytokines and chemokines (Saxelin et al. 2005). Different strains or species of probiotics elicit varied cytokine response, which signify the strain variation in the efficacy of probiotics. For example, VSL#3 which is mixture of eight different strains upregulates production of IL-10 and downregulates IL-12 secretion by dendrite cells (DC’s). LPS mediated upregulation of IL-12 is also diminished by VSL#3, which also regulates the production of IL-10. Antigen presenting cells primed by probiotics can differentiate naïve T cells into different T cell populations depending upon the cytokines. *Lactobacillus rhamnosus* GG has been proposed for atopic diseases in allergic children, as it skews T cell population from Th-2 type of response towards Th-1 type with low grade of inflammation (Marschan et al. 2008). Probiotic bacteria are also known to increase the adaptive immune response and antibody formation. For example, *Bifidobacterium* and *Lactobacillus* strains increase the synthesis of IgA by mucosal lymphoid cells in patients with Crohn’s disease (Malin et al. 1996).
1.1.2 Strengthening of mucosal barrier
Pathogens disrupt gut mucosal barrier and increase the permeability of mucosal lining. Probiotic bacteria are known to prevent such damage to mucosal wall by improving the cell survival and proliferation. For example, *L. acidophilus* prevents damage in tight junctions and decrease in pathogen induced transepithelial resistance of the cell monolayer (Saxelin *et al.* 2005).

1.1.3 Competitive pathogen exclusion
Probiotics have been proven to be preventive in diarrhoeal diseases, which include acute bacterial, viral and antibiotic associated diarrhoea. *Lactobacillus* GG, *Lactobacillus reuteri*, *Saccharomyces boulardii*, *Bifidobacterium* are some of the examples of probiotic microorganisms which have shown significant benefit in diarrhoeal cases. Probiotics might prevent infection by competing with pathogenic bacteria or viruses for binding sites on epithelium (Parvez *et al.* 2006). Probiotics are also known to inhibit the growth of pathogens by secreting antimicrobial molecules like bacteriocins. *E. coli* strains in cell culture monolayer was found to be reduced when certain strains of probiotic *Lactobacillus* sp. were added before pathogen infection.

1.1.4 Suppression of intestinal inflammation
Changes in gut microbiota have been implicated in number of inflammatory diseases of gut. In case of ulcerative colitis there is marked reduction in total number of lactobacilli when compared to other microbes in remission phase (Saxelin *et al.* 2005). Bacteria in mucosal biopsies of patients with active inflammatory diseases like Crohn’s disease or ulcerative colitis were detected by single strand conformation analysis and real time polymerase chain reaction. This study showed that diversity of microbiota was reduced significantly in patients with IBD (Inflammatory Bowel Disease). There is a significant loss of bacteria belonging to the genera *Bacteroides*, *Eubacterium* and *Lactobacillus*. In mouse model of colitis, some species of *Lactobacillus* and *Bifidobacterium* have established beneficial activity by reducing inflammation (Saxelin *et al.* 2005).
1.2 *Lactobacillus sp. as a probiotic microorganism*

Lactobacilli are Gram positive rods that fall into the group of Lactic acid bacteria (LAB). They are generally recovered from areas where there is abundant supply of rich carbohydrate sources. Therefore, they harbour at diverse ecological niches such as mucosal membrane of humans and animals (oral cavity, intestine and vagina), on plants and materials of plant origin, in manure and manmade habitats such as sewage and fermenting or spoiling food (Bernardeau et al. 2008). Lactobacilli have the longest history of use as probiotics and are also widely known for their fermentative role in foods composed of vegetables, meat and particularly dairy products.

1.2.1 Health Benefits and Mechanisms of action of Lactobacilli on Host

Lactobacilli have been widely recommended for enteric infections and post antibiotic syndromes. Clinical studies have also highlighted the efficacy of some lactobacilli in acute infectious diarrhoea and prevention of antibiotic associated diarrhoea. Among intestinal disorders, use of lactobacilli could prevent colorectal cancer and can be used for the treatment of inflammatory bowel disease. Lactobacillus strains are also known to have role in prevention, treatment of urogenital disease and bacterial vaginosis in women. It is also preventative of atopic disease, food hypersensitivity and the prevention of dental caries (Lebeer et al. 2008). The clinical evidences of health benefits of lactobacilli vary from one study to another because of difference in clinical settings and probiotic strains used.

The basic mechanisms of health benefits conferred by lactobacilli includes i) pathogen inhibition and restoration of microbial homeostasis ii) enhancement of epithelial barrier function iii) modulation of immune responses. Since the host response to lactobacilli varies from strain to strain, these mechanisms cannot be generalised for all the strains (Fig 1.1).

i) Pathogen inhibition and microbial homeostasis: The anti-pathogenic effect of probiotics could be due to competition for nutrients, production of antimicrobials and/or competitive exclusion. Metabolic interactions between the species lead to symbiotic relationships where in primary degraders of carbohydrates can lead to release of polysaccharides which can be later on fermented by LAB. Apart from competitive advantage,
lactobacilli produce a variety of compounds that have antimicrobial activity against bacteria and viruses. Lactic acid is one of the key antimicrobial compounds produced by LAB and is known to be inhibitory to virulence factors associated with pathogens like *Salmonella* (Lebeer *et al.* 2008). Bacteriocins are also another class of antimicrobials secreted by many lactobacilli. These antimicrobials are small peptides with high isoelectric point and may act by inducing membrane permeabilization and subsequent leakage of molecules from target bacteria. Hydrogen peroxide ($\text{H}_2\text{O}_2$) has also been implicated for antimicrobial activity especially in vagina of healthy women (Servin 2004). It has been reported that $\text{H}_2\text{O}_2$ is responsible for anti- *Salmonella* activity exhibited by *L. johnsonii* NCC533 (Pridmore *et al.* 2008). Pathogens can also be excluded by competing for the same binding site with lactobacilli. Intestinal pathogen like *E. coli* uses oligosaccharide receptor site in the gut and probiotic could use the same attachment site for mucosal binding (Le Bouguenec 2005). This anti-pathogenic activity observed during this kind of interaction is known as competitive exclusion.

ii) **Enhancement of epithelial barrier function:** - Epithelial barrier is compromised in number of clinical states of IBD, enteric infection, food allergy etc. Probiotic lactobacilli are known to strengthen the epithelial barrier by various mechanisms like induction of mucin secretion, enhancement of tight junction functioning and prevention of apoptosis. Cell surface associated factors like LTA (Lipoteichoic acid) from *L. johnsonii* La1 could abolish the effect of *E. coli* and Lipopolysaccharide induced IL-8 release by HT-29 cells. Direct contact with epithelial cells could also prevent apoptosis and has anti inflammatory role by activation of mitogen activated protein kinases (MAPK) and Akt. Secreted proteins from lactobacilli are also known to stabilize tight junctions, for example VSL#3 a probiotic mixture which includes *L. casei, L. plantarum, L. acidophilus* and *L. delbrueckii* secretes proteinaceous soluble factor (>50 kDa) which strengthens tight junctions. Peptides from *L. rhamnosus* GG activate MAPK p38 and JNK for which activate heat shock proteins in intestinal epithelial cells (Tao *et al.* 2006).
Modulation of immune response: Intestinal epithelial barrier plays an important role in maintenance of immune balance. The major cell types which are involved in immune homeostasis includes intestinal epithelial cells, dendritic cells (DC’s) and macrophages. Innate immune response leads to production of antimicrobial compounds like defensins, nitric oxide etc. The adaptive immune responses against commensal, probiotic and pathogenic bacteria are mainly carried at mucosal lymphoid follicles present all over the gut and mainly called as gut-associated lymphoid tissue (GALT). Lactobacilli are also known to prime immunoregulatory responses and induce regulatory DC’s and T cells. Macrophages when exposed to *Lactobacillus rhamnosus* GG reduced the level of TNF-α production. Lactobacilli differ significantly in their immunomodulatory role and it varies from strain to strain. Some strains have higher capacity to elicit IL-12 and TNF-α and other strains are less potent inducers. Monocytes produced higher level of IL-12 and TNF-α in response to *L. plantarum* in comparison to *E. coli*. However, in contrast to monocytes, DCs secrete large amount of IL-12, TNF-α, IL-6 and IL-10 in response to *E. coli* than to *L. plantarum*. The probiotic mixture VSL#3 is also known to ameliorate Th-1 mediated colitis by induction of TGF-β (Di Giacinto et al. 2005). A research article Herich and Levkut 2002, suggests that when gnotobiotic rats are given *L. plantarum* in addition to *E. coli* they show lower count of *E. coli* in the small intestine and caecum, in comparison to the rats fed with only *E. coli*. These studies suggest that *Lactobacillus* strains elicit differential immune response i.e. towards pro- or anti-inflammatory depending upon the specific bacterial strain applied, the specific immune cell used and the specific experimental design.

### 1.3 *Saccharomyces cerevisiae* as probiotic microorganism

*Saccharomyces cerevisiae* has been known to thrive in diverse environments such as plants, animals, soil and water. *Saccharomyces cerevisiae* has also been reported in fermentation of several food preparations and beverages. Faeces of humans and animals can also be exploited for isolation of probiotic yeast strains (Liong 2011). Recently there is increased interest in developing probiotic yeast for human and
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animal use. Among yeast strains *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae var boulardii* are the only yeast strains commercialized for human consumption (Liong 2011). Like *Lactobacillus* spp., *Saccharomyces cerevisiae* has also attained GRAS i.e. generally regarded as safe status from Food and Drug Administration.

In comparison to prokaryotic probiotics, yeast spp. are little less explored for their probiotic attributes. Moreover, there are marked differences in their mode of action and their properties observed both at *in vitro* and *in vivo* levels. One of the advantages of using yeast over bacterial probiotics is associated with transfer of antibiotic resistance genes. There are chances of transferring antibiotic resistant genes from bacterial probiotics to pathogens in the host gut. However, most of the yeast lack such plasmid encoded genes and there are no reports which discusses about the transfer of genetic material from yeast to bacteria (Liong 2011). Therefore, probiotic yeast can be a safe alternative during antibiotic treatment. Yeast also has a unique advantage over bacterial probiotics because of their robust size and nutrition adaptability, they can utilize broad range of substrates, effectivity against broad range of pathogens and produce several other useful metabolites.

There are several beneficial effects of *Saccharomyces cerevisiae* which have been attributed to human health and well being. Food supplementation with *Saccharomyces cerevisiae* has also lead to enhanced body weight gain with increase in probiotic uptake (Lutful Kabir 2009). *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae var boulardii* have found to be effective in treatment and prevention of various type of diarrhoea including diarrhoea caused by pathogens, antibiotic associated diarrhoea and acute diarrhoea in children. It has also been found effective in lactose intolerance, vaginal yeast infections and food allergies (Liong 2011).

### 1.3.1 Clinical efficacy of *Saccharomyces boulardii*

Several beneficial effects of *Saccharomyces boulardii* on human health have been reported in clinical studies conducted last decade. It has been found very effective in the treatment of antibiotic associated diarrhoea (AAD), traveller’s diarrhoea, *Clostridium difficile* and *Helicobacter pylori* infection (Czerucka et al. 2007). In spite of multi-factorial benefits derived from *S. boulardii*, there is increased demand for new strains for other clinical and industrial applications. Hence, this study was
designed to isolate and characterise promising probiotic yeast strains from ayurvedic fermented biomedicine to boost its applicability as an adjunct probiont.

The mechanism of action of *S. cerevisiae* on host physiology varies from host to host and it can exert beneficial effect by a variety of distinct and overlapping mechanisms. This can be broadly classified into 1) Trophic effect on intestinal mucosa 2) Immunomodulation and anti-inflammatory role 3) Maintenance of gut barrier integrity 4) Inhibition of pathogens (Fig 1.1).

i) Trophic effect on intestinal mucosa: *Saccharomyces cerevisiae* helps in restoration of intestinal homeostasis. It produces an array of enzymes which are not produced by host and helps in breakdown of complex macromolecules. For instance, feeding trials in humans and rats have shown that there is a marked increase in secretion of enzymes such as sucrose-isomaltase, lactase, maltase-glucoamylase, α-glucosidase and alkaline phosphatase (Buts et al. 1986). Yeasts are also known to modify luminal short-chain fatty acids (SCFAs) concentration. As per one report, administration of *S. cerevisiae* var. *boulardii* increased the total SCFA, without altering faecal microflora. This further helps in preventing enteric diarrhoea (Moslehi-Jenabian et al. 2010). Moreover, it has also been shown that *S. cerevisiae* var. *boulardii* can prevent the reaction to food antigens by endoluminal proteolysis which further helps in absorption of completely or incompletely degraded proteins or antigens (Moslehi-Jenabian et al. 2010). Polyamines (spermine and spermidine) of *S. cerevisiae / S. cerevisiae* var. *boulardii* exert trophical effect on the intestinal mucosa (Buts et al. 1994).

ii) Immunomodulatory and anti-inflammatory role of *S. cerevisiae*: *S. cerevisiae* are known to possess immune stimulation activity at innate and adaptive level. Elicitation of immune response can be because of biologically active substances in yeast (Martins et al. 2007a). This majorly includes β-glucans, α-mannans, mannoproteins and chitins. These components elicit inflammatory response by stimulating the immune system. One study reports that rats treated with high dose of *S. cerevisiae* could elicit increased secretion of secretory IgA in duodenal fluid, villus and crypt cells (Buts et al. 1990). *S. cerevisiae* var *boulardii* increases
sIgA and IL-10 production, in comparison to bacterial probiotics like *Bifidobacterium animalis, E. coli* and *Lactobacillus casei*. *S. cerevisiae var boulardii* have also shown to elicit differential cytokine production and phagocytic activity. In human colocytes, *S. cerevisiae var boulardii* stimulated the production of peroxisome proliferator activated receptor gamma (PPAR-γ), which further helps in the reduction of pro-inflammatory response. Alteration of inducible nitric oxide synthase (iNOS) has been implicated in inflammatory bowel disease (IBD). Treatment with *S. cerevisiae var boulardii* could alleviate iNOS levels and can act as an therapeutic alternative (Girard *et al.* 2005). Low molecular weight soluble protein (<1 kDa) from *S. cerevisiae* blocks NF-κB activation and NF-κB mediated IL-8 gene expression in intestinal epithelial cells and monocytes.

**iii)** Maintenance of gut barrier integrity: Several strains of *S. cerevisiae* and *S. cerevisiae var boulardii* increased the gut barrier integrity as observed by increase in transepithelial resistance (TER) in Caco2 (human epithelial adenocarcinoma cell lines) (Klingberg *et al.* 2008). T84 cells when infected with enteropathogenic *E. coli* reduced TER and disrupted the distribution of tight junction protein Zonula occludens (ZO-1), while treatment with *S. cerevisiae var boulardii* could reduce the effect of this infection (Czerucka *et al.* 2000). Similar to this observation, *S. cerevisiae var boulardii* treatment ameliorated the disruption of tight junction proteins claudin-1 and ZO-2 level by *Shigella* infection.

**iv)** Inhibition to pathogens: Unlike bacterial probiotics, eukaryotic probiotics do not produce antimicrobial metabolites to exert their inhibitory action against pathogens (Martins *et al.* 2009). Some reports of eukaryotic probiotics indicate successful killing of various pathogens like *E. coli, Salmonella typhi, Shigella dysentriae* and *Clostridium difficile* (Czerucka *et al.* 2007). *S. cerevisiae var boulardii* competes with enteropathogens for food and mucosal receptors in the gut, consequently prevents the pathogens to colonize host-gut (Filho-Lima *et al.* 2000). For example, *S. cerevisiae* modifies the surface receptors in Vero cells in such a way that there is marked reduction in the adhesion of *C. difficile* by its proteolytic activity or
by steric hindrance (Tasteyre et al. 2002). In another example, \textit{S. cerevisiae var boulardii} attenuated the adherence of \textit{Citrobacter rodentium} which in turn reduced \textit{C. rodentium} induced colitis in mice (Wu et al. 2008). Secreted proteins from \textit{S. cerevisiae var boulardii} have also been shown to neutralize bacterial toxins from \textit{Clostridium difficile} (Moslehi-Jenabian et al. 2010).
Figure 1.1 Different Mode of Action of Probiotic Lactobacillus sp. and yeast on intestinal lumen
1.4 Sources for isolation of probiotic strains

Metchnikoff emphasised on the concept of use of lactic acid bacteria in dairy products. Later on, Scientist’s also pointed the use of lactic acid bacteria of intestinal origin for human application, as these strains can suit to intestinal environment and can reach highest density in the intestinal ecosystem (Lee et al. 2008). Due to conventional usage of lactic acid cultures in fermented products, some researchers emphasised the incorporation of probiotic in fermented formulations, which can have varied nutritional and therapeutic benefit to the consumers (Lilly and Stillwell 1965). Recently, Dairy products gained a huge success and acceptance in commercial market. Conventionally these products hold the record of being healthy and are known to the consumer due to their content of viable fermenting microorganisms (Heller 2001). The most frequently used bacteria in these products include Lactobacillus and Bifidobacterium (Adel M. Mahasneh and Abbas 2010). The health benefits of these sources encouraged researchers to explore novel probiotic bacteria in traditionally fermented foods which are associated with good health of people who consume such foods (Salminen et al. 1998). These strains may have an added advantage in revealing their taxonomic characteristics and obtaining strains with new functional traits, which can also be useful for probiotic applications (Adel M. Mahasneh and Abbas 2010).

Moreover, traditionally fermented foods are processed through naturally occurring microorganisms. Modern molecular tools can be used to define the starter culture to ensure consistency and quality of final product. Microorganisms involved in these fermented foods do not possess health risk and therefore they fall under the category of “GRAS” (generally recognised as safe). The incorporation of probiotics in traditional fermented food has lead to the development of novel type of products. Some of the products have also the advantage of prebiotic component present in traditionally fermented food. A prebiotic is a food ingredient that is not hydrolysed by human digestive enzymes and has beneficial effect on host by selectively stimulating the growth of beneficial microbes. Prebiotics include non digestible carbohydrates, peptides, proteins, and certain lipids. A food product which constitutes probiotics and prebiotics are termed as synbiotic. In most of these fermented products, process of fermentation is either parallel or acts in a sequential manner, with a changing dominant flora. The common fermenting bacteria are species
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of *Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus* and *Bacillus* (Gupta and Abu-Ghannam 2012).

Among traditionally fermented foods, cereal based fermentation products constitute major source of dietary nutrients all over the world. Cereal based fermentation products are considered to be good substrates for probiotics as well. Among Asian and African countries, cereal based fermented products have been in use for centuries. *Idli* (steam cooked fermented paste) and *Dosa* (fermented paste based fried as pancake) are very popular in India, the fermenting microorganisms involved during this fermentation process mainly constitute *Leuconostoc mesenteroides, Streptococcus faecalis, Lactobacillus delbrueckii, Lactobacillus fermentii, Lactobacillus lactis* and *Pediococcus cerevisiae*.

Now a days, plant based fermented foods like fruit juice based functional beverages i.e. fortified with probiotic and prebiotic ingredients are gaining popularity. However, these kinds of products have issue of consumer dissatisfaction because of its poor sensory characteristics in comparison to conventional juices (Gupta and Abu-Ghannam 2012). If the information regarding health benefits are provided then the consumer preference of functional beverages goes up. In a report Rakin et al. 2004, it has been observed that beetroot juice fermentation with *Lactobacillus* and addition of yeast autolysate, had optimum properties of pigments, vitamins and minerals.

Recently, herbal sources or extracts are also being tapped for isolation of *Lactobacillus* sp. (Cakir 2010) and some trials also suggests that combination of herbs with probiotics can have significant improvement in the health of chicken (Alcicek et al. 2004; Jung et al. 2010), fish (Harikrishnan et al. 2011) and piglets (Zangeronimo et al. 2011). However, only few studies have highlighted the efficacy of herbs in conjunction with probiotics for beneficial attributes in humans. Reddy et al. highlighted that herbal drugs in conjunction with probiotics have enhanced its effect in comparison to the conventional therapy (Reddy et al. 2000).
1.5 Herbal biomedicines: a source for isolation of microbes with probiotic attributes

There is a huge upsurge in demand of herbal medicine which includes herbal products and raw materials. In the global market, annual growth rate has been predicted to be in between 5% and 15% (Citarasu 2010). In an estimate, the total herbal drug market was around US $62 billion and by the year 2050, it is expected to reach US $5 trillion (Citarasu 2010). Herbal medicines are also very popular in European markets and have significant contribution in gross pharmaceutical market (Bodeker and Kronenberg 2002). In Indian context, botanical trade accounts for US $10 billion per annum and exports around US $1.1 billion (Citarasu 2010). United States is the largest market for Indian exports with a share of nearly 50%.

In India, herbal biomedicines have been practised for thousands of years, under the realm of Ayurvedic sciences. Ayurveda term is derived from “Ayur” which means life and “veda” which means knowledge. These herbal medicines are of various types, including herbal teas, infusions, decoctions, tinctures, capsules, powders, infused oils, ointments, creams, and lotions etc. along with arishta (fermented decoctions) and asavas (fermented infusions). As reported recently asava and arishta are very popular dosage forms of Ayurvedic medicines. These preparations of asavas and arishta are also referred as medicinal wines, as that involve fermentation process to generate alcohol (Sushruta and Khale 2011). There are multiple benefits of these medicinal preparations like better keeping quality, effective drug extraction from herbal preparations and enhanced therapeutic properties (Sekar and Mariappan 2008).

1.5.1 Preparation of Asava and Arishta

The general method of extraction of drugs from medicinal plants in asavas and arishtas are decoction and infusions. In the preparation of Decoction, crude drug is boiled in water, then cooled and filtered. This process helps in extracting water soluble and heat stable components. In the process of preparation, infusions of crude drug are macerated with cold or boiling water. After this, prescribed quantity of honey, jaggery and/or sugar are added. The detailed description about the traditional procedure and equipments required are mentioned in these articles (Sekar and
Fermentation is carried out in large wooden vats.

The fermentation is brought about by addition of *dhataki* flowers, which harbours wild species of yeast. These flowers are added to the contents and mixed well for ensuring the uniform distribution of yeast. The inoculum of wild yeast can also come from other ingredients like honey and raisins (gum) etc. The vessels are then sealed and kept in a cool dark place for fermentation process. The duration of fermentation process may vary for different season and may take upto 30 to 60 days. The filtered medicine is then dispensed in different batches in stoppered glass bottles. The dosage of consumption is different for different drugs and also depends upon the property of drug. According to one report, there are more than 79 products of asava and arishta. These herbal biomedicines are used for the treatment of various complications and problems in digestive diseases, paediatrics, nervous system, respiratory system, reproductive system, immune system, general illness and infectious diseases (Sekar and Mariappan 2008).

Therefore, asava and arishta based products have huge market potential and medical relevance. On the other hand, repetitive usage of antibiotics has led to the evolution of antibiotic resistant microorganisms. Hence, understanding the role of microorganisms in these biomedical fermentative processes could have dual advantage of revealing taxonomic attributes and procuring strains with new functional traits that can be exploited for probiotic applications. Since the nature of microorganisms present in these fermentation processes and their probiotic potential has not been explored, keeping this in thought, we have attempted to explore following microbiological aspects in these herbal biomedicines.

**Objectives of the study include:**

1) Assessment of microbial community structure in herbal biomedicines
2) Characterisation of *Lactobacillus plantarum* for probiotic attributes
3) Selection of yeast for probiotic attributes and stability of potential probionts in Kutajarista