DIETARY MODULATION OF THE HUMAN COLONIC MICROBIOTA THROUGH PLANT-DERIVED PREBIOTIC COMPOUNDS

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Submitted in complete fulfilment for the Degree of Master of Technology (Biotechnology) in the Department of Biotechnology, Durban University of Technology, Durban, South Africa

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Preface

This study represents original work by the author and has not been submitted in any form to another university or institute. Where use was made of others, it has been duly acknowledged in the text.

The research described in this thesis was carried out at the Department of Biotechnology, Durban University of Technology, M. L. Sultan Campus, under the supervision of Professor Odhav.

_____

M. A. Kassim

2007

Acknowledgements

I would like to extend my sincere thanks and gratitude to:

My Supervisor, Prof. B. Odhav for all her expertise and constructive criticism throughout the course of this project,

My co-supervisor, Prof. H. Baijnath for his time and effort in obtaining plant material during the course of this project,

Ms. D. E. Stephens, Dr. K. Permaul and Mr. V. Mohanlall, for their assistance and guidance throughout the course of this project,

Staff and students at the Department of Biotechnology, Durban University of Technology for their general support, encouragement and assistance in leaf collecting,

My family, for all their support and encouragement throughout this project,

The National Research Foundation (NRF) for funding this project.

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List of Abbreviations

AOAC-Association of Analytical Chemists

AUC-Area under the curve

CFU-Colony Forming Units

DP-Degree of Polymerisation

DUT-Durban University of Technology

FOS-Fructooligosaccharides

GI-Gastrointestinal

GRAS-Generally Regarded As Safe

HPLC-High Performance Liquid Chromatography

r- Pearson's Co-efficient of Correlation

SCFA-Short Chain Fatty Acids

TFTC-Too Few To Count

TNTC-Too Numerous To Count

Abstract

The human gut microbiota play a major role in host health, and attempts are being made to manipulate the composition of the gut microbiota-increase the composition of bacterial groups, such as lactobacilli and bifidobacteria that are perceived as exerting health promoting properties. These bacteria defined as food supplements (probiotics) beneficially affect the host by improving the intestinal microbial balance, and have been used to change the composition of the colonic microbiota. However, such changes may be transient, and the implantation of exogenous bacteria therefore becomes limited. In contrast, prebiotics are naturally occurring carbohydrates that are classified as non-digestible oligosaccharides present in edible plants. These carbohydrates enter the colon as intact compounds, elicit systemic physiological functions and act as fermentable substrates for colonic microflora-influencing the species composition and metabolic characteristics of intestinal microflora providing important health attributes. Currently, a widely marketed prebiotic, inulin is extracted from plants of the family Asteraceae. There are many unexploited plants that are regularly consumed and that may have a prebiotic effect or can have very high levels of inulin which could make them commercially viable. In this study, we investigated prebiotic compounds, especially inulin from locally growing, non-commercialised leafy plants. The aqueous extracts of 22 plants from the families Asparagaceae, Alliaceae, Asteraceae, Solanaceae, Cucurbitaceae, Amaranthaceae, Acanthaceae, Polygonaceae, Portulaceae, Fabaceae, Chenopodiaceae, Pedaliaceae and Apiaceae from Kwa-Zulu Natal were investigated for a prebiotic effect using a modified batch-culture technique with *Lactobacillus bulgaricus, Lactobacillus lactis, Lactobacillus reuteri and Bifidobacterium longum*, four common probiotics and the inulin content of the plants was determined using high performance liquid chromatography. Of the 22 plants studied, *Solanum nigrum, Amaranthus spinosus, Amaranthus hybridus, Asystasia gangetica, Senna occidentalis, Cerathoteca triloba, Asparagus sprengeri, Tulbaghia violacea, Sonchus oleraceus* and *Taraxacum officinale* exhibited a prebiotic effect. The prebiotic effect of the *Taraxacum officinale, Sonchus oleraceus* and *Asparagus sprengeri* extracts on *L. lactis* and *L. reuteri* was higher than or equivalent to inulin-a commercial prebiotic. In this study, *Sonchus oleraceus* exhibited the best prebiotic effect-was the only plant to stimulate all the probiotics including *B. longum*. Of all the plants analysed, *Asparagus sprengeri* tuber contained the highest amount of inulin (3.55%).

Key Words: Bifidobacteria, Fructan, Inulin, Lactobacilli, Oligofructose, Prebiotic

Chapter One:

Introduction and Literature Review

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

Introduction

As the medical and economical burden of acute and chronic gut disorders increase, functional foods that target particular genera of bacteria (esp. Lactobacilli and Bifidobacteria) have potential of being manipulated to overcome some of these ailments. Taking the view that positive components of the gut flora already exist in the intestinal tract, the prebiotic concept has been developed. Here, dietary carbohydrates have a selective metabolism within the gut flora thereby shifting the community towards a more advantageous structure. Currently the most widely used prebiotic commercially is inulin, which is known to have a prebiotic effect. Inulin is produced naturally in over 36000 plants world wide.

Currently the main commercial source of inulin is from the roots of *Cichorium intybus*, commonly known as Chicory of the family Asteraceae. The aim of this study was to look for alternative sources of inulin from various plants namely *Solanum nigrum, Physalis viscosa, Momordica balsamina, Amaranthus spinosus, Amaranthus hybridus, Amaranthus dubius, Asystasia gangetica, Justicia flava, Emex australis, Oxygonum sinuatum, Bidens pilosa, Galinsoga parviflora, Portulaca oleracea, Senna occidentalis, Chenopodium album, Ceratotheca triloba, Centella asiatica, Asparagus sprengeri, Tulbaghia violacea, Sonchus oleraceus, Taraxacum officinale* and *Cleome monophylla*.

These indigenous plants were chosen because they occur commonly in the Kwa-Zulu Natal area, are regularly consumed and known to cure a variety of ailments traditionally. In this study, the ability of the selected plant extracts to stimulate the growth of four common probiotic strains viz. *Lactobacillus lactis, Lactobacillus bulgaricus, Lactobacillus reuteri* and *Bifidobacterium longum* was determined using a modified batch culture technique. The inulin content of the extracts was determined using high performance liquid chromatography (HPLC) techniques.

In the first chapter of this dissertation, the literature reviews the normal gut flora and the factors that affect it. This is followed by the prebiotic concept. A review of the current knowledge of the plants used in this study is also given. Chapter Two describes the methods that were used to

investigate plants that could have a prebiotic effect on *L. lactis*, *L. bulgaricus*, *L. reuteri* and *B. longum*. The experiments involved testing the effect of the aqueous extracts of twenty two plants on the growth of the above probiotic cultures in MRS broth, over a 96 hour period. Further analyses involved the determination of the inulin content of the plants using HPLC. Statistical analysis was used to correlate the relationship between the prebiotic effect of the plants and their inulin content. Chapter Three covers the results obtained and Chapter Four discusses these results in context with what is known and what we found. Chapter Five consists of the conclusion. A detailed outline of this dissertation may be found on the contents pages.

Literature review

1.1 Probiotics

1.1.1The human gut flora

Dietary modulation of the human gut flora has been carried out for many years. In fact, the first records of the intake of bacterial drinks by humans are over 2000 years old. However, at the beginning of this century probiotics were first put onto a scientific basis by the work of Metchnikoff at the Pasteur Institute in Paris who observed longevity in Bulgarian peasants and associated this with their elevated intake of soured milks—which contained what is now known as probiotics. During these studies, he hypothesised that the normal gut microflora could exert adverse effects on the host and that consumption of certain bacteria could reverse this effect (Metchnikoff, 1907).

Subsequent research (Brady *et al*., 2000; Roberfroid, 2000; Servin, 2004; Tomasik & Tomasik, 2003) has been directed towards the use of intestinal isolates of bacteria as probiotics. Probiotic bacteria, by definition, are living microbial food ingredients which have a beneficial effect on human health (Salminen *et al*., 1998). They consist mainly of lactic acid producing bacteria, Lactobacilli and Bifidobacteria however *Bacillus* spp. and fungi such as *Saccharomyces* spp. and *Aspergillus* spp have also been included.

1.1.2 Lactobacilli

Lactobacilli are Gram-positive, non-spore forming rods, catalase negative, usually non-motile and do not reduce nitrate. As glucose fermenters, they can be divided into biochemical subgroups

on the basis of the metabolic route by which glucose is metabolised, either homofermentative or heterofermentative. The *Lactobacillus* population of the human gastrointestinal system consists of various species, subspecies and biotypes of the genera, of which the most frequently isolated lactobacilli belong to *L. acidophilus, L. salivarius, L. casei, L. plantarum, L. fermentum, L. lactis, L. bulgaricus, L. reuteri* and *L. brevis* (Mikelsaar *et al*., 1998). Lactobacilli have GRAS status (Salminen *et al*., 1998). According to 16S *r*RNA sequencing data, lactobacilli may further be divided into three distinct 16S *r*RNA groups (Collins *et al*., 1991).

1.1.3 Bifidobacteria

Bifidobacteria are Gram-positive, non-spore forming rods, with distinct cellular bi-furcating or club-shaped morphologies. The Bifidobacterial population of the human gastrointestinal system consists of various species, subspecies and biotypes of the genera, of which the most frequently isolated bifidobacteria belong to *B. longum, B. bifidum, B. breve* and *B. adolescentis* (Trojanova *et al*., 2004). Since bifidobacteria constitute up to 25% of the gut microflora in some adult individuals, they make a significant contribution to carbohydrate fermentation in the colon. Hexoses are fermented by the fructose-6-phosphate, or 'bifidus' shunt (de Vries & Stouthammer, 1968), which is characterised by the presence of the key enzyme fructose-6-phosphate phosphoketolase.

1.1.4 Limitations of the probiotic approach

Despite the very widespread use of probiotics, the approach may have some difficulties. The bacteria used are usually anaerobic and do not relish extremes of temperature. To be effective, probiotics must be capable of being prepared in a viable manner and on a large scale (e.g. for industrial purposes), whilst during use and under storage the probiotic should remain viable and stable, be able to survive in the intestinal ecosystem and the host should gain beneficially from harbouring the probiotic (Rastall, 2004; Tomomatsu, 1994).

It is therefore proposed that the exogenous bacteria reach the intestine in an intact and viable form, establish therein and exert their advantageous properties. In order to do so, the bacteria are confronted by a number of physical and chemical barriers in the gastrointestinal tract. These include gastric acidity, bile acid secretion and pancreatic enzymes. Moreover, on reaching the

colon the probiotic may be in some sort of stressed state that would probably compromise chances of survival (Ahmed, 2003).

1.1.5 Risks of the probiotic approach

The probiotic definition requires that the efficacy and safety of probiotics be verified, and thus the assessment of this constitutes an important part of their characterisation for human use (Ahmed, 2003). Currently, the most realistic and effective manner to fortify probiotics is to selectively stimulate the probiotics already present in the gut through increasing their metabolic capacity. This is the prebiotic concept.

The ability of specific probiotic strains to survive gastric conditions and to adhere strongly to the intestinal mucosa following oral administration may entail a risk of bacterial translocation, bacteraemia and sepsis. For current probiotic foods, such risks have been reported rarely, but some cases of bacteraemia in subjects with serious underlying diseases are known (Salminen *et al*., 2002).

This research is therefore directed towards manipulating the bacteria already present in the gut by metabolic manipulation of the gut microflora.

1.2 Introducing the concept of prebiotics

1.2.1 Definition of a prebiotic

A prebiotic was first defined as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson & Roberfroid, 1995). A prebiotic substrate is selectively utilised by beneficial components of the indigenous gut flora but does not promote potential pathogens such as toxin-producing Clostridia, proteolytic Bacteroides and toxigenic *Escherichia coli.* In this manner, a 'healthier' microflora composition is obtained whereby the Bifidobacteria and/or Lactobacilli become predominant in the intestine and exert possible health-promoting effects (Blaut, 2002; van Loo *et al*., 1999). As diet is the main factor controlling the intestinal microflora, it is possible to modulate the composition of the microflora through foods.

1.2.2 Classification of a food ingredient as a prebiotic

A prebiotic nature has however been attributed to many food components without due consideration to the criteria required. In particular, almost every food oligosaccharide and polysaccharide (including dietary fibre) has been claimed to have prebiotic activity, but not all dietary carbohydrates are prebiotics. There is, therefore, a need to establish clear derivatives for classifying a food ingredient as prebiotic (Blaut, 2002; Fooks & Gibson, 2002; van Loo *et al*., 1999). Such classification requires a scientific demonstration that the food component or ingredient:

- Resists host digestion, absorption and adsorption processes
- Is fermented by the microflora colonising the gastrointestinal system
- Selectively stimulates the growth and/or the activity of one or a limited number of bacteria within the gastrointestinal system

The premise behind prebiotics is therefore to stimulate certain indigenous bacteria resident in the gut rather than introducing exogenous species as is the case with probiotics. Ingesting a diet containing non-digestible carbohydrates that are selectively fermented by indigenous beneficial bacteria is the principle behind the prebiotic.

Any dietary component that reaches the colon intact is a potential prebiotic; however, most of the interest in the development of prebiotics is aimed at non-digestible oligosaccharides. It is also possible to take in prebiotics more naturally through the diet. Many fruit and vegetables contain prebiotic oligosaccharides such as inulin. Examples are Onion, Garlic, Banana, Asparagus, Leek, Jerusalem artichoke and Chicory (van Loo *et al*., 1995; 1999).

1.2.3 Common prebiotic compounds

As the majority of bacteria resident in the gut microflora are present in the colon, prebiotics are usually directed towards lower gut bacteriology. Examples of oligosaccharides for which a prebiotic effect has been suggested are: Lactulose, Galactooligosaccharides, Soybean

oligosaccharides, Lactosucrose, Isomalto-oligosaccharides, Glucooligosaccharides and Fructooligosaccharides (Gibson *et al*., 2000).

As mentioned, current prebiotics seem to be mainly confined to oligosaccharides that are non-digestible in the upper gut, and confer the degree of fermentation selectivity that is required, for example, specifically towards bifidobacteria.

Non-digestible carbohydrates (referred to as dietary fibre) of plant origin are the main substrates of probiotic organisms and include resistant starch as well as non-starch polysaccharides such as cellulose, hemicellulose, pectin and inulin, of which inulin is used on a commercial scale (Blaut, 2002; van Loo *et al*., 1995; 1999).

1.2.3.1 Inulin as a prebiotic

Inulin is a naturally occurring oligosaccharide (several simple sugars linked together) produced by many types of plants and belongs to a class of carbohydrates known as fructans. Fructan is a general term used for naturally occurring plant oligosaccharides and polysaccharides and refers to any carbohydrate compound in which one or more fructosyl–fructose links comprise the majority of glycosidic bonds (Carabin & Gary Flamm, 1999; Ritsema & Smeekens, 2003; van Loo *et al*., 1999).

Within the inulin-type fructans are two general groups of materials, inulin and its subsets, oligofructose and fructooligosaccharides (FOS) (Carabin & Gary Flamm, 1999). Inulin, as a plant-derived carbohydrate, has the benefits of soluble dietary fibre. It is not digested or absorbed in the small intestine, but is fermented in the colon by the beneficial probiotics lactobacilli and bifidobacteria (Molina *et al*., 2005).

Functioning as a prebiotic, inulin has been associated with enhancing the gastrointestinal and immune systems. In addition, it has been shown to increase the absorption of calcium and magnesium, influence the formation of blood glucose, and reduce the levels of cholesterol and serum lipids (Molina *et al*., 2005). Therefore, inulin obtained from several Asteraceae plants (Jerusalem artichoke, Artichokes, Chicory, Dahlias, and Dandelions) is a subject of interest in

many food research programs. Commercially available inulins are obtained mainly from Chicory, Jerusalem artichoke, and Dahlia. Inulin is extracted from Chicory root with hot water, and subsequently purified by technology that is commonly used in the starch industry (van de Wiele *et al*., 2004). These inulins are distributed with several trade names such as Raftiline® or Fibruline®.

Inulin has specific technological assets, being soluble upon heating and bland in taste. It can be blended into a large number of different food products which retain their intrinsic flavour without alteration of texture and appearance. Inulin helps to provide body, good mouth-feel and appearance, thus it can be used as a fat replacer for the emerging sector of lower energy food products (Franck, 2002).

It must be emphasised that inulin or oligofructose cannot be quantitatively measured in the soluble fibre fraction of foods obtained by the standard Association of Analytical Chemists (AOAC) dietary fibre method. Therefore, β-fructans have to be directly determined in samples (Tungland, 2000).

Inulin (Figure 1.1) is a polydisperse β-2-1 fructan. The fructose units (F) in this mixture of linear fructose polymers and oligomers are each linked by β-2-1 bonds. A glucose molecule (G) typically resides at the end of each fructose chain and is linked by an α-1-2 bond, as in sucrose. The unique aspect of the structure of inulin is its β-2-1 bonds. These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary benefits (Tomasik & Tomasik, 2003).

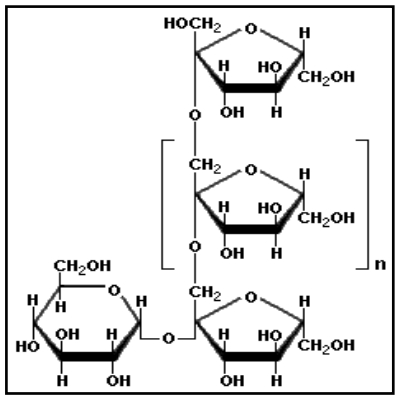

Fig. 1.1 Chemical structure of inulin (Tungland, 2000)

The prebiotic properties of carbohydrates are likely to be influenced by the following factors (Manning & Gibson, 2004):

(i) Monosaccharide composition-Recognised prebiotics are built primarily from glucose, galactose, xylose and fructose. The prebiotic potential of oligosaccharides composed of other monosaccharides is not known at the present time.

(ii) Glycosidic linkage-The linkage between the monosaccharide residues is a crucial factor in determining both selectivity of fermentation and digestibility in the small intestine. Fermentation of fructooligosaccharide prebiotics is selective because of a cell associated β-fructofuranosidase in the bifidobacteria.

(iii) Molecular weight-Polysaccharides are generally not prebiotic in their metabolism but oligosaccharides are (Wang & Gibson, 1993). Inulin has the highest molecular weight, but most of the carbohydrate in inulin has a degree of polymerisation (DP) less than 25, with an average of about 14 (De Leenheer,1994).

1.2.3.2 Inulin as a prebiotic-Safety and tolerance

Today, only inulin from Chicory roots is commercialised as a purified food ingredient. The Chicory roots that are used are of the same species (*Cichorium intybus*) that has been used for many years to produce the coffee substitute. At the moment, no genetically modified organism-derived Chicory roots are used (Carabin & Garry Flamm, 1999; Coussement, 1999).

Among the several commercial inulin types available, all have a very high purity; they differ with regard to their powder characteristics and carbohydrate composition. Standard inulin, as it is extracted from Chicory roots, always contains a small amount of sugars (up to 10%). These sugars are present in the root and are not a result of processing (Coussement, 1999; van Loo *et al*., 1995). Thanks to their plant origin, together with the use of modern processing techniques, commercial inulin can easily meet today's high microbiological standards for food ingredients (Franck, 2002).

1.2.3.2.1 Safe for human consumption

Inulin is present in the daily diet of many of the world's populations (Van Loo *et al*., 1995). This presence is not a matter of trace amounts; several grams per day may be ingested through the normal diet. Inulin is a macronutrient. It is used either as a supplement to foods or as a macronutrient substitute. As a supplement to food, it is added mainly for its nutritional properties.

Adding inulin or oligofructose increases the dietary fibre content of the food. Such additions are usually in the range of three to six grams per portion, in extreme cases up to ten grams. In other applications, inulin is added to allow a specific nutritional claim such as that regarding the bifidogenic activity. In these foods, typical levels are approximately three to eight grams per portion (Briet *et al*., 1995; Carabin & Garry Flamm, 1999). The average dietary intake of inulin by humans is estimated to be one to four grams per day (Roberfroid, 2002).

No evidence of treatment-related toxicity, carcinogenicity, or genotoxicity has been observed from standard toxicity tests conducted at doses far above anticipated human exposure. With regards to their food use, the real issue is not that of safety, but rather of gastrointestinal (GI) tolerance. Signs of intolerance in the form of abdominal pain and bloating, flatulence, and/or osmotic diarrhoea have been seen with intakes above 20–30 g (Carabin & Garry Flamm, 1999).

1.2.3.2.2 Legal and regulatory status

Inulin derived from various natural sources, such as Chicory root, Dahlia and Jerusalem artichoke is legally classified as a food or natural food ingredient, and has non additive status. In the United States, inulin has food ingredient status and is Generally Regarded As Safe (GRAS). As stated in GRAS policy, inulin can be used without any significant restrictions for all intended food categories, unless the food is standardised and the standard does not permit its use (Tungland, 2000).

1.3 Potential food applications

1.3.1 Prebiotics in South Africa

In South Africa, inulin has been given food ingredient status (Tungland, 2000). As prebiotics exploit the use of non-viable dietary components to improve gut health, the range of foods into

which they can be added is much wider than that for probiotics, where culture viability needs to be maintained. This has the advantage that heat stability, or exposure to oxygen is not an issue. As such, virtually any carbohydrate containing food is susceptible to supplementation.

The identified range of prebiotic containing products manufactured in South Africa includes three fortified infant foods, seven yoghurt products selected from a variety of dairy products containing live cultures targeted at children and adults, and 16 probiotic supplements of which three are targeted at infants/children and 13 at adults. A combination of probiotics and prebiotics was found in six supplements, of which only one is targeted at children, two energy drinks and one dairy product (Brink *et al*., 2005).

Sixteen food items naturally containing or fortified with prebiotics, including two supplements, two breakfast cereals, 11 nutritional drinks and one muesli bar, were identified. Four of these are targeted at infants/children, four at children and adults, and eight at adults only (Brink *et al*., 2005).

The latest trend in the functional food market is to combine probiotics with prebiotics to enhance the effect of probiotics (Menrad, 2003). However, prebiotics also have health benefits that are not related to the simultaneous intake of probiotics. Inulin and fructooligosaccharides are among the most common prebiotics included in breakfast cereals and nutritional drinks or used in combination with probiotics in nutritional supplements (Roberfroid, 2001).

1.4 Health-related aspects and applications

At present, most prebiotics are selected on the basis of their ability to promote the growth of lactic-acid-producing microorganisms. Inulin is the most common prebiotic. The possible benefits of inulin to human health have now been studied for more than a decade.

These benefits may be summarised as follows (Den Hond *et al*., 2000; Delzenne, 2003).

- Feeding with inulin may lead to a prebiotic effect, allowing an (re)equilibrium of the colonic microbiota

- The production of short-chain fatty acids allows oligosaccharides to play a role in cell proliferation in normal or altered colonic cells (butyrate)
- Lower pH, with consequences on cation absorption
- Reach the liver, to play a role in lipid and glucose homeostasis

A number of benefits can be ascribed to prebiotic intake and some areas of interest are described below:

1.4.1 Protection against colon cancer

Many common diseases of the human large bowel arise in the distal colon, particularly colon cancer (Rowland, 1992). Prebiotics have been postulated to be protective against the development of colon cancer (Bouhnik *et al*., 1996; Buddington *et al*., 1996; Hylla *et al*., 1998; Reddy *et al*., 1997; Rowland & Tanaka, 1993). In addition, it is thought that tumours arise 100 times more often in the large intestine compared to the small intestine (Morotomi *et al*., 1990). For this reason, many researchers believe that the colonic microflora has an important role to play in the development of bowel cancer (Rowland, 1998). It is known that several species of bacteria commonly found in the colon produce carcinogens and tumour promoters from the metabolism of food components.

Interest in a diet-mediated intervention towards colon cancer arises due to the slow, progressive nature of the disease and the fact that we can influence colonic microbiology by diet. It is thought that prebiotics may protect against the development of colon cancer through at least two mechanisms:

(i) Production of protective metabolites. Butyrate is a common fermentation end product and is known to stimulate apoptosis in colonic cancer cell lines and it is also the preferred fuel for healthy colonocytes (Kim *et al*., 1982; Prasad, 1980). For these reasons, it is generally believed that it is desirable to increase the level of butyrate formed in the large gut. Some prebiotics are known to have this effect (Videla *et al*., 2001).

(ii) Subversion of colonic metabolism away from protein and lipid metabolism. It is possible that prebiotics would induce a shift in bacterial metabolism in the colon towards more benign end products. An obvious target would be to shift the metabolism of Clostridia and Bacteroides away from proteolysis to a saccharolysis.

Lactic acid bacteria are believed to have inhibitory effects on several bacteria that produce carcinogenic enzymes and are themselves non-producers. Moreover, prebiotics may indirectly modify the activities of enzymes produced by the lactic acid bacteria that are involved in carcinogenesis, such as azoreductases, nitroreductases, β-glucuronidase, etc. (Reddy, 1998).

1.4.2 Antibacterial activity-effects on pathogens

Many mechanisms have been postulated by which lactobacilli and bifidobacteria could produce antimicrobial activity. In addition to their competitive inhibition of the epithelial and mucosal adherence of pathogens and inhibition of epithelial invasion by pathogens, lactobacilli and bifidobacteria also show antimicrobial activity by producing antimicrobial substances and/or stimulating mucosal immunity (Servin, 2004).

(i) Production of acids-By producing metabolites such as acetic and lactic acids, and thus lowering the pH, a large number of lactobacilli inhibit the growth of bacterial pathogens (Vandenbergh, 1993). *Lactobacillus* strains isolated from the human digestive tract have been found to inhibit the growth of four species known to be anaerobic bacterial etiologic agents of gastroenteric infections, *Helicobacter pylori*, *Campylobacter jejuni*, *Campylobacter coli* and *Clostridium dificile* (Strus *et al*., 2001).

(ii) Production of biosurfactants-*Lactobacillus fermentum* RC-14 and the biosurfactants it secretes have been reported to inhibit infections of surgical implants in rats (Gan *et al.*, 2002), caused by *Staphylococcus aureus,* a major cause of community and hospital acquired infections. In addition, *Lactobacillus* strains have been found to be highly adherent to catheters, and strain RC-14, together with the biosurfactant it produces, have been reported to produce significant inhibition of the adherence of *Staphylococcus aureus* to surgical implants (Reid *et al*., 1994).

1.4.3 Improved calcium absorption

There has been increasing interest in recent years in the possibility of increasing mineral (particularly calcium) absorption through the consumption of prebiotics. Although the small intestine is the principal site of calcium absorption in humans, it is thought that significant amounts are absorbed throughout the length of the gut, consequently, maximising of colonic effects is desirable (Scholz-Ahrens *et al*., 2001).

Several mechanisms have been postulated for increased calcium absorption induced by prebiotics (Fairweather-Tait & Johnson, 1999). They include the following:

(i) Fermentation of prebiotics such as inulin results in a significant production of short chain fatty acids (SCFA), leading to a reduction in lumenal colonic pH. This is likely to increase calcium solubility and overall levels in the gut.

(ii) Phytate (myoinositol hexaphosphate) is a component of plants that reaches the colon largely intact (Cummings *et al*., 1979). It also forms stable, insoluble complexes with divalent cations, such as calcium, rendering them unavailable for transport. Fermentation results in bacterial metabolism of phytate, thereby liberating calcium.

(iii) It is postulated that a calcium exchange mechanism operates in the colon. In this system, SCFAs enter the colon in a protonated form and then dissociate in the intracellular environment. The liberated proton is then secreted into the lumen in exchange for a calcium ion (Fairweather-Tait & Johnson, 1999).

1.4.4 Immunological effects

Besides its role as a barrier to potential pathogens, the intestinal flora is thought to protect the host by priming the immunological defence mechanism. Scientific attention is increasingly being focussed on the mechanism of the innate immune response of the host to various components of the autochthonous microbiota, including lactobacilli and bifidobacteria. The interdependency between the epithelium and adjacent lymphoid cells is such that the epithelium is considered to

have a central role in the mucosal immune system and is an active participant in both the afferent and efferent limbs of the mucosal immune response (Shanahan, 1999).

Lactic acid bacteria are also thought to stimulate both non-specific host defence mechanisms and certain types of cells involved in the specific immune response. The result is often increased phagocytic activity and/or elevated immunological molecules such as secretory IgA, which may affect pathogens such as *Salmonella* and Rotavirus. Most attention in this respect has been diverted towards the intake of probiotics (lactic acid bacteria) and interactions between cell wall components and immune cells (Manning & Gibson, 2004).

As prebiotics serve a similar end point to lactic acid bacteria (i.e. improved composition of the gut microflora) similar effects may occur through their intake. A recent animal study has shown that prebiotics had an effect on immune functions (Swanson, 2002). All of these effects are generally considered to promote health and are the reason that inulin is one of the most promising functional food ingredients discovered to date (Meier & Gassull, 2004).

1.5 Inulin producing plant species

Inulin producing plant species (Table 1.1) are found in several monocotyledonous and dicotyledonous families, including Alliaceae, Fabaceae, and Asteraceae. However only one inulin containing plant species, (Chicory, *Cichorium intybus*), is used to produce inulin industrially (Roberfroid, 2000; Franck, 2002). In this study, a further 22 plants were explored for inulin containing properties.

Table 1.1: Plants containing high amounts of inulin

Scientific name	Family	English name
Taraxacum officinale	Asteraceae	Dandelion
Dioscorea sp.	Dioscoreaceae	Wild yam
Helianthus tuberosus	Asteraceae	Jerusalem artichokes
Cichorium intybus	Asteraceae	Chicory
Pachyrhizus erosus	Fabaceae	Jicama
Arctium lappa	Asteraceae	Burdock
Allium cepa	Alliaceae	Onion
Allium sativum	Alliaceae	Garlic

Agave sp.	Agavaceae	Agave
Asparagus officinalis	Asparagaceae	Asparagus
Dahlia sp.	Asteraceae	Dahlia

[¤]van Loo *et al*., 1995

1.5.1 Overview of plants used in this study

The rationale for selecting these particular plants is that a number of plants from the families Asparagaceae, Alliaceae, and Asteraceae growing in the Northern Hemisphere (America) are known to contain inulin (van Loo *et al*., 1995).

Other leafy vegetables from the families Solanaceae, Asteraceae, Cucurbitaceae, Apiaceae, Acanthaceae, Pedaliaceae, Chenopodiaceae, Fabaceae, Amaranthaceae, Polygonaceae and Portulaceae are consumed regularly in Kwa-Zulu Natal (South Africa), and could be a potential source of inulin. The plants used in this study are illustrated in figures 1.2-1.23.

Previously (Hedrick, 1972; Odhav *et al*., 2007), the carbohydrate content of these plants was reported and these values are shown in Table 1.2.

Table 1.2: Carbohydrate content of selected indigenous plants

Scientific Name	**Family**	**English Name**	**Zulu Name**	**Carbohydrate content (grams per 100 g)[¤]**
Solanum nigrum	Solanaceae	Common nightshade	Umsobo	9.03
Physalis viscosa	Solanaceae	Grapeground cherry	Uqadolo	9.81
Momordica balsamina	Cucurbitaceae	Balsam apple	Inkaka	6.82
Amaranthus spinosus	Amaranthaceae	Prickly amaranth	Imbuya	1.16
Amaranthus hybridus	Amaranthaceae	Cocks-comb	Imbuya	6.09
Amaranthus dubius	Amaranthaceae	Wild spinach	Imbuya	7.86
Asystasia gangetica	Acanthaceae	Hunter's spinach	Isihobo	8.27
Justicia flava	Acanthaceae	Yellow justicia	Impela	8.77
Emex australis	Polygonaceae	Cape spinach	Inkunzane	2.73
Oxygonum sinuatum	Polygonaceae	Double thorn	Umkandu	2.85
Bidens pilosa	Asteraceae	Blackjack	Ilenjane	3.72
Galinsoga parviflora	Asteraceae	Gallant soldier	Ushukeyana	5.29

Portulaca oleracea	Portulacaceae	Purslane	Amadilika	2.65
Senna occidentalis	Fabaceae	Coffee senna	Isinyembane	9.37
Chenopodium album	Chenopodiaceae	Fat hen	Imbikilicane	8.34
Ceratotheca triloba	Pedaliaceae	Wild foxglove	Udonqa	8.28
Centella asiatica	Apiaceae	Marsh pennywort	Icukudwane	3.81
Asparagus sprengeri	Asparagaceae	Asparagus fern	Isigoba	*
Tulbaghia violacea	Alliaceae	Society garlic	Isihaqa	*
Sonchus oleraceus	Asteraceae	Sowthistle	*	45
Taraxacum officinale	Asteraceae	Dandelion	*	9.2
Cleome monophylla	Capparidaceae	Spindlepod	Isiwisaesilu-hlaza	3.40

* Not recorded
▫ Hedrick, 1972; Odhav *et al*., 2007

Solanum nigrum (Fig.1.2) belongs to the family Solanaceae. It is used as an antiphlogistic, diaphoretic, diuretic, emollient, antiperiodic, febrifuge, narcotic, purgative, sedative and as a poultice in treatment of cancerous sores, leukoderma and wounds. Its toxicity is not clear but the green berries contain solanine which has been associated with poisoning. It is regularly consumed by the local community who use the leaves as potherbs and as a relish. The fruit of this plant is a delicacy (Beekrum, 2003).

Fig. 1.2 *Solanum nigrum*

Physalis viscosa (Fig. 1.3) also belongs to the family Solanaceae. The plant is used as a tonic, laxative, diuretic and a sedative. The juice of the berries is beneficial in several urinary disorders and in inflammatory diseases. No toxicity data for this plant is reported. Although this plant is regarded as a famine food, in the Transkei region the leaves, fruit and berries are edible (Beekrum, 2003).

Fig. 1.3 *Physalis viscosa*

Momordica balsamina (Fig. 1.4), which belongs to the family Cucurbitaceae, is used for liver disorders, blood cleansing, ulcers of the stomach and duodenum, inflammations, insomnia, marsh fever, urinary tract infections, and for bile disorders. It is also used as a purgative, emetic, bitter stomachic and as a wash for fever and yaws. No toxicity reports are available. The leaves and the ripe fruit of *M. balsamina* are consumed regularly amongst the African population. It is cooked as spinach, with nuts or maize meal, and is also used as a relish in soup (Beekrum, 2003).

Fig. 1.4 *Momordica balsamina*

*Amaranthus spinosus (*Fig. 1.5) belongs to the family Amaranthacaeae. It is used as an astringent, a diaphoretic agent, a diuretic, an emollient, a febrifuge and as a purgative. It is also used to treat snakebites, ulcerated mouths, vaginal discharges, nosebleeds, and wounds. No members of this genus are known to be poisonous. It is usually consumed raw or cooked as spinach (Beekrum, 2003).

Fig. 1.5 *Amaranthus spinosus*

Amaranthus hybridus (Fig. 1.6) belongs to the family Amaranthacaeae. It is used for the treatment of intestinal bleeding, diarrhoea, and excessive menstruation. No members of this genus are known to be poisonous. It is used as a relish, and is consumed by mixing it with maize meal. It is also cooked as spinach, added to soups, or eaten raw. Tea made from leaves is an astringent (Beekrum, 2003).

Fig. 1.6 *Amaranthus hybridus*

Amaranthus dubius (Fig.1.7) belongs to the family Amaranthacaeae and is a popular spinach. These species are harvested in summer as many times as possible. They are not stored for later use and are therefore cooked immediately after harvesting. They are used as potherbs, cooked and eaten as spinach. Seeds are nutritious. The whole plant is used to alleviate stomach pains. There are no known toxicity effects (Beekrum, 2003).

Fig. 1.7 *Amaranthus dubius*

Asystasia gangetica (Fig. 1.8) belongs to the family Acanthaceae. It is indigenous to Africa and is rarely consumed. It is used to ease childbirth pains, facilitate labour, stiff neck, nose bleeding, stomach aches, fever, aches, epilepsy, heart pains and urethral discharge. This plant is not toxic (Beekrum, 2003).

Fig. 1.8 *Asystasia gangetica*

Justicia flava (Fig. 1.9) belongs to the family Acanthaceae. It is semi-cultivated and eaten as a vegetable. It is used to treat stomach ache and diarrhoea and is also used to treat fevers and yaws. Leaves are used as emetics and eye lotions. No toxicity reports are available (Beekrum, 2003).

Fig. 1.9 *Justicia flava*

Emex australis (Fig. 1.10) belongs to the family Polygonaceae. It is widespread in South Africa and available throughout the year; therefore, there is no need to store this plant. It is used to treat gastrointestinal disorders, colic, biliousness and dyspepsia. It contains oxalic acid, which can be toxic. It is cooked as spinach (Beekrum, 2003).

Fig. 1.10 *Emex australis*

Oxygonum sinuatum (Fig. 1.11) belongs to the family Polygonaceae. The leaf sap is used for cough and bronchial complications. It is used for gastric ulcers, malaria and hepatitis. No toxicity data of this plant is available (Beekrum, 2003).

Fig. 1.11 *Oxygonum sinuatum*

Bidens pilosa (Fig. 1.12) belongs to the family Asteraceae. The leaves of these plants are harvested in summer by the local community and stored during the winter months. Leaves have anti-inflammatory, styptic, and anti-rheumatic properties. Substances isolated are bactericidal and fungicidal. A juice made from the leaves is used to dress wounds. Some compounds from this plant are phototoxic. Substances isolated from leaves can cause burns on human skin in the presence of sunlight at concentrations as low as 10 ppm. It is used as a potherb, tea, salads, soups, and stews. It is also used as relish, with peanut butter (Beekrum, 2003).

Fig. 1.12 *Bidens pilosa*

Galinsoga parviflora (Fig. 1.13) belongs to the family Asteraceae. When rubbed onto the body, the plant is useful in treating nettle stings. An extract of the fresh leaves is used to dress wounds and cuts. No toxicity reports are available. The leaves are eaten as a potherb, raw or cooked and as flavouring in soups (Beekrum, 2003).

Fig. 1.13 *Galinsoga parviflora*

Portulaca oleracea (Fig 1.14) belongs to the family Portulacaceae and is either consumed raw or cooked by the local community. It is eaten as a salad or vegetable. The whole plant is used to treat bacillary dysentery, diarrhoea, haemorrhoids and enterorrhagia. It has antibacterial, antiscorbutic, depurative, diuretic and febrifuge properties. This plant contains oxalic acid which causes in coordination of gait and tetanic conditions in sheep (Beekrum, 2003).

Fig. 1.14 *Portulaca oleracea*

Senna occidentalis (Fig. 1.15) belongs to the family Fabaceae and is considered as a famine food by the local community. Young leaves are eaten and the roasted seed is used as a coffee substitute. It is used for stomach pains, biliousness, fevers, jaundice, ringworms, sore throats and wounds. Dried leaves are used for lumbago and haemorrhoids whilst fresh leaves are used to treat eczema, abscesses and skin diseases. Un-roasted seeds are toxic and leaves are toxic if large quantities are consumed (Beekrum, 2003).

Fig. 1.15 *Senna occidentalis*

Chenopodium album (Fig 1.16) belongs to the family Chenopodiaceae. It is cooked as spinach, and is often added to maize meal and eaten as porridge. Seeds are dried, ground into a meal and used as gruel, or eaten raw when added to salads. Leaves have antihelminthic, antiphlogistic, anti-rheumatic, mildly laxative and odontalgic properties. It is applied as a wash or poultice to bug bites, sunstroke, rheumatic joints and swollen feet. It contains small amounts of saponins. Cooking the plant reduces the oxalic acid content (Beekrum, 2003).

Fig. 1.16 *Chenopodium album*

Ceratotheca triloba (Fig. 1.17) belongs to the family Pedaliaceae and has an unpleasant scent when harvested. However, when cooked the scent disappears. It is sweet tasting and is used as relish or spinach. It is used in the treatment of painful menstruation, stomach cramps, nausea, fever and diarrhoea. It is also used to relieve gastric disorders. It is not toxic (Beekrum, 2003).

Fig. 1.17 *Ceratotheca triloba*

Centella asiatica (Fig. 1.18) belongs to the family Apiaceae. This plant is cooked by the local community as spinach and eaten with maize meal. Leaves are dried and used as famine food. It is also used in salads and curries. It is a diuretic herb that clears toxins, reduces inflammations and fevers, improves healing and immunity, improves the memory and has a balancing effect on the nervous system. It is used in the treatment of wounds, chronic skin conditions, venereal diseases, malaria, varicose veins, ulcers, nervous disorders and for the treatment of senility. In large doses, this plant is a stupefying narcotic, sometimes producing cephalagia or vertigo with a tendency to lapse into a coma (Beekrum, 2003).

Fig. 1.18 *Centella asiatica*

Asparagus sprengeri (Fig. 1.19) is a member of the family Asparagaceae, the origin of Asparagus is believed to be the eastern Mediterranean region. However, it grows wild in Europe, the Caucasus and western Siberia, and occurs now worldwide as a crop plant. In Africa Asparagus is mainly grown in southern Africa (South Africa, Lesotho) and North Africa (Tunisia), whereas in tropical Africa it is found in the highlands of eastern Africa (Kenya, Uganda, Zimbabwe), (Nichols, 2004).

The major product of Asparagus is the tender young expanded shoots (spears) which are eaten lightly cooked. The spears are also processed either by canning (or bottling) in brine or by deep-freezing. They are harvested prior to emergence as white Asparagus, or after emergence when 18–25 cm tall as green Asparagus (Benson, 1999).

Figure 1.19 *Asparagus sprengeri* tuber

Tulbaghia violacea, (Fig. 1.20) a member of the family Alliaceae occurs in Malawi, Botswana, Zimbabwe and Mozambique; also in South Africa, Swaziland and Lesotho. *T. violacea* comprises 22 species and is confined to southern Africa, north to Tanzania and Angola.

In Zimbabwe and South Africa the leaves of *T. violacea* are cooked as a relish, alone or with leaves of other plants. The rhizome is scraped clean and boiled with meat in stews or roasted as a vegetable. Young leaves are chopped and used to flavour soups, stews, pickles and omelettes as a substitute for shallot. In South Africa the bruised rhizome is used in baths for the relief of fever, rheumatism or paralysis. Small doses are used as a laxative (van der Burg, 2004).

Figure 1.20 *Tulbaghia violacea*

Sonchus oleraceus, (Fig. 1.21) a member of the family Asteraceae, is native to Eurasia and northern Africa. It is currently a cosmopolitan weed. *Sonchus* comprises about 60 species, of which 17 have been recorded in tropical Africa (Schippers, 2004).

Throughout Africa, the primary use of *S. oleraceus* is as a cooked leafy vegetable, but it is also eaten raw. The tender leaves are eaten as a salad and some people also eat the juicy root. In Uganda the Langi people first dry the leaves and later boil and mash them to be added to beans or made into a sauce that is eaten with a staple food. The leaves are said to clear infections, are used as a sedative, stomachic, diuretic and to treat liver diseases, including hepatitis. *S. oleraceus* has the highest carbohydrate content (45 g/100 g) (Hedrick, 1972) of all the plants studied, as can be seen in Table 1.2.

Figure 1.21 *Sonchus oleraceus*

Taraxacum officinale (Fig. 1.22) (common Dandelion) a member of the family Asteraceae, is believed to be native to Europe however it is naturalised in many parts of the world.

Dried and ground roots are used for non-caffeinated, coffee-like beverages, as a flavouring agent in coffee and cocoa, and as an addition to salad dishes. Dandelion wine can be made from the leaves and flower heads. Young, tender leaves are used in salads and soups.

The bitter plant resin found in both roots and above-ground parts contains taraxacin, taraxerin, taraxerol, taraxasterol, inulin, gluten, gum, potash, choline, levulin, and putin. The plant itself is nutritious, being high in vitamins A, C, and niacin (Simon *et al*., 1984).

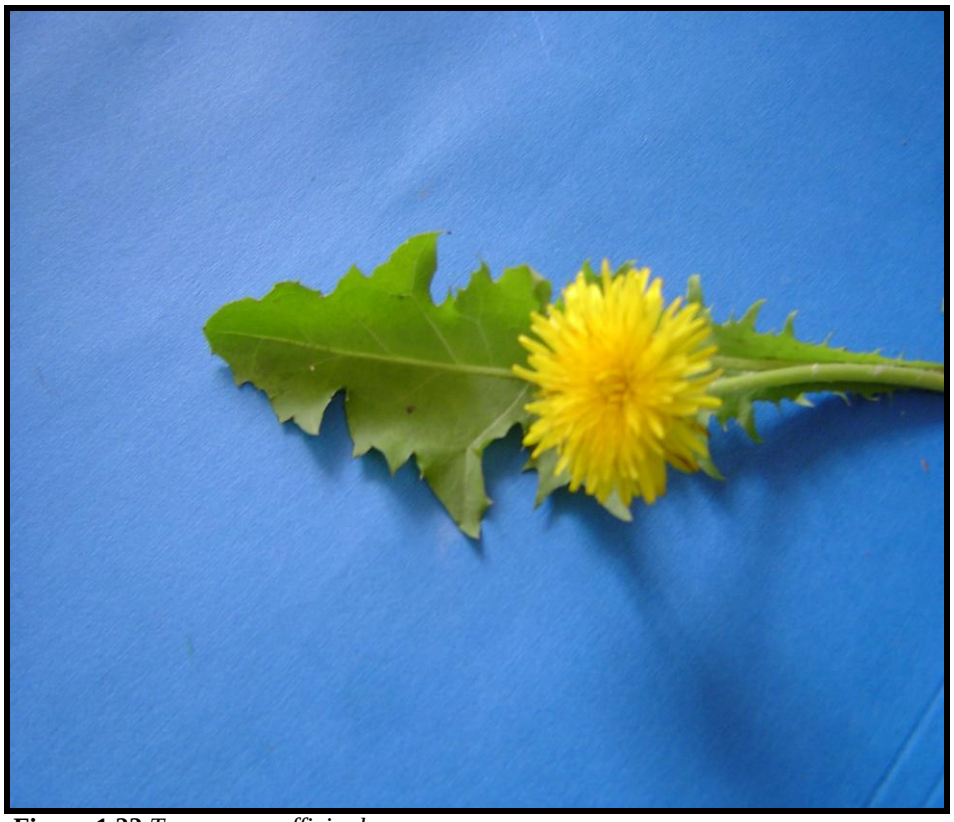

Figure 1.22 *Taraxacum officinale*

Cleome monophylla (Fig 1.23) belongs to the family Capparidaceae. The leaves are cooked as spinach and the pungent seeds are used as a mustard substitute. The pounded root is put on the lips to restore consciousness when in a faint (restorative properties). The flowers are hermaphrodite (has both male and female organs) and for optimum growth, this plant requires light (sandy) and well drained soils (Huxley, 1992).

Fig. 1.23 *Cleome monophylla*

1.5.2 Inulin in plants

The most prominent storage carbohydrate in the plant kingdom is starch; nevertheless, fructan (a fructose polymer) is used as a storage compound in approximately 15% of flowering plant species (van Loo *et al*., 1999). Unlike starch, fructans are water-soluble and are believed to be localised in the vacuole (Darwen & John, 1989; Wiemken *et al*., 1986).

Fructans occur in many economically important crops, for example, Asparagus, Onion, Chicory, Wheat, Rye, Oats, and grasses, but are absent in species such as Tobacco and Potato. The focus of this study was the inulin-type of fructans that consist of linear β-2-1 linked fructofuranosyl units found in Asteraceae species such as Chicory, *Sonchus oleraceus* and *Taraxacum officinale* (Dandelion) (Michiels *et al*., 2004; Bogacheva *et al*., 1999). Plant fructans have many different structures and chain lengths, ranging from three up to a few hundred fructose units.

1.5.2.1 Inulin production in plants

In inulin producing plants, fructan is synthesised from sucrose by the action of two or more different fructosyltransferases. According to the classical model of Edelman & Jefford (1968), two enzymes are involved in the synthesis of the most simple form of fructan, inulin. The first enzyme, sucrose 1-fructosyl transferase (1-SST), initiates *de novo* fructan synthesis by catalysing the transfer of a fructosyl residue from sucrose to another sucrose molecule, resulting in the formation of the trisaccharide, 1-kestose (G1-2F1-2F) (Vijn & Smeekens, 1999).

The second enzyme, fructan: fructan 1-fructosyl transferase (1-FFT), transfers fructosyl residues from a fructan molecule with a DP of ≥3, to another fructan molecule or to sucrose. The action of 1-SST and 1-FFT results in the formation of a mixture of fructan molecules with different chain lengths (van den Ende *et al*., 2000).

Fructans are often stored in specialised organs, for example in the taproot of Chicory (*Cichorium intybus*), the tubers of Dahlia (*Dahlia variabilis*), and the bulbs of Tulip (*Tulipa gesneriana*) and Onion (*Allium cepa).*

1.6 Metabolism of inulin by Lactobacilli and Bifidobacteria

1.6.1 Lactobacilli

Data on the growth of members of the colonic microbiota, other than bifidobacteria, on inulin-type fructans have been reported (Duncan *et al*., 2003). It has been demonstrated that certain *Lactobacillus* strains, including strains of *Lactobacillus acidophilus* and *Lactobacillus paracasei*, are able to grow on these prebiotics (Cebeci & Gurakan, 2003). Further, an operon involved in oligofructose utilisation has been described for *L. acidophilus* NCFM (Barrangou *et al*., 2003).
Some *in vivo* studies with animal models or clinical trials have demonstrated an increase of the number of lactobacilli when inulin-type fructans are applied (Kleessen *et al*., 2001), but in other reports the number of lactobacilli remains stable after administration of such prebiotics (Gibson *et al*., 1997).

Among the lactobacilli, *L. lactis* is considered an important probiotic. Despite the interest in *L. lactis* as a probiotic, little is known about the physiology and genetics of this microorganism. Lactobacilli are extremely fastidious organisms, adapted to complex organic substrates. They require not only carbohydrates as an energy and carbon source, but also nucleotides, amino acids, and vitamins for their growth in a defined medium (Hebert *et al*., 2000; 2004).

The overall activity of this bacterial group enhances the health of their host. Lactobacilli utilise carbohydrates by lactic acid fermentation, which constitutes their main metabolic trait. This bacterial group is also generally involved in peptide and amino acid metabolism by means of a complex set of peptidases and amino acid converting enzymes (Hammes & Hertel, 2002). The need for essential minerals, such as manganese and magnesium, has also been documented (Loubiere *et al*., 1997).

Magnesium and manganese are recognised as important factors for significant growth of both *L. bulgaricus* and *L. lactis*. A combination of magnesium and manganese in the growth medium greatly enhanced glutamate uptake and stimulated the growth of *L. bulgaricus* and *L. lactis* (de Giori *et al*., 2002).

In vitro fermentation of inulin by human colonic bacteria, mainly lactobacilli and bifidobacterium, produces lactate and short chain carboxylic acids, mostly acetate. Consequently, the bacterial metabolism of these substrates causes a marked decrease in the culture medium pH (Trojanova *et al*., 2004).

Lactobacilli show an absolute requirement for essential nutrients, including amino acids arginine, glutamic acid, isoleucine, leucine, tryptophan, tyrosine, cysteine, and valine (Hebert *et al*., 2000; Loubiere *et al*., 1997). Frequently, their transport occurs across the cell membrane against concentration gradients using an active system consisting of coupling of the carrier to the metabolic machinery. General models have been proposed, which involve chemiosmotic coupling to ion gradients or direct chemical coupling of the transport to ATP or intermediates of oxidative phosphorylation (Akiyama *et al*., 2001).

The 1.2-and 2.6-β-linkages making up inulin are resistant to mammalian digestive enzymes, such as the disaccharidases (sucrase, maltase, isomaltase, or lactase) of intestinal mucosa and alpha amylase of pancreatic homogenates (Oku *et al*., 1984). Inulin reaches the colon virtually un-altered. The fermentation of fructooligosaccharides has been studied in some detail (Rastall & Maitin, 2002).

The current paradigm is that such bacteria possess inducible cell associated β-fructofuranosidases which liberate monomeric fructose molecules that are then transported into the bacterial cell (Gibson, 2004). Endo and exoinulinases hydrolyse linear β-2-1-linked fructose polymers of inulin initiated by a glucose unit. Most inulinases are β-fructosidases and split-off fructose moieties from the non-reducing end of the inulin molecule or from certain sugars displaying a fructose unit at the terminal β-2-1-position (Molina *et al*., 2005).

These enzymes can also be denominated as 2.1-β-D-fructan-fructano hydrolases. Inulinases with β-fructosidase activity are encountered in plants and microbes (Dysseler & Hoffem, 1995). Probiotics have highly active intracellular 2.1.β-D-fructan-fructohydrolase enzymes and are known to produce inulinase enzymes when growing on fructooligosaccharides, such as inulin (McKellar *et al*., 1993).

During the fermentation of inulin, energy is provided for bacterial proliferation and cell mass, and, mainly CO_2, medial H_2 and relatively no CH_4 is produced (Wang & Gibson, 1993). In addition to these fermentation products, short chain fatty acids, acetate, propionate and butyrate are also formed along with L-+-lactate (Hartemink & Rombout, 1997).

1.6.2 Bifidobacteria

It is well known that sugar metabolism by lactic acid bacteria is species and even strain dependent (Poolman, 1993). Many differences in prebiotic fermentation patterns are described for lactic acid bacterial strains, in particular for probiotic bifidobacterium strains (Bielecka *et al*., 2002; Crittenden *et al*., 2002; Hopkins *et al*., 1998). However, data on the kinetics of bifidobacteria grown on inulin type fructans is scarce (Durieux *et al*., 2001; Hopkins *et al*., 1998).

The human large intestine is a complex ecosystem in which several hundreds of different bacterial species reside (Gibson *et al*., 1997). This microflora metabolises non digestible carbohydrates such as inulin to a variety of products such as short chain fatty acids (e.g. acetic acid, propionic acid and butyric acid), other organic acids (e.g. lactic acid, succinic acid and pyruvic acid), and gases (e.g. H_2, H_2S and CO_2).

Bifidobacteria are a predominant group of colonic microflora that can account for up to 25% of the total number of bacteria present (Macfarlane & Macfarlane, 1997). Due to their heterofermentative nature, bifidobacteria can produce lactic acid and ethanol as well as several short chain fatty acids such as acetic and formic acids. Some investigators also mention the production of small amounts of carbon dioxide and succinic acid by bifidobacterium strains (Ballongue, 1998).

The most common prebiotic for bifidobacteria is inulin (Playne & Crittenden, 1996). This prebiotic stimulates the specific growth of bifidobacteria, which is the so called bifidogenic effect (Roberfroid *et al*., 1998) but strain and species differences occur in bifidobacterial carbohydrate utilisation patterns (Crittenden *et al*., 2002; Hopkins *et al*., 1998).

Inulin and oligofructose are two examples of fructans of the inulin type. The β (2-1) linkages of these fructans prevent their digestion in the upper part of the gastrointestinal tract and are responsible for their reduced calorific value and dietary fibre like effects (Roberfroid *et al*., 1998). Once they arrive in the colon, these fructans are selectively metabolised by bifidobacteria and lactobacilli producing β-fructofuranosidases that hydrolyse these bonds. However, the mechanism of hydrolysis for lactobacilli and bifidobacteria differs.

1.6.2.1Genomic adaptation of *B. longum* NCC 2705 for utilisation of a diversity of complex carbohydrates (Schell *et al*., 2006)

Schell & colleagues (2006) most striking observation was that *B. longum* has an excessive number of genes associated with oligosaccharide metabolism, comprising >8% of the genome. The amplification of some of these by gene duplication and apparent horizontal acquisition of others suggests that *B. longum* has been subjected to a strong environmental pressure to amplify the level and diversity of its metabolic capabilities, perhaps in response to competition for varied substrates in the gastrointestinal tract ecosystem.

The apparent absence of pectinases, cellulases, and α- and β-amylases in *B. longum* contrasts sharply with its numerous other glycosyl hydrolases. These sometimes novel glycosyl hydrolases appear to attack a wide spectrum of heterogenous, less common linkages found in plant polymers such as hemicelluloses, arabinogalactans, arabinoxylans, gums, inulins, galactomannans, and branched starches (limit dextrins).

This observation substantiates previous studies of nutrient utilisation by bifidobacteria that foreshadowed this extensive ability. The persistence of *B. longum* in the colon may result from its adaptation to catabolise the substrates that are poorly digested by the host or other gastrointestinal tract microorganisms, which instead focus on utilisation of sugars and the more abundant uniform polymers like pectins and linear starch.

Interestingly, previous work showed that high molecular weight carbohydrate concentrations were lower in the colon than in the upper located ileum, implying that complex carbohydrates are largely broken down in the colon. Consistent with this proposal, *B. longum* also has numerous

high-affinity MalEFG-type oligosaccharide transporters, but only one PTS-type sugar transporter, the more common type of carbohydrate transporter in *E. coli*, and other less dominant GI tract bacteria.

Chapter Two:

Materials and Methods

CHAPTER TWO: MATERIALS AND METHODS

Introduction

This section describes the methods that were used to investigate plants that could have a prebiotic effect on *Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus reuteri* and *Bifidobacterium longum*. The experiments involved testing the effect of the aqueous extracts of twenty two plants on the growth of the above probiotic cultures in MRS broth, over a 96 hour period. Further analyses involved the determination of the inulin content of the plants using HPLC. Statistical analysis was used to correlate the relationship between the prebiotic effect of the plants and their inulin content.

The plants used in the study were selected based on the information gathered from reviewing literature. A number of these plants are mainstays in the diets of rural and urban households across most of Africa, including South Africa and are also used as traditional medicines (Fennell *et al*., 2004; Gockowski *et al*., 2003; Maundu *et al*., 1999).

All the reagents used in this study were of analytical grade and were purchased from Sigma (Germany) and Merck (Darmstadt, Germany). The ROGOSA agar medium (Appendix 3) and MRS broth (Appendix 1) used were purchased from Merck (Darmstadt, Germany) and Biolab, Merck (Gauteng). *L. lactis* and *L. bulgaricus* were obtained from the Durban University of Technology (DUT) culture collection, whilst *L. reuteri* and *B. longum* were isolated from Reuteri™ and Probiflora™-two commercially available probiotic supplements.

2.1 Collection and preparation of plant material

The twenty two plants used in this study were identified by a botanist, Professor H. Baijnath of the School of Botany and Zoology, University of Kwa-Zulu Natal (Westville Campus) using taxonomic keys. The plants were brought to the laboratory in refuse bags and were photographed on arrival. The plants are illustrated in Chapter One, Figures 1.2 to 1.23. Pertinent details of each of the plants i.e., the scientific name, family, English and Zulu names, location and plant parts used are listed in Table 2.1.

Table 2.1: Details of plants analysed

Scientific Name	Family	English Name	Zulu Name	Location	Part used
Solanum nigrum	Solanaceae	Common nightshade	Umsobo	Reservoir Hills	Leaf
Physalis viscosa	Solanaceae	Grapeground cherry	Uqadolo	Park Rynie	Leaf
Momordica balsamina	Cucurbitaceae	Balsam apple	Inkaka	National Botanical Institute, Durban	Leaf
Amaranthus spinosus	Amaranthaceae	Prickly amaranth	Imbuya	Reservoir Hills	Leaf
Amaranthus hybridus	Amaranthaceae	Cockscomb	Imbuya	Reservoir Hills	Leaf
Amaranthus dubius	Amaranthaceae	Wild spinach	Imbuya	Reservoir Hills	Leaf
Asystasia gangetica	Acanthaceae	Hunter's spinach	Isihobo	Reservoir Hills	Leaf
Justicia flava	Acanthaceae	Yellow justicia	Impela	Reservoir Hills	Leaf
Emex australis	Polygonaceae	Cape spinach	Inkunzane	Reservoir Hills	Leaf
Oxygonum sinuatum	Polygonaceae	Double thorn	Umkandu	Reservoir Hills	Leaf
Bidens pilosa	Asteraceae	Blackjack	Ilenjane	Reservoir Hills	Leaf
Galinsoga parviflora	Asteraceae	Gallant soldier	Ushukeyana	Reservoir Hills	Leaf
Portulaca oleracea	Portulacaceae	Purslane	Amadilika	Verulam	Leaf
Senna occidentalis	Fabaceae	Coffee senna	Isinyembane	Reservoir Hills	Leaf
Chenopodium album	Chenopodiaceae	Fat hen	Imbikilicane	Reservoir Hills	Leaf
Ceratotheca triloba	Pedaliaceae	Wild foxglove	Udonqa	Reservoir Hills	Leaf
Centella asiatica	Apiaceae	Marsh pennywort	Icukudwane	Reservoir Hills	Leaf
Asparagus sprengeri	Asparagaceae	Asparagus fern	Isigoba	Ex-horticulture	Tuber
Tulbaghia violacea	Alliaceae	Society garlic	Isihaqa	Ex-horticulture	Leaf & Bulb
Sonchus oleraceus	Asteraceae	Sowthistle	*	Chatsworth	Leaf & Root
Taraxacum officinale	Asteraceae	Dandelion	*	Chatsworth	Leaf & Root
Cleome monophylla	Capparidaceae	Spindlepod	Isiwisa esiluhlaza	Reservoir Hills	Leaf

* Not recorded

Two kilograms of fresh healthy looking leaves were harvested from all the plants except for *Asparagus sprengeri* for which 500 g of tubers were harvested, and roots and leaves were harvested from *Sonchus oleraceus* and *Taraxacum officinale*, and leaves and bulbs were harvested from *Tulbaghia violacea*.

The harvested material was placed in trays in a 30°C oven with built in extractor fan at the University of Kwa-Zulu Natal (Westville Campus) until they had dried. The dried material was then milled using a Salton blender to obtain a powder-like texture and stored in glass bottles in a dark cupboard.

Aqueous extraction of compounds was carried out according to the procedure outlined by Trojanova & co-workers (2004), with slight modifications. Dried ground plant material (1 g) was stirred in 5 ml of distilled water (85°C) for 20 minutes. The solution was then filtered with a Whatman no. 1 filter paper. For HPLC, the extract was cooled to 60°C and used immediately and for batch culture experiments, the extract was cooled before use.

2.2 Bacterial Cultures

Lactobacillus lactis (Culture collection, DUT), *Lactobacillus bulgaricus* (Culture collection DUT), *Lactobacillus reuteri* (ATCC 55730) and *Bifidobacterium longum* (Rosell-175 ME) were used for testing all the plant extracts.

Lactobacillus lactis and *Lactobacillus Bulgaricus* were obtained from the Durban University of Technology culture collection.

Lactobacillus reuteri (ATCC 55730) was isolated from Reuteri™ (Ithebe Pharmaceuticals, Claremont, South Africa). One drop of the Reuteri™ suspension was inoculated on ROGOSA agar plates and incubated aerobically at 37°C for 48 hours. After incubation, the identification of *L. reuteri*, which is a gram positive, non spore forming rod shaped organism was confirmed via morphological characteristics and microscopy (gram stain).

Bifidobacterium longum (Rosell-175 ME) used in this study was isolated from Probiflora™ (NutriLida Health Care, Johannesburg, South Africa). One Probiflora™ tablet was dissolved in 10 ml of sterile distilled water. After the tablet had dissolved, a loop full of the suspension was inoculated on ROGOSA agar plates and incubated anaerobically at 37°C for 48 hours. After incubation, the identity of *B. longum*, which is a gram positive, pleomorphic rod shaped organism was confirmed via morphological characteristics and microscopy (gram stain).

All probiotic strains were stored in Microbanks (Pro-Lab Diagnostics, Toronto, Canada) according to the manufacturer's instructions at -80°C. When cultures were needed one bead was removed and plated out on a ROGOSA agar plate. *L. bulgaricus*, *L. lactis* and *L. reuteri* were grown aerobically at 37°C whilst *B. longum* was grown anaerobically at 37°C in an anaerobic jar (Anaerocult® A, Merck, Darmstadt, Germany).

2.3 Evaluation of the effect of the extracts on the growth of selected probiotics using a Batch culture Technique

This was carried out according to the procedure outlined by Molina & co-workers (2005), with slight modifications.

MRS broth (Biolab; Merck, Gauteng) (5 ml) was inoculated with 5 loops-full of either *L. bulgaricus*, *L. reuteri*, *L. lactis* or *B. longum* and incubated overnight at 37°C under aerobic conditions for Lactobacilli and anaerobic conditions for Bifidobacteria.

The overnight culture was standardised to 0.8 nm @ 620 nm using a Varian-Carey UV-Vis spectrophotometer by diluting the culture (if necessary) with sterile MRS broth until the desired absorbance (sufficient to give a concentration of 10^5 cfu/ml at the beginning of the experiment) was obtained.

MRS broth (195 ml) was prepared in a flask. Five millilitres of plant extract was added to the MRS broth and mixed thoroughly. A positive control made up of 1 g Chicory inulin (Sigma; Germany) dissolved in 5 ml of distilled water, and a negative control with 5 ml MRS broth was also used. Twenty five millilitres of each was transferred to five sterile 100 ml Schott bottles.

Each bottle was then inoculated with 250 µl of the test organism, capped, mixed and incubated either aerobically or anaerobically at 37°C.

The effect of the extracts on the growth of the selected probiotics was evaluated at 0, 24, 48, 72 and 96 hours by removing one bottle and testing 1 ml for total number of bacteria. Bacteria were enumerated by serially diluting to 10^{-7} (in triplicate) on ROGOSA agar plates using the spread plate technique. The plates were then incubated either aerobically or anaerobically at 37°C for 48 hours and the colony forming units (CFU) were counted using a colony counter.

Plates containing between 30 and 300 cfu/ml were counted. The average of three replicates and their standard deviation was then calculated. Those plates with less then 30 cfu/ml were considered too few to count (TFTC) whilst those with more then 300 cfu/ml were considered too numerous to count (TNTC).

The total growth/growth response obtained over the 96 hour period was calculated using the area under the curve (AUC). Plants that performed better than the median for the negative control were regarded as having a prebiotic effect.

2.3.1 Statistical analysis

The AUC was calculated for all the samples using the Trapezoidal rule. The data was given as average CFU at discrete time points from Time 0 hours to 96 hours. Each time segment is considered a trapezoid and the area is given by the segment width and the average number of CFU within the segment width. The total area under the curve is the sum of the areas of the individual segments.

To make sure that the bacteria were comparable, the percentage change from baseline in number of bacteria was calculated, and this percentage change was used in the calculation of the AUC.

Correlation analysis was used to describe the degree of strength by which one variable is linearly related to another (inulin content of the plant extracts related to the prebiotic effect). This measure is based on a scale between -1 and +1. If an inverse relationship exists, then Pearson's

coefficient of correlation (*r*) will fall between 0 and -1. If there is a direct relationship, then *r* will fall between 0 and +1. If there is no relationship between the two variables, then *r*=0.

The Wilcoxon rank sum test was used to determine whether there was a significant difference ($p<0.05$) between the median AUC of the plant extracts and the medians of the positive and negative controls.

2.4 Quantification of inulin

Inulin content of the extracts was determined using the procedure outlined by Vendrell-Pascuas & colleagues (2000), using HPLC.

It is generally accepted that the most quantitative method for the measurement of inulin-type fructans, involve enzymatic hydrolysis of all the fructan materials to glucose and fructose, followed by the measurement of these sugars by HPLC. This method involved hydrolysis of the extract with an inulinase enzyme, and determination of the released fructose by HPLC. The method incorporated a sample blank (without inulinase hydrolysis) from each sample to subtract contributions of free fructose and fructose from sucrose.

2.4.1 Preparation of the extract

After filtration (Whatman no. 1), the extract was cooled to 60°C and 2 ml of the extract was removed for analysis. This was then divided into two aliquots of 1 ml each. To one tube, 200 µl of Inulinase enzyme (Sigma) (17.5 units/ml) was added, and to the second tube, 200 µl of distilled water was added. These tubes were then incubated in a shaking water bath at 50°C for 30 min for total digestion of inulin.

The hydrolysed extracts were then cooled to room temperature and passed through a C_{18} Sep-Pak Plus cartridge (Waters Corp. Wilford, Mass USA), previously conditioned with 10 ml methanol (HPLC grade) and 10 ml water (MillQ. R.G. Millipore, USA). The filtered extracts were then transferred to 1.5 ml eppendorf tubes and stored in darkness at -20°C until HPLC analysis was performed. All the samples were filtered through a 0.45 µm nylon filter (Scheicher & Schuell, Germany) before injection into the HPLC system.

2.4.2 HPLC Analysis

2.4.2.1 Instrumentation

HPLC was conducted using a Model L-7100 pump system, and a Model L-7200 autosampler (injector with 20 µl sample loop) a Model L-7490 refractive index detector, and a D-7000 Multi-HSM manager which acquired data from the refractive index detector. The analytical column used was a Li Chrospher® NH_2 (250x4 mm, 5 µm particle size). All were from Merck (Darmstadt, Germany).

2.4.2.2 Chromatographic conditions

Chromatographic separation was achieved with a mobile phase of acetonitrile–water (80:20, v/v). The flow-rate of the eluent was 1.8 ml/min. The volume of the sample injected was 20 µl (filling the loop completely); 15 min were needed to complete the analysis. All measurements were done at room temperature. Peak areas were used for quantitative analysis. Calibration curves were prepared between four levels from 100, 50, 25 and 12.5 mg/ml of fructose (Appendix 4), glucose (Appendix 6) and sucrose (Appendix 5) (HPLC grade) from Sigma (Steinham, Germany) in water.

2.4.2.3 Calculation and expression of results

The amount of inulin dietary fibre (IN) was calculated using the formula:

$$\%IN = [A \times (F_1 - F_2) / P] \times 100$$

for a sample containing no sucrose and ;

$$\%IN = [A \times (F_1 - F_2 - F_3) / P] \times 100$$

for a sample containing sucrose, with $F_3 = S/B$ where: F_1 is concentration of the total fructose (g/l); F_2 is concentration of the free fructose (g/l); F_3 is concentration of fructose from sucrose (g/l); S is concentration of sucrose (g/l); P is mean mass (g/l) of the test samples; A=1.03 (empirical conversion factor for fructose to inulin, obtained from different dilutions of inulin hydrolysed with Fructozyme enzyme and quantified using rhamnose as internal standard); B=2.13 (empirical conversion factor for fructose to sucrose, obtained from different dilutions of sucrose hydrolysed with Inulinase enzyme and quantified using rhamnose as internal standard).

Chapter Three:

Results

CHAPTER THREE: RESULTS

The effect of the plant extracts on the growth of *Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus reuteri* and *Bifidobacterium longum* was studied in MRS broth and also compared to inulin that is currently commercialised. The effect was studied using a Batch culture technique and the probiotic cultures were grown over a 96 hour period. Samples were taken at times 0, 24, 48, 72 and 96 hours (in triplicate) to enumerate the bacteria (measured in CFU). The overall increase in growth of the probiotics over the 96 hour period was confirmed statistically using the Trapezoidal rule. Further analyses involved the determination of the inulin content of the plants using HPLC. Statistical analysis was used to correlate the relationship between the prebiotic effect of the plants and their inulin content.

3.1 Effect of the plant extracts on the growth of the probiotics

3.1.1 Effect on *Lactobacillus lactis*

The growth of *L. lactis* over a period of 96 hours in the presence of the plant extract compared to when no plant extract was added (MRS broth only) is shown in figures 3.1 to 3.25 and is summarised in Table 3.1. Inulin stimulated the growth of *L. lactis*, as can be seen in figure 3.26.

L. lactis grew differently with each of the extracts over the 96 hour period and in some cases there was also an inhibitory effect as growth had decreased, as can be seen in Table 3.1. *Amaranthus dubius* (leaves) and *Galinsoga parviflora* (leaves) did not stimulate the growth of *L. lactis*. The growth obtained in the presence of these extracts was lower than the growth obtained in MRS broth only. *Physalis viscosa* (leaves) only stimulated the growth of *L. lactis* at time 72 hours, thereafter; the growth had decreased-the prebiotic effect of this plant was inconsistent and not long lasting.

Asparagus sprengeri (tuber), *Sonchus oleraceus* (leaves and roots) and *Taraxacum officinale* (leaves and roots) stimulated the growth of *L. lactis*. The prebiotic effect for these plants was longer lasting-resulting in higher growth compared to that when no plant extract was added at time 96 hours.

The overall increase in growth was confirmed using statistical analysis (AUC over 96 hour period). It was found that *Solanum nigrum* (leaves), *Amaranthus spinosus* (leaves), *Amaranthus hybridus* (leaves), *Asystasia gangetica* (leaves), *Oxygonum sinuatum* (leaves), *Senna occidentalis* (leaves), *Asparagus sprengeri* (tuber), *Tulbaghia violacea* (leaves and bulb), *Sonchus oleraceus* (leaves and roots), *Taraxacum officinale* (leaves and roots) and *Cleome monophylla* (leaves) had a higher growth response than that of MRS broth only (Figure 3.27) and thus had an overall increase in the number of organisms over the 96 hour period.

A comparison of the growth of *L. lactis* in the plant extracts with commercial inulin (positive control) showed that *Asparagus sprengeri* (tuber), *Tulbaghia violacea* (leaves), *Sonchus oleraceus* (leaves) and *Taraxacum officinale* (leaves and root) had better growth than commercial inulin. This is represented in figure 3.28.

Table 3.1: Comparison of the growth of *L. lactis* in the presence of the plant extracts relative to MRS broth only at times 48, 72 and 96 hours

Plant	**Time (hours)**		
	48	**72**	**96**
A. hybridus	-	-	-
A. dubius	-	-	-
A. spinosus	+	+	+
S. nigrum	+	-	-
G. parviflora	-	-	-
C. asiatica	-	-	+
J. flava	-	-	-
P. viscosa	-	+	-
A. sprengeri	+	+	+
S. oleraceus leaves	+	+	+
S. oleraceus roots	+	+	+
T. officinale leaves	+	+	+
T. officinale roots	+	+	+
T. violacea leaves	+	+	-
T. violacea bulbs	-	+	+
M. balsamina	+	+	+
C. monophylla	+	+	-

C. triloba	+	+	-
E. australis	-	-	+
S. occidentalis	-	-	+
A. gangetica	+	-	+
O. sinuatum	+	-	-
B. pilosa	-	-	-
P. oleracea	-	-	-
C. album	-	-	-

A (+) symbol indicates a CFU value higher than that obtained from *L. lactis* grown in MRS broth only. A (-) symbol indicates a CFU value lower than that obtained from *L. lactis* grown in MRS broth only.

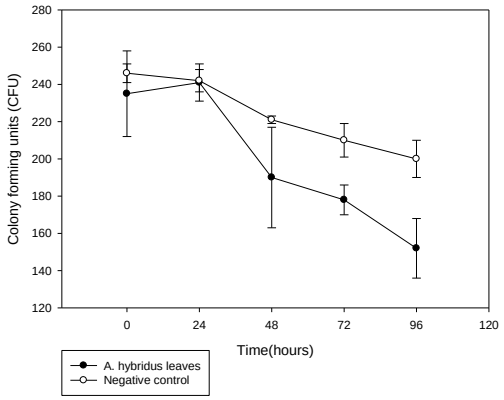

Fig. 3.1 Growth of *L. lactis* with aqueous extract of *A. hybridus* compared to negative control (MRS broth only)

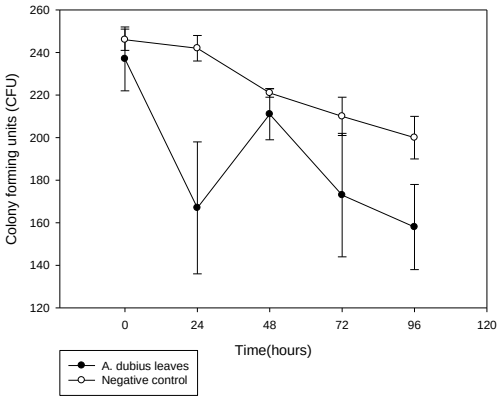

Fig. 3.2 Growth of *L. lactis* with aqueous extract of *A. dubius* compared to negative control (MRS broth only)

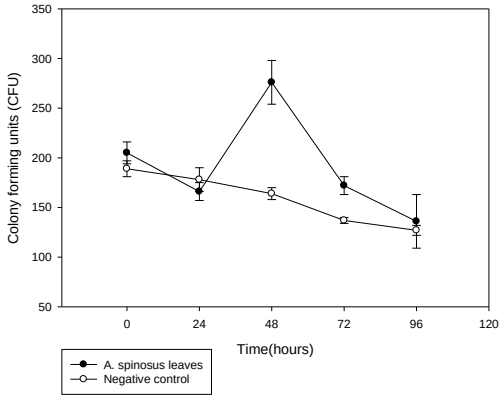

Fig. 3.3 Growth of *L. lactis* with aqueous extract of *A. spinosus* compared to negative control (MRS broth only)

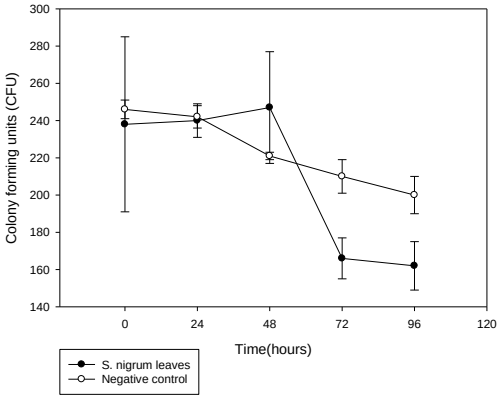

Fig. 3.4 Growth of *L. lactis* with aqueous extract of *S. nigrum* compared to negative control (MRS broth only)

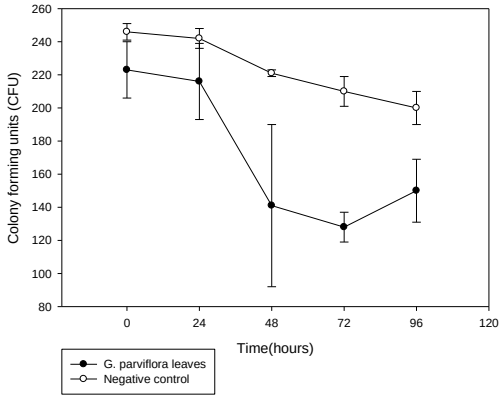

Fig. 3.5 Growth of *L. lactis* with aqueous extract of *G. parviflora* compared to negative control (MRS broth only)

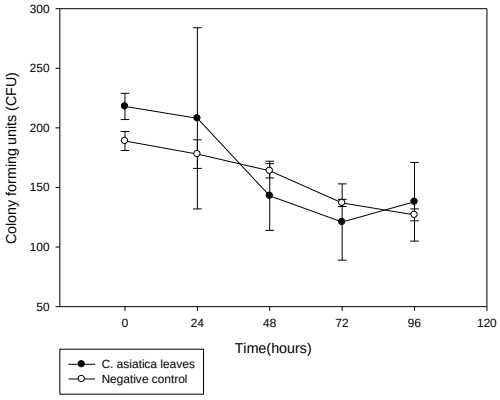

Fig. 3.6 Growth of *L. lactis* with aqueous extract of *C. asiatica* compared to negative control (MRS broth only)

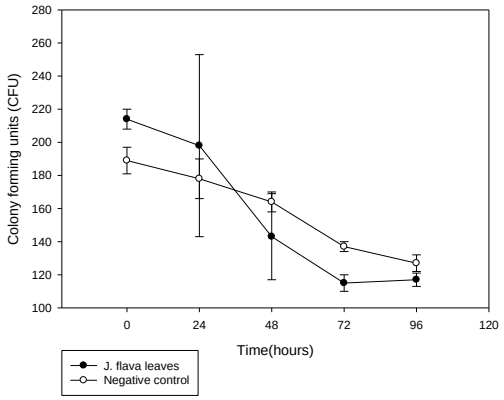

Fig. 3.7 Growth of *L. lactis* with aqueous extract of *J. flava* compared to negative control (MRS broth only)

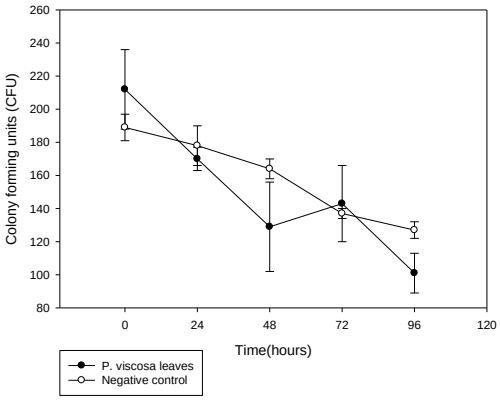

Fig. 3.8 Growth of *L. lactis* with aqueous extract of *P. viscosa* compared to negative control (MRS broth only)

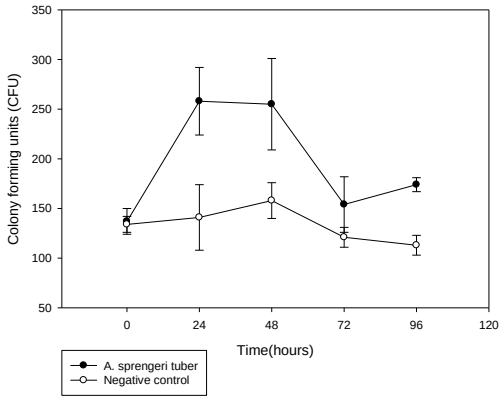

Fig. 3.9 Growth of *L. lactis* with aqueous extract of *A.sprengeri* compared to negative control (MRS broth only)

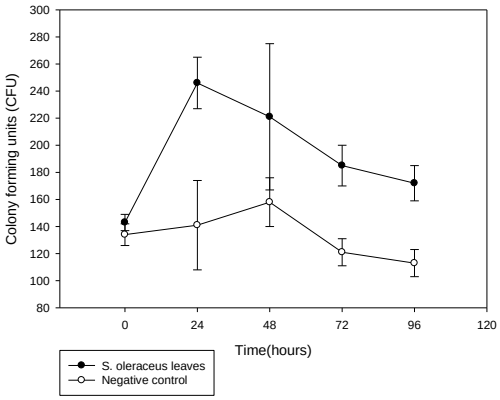

Fig. 3.10 Growth of *L. lactis* with aqueous extract of *S. oleraceus* leaves compared to negative control (MRS broth only)

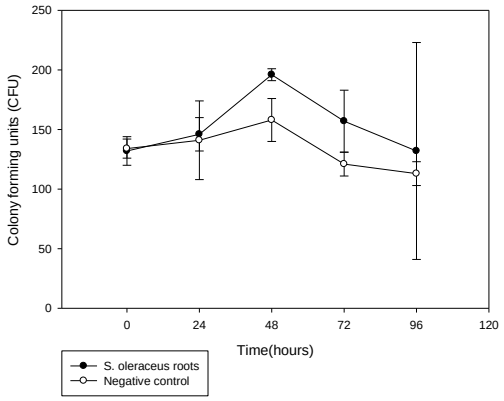

Fig. 3.11 Growth of *L. lactis* with aqueous extract of *S. oleraceus* roots compared to negative control (MRS broth only)

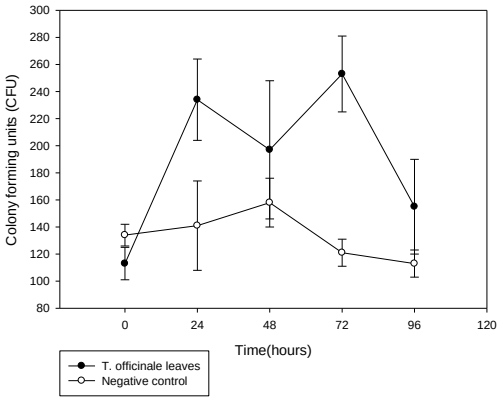

Fig. 3.12 Growth of *L. lactis* with aqueous extract of *T. officinale* leaves compared to negative control (MRS broth only)

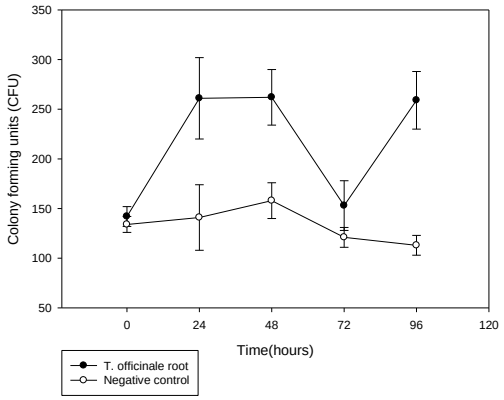

Fig. 3.13 Growth of *L. lactis* with aqueous extract of *T. officinale* roots compared to negative control (MRS broth only)

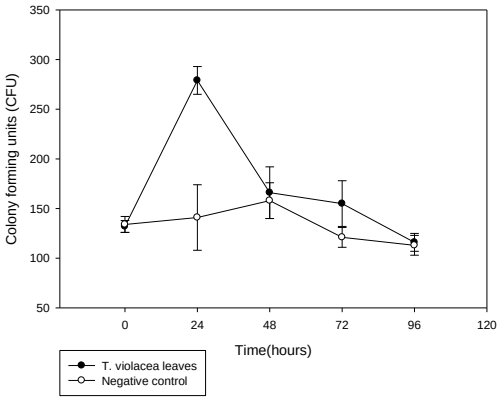

Fig. 3.14 Growth of *L. lactis* with aqueous extract of *T. violacea* leaves compared to negative control (MRS broth only)

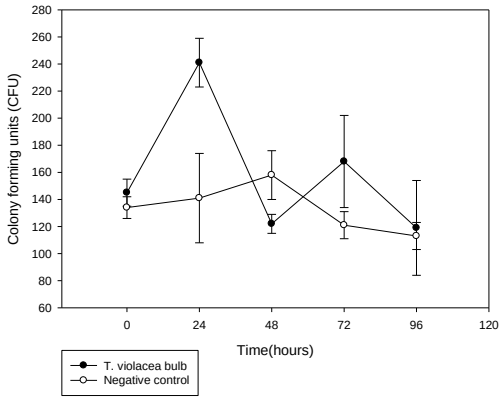

Fig. 3.15 Growth of *L. lactis* with aqueous extract of *T. violacea* bulbs compared to negative control (MRS broth only)

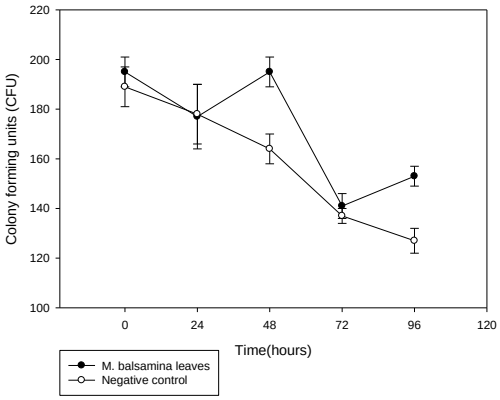

Fig. 3.16 Growth of *L. lactis* with aqueous extract of *M. balsamina* compared to negative control (MRS broth only)

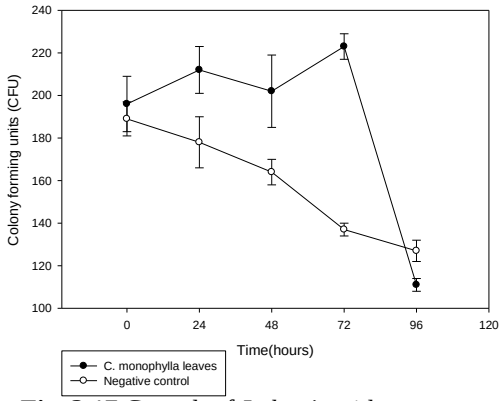

Fig. 3.17 Growth of *L. lactis* with aqueous extract of *C. monophylla* compared to negative control (MRS broth only)

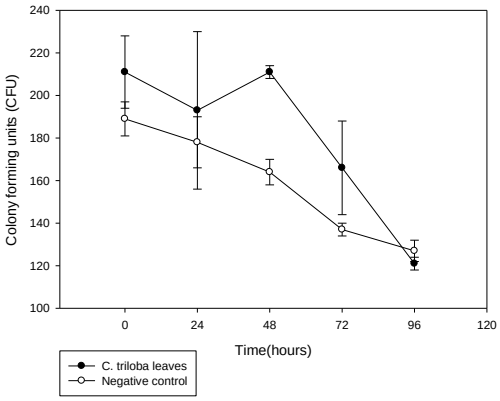

Fig. 3.18 Growth of *L. lactis* with aqueous extract of *C. triloba* compared to negative control (MRS broth only)

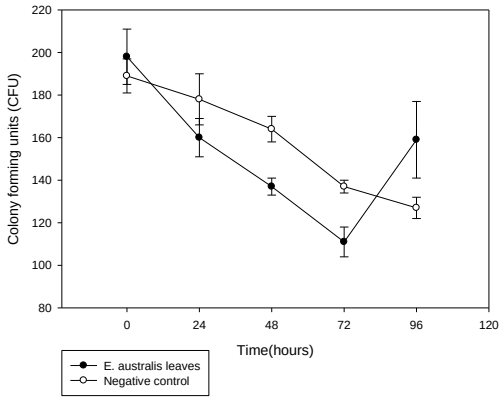

Fig. 3.19 Growth of *L. lactis* with aqueous extract of *E. australis* compared to negative control (MRS broth only)

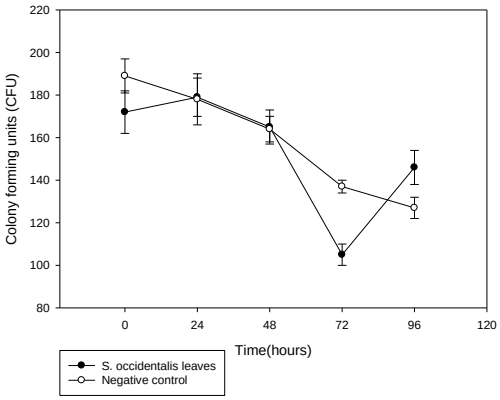

Fig. 3.20 Growth of *L. lactis* with aqueous extract of *S. occidentalis* compared to negative control (MRS broth only)

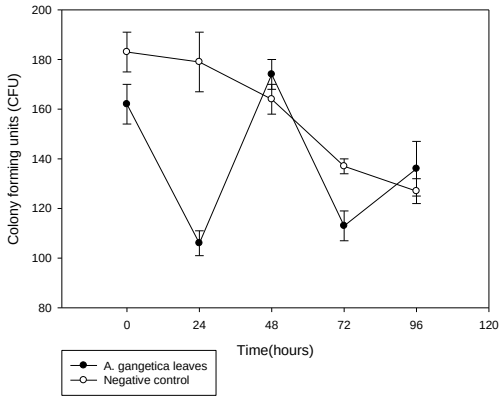

Fig. 3.21 Growth of *L. lactis* with aqueous extract of *A. gangetica* compared to negative control (MRS broth only)

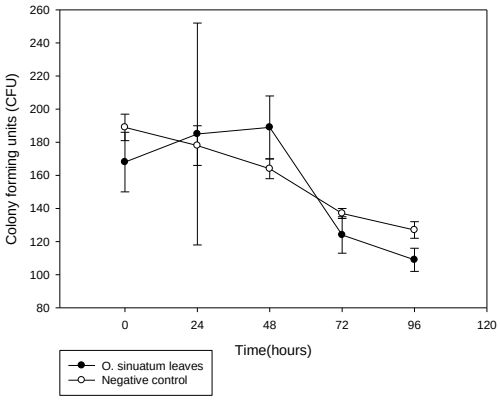

Fig. 3.22 Growth of *L. lactis* with aqueous extract of *O. sinuatum* compared to negative control (MRS broth only)

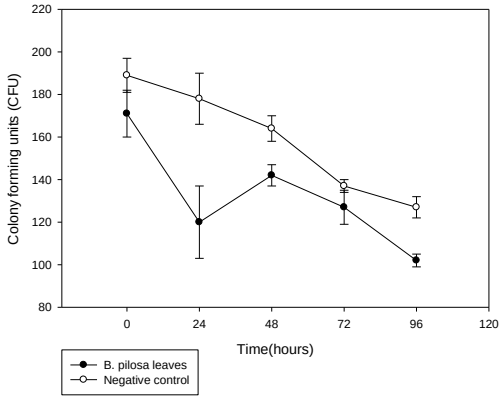

Fig. 3.23 Growth of *L. lactis* with aqueous extract of *B. pilosa* compared to negative control (MRS broth only)

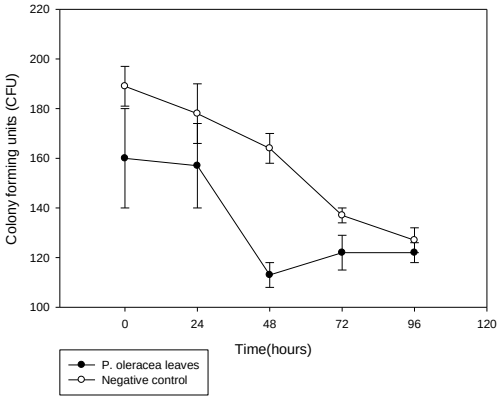

Fig. 3.24 Growth of *L. lactis* with aqueous extract of *P.oleracea* compared to negative control (MRS broth only)

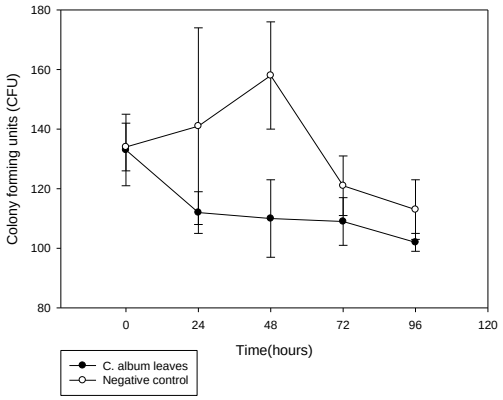

Fig. 3.25 Growth of *L. lactis* with aqueous extract of *C. album* compared to negative control (MRS broth only)

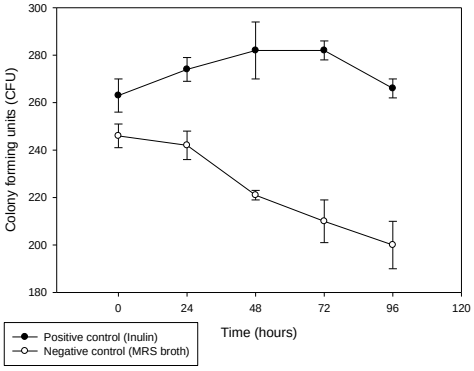

Fig. 3.26 Growth of *L. lactis* in the presence of commercial inulin compared to negative control (MRS broth only)

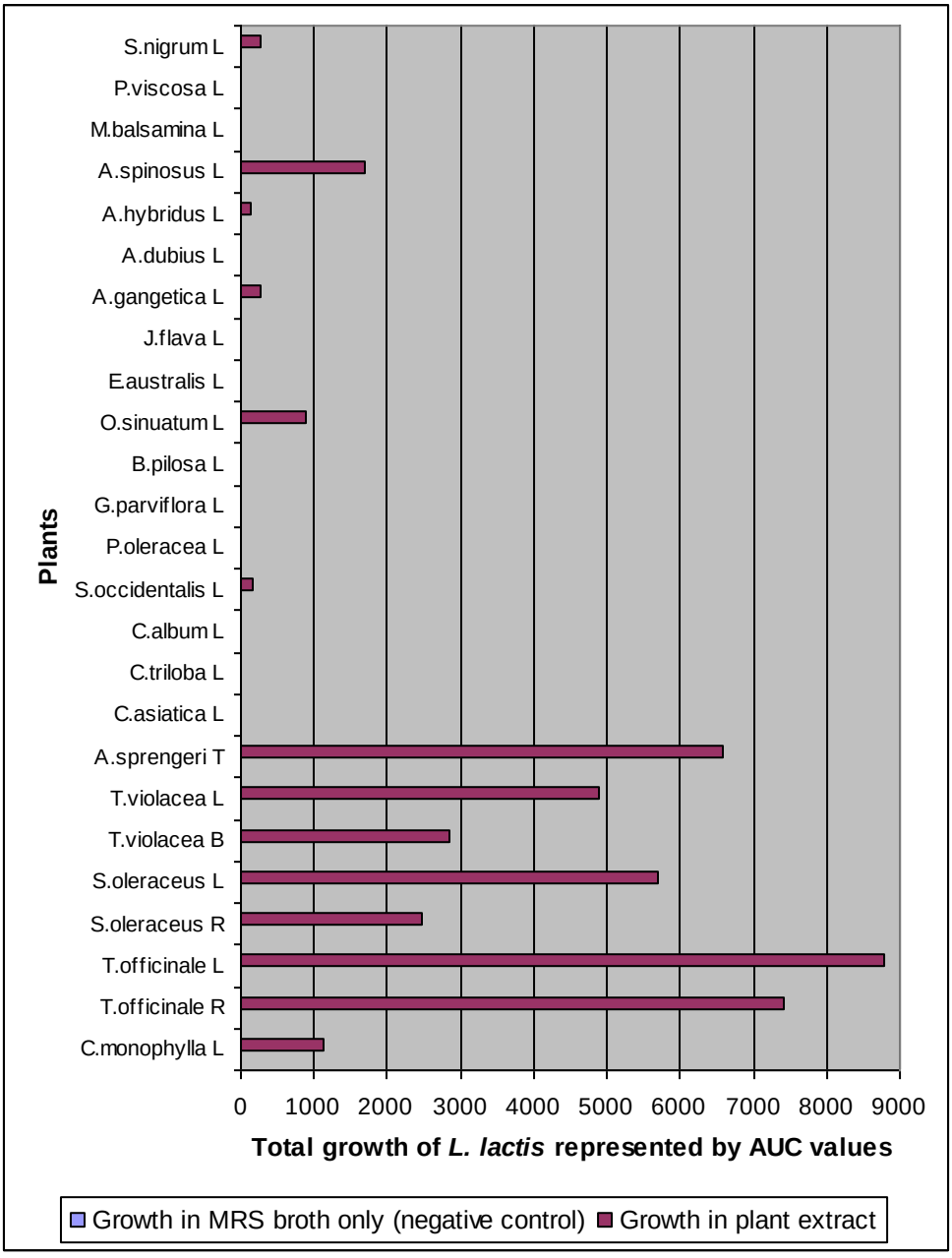

Fig.3.27 Comparison of the growth response of *L. lactis* over 96 hour period for 25 plant extracts to that when no plant extract added (negative control)

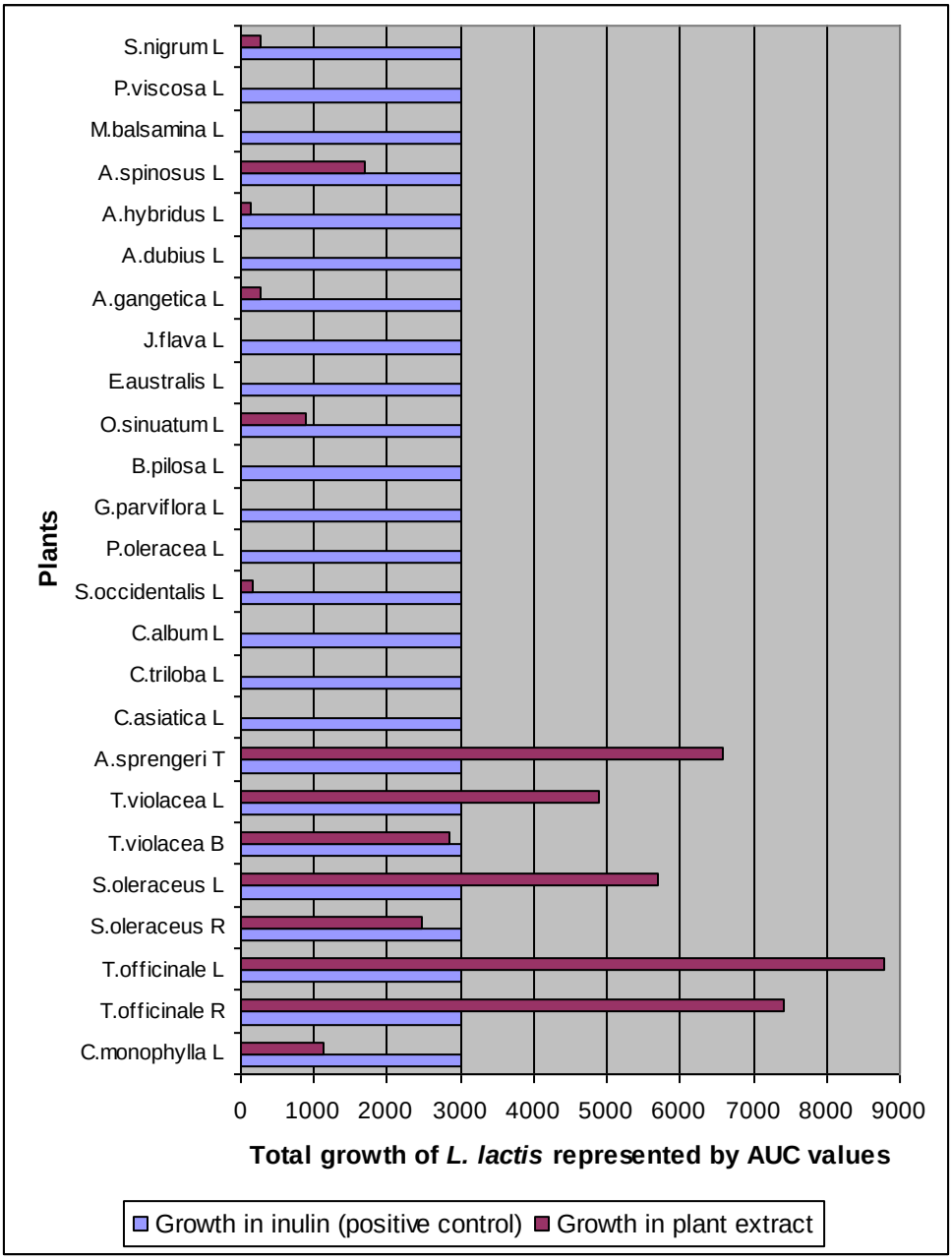

Fig.3.28 Comparison of the growth response of *L. lactis* over 96 hour period for 25 plant extracts to that of commercial inulin (positive control)

3.1.2 Effect on *Lactobacillus bulgaricus*

The growth of *L. bulgaricus* over a period of 96 hours in the presence of the plant extract compared to when no plant extract was added (MRS broth only) is shown in figures 3.29 to 3.53 and summarised in Table 3.2. Inulin, as expected, stimulated the growth of *L. bulgaricus* (figure 3.54).

The growth of *L. bulgaricus* (measured in CFU) obtained in the presence of the plant extract after each 24 hour interval varied-certain extracts promoted higher growth consistently throughout the 96 hour period, whilst others only promoted high growth at a specific time, after which the growth was inhibited, as can be seen in Table 3.2. Plants from the family Amaranthaceae i.e. *Amaranthus hybridus* (leaves) and *Amaranthus dubius* (leaves) stimulated the growth of *L. bulgaricus* over the 96 hour period. The growth obtained in the presence of these plant extracts was higher than that obtained when no plant extract was added (MRS broth only)-the stimulation was consistent for the entire 96 hour period. The stimulation by *Portulaca oleracea* (leaves) was found to be inconsistent-the highest growth was achieved only at time 72 hours, thereafter the growth had decreased.

Statistical analysis (AUC over 96 hour period) was used to confirm the overall increase in growth. Of the 22 plants studied, *Solanum nigrum* (leaves), *Momordica balsamina* (leaves), *Amaranthus spinosus* (leaves), *Amaranthus hybridus* (leaves), *Amaranthus dubius* (leaves), *Asystasia gangetica* (leaves), *Justicia flava* (leaves), *Galinsoga parviflora* (leaves), *Senna occidentalis* (leaves), *Ceratotheca triloba* (leaves), *Centella asiatica* (leaves), *Asparagus sprengeri* (tuber), *Tulbaghia violacea* (leaves and bulb), *Sonchus oleraceus* (leaves and roots), *Taraxacum officinale* (leaves and roots) and *Cleome monophylla* (leaves) had a higher growth response for *L. bulgaricus* as shown in figure 3.55.

A comparison of the growth of *L. bulgaricus* in the plant extracts and commercial inulin (positive control) showed that none of the plant extracts had a prebiotic effect higher than that of inulin for *L. bulgaricus*, as can be seen in figure 3.56.

Table 3.2: Comparison of the growth of *L. bulgaricus* in the presence of the plant extracts relative to MRS broth only at times 48, 72 and 96 hours

Plant	**Time (hours)**		
	48	**72**	**96**
A. hybridus	+	+	+
A. dubius	+	+	+
A. spinosus	+	+	-
S. nigrum	+	+	-
G. parviflora	+	-	-
C. asiatica	-	-	-
J. flava	+	-	-
P. viscosa	-	-	-
A. sprengeri	-	+	+
S. oleraceus leaves	+	+	+
S. oleraceus roots	+	+	+
T. officinale leaves	+	+	+
T. officinale roots	+	+	+
T. violacea leaves	-	+	+
T. violacea bulbs	+	+	+
M. balsamina	+	+	-
C. monophylla	+	+	+
C. triloba	+	+	+
E. australis	+	+	+
S. occidentalis	+	-	-
A. gangetica	+	-	-
O. sinuatum	-	-	-
B. pilosa	-	-	-
P. oleracea	-	+	-
C. album	-	-	-

A (+) symbol indicates a CFU value higher than that obtained from *L. bulgaricus* grown in MRS broth only. A (-) symbol indicates a CFU value lower than that obtained from *L. bulgaricus* grown in MRS broth only.

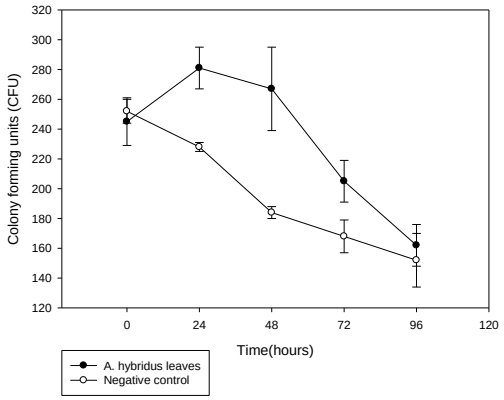

Fig. 3.29 Growth of *L. bulgaricus* with aqueous extract of *A. hybridus* compared to negative control (MRS broth only)

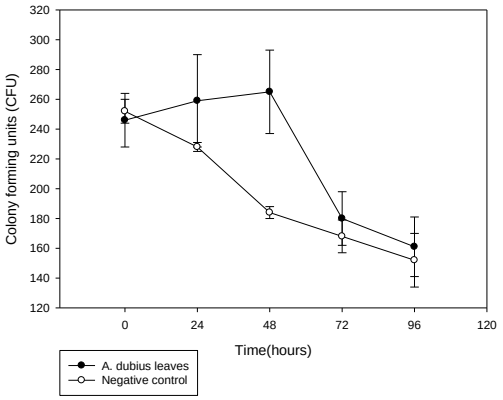

Fig. 3.30 Growth of *L. bulgaricus* with aqueous extract of *A. dubius* compared to negative control (MRS broth only)

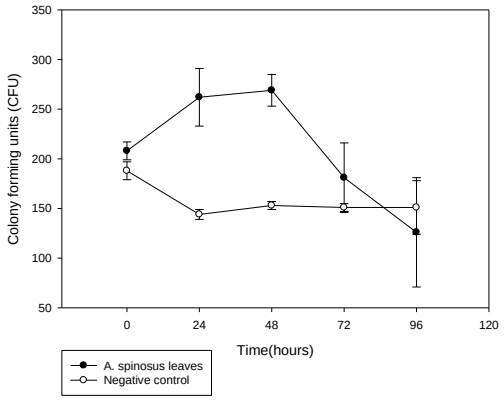

Fig. 3.31 Growth of *L. bulgaricus* with aqueous extract of *A. spinosus* compared to negative control (MRS broth only)

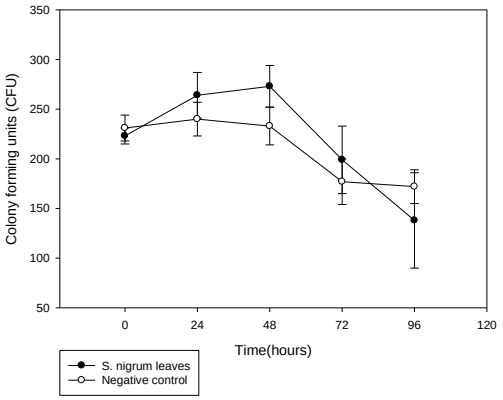

Fig. 3.32 Growth of *L. bulgaricus* with aqueous extract of *S. nigrum* compared to negative control (MRS broth only)

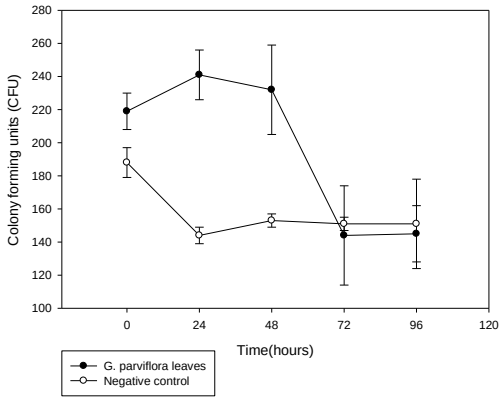

Fig. 3.33 Growth of *L. bulgaricus* with aqueous extract of *G. parviflora* compared to negative control (MRS broth only)

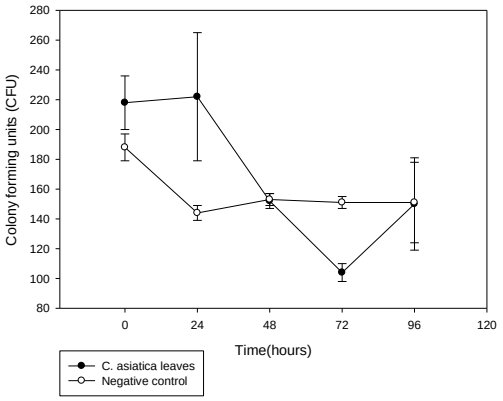

Fig. 3.34 Growth of *L. bulgaricus* with aqueous extract of *C. asiatica* compared to negative control (MRS broth only)

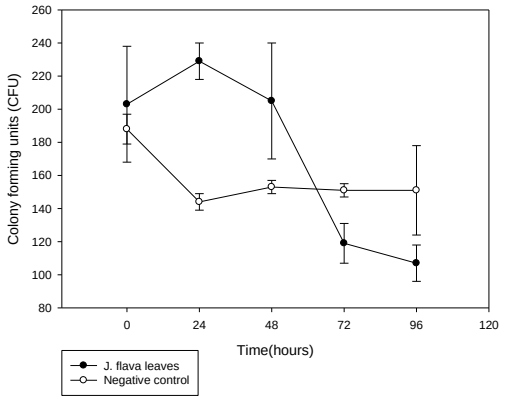

Fig. 3.35 Growth of *L. bulgaricus* with aqueous extract of *J. flava* compared to negative control (MRS broth only)

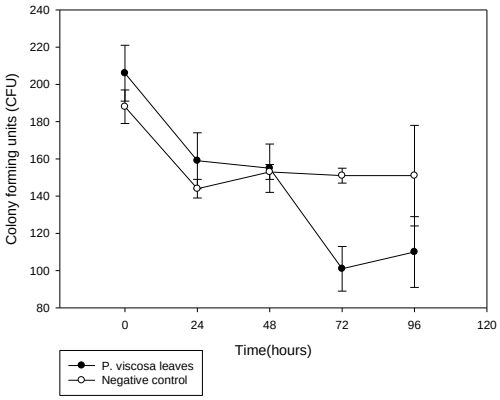

Fig. 3.36 Growth of *L. bulgaricus* with aqueous extract of *P. viscosa* compared to negative control (MRS broth only)

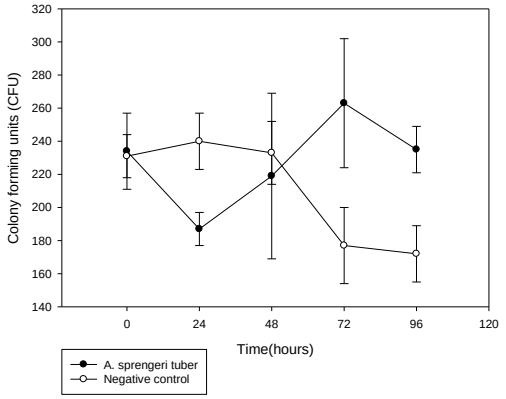

Fig. 3.37 Growth of *L. bulgaricus* with aqueous extract of *A. sprengeri* compared to negative control (MRS broth only)

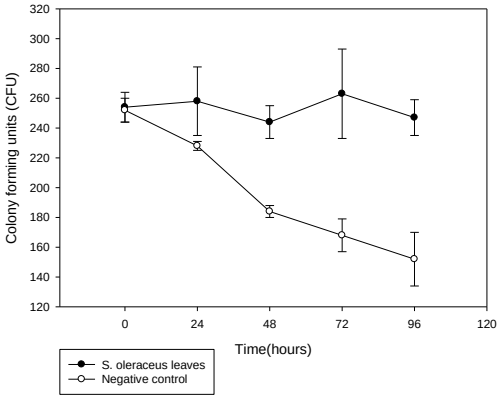

Fig. 3.38 Growth of *L. bulgaricus* with aqueous extract of *S. oleraceus* leaves compared to negative control (MRS broth only)

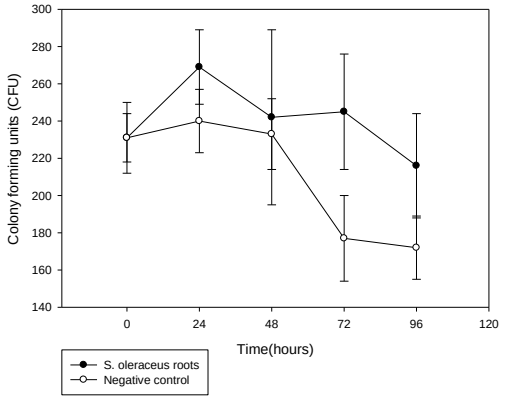

Fig. 3.39 Growth of *L. bulgaricus* with aqueous extract of *S. oleraceus* roots compared to negative control (MRS broth only)

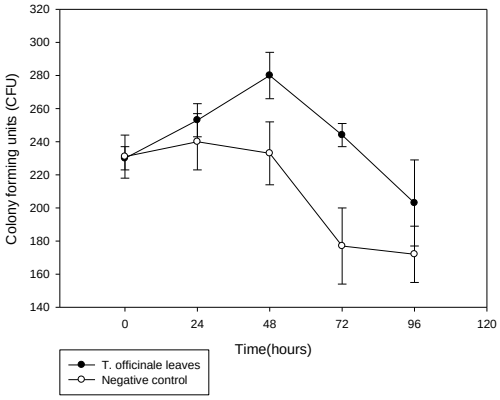

Fig. 3.40 Growth of *L. bulgaricus* with aqueous extract of *T. officinale* leaves compared to negative control (MRS broth only)

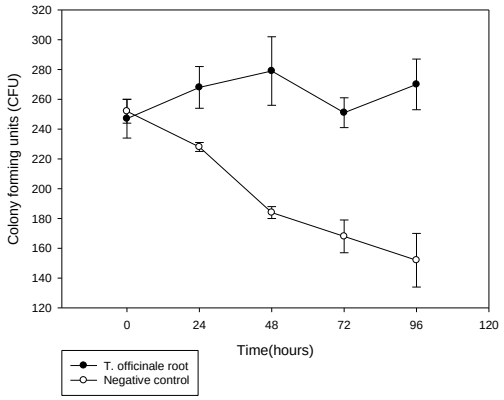

Fig. 3.41 Growth of *L. bulgaricus* with aqueous extract of *T. officinale* root compared to negative control (MRS broth only)

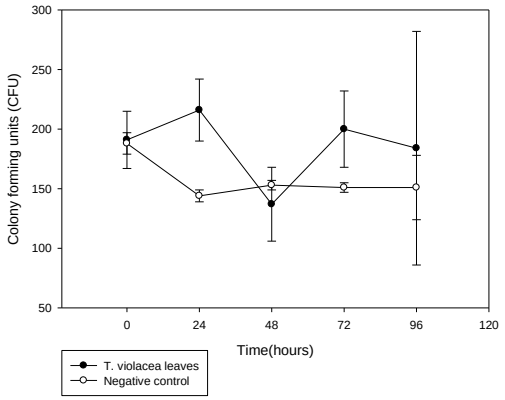

Fig. 3.42 Growth of *L. bulgaricus* with aqueous extract of *T. violacea* leaves compared to negative control (MRS broth only)

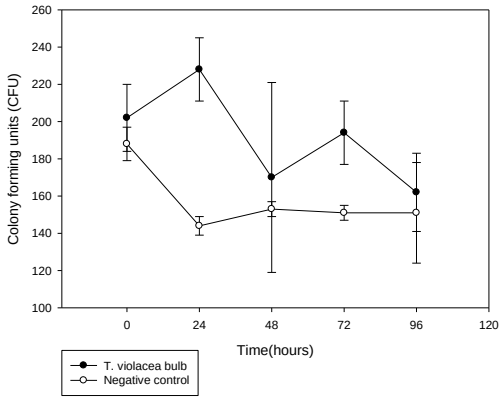

Fig. 3.43 Growth of *L. bulgaricus* with aqueous extract of *T. violacea* bulbs compared to negative control (MRS broth only)

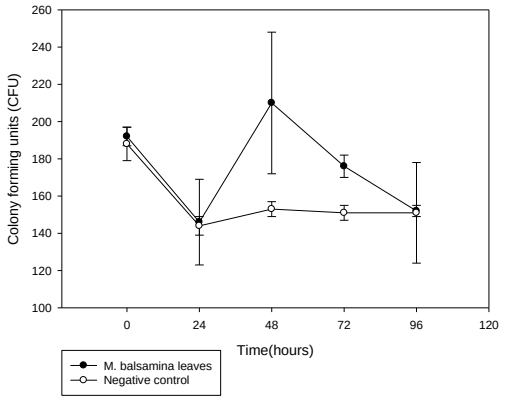

Fig. 3.44 Growth of *L. bulgaricus* with aqueous extract of *M. balsamina* compared to negative control (MRS broth only)

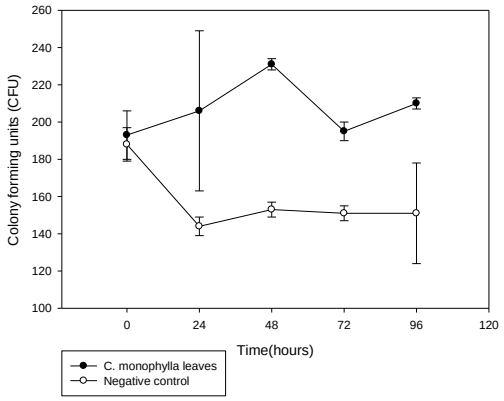

Fig. 3.45 Growth of *L. bulgaricus* with aqueous extract of *C. monophylla* compared to negative control (MRS broth only)

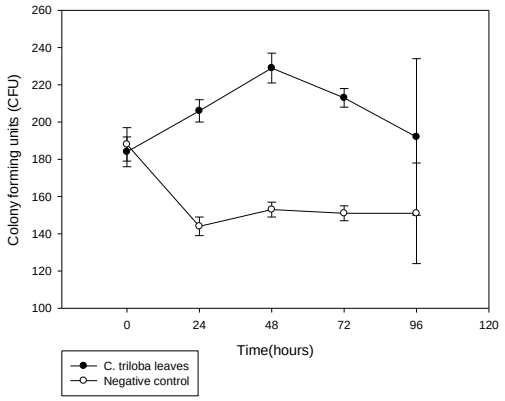

Fig. 3.46 Growth of *L. bulgaricus* with aqueous extract of *C. triloba* compared to negative control (MRS broth only)

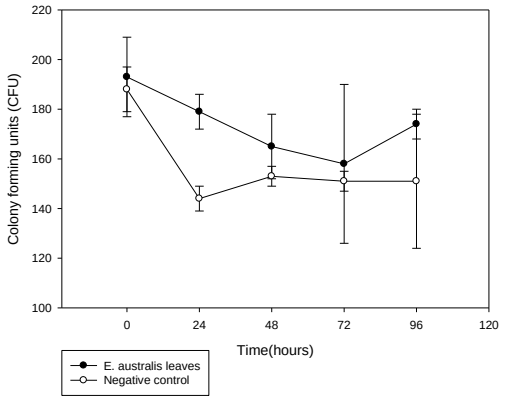

Fig. 3.47 Growth of *L. bulgaricus* with aqueous extract of *E. australis* compared to negative control (MRS broth only)

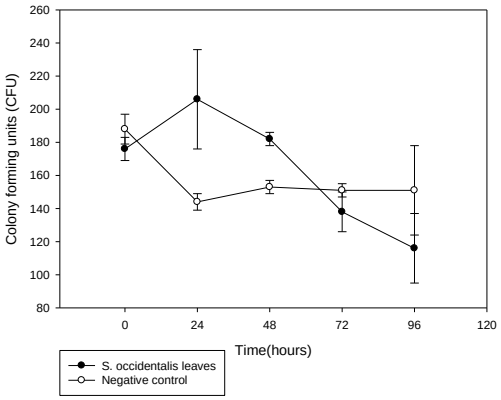

Fig. 3.48 Growth of *L. bulgaricus* with aqueous extract of *S. occidentalis* compared to negative control (MRS broth only)

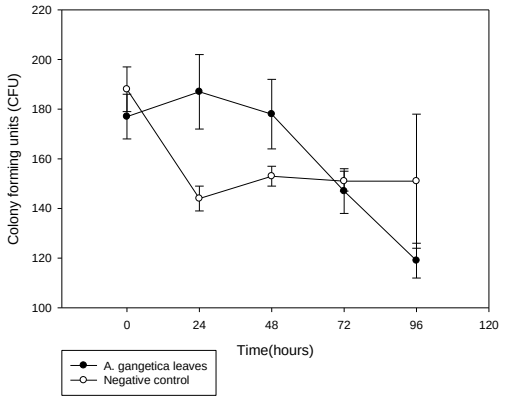

Fig. 3.49 Growth of *L. bulgaricus* with aqueous extract of *A. gangetica* compared to negative control (MRS broth only)

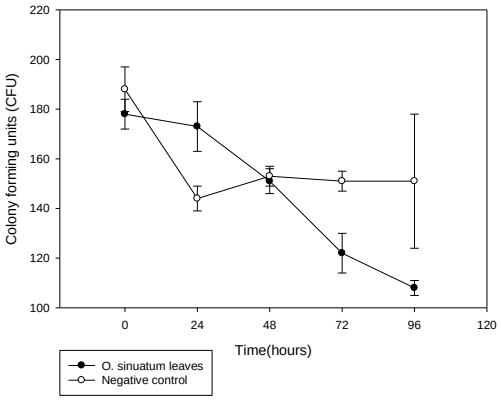

Fig. 3.50 Growth of *L. bulgaricus* with aqueous extract of *O. sinuatum* compared to negative control (MRS broth only)

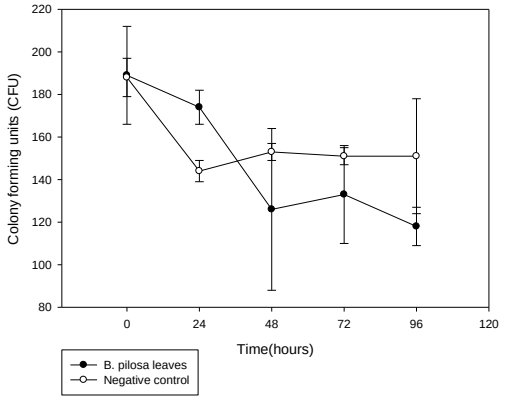

Fig. 3.51 Growth of *L. bulgaricus* with aqueous extract of *B. pilosa* compared to negative control (MRS broth only)

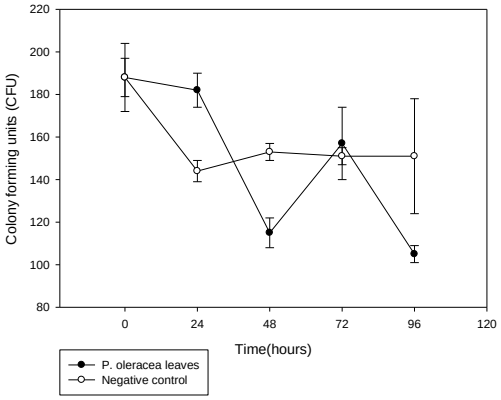

Fig. 3.52 Growth of *L. bulgaricus* with aqueous extract of *P. oleracea* compared to negative control (MRS broth only)

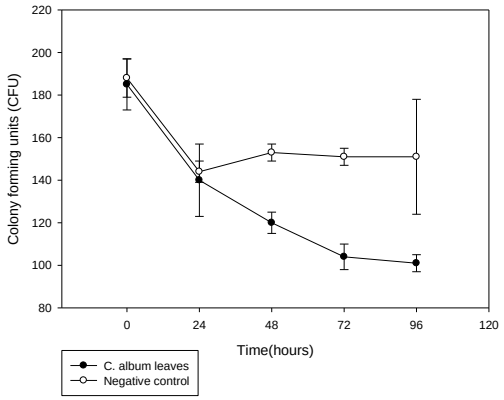

Fig. 3.53 Growth of *L. bulgaricus* with aqueous extract of *C. album* compared to negative control (MRS broth only)

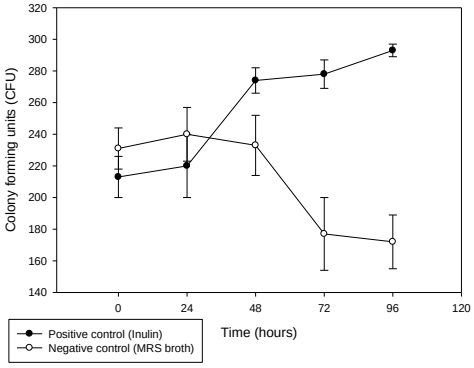

Fig. 3.54 Growth of *L. bulgaricus* in the presence of commercial inulin compared to negative control (MRS broth only)

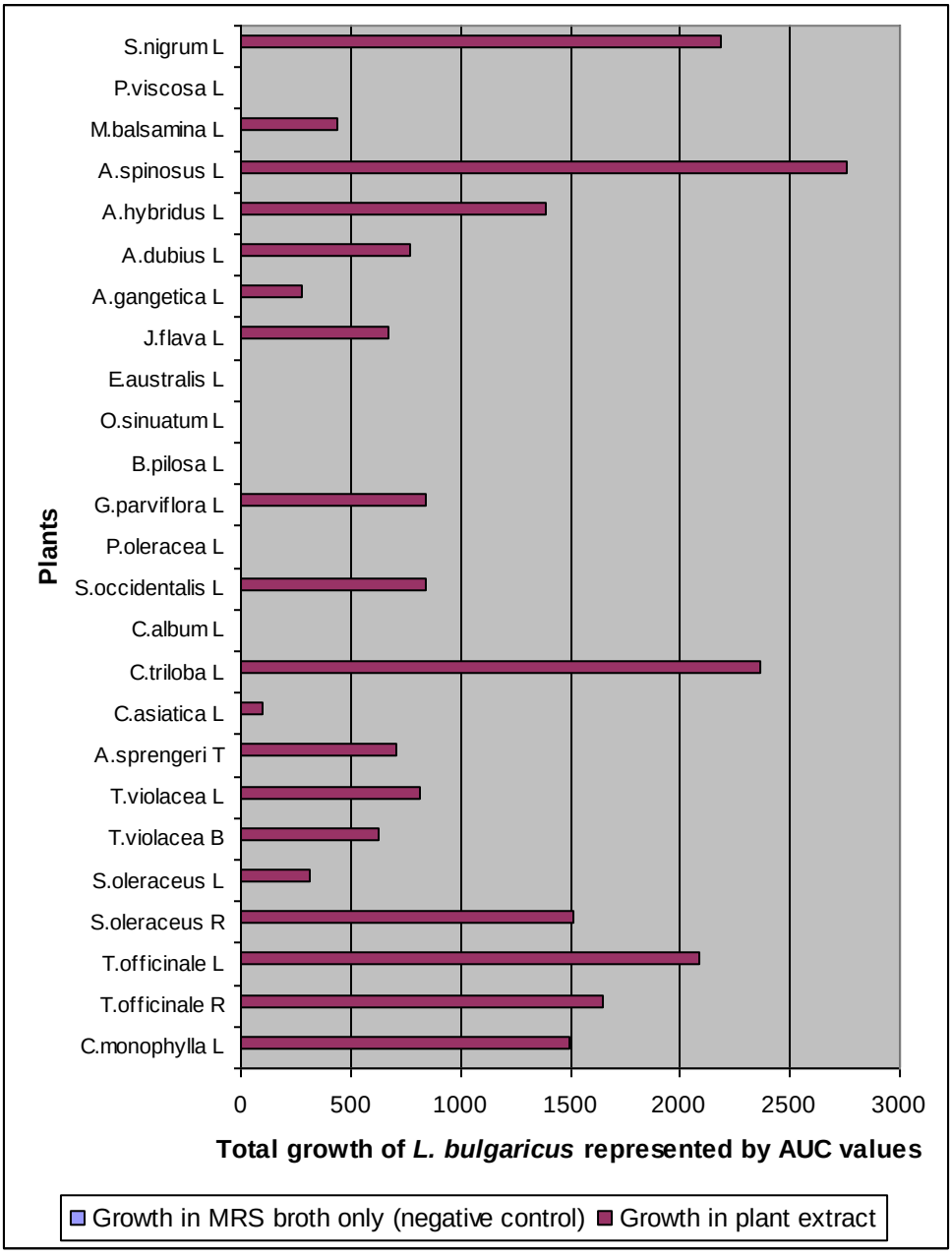

Fig. 3.55 Comparison of the growth response of *L. bulgaricus* over 96 hour period for 25 plant extracts to that when no plant extract was added (negative control)

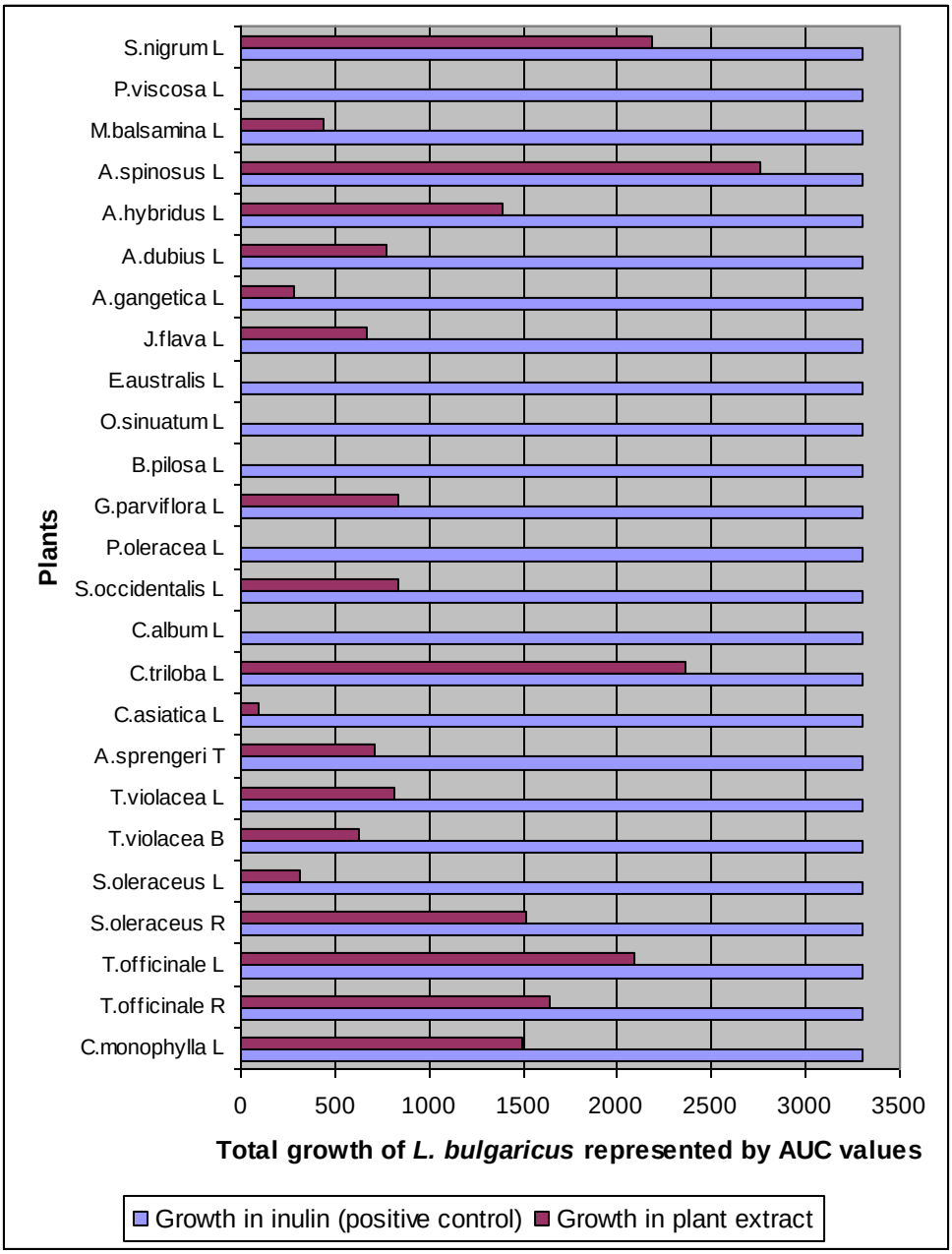

Fig. 3.56 Comparison of the growth response of *L. bulgaricus* over 96 hour period for 25 plant extracts to that of commercial inulin (positive control)

3.1.3 Effect on *Lactobacillus reuteri*

The growth of *L. reuteri* over a period of 96 hours in the presence of the plant extract compared to absence of plant extract (MRS broth only) is shown in figures 3.57 to 3.62 and summarised in Table 3.3. *L. reuteri* was only tested against those extracts which exhibited the best prebiotic effect on both *L. lactis* and *L. bulgaricus* in this study. The growth of *L. reuteri* in the presence of commercial inulin (positive control) compared to MRS broth only is shown in figure 3.63. Inulin stimulated the growth of *L. reuteri*.

L. reuteri grew differently with each of the extracts over the 96 hour period and in some cases there was also an inhibitory effect as growth had decreased, as can be seen in Table 3.3. *Chenopodium album* (leaves), *Sonchus oleraceus* (leaves and roots), *Taraxacum officinale* (leaves and roots) and *Tulbaghia violacea* (leaves) stimulated the growth of *L. reuteri*-the growth obtained in the presence of the plant extracts was higher then that when no plant extract was added. The stimulation was found to be consistent throughout the 96 hour period for *Chenopodium album* (leaves), *Sonchus oleraceus* (leaves and roots) and *Taraxacum officinale* (leaves and roots)-exhibiting a longer-lasting prebiotic effect.

The overall increase in growth was confirmed using statistical analysis (AUC over 96 hour period). Of the six extracts against which *L. reuteri* was tested, *Chenopodium album* (leaves), *Sonchus oleraceus* (leaves and roots) and *Taraxacum officinale* (leaves and roots) had a higher growth response for *L. reuteri* to that when no plant extract was added as depicted in figure 3.64. A comparison of the growth of *L. reuteri* in the plant extracts with commercial inulin (positive control) revealed that *Taraxacum officinale* (root) exhibited a prebiotic effect higher than that of inulin as shown in figure 3.65.

Table 3.3: Comparison of the growth of *L. reuteri* in the presence of the plant extracts relative to MRS broth only at times 48, 72 and 96 hours

Plant	**Time (hours)**		
	48	**72**	**96**
T. officinale leaves	+	+	+
T. officinale roots	+	+	+
S. oleraceus leaves	+	+	+
S. oleraceus roots	+	+	+
T. violacea leaves	-	+	+
C. album	+	+	+

A (+) symbol indicates a CFU value higher than that obtained from *L. reuteri* grown in MRS broth only. A (-) symbol indicates a CFU value lower than that obtained from *L. reuteri* grown in MRS broth only.

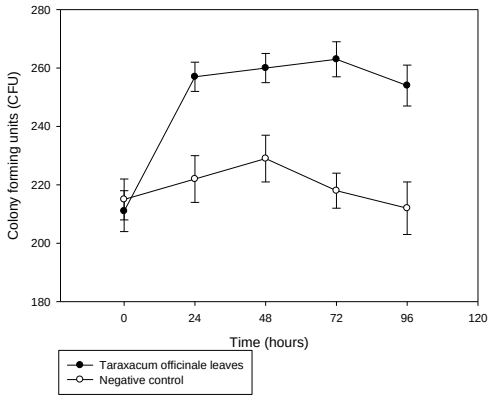

Fig. 3.57 Growth of *L. reuteri* with aqueous extract of *T. officinale* leaves compared to negative control (MRS broth only)

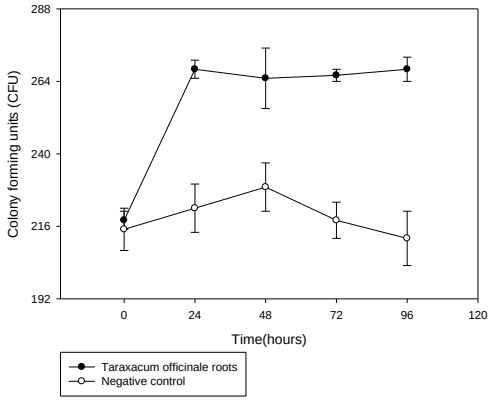

Fig. 3.58 Growth of *L. reuteri* with aqueous extract of *T. officinale* roots compared to negative control (MRS broth only)

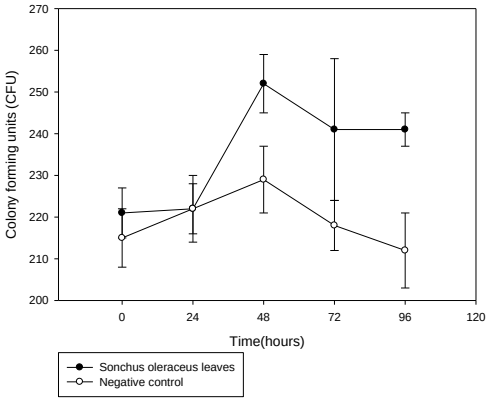

Fig. 3.59 Growth of *L. reuteri* with aqueous extract of *S. oleraceus* leaves compared to negative control (MRS broth only)

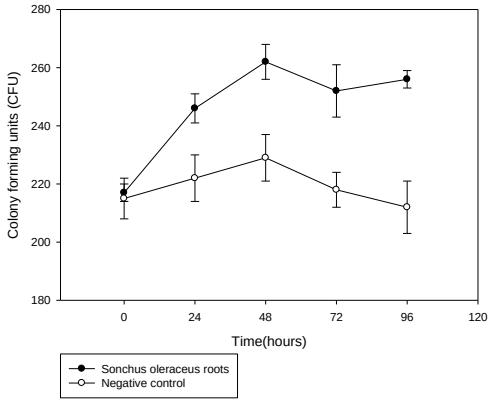

Fig. 3.60 Growth of *L. reuteri* with aqueous extract of *S. oleraceus* roots compared to negative control (MRS broth only)

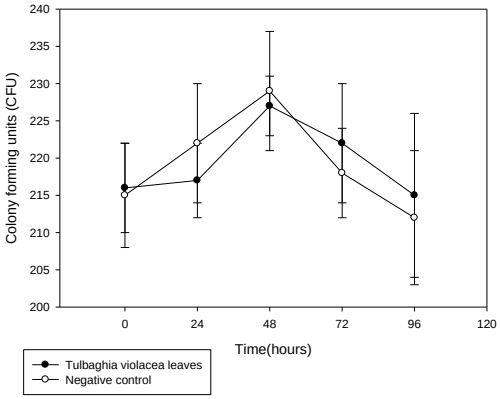

Fig. 3.61 Growth of *L. reuteri* with aqueous extract of *T. violacea* leaves compared to negative control (MRS broth only)

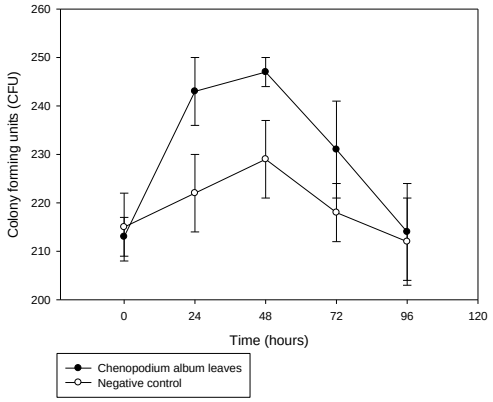

Fig. 3.62 Growth of *L. reuteri* with aqueous extract of *C. album* leaves compared to negative control (MRS broth only)

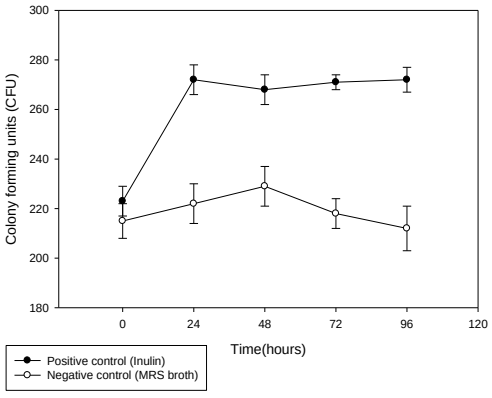

Fig. 3.63 Growth of *L. reuteri* in the presence of commercial inulin compared to negative control (MRS broth only)

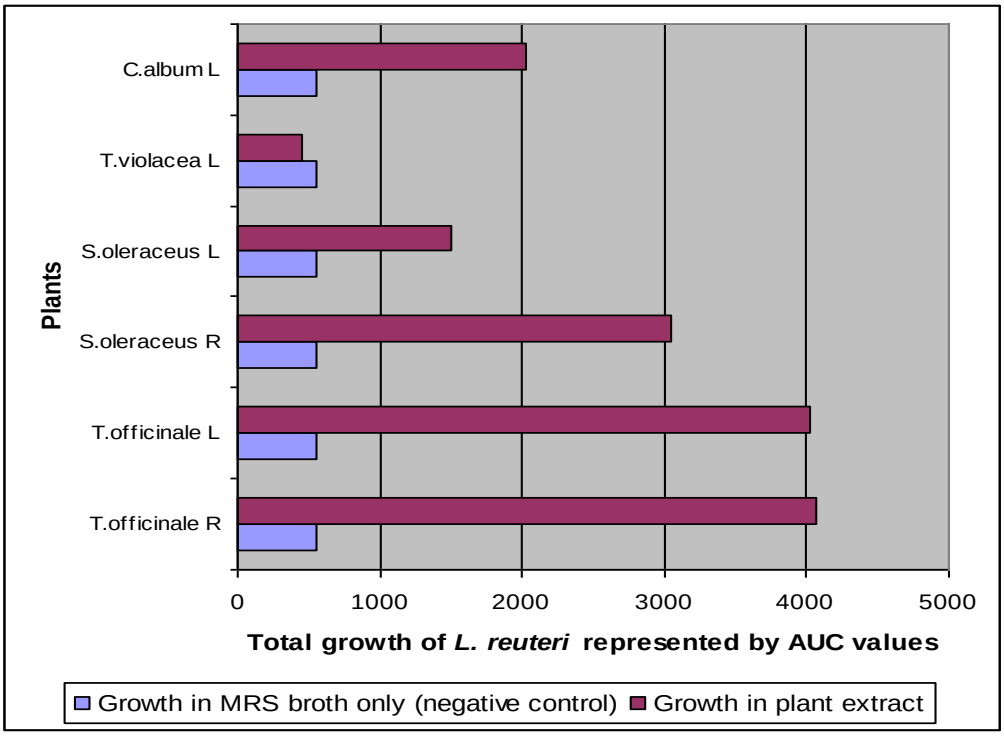

Fig. 3.64 Comparison of the growth response of *L. reuteri* over 96 hour period for plant extracts to that when no plant extract was added (negative control)

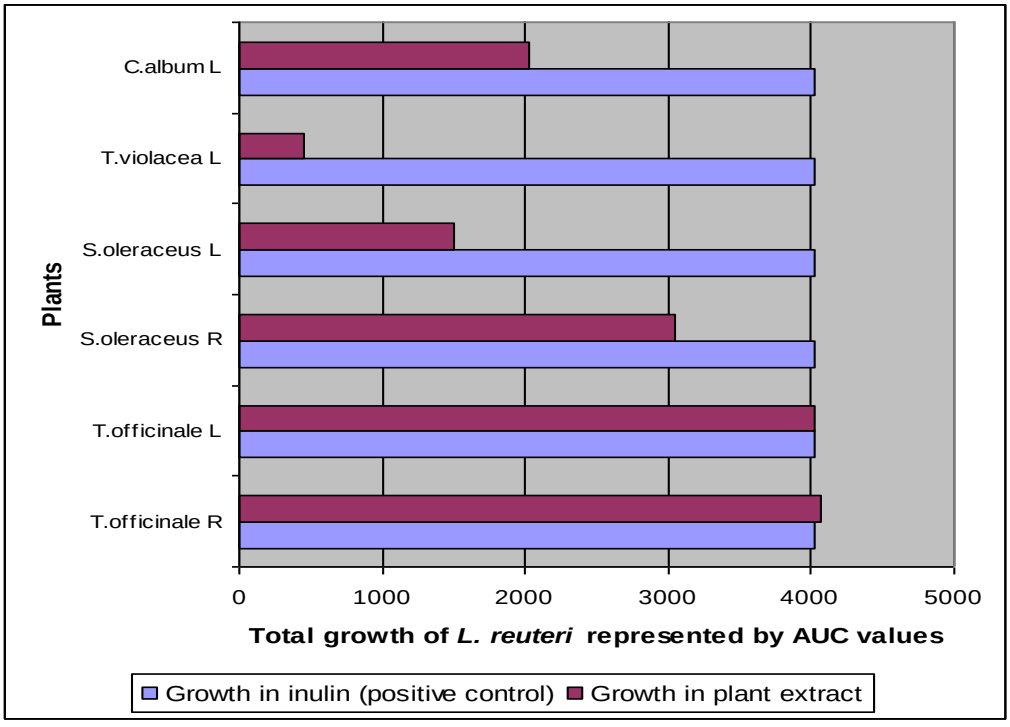

Fig. 3.65 Comparison of the growth response of *L. reuteri* over 96 hour period for plant extracts to that of commercial inulin (positive control)

3.1.4 Effect on *Bifidobacterium longum*

The growth of *B. longum* over a period of 96 hours in the presence of the plant extract compared to when no plant extract was added (MRS broth only) is shown in figures 3.66 to 3.90 and summarised in Table 3.4. The growth of *B. longum* was stimulated by commercial inulin, as can be seen in figure 3.91.

B. longum grew differently with each of the extracts over the 96 hour period and in some cases there was also an inhibitory effect as growth had decreased, as can be seen in Table 3.4. Stimulation of growth by *Momordica balsamina* (leaves) and *Oxygonum sinuatum* (leaves) for example was found to be inconsistent, the highest growth was achieved at time 72 hours, thereafter, the growth of *B. longum* had decreased. Plants from the family Asteraceae i.e. *Sonchus oleraceus* (leaves and roots) stimulated the growth of *B. longum* and gave a higher growth of *B. longum* at the end of the 96 hour period.

The overall increase in growth was confirmed using statistical analysis (AUC over 96 hour period). It was found that *Sonchus oleraceus* (leaves and roots) had a higher growth response for *B. longum* when compared to the growth obtained when no plant extract was added as depicted in figure 3.92.

A comparison of the growth of *B. longum* in the plant extracts and commercial inulin (positive control) revealed that none of the plants exhibited a prebiotic effect higher than that of the positive control as shown in figure 3.93.

Table 3.4: Comparison of the growth of *B. longum* in the presence of the plant extracts relative to MRS broth only at times 48, 72 and 96 hours

Plant	**Time (hours)**		
	48	**72**	**96**
A. gangetica	-	-	-
C. triloba	-	-	-
B. pilosa	-	-	-
C. album	-	-	-
S. occidentalis	-	-	-
E. australis	-	-	-
M. balsamina	-	+	-
P. viscosa	-	+	-
O. sinuatum	-	+	-
P. oleracea	-	+	-
J. flava	-	-	-
C. asiatica	-	-	-
A. dubius	-	-	-
A. hybridus	+	-	-
A. spinosus	-	-	-
S. nigrum	-	-	+
G. parviflora	+	-	-
C. monophylla	+	-	-
T. officinale roots	+	-	+
T. officinale leaves	-	-	+
S. oleraceus roots	+	-	+
S. oleraceus leaves	-	-	+
T. violacea bulbs	-	-	+
T. violacea leaves	-	-	-
A. sprengeri	+	+	+

A (+) symbol indicates a CFU value higher than that obtained from *B. longum* grown in MRS broth only. A (-) symbol indicates a CFU value lower than that obtained from *B. longum* grown in MRS broth only.

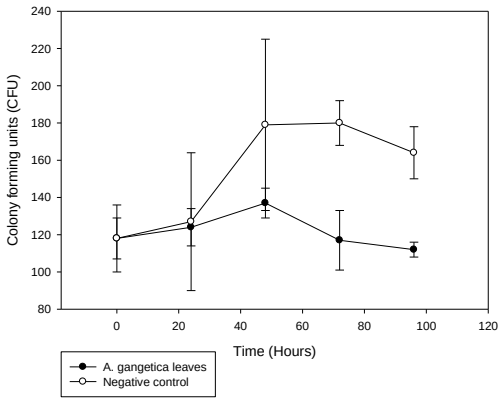

Fig. 3.66 Growth of *B. longum* with aqueous extract of *A. gangetica* compared to negative control (MRS broth only)

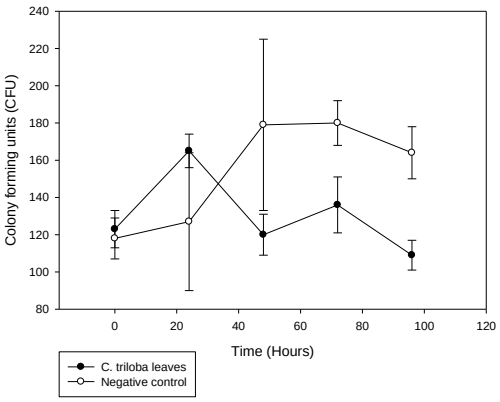

Fig. 3.67 Growth of *B. longum* with aqueous extract of *C. triloba* compared to negative control (MRS broth only)

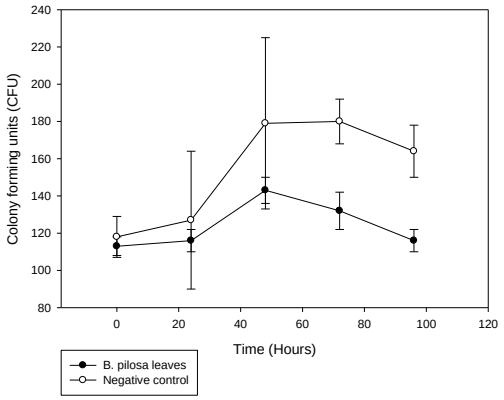

Fig. 3.68 Growth of *B. longum* with aqueous extract of *B. pilosa* compared to negative control (MRS broth only)

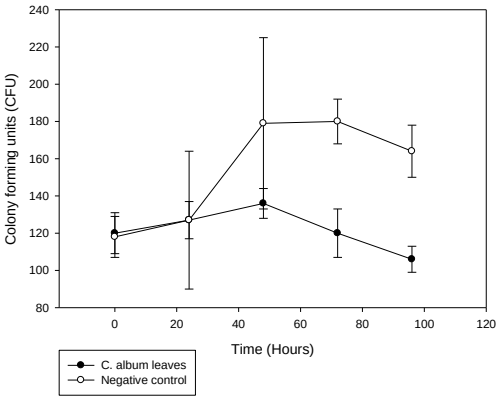

Fig. 3.69 Growth of *B. longum* with aqueous extract of *C. album* compared to negative control (MRS broth only)

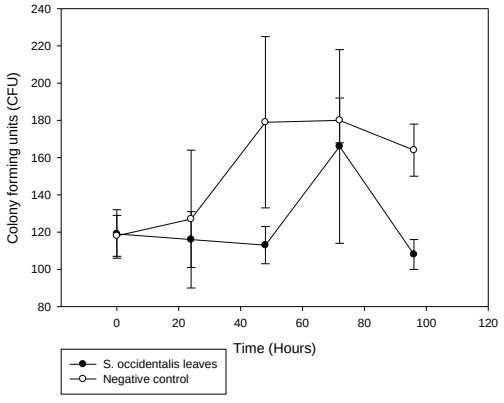

Fig. 3.70 Growth of *B. longum* with aqueous extract of *S. occidentalis* compared to negative control (MRS broth only)

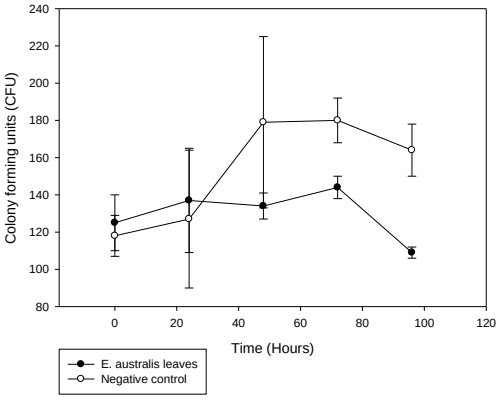

Fig. 3.71 Growth of *B. longum* with aqueous extract of *E. australis* compared to negative control (MRS broth only)

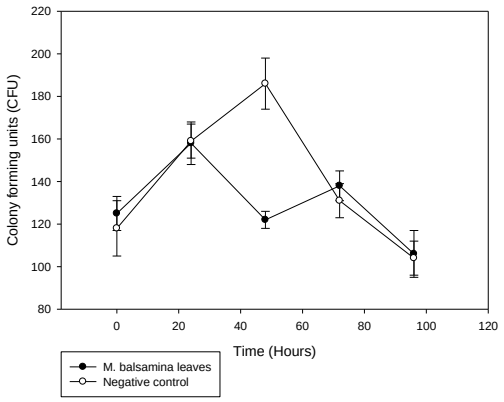

Fig. 3.72 Growth of *B. longum* with aqueous extract of *M. balsamina* compared to negative control (MRS broth only)

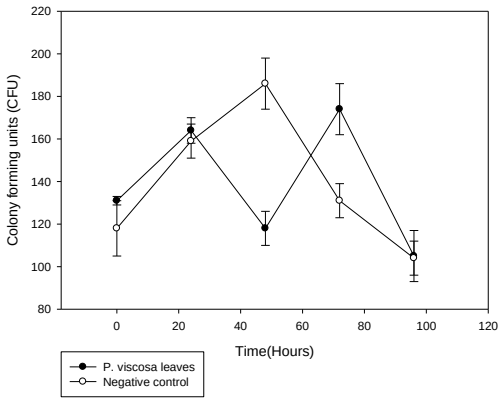

Fig. 3.73 Growth of *B. longum* with aqueous extract of *P. viscosa* compared to negative control (MRS broth only)

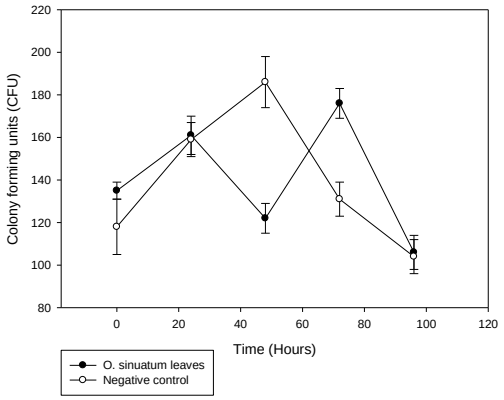

Fig. 3.74 Growth of *B. longum* with aqueous extract of *O. sinuatum* compared to negative control (MRS broth only)

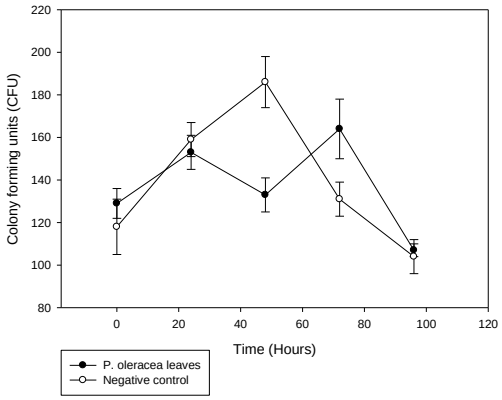

Fig. 3.75 Growth of *B. longum* with aqueous extract of *P. oleracea* compared to negative control (MRS broth only)

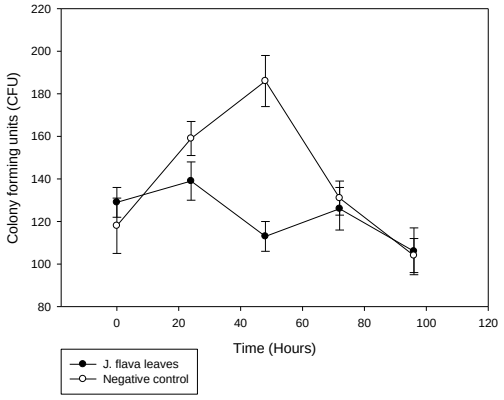

Fig. 3.76 Growth of *B. longum* with aqueous extract of *J. flava* compared to negative control (MRS broth only)

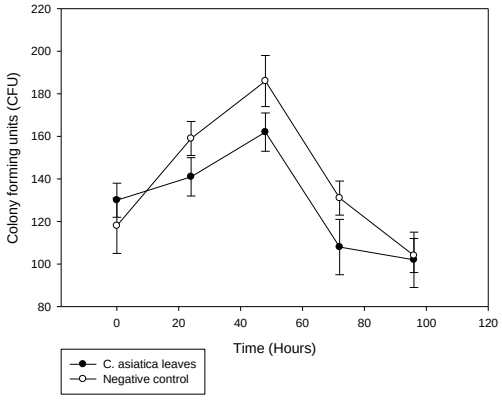

Fig. 3.77 Growth of *B. longum* with aqueous extract of *C. asiatica* compared to negative control (MRS broth only)

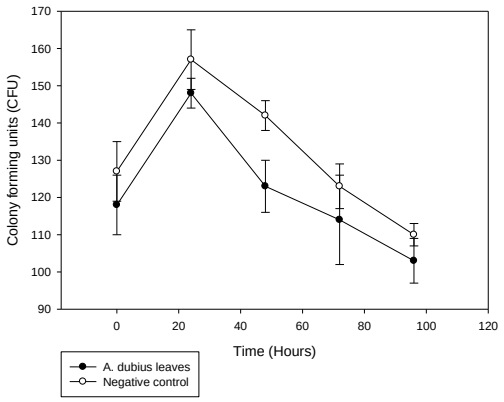

Fig. 3.78 Growth of *B. longum* with aqueous extract of *A. dubius* compared to negative control (MRS broth only)

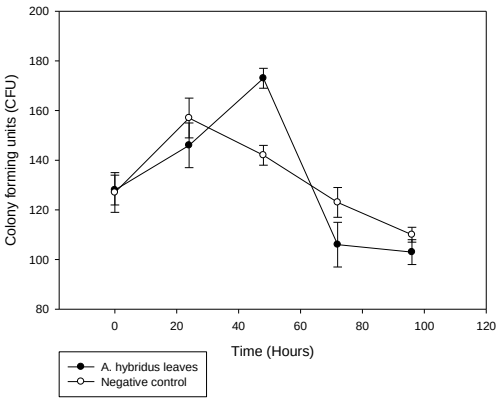

Fig. 3.79 Growth of *B. longum* with aqueous extract of *A. hybridus* compared to negative control (MRS broth only)

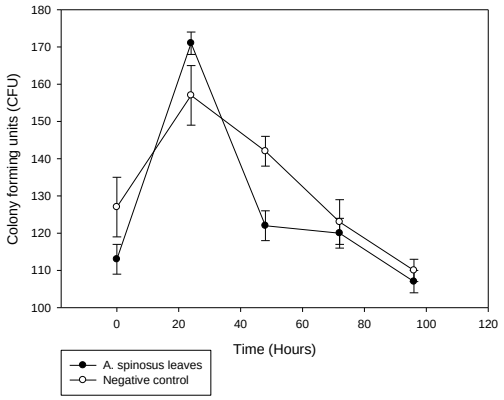

Fig. 3.80 Growth of *B. longum* with aqueous extract of *A. spinosus* compared to negative control (MRS broth only)

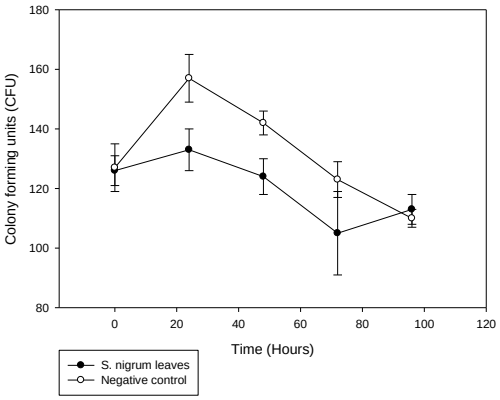

Fig. 3.81 Growth of *B. longum* with aqueous extract of *S. nigrum* compared to negative control (MRS broth only)

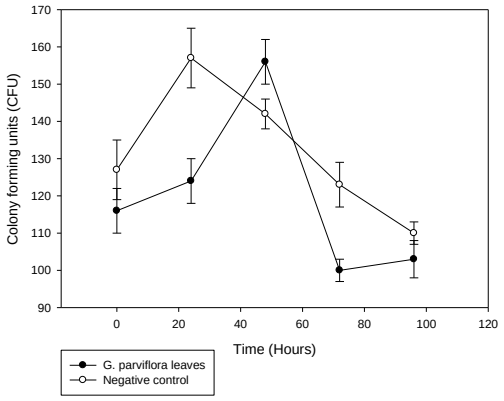

Fig. 3.82 Growth of *B. longum* with aqueous extract of *G. parviflora* compared to negative control (MRS broth only)

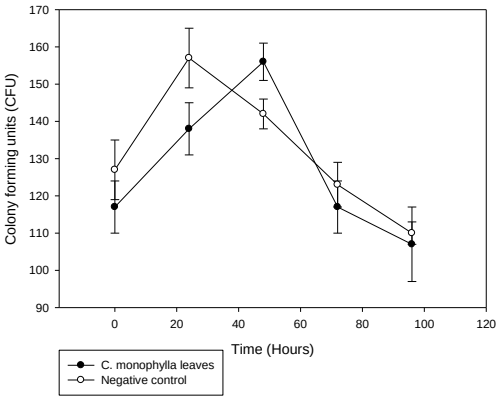

Fig. 3.83 Growth of *B. longum* with aqueous extract of *C. monophylla* compared to negative control (MRS broth only)

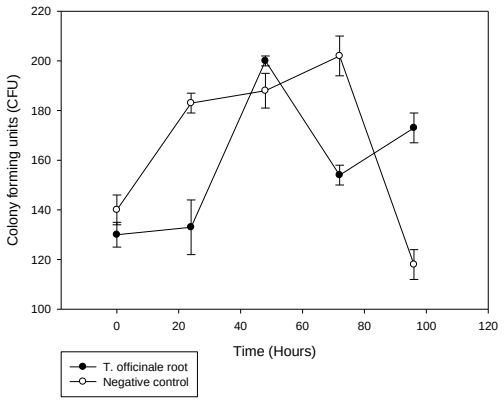

Fig. 3.84 Growth of *B. longum* with aqueous extract of *T. officinale* root compared to negative control (MRS broth only)

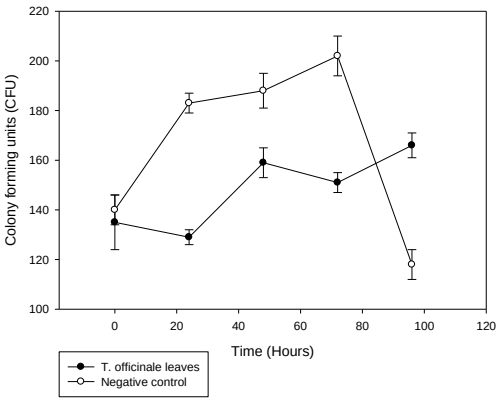

Fig. 3.85 Growth of *B. longum* with aqueous extract of *T. officinale* leaves compared to negative control (MRS broth only)

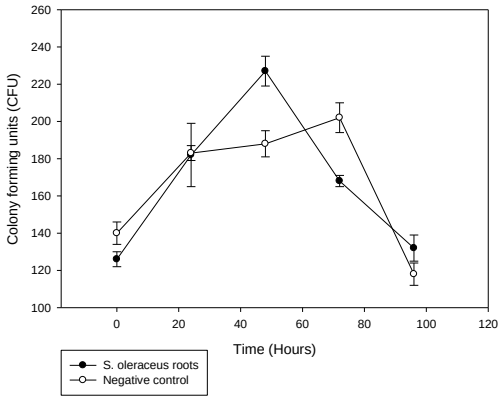

Fig. 3.86 Growth of *B. longum* with aqueous extract of *S. oleraceus* roots compared to negative control (MRS broth only)

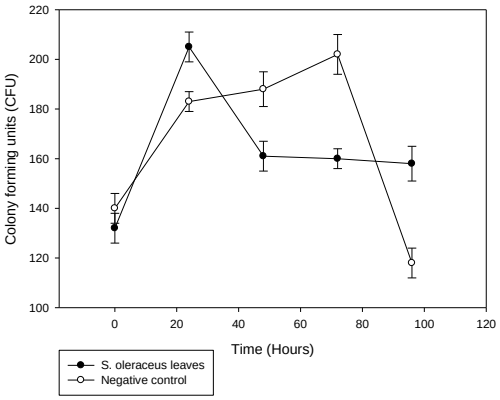

Fig. 3.87 Growth of *B. longum* with aqueous extract of *S.oleraceus* leaves compared to negative control (MRS broth only)

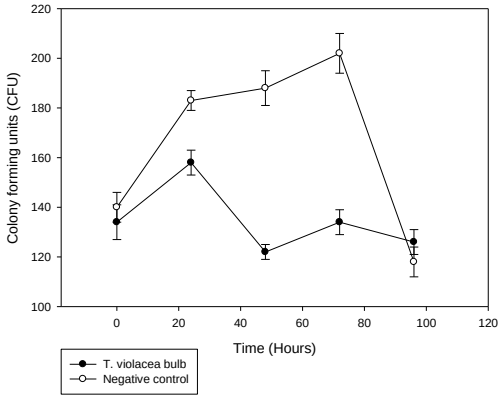

Fig. 3.88 Growth of *B. longum* with aqueous extract of *T. violacea* bulbs compared to negative control (MRS broth only)

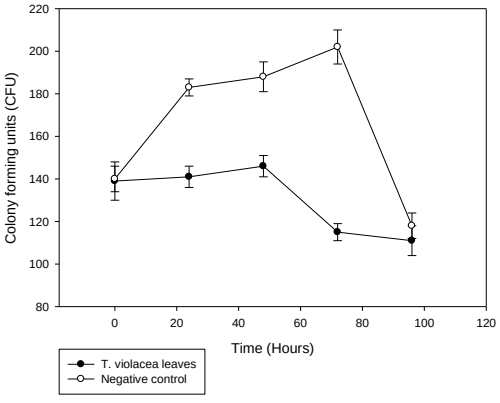

Fig. 3.89 Growth of *B. longum* with aqueous extract of *T. violacea* leaves compared to negative control (MRS broth only)

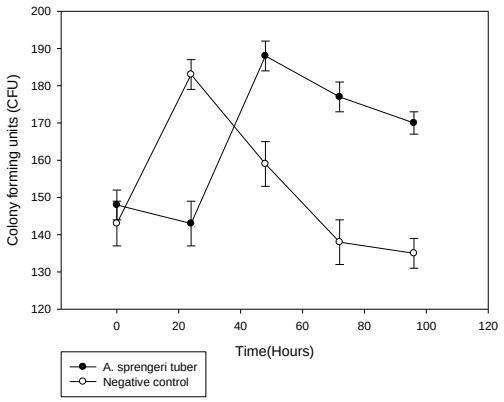

Fig. 3.90 Growth of *B. longum* with aqueous extract of *A. sprengeri* tuber compared to negative control (MRS broth only)

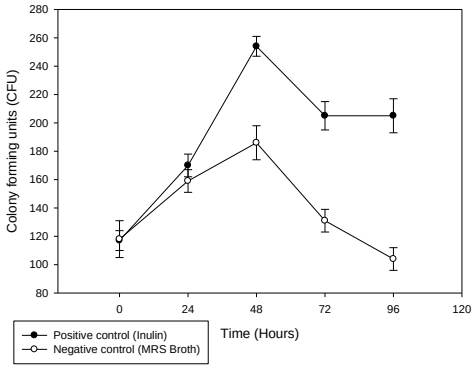

Fig. 3.91 Growth of *B. longum* in the presence of commercial inulin compared to negative control (MRS broth only)

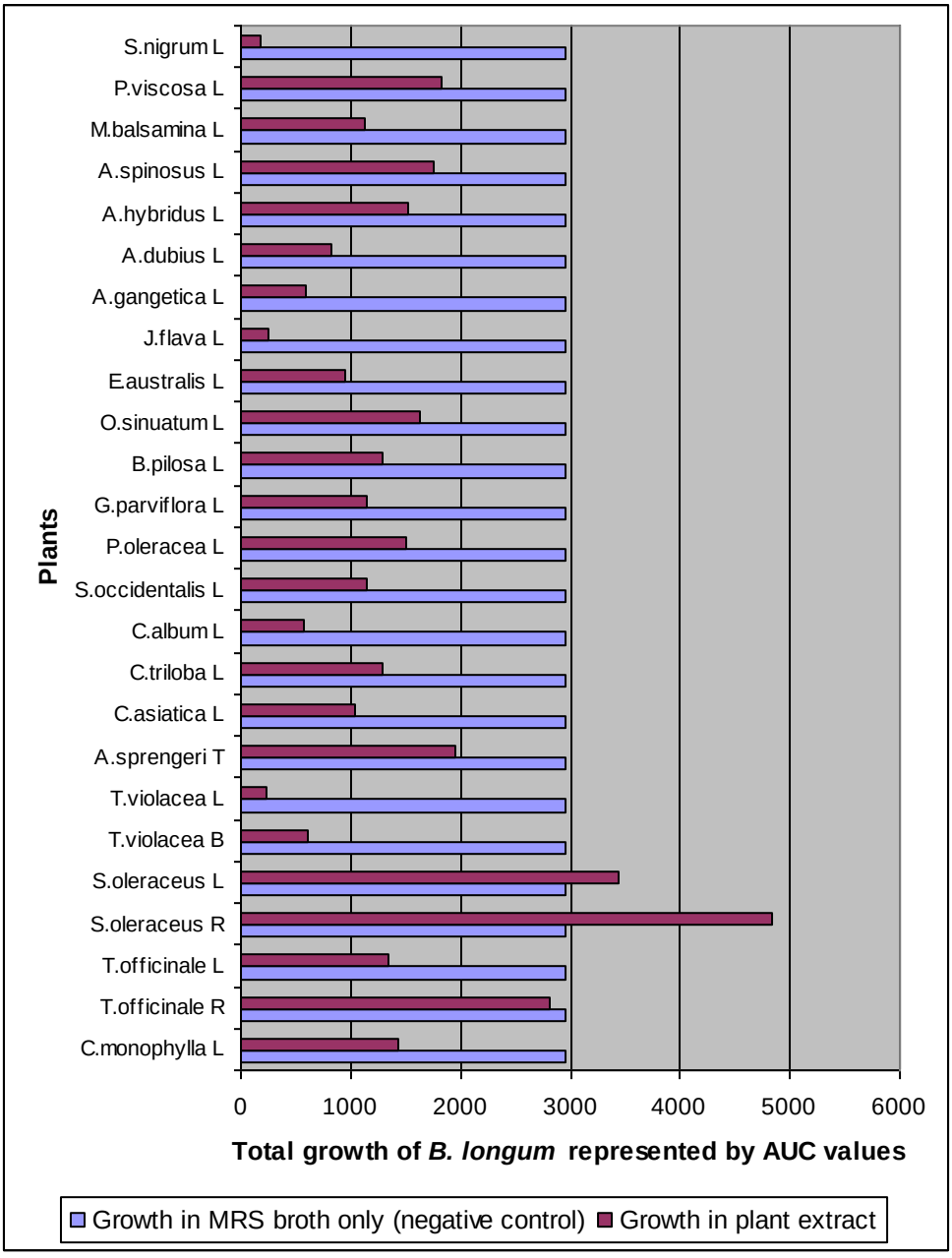

Fig. 3.92 Comparison of the growth response of *B. longum* over 96 hour period for 25 plant extracts to that when no plant extract was added (negative control)

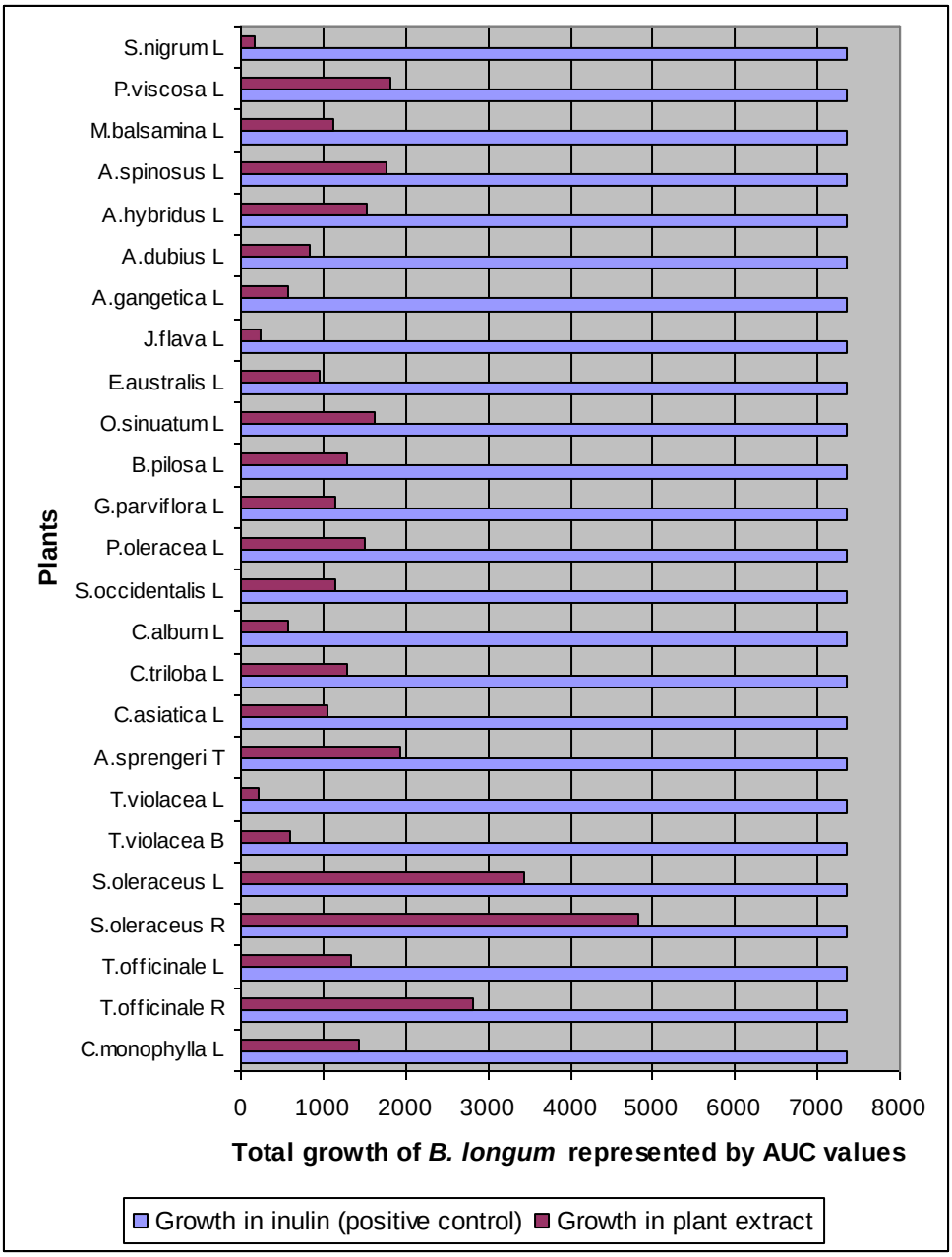

Fig. 3.93 Comparison of the growth response of *B. longum* over 96 hour period for 25 plant extracts to that of commercial inulin (positive control)

3.2 Inulin content of the plants

The inulin content was only measured in those plants which exhibited a prebiotic effect in this study. The quantity of inulin in the plant extracts was then correlated with the growth of the probiotic bacteria.

An HPLC method was used for the quantification of these β-Fructans in the plant extracts. This method included an extraction process with hot water, followed by hydrolysis with inulinase enzyme, and determination of the released fructose by HPLC with refractive index detection.

The method had to be optimised for the extracts tested in this study. We found that the amount of sample needed for the analysis was very low (1 g) due to the high sensitivity of the HPLC system. Standards such as glucose (Appendix 4), fructose (Appendix 5) and sucrose (Appendix 6) in varying concentrations were tested for their ability to be detected and to quantify fructose correctly. The hydrolysis conditions were 50°C for 30 minutes as described. Under these conditions total hydrolysis of inulin occurs as shown in the chromatograms (Figures 3.94 to 3.101) below.

It is generally accepted that the most quantitative method for the measurement of inulin type fructans involve enzymatic hydrolysis of all fructan materials to fructose and glucose using inulinase, followed by measurement of these released sugars by HPLC.

Figure 3.94 shows a chromatogram of a 1% inulin solution (Appendix 2) without the addition of inulinase. No fructose, glucose or sucrose was detected in this sample. Figure 3.95 shows a chromatogram of a 1% inulin solution after hydrolysis with inulinase. The quantity of fructose obtained in this figure represents the total amount of fructose present in the 1% inulin solution. Figures 3.96, 3.98 and 3.100 show chromatograms of the *Sonchus oleraceus* root, *Asparagus sprengeri* tuber and *Taraxacum officinale* root without the addition of inulinase. The quantity of fructose obtained in these figures represents the amount of free fructose in the samples. Figures 3.97, 3.99 and 3.101 show chromatograms of the *Sonchus oleraceus* root, *Asparagus sprengeri* tuber and *Taraxacum officinale* root after hydrolysis with inulinase. The quantity of fructose

obtained in these figures represents the total amount of fructose in the sample. The retention time for fructose varied between 3.60 and 3.92 minutes. The resolution was satisfactory.

For those plant samples that contained sucrose, a correction had to be made since the inulinase also splits sucrose, producing glucose and fructose. This problem was solved by using a duplicate sample of identical weight but without inulinase being added and followed likewise by HPLC analysis. The free fructose (F_2) and the fructose originating from sucrose (F_3) could thus be assessed and subtracted also from the amount of fructose (F_1) obtained by the inulinase digestion.

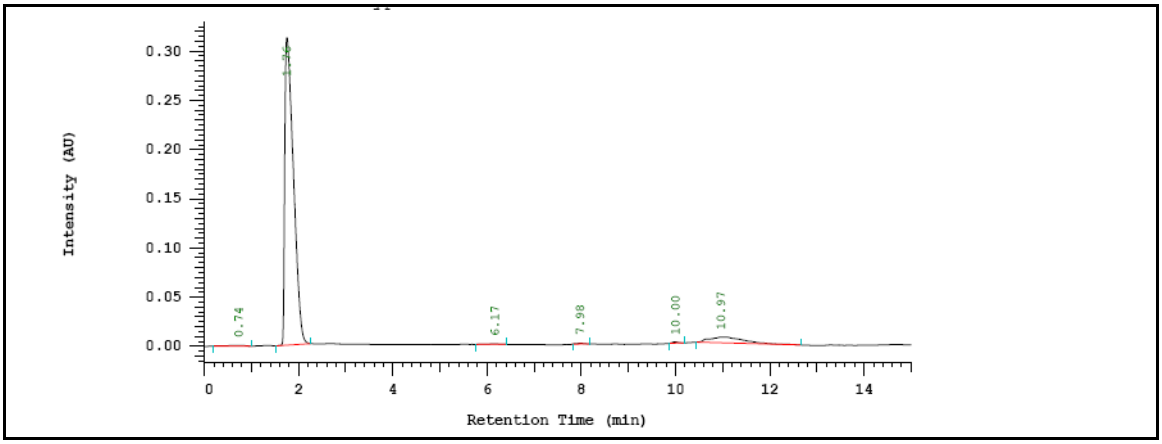

Fig. 3.94 Chromatogram of 1% Inulin solution without inulinase hydrolysis

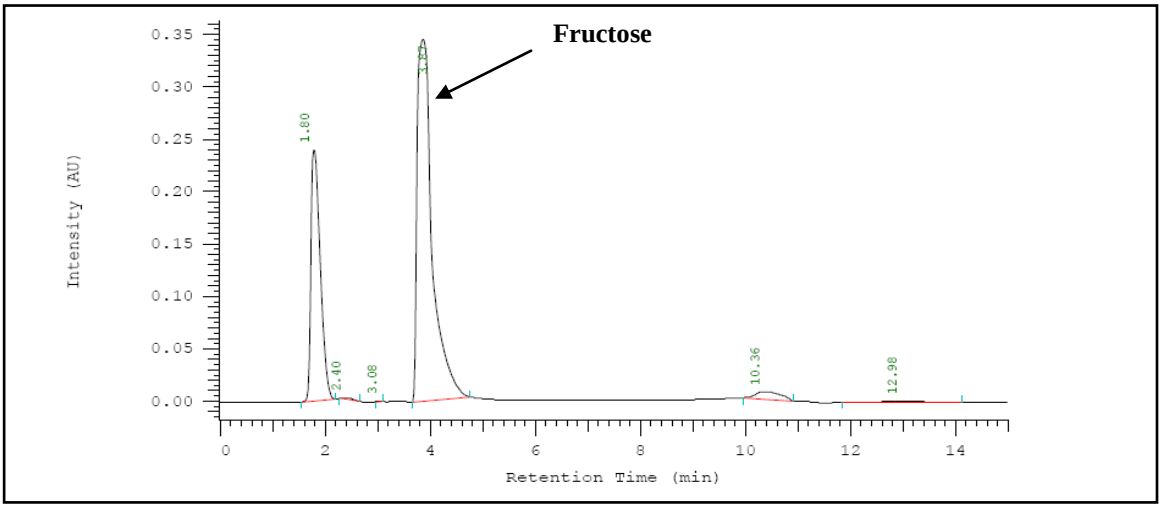

Fig. 3.95 Chromatogram of 1% Inulin solution after hydrolysis with inulinase

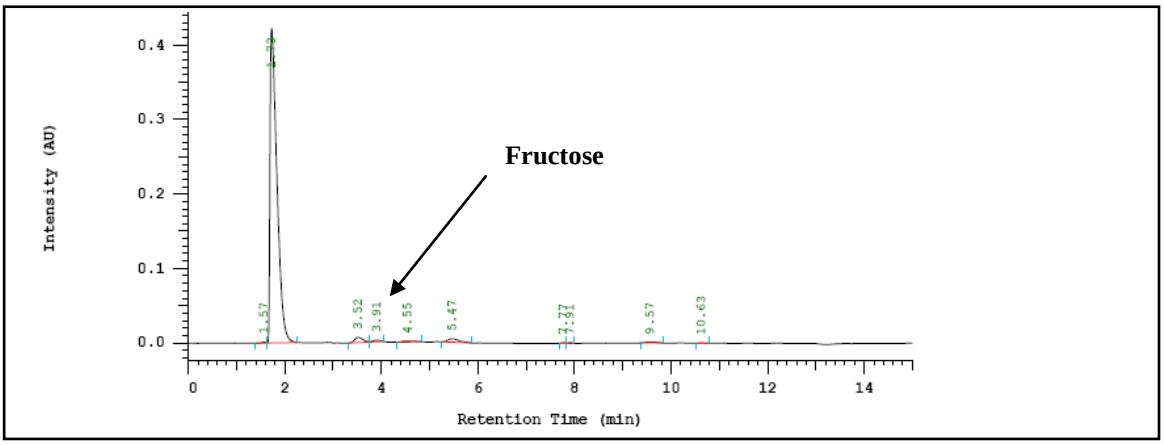

Fig. 3.96 Chromatogram of *Sonchus oleraceus* root without inulinase hydrolysis

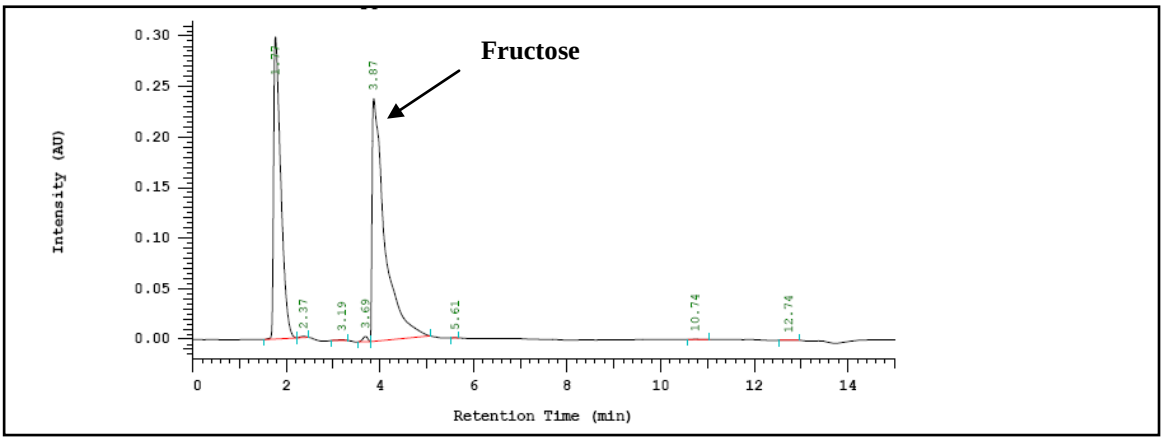

Fig. 3.97 Chromatogram of *Sonchus oleraceus* root after hydrolysis with inulinase

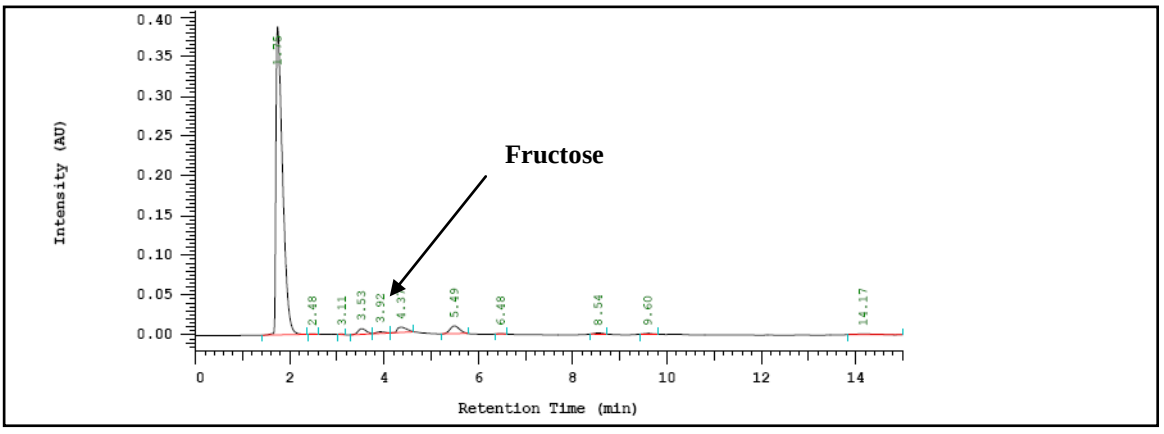

Fig. 3.98 Chromatogram of *Asparagus sprengeri* tuber without inulinase hydrolysis

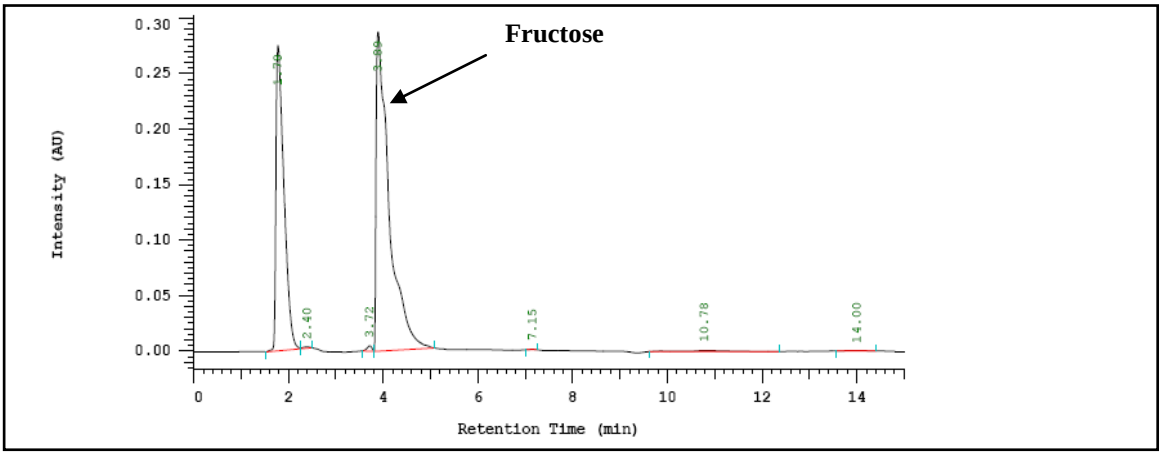

Fig. 3.99 Chromatogram of *Asparagus sprengeri* tuber after hydrolysis with inulinase

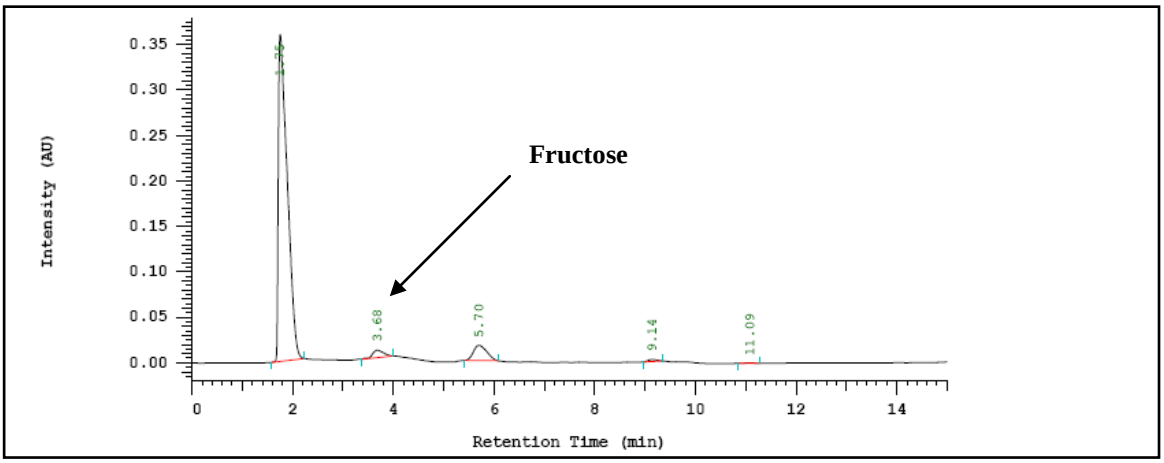

Fig. 3.100 Chromatogram of *Taraxacum officinale* root without inulinase hydrolysis

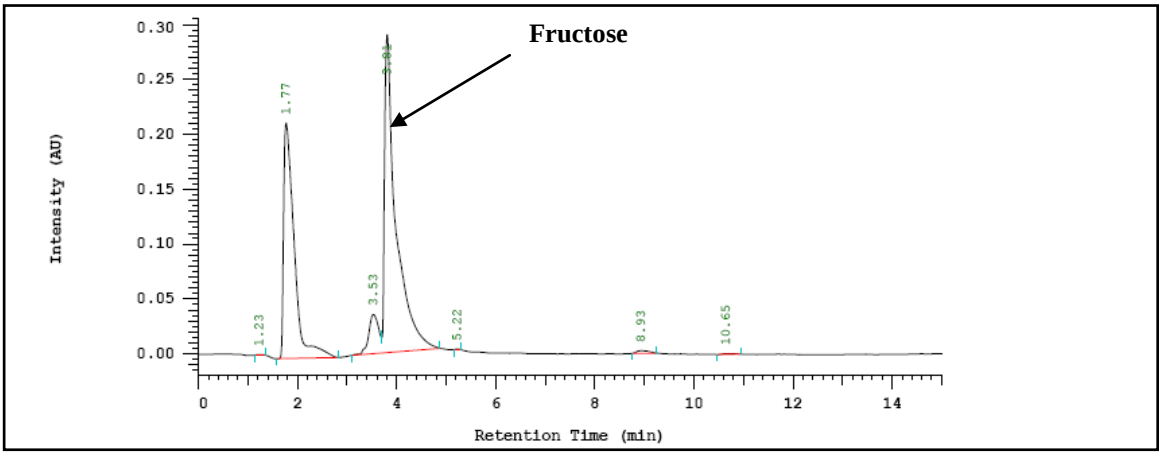

Fig. 3.101 Chromatogram of *Taraxacum officinale* root after hydrolysis with inulinase

The inulin content of the plant extracts varied according to family and the different plant parts (leaves, roots, bulbs, tubers) analysed, as can be seen in Table 3.5. It was found that the leaves contained more inulin then underground parts (roots or bulbs) for *T. violacea* (3.29%/3.02%), *S. oleraceus* (3.02%/2.94%) and *T. officinale* (2.99%/2.87%). Plants of the family Solanaceae, such as *Solanum nigrum* had an inulin content which ranged from 2.79 to 2.81%, and those from the family Amaranthaceae such as *Amaranthus spinosus* and *Amaranthus dubius* had an inulin content which ranged from 2.60 to 2.74%.

Plants from the family Asteraceae i.e. *Galinsoga parviflora*, *Sonchus oleraceus* and *Taraxacum officinale* had an inulin content which ranged between 2.77 and 3.02%.

Of all the plants studied, *Asparagus sprengeri* (tuber) of the family Asparagaceae had the highest inulin concentration (3.55%).

Table 3.5: Percentage inulin concentration of plant extracts

Scientific Name	Family	Plant part tested	Inulin content (%)
Solanum nigrum	Solanaceae	Leaf	2.79
Physalis viscosa	Solanaceae	Leaf	2.81
Momordica balsamina	Cucurbitaceae	Leaf	2.80
Amaranthus spinosus	Amaranthaceae	Leaf	2.60
Amaranthus hybridus	Amaranthaceae	Leaf	2.77
Amaranthus dubius	Amaranthaceae	Leaf	2.74
Justicia flava	Acanthaceae	Leaf	2.92
Galinsoga parviflora	Asteraceae	Leaf	2.77
Ceratotheca triloba	Pedaliaceae	Leaf	3.11
Asparagus sprengeri	Asparagaceae	Tuber	3.55*
Tulbaghia violacea	Alliaceae	Leaf	3.29
		Bulb	3.02
Sonchus oleraceus	Asteraceae	Leaf	3.02
		Root	2.94
Taraxacum officinale	Asteraceae	Leaf	2.99
		Root	2.87
Cleome monophylla	Capparidaceae	Leaf	2.48

*indicates plant containing highest amount of inulin

3.3 Correlation of prebiotic effect of the plant extracts and quantity of inulin

The prebiotic effect of the plant extracts relative to the inulin content of the extracts is shown in Table 3.6.

Correlation analysis is used to describe the degree of strength by which one variable is linearly related to another. This measure is based on a scale between -1 and +1. If an inverse relationship exists, then Pearson's coefficient of correlation (r) will fall between 0 and -1. If there is a direct relationship, then r will fall between 0 and +1. If there is no relationship between the two variables, then r=0.

For *L. lactis*, there was a direct relationship (r=0.5278) between the growth response (rep. by AUC values) of the organism and the inulin content of the extracts indicating that a high inulin concentration gave a higher increase in the growth of *L. lactis*.

An inverse relationship (r=-0.237) between the growth response (rep. by AUC values) of the organism and inulin content of the extracts was observed for *L. bulgaricus*, indicating that lower inulin concentrations gave a higher increase in the growth of *L. bulgaricus*.

There was no correlation (r=0.0347) between the growth response (rep. by AUC values) of the organism and the inulin content of the extracts for *B. longum*-no pattern was observed.

The growth response (rep. by AUC values) of all the probiotic cultures was then combined and correlated with the inulin content of the extracts. A direct relationship (r=0.1475) between the combined growth response and the inulin content of the extracts was observed, indicating a slight pattern-the inulin content of the extracts did affect the growth of the combined organisms, and it can therefore be concluded that the higher the inulin concentration in an extract, the higher the overall increase in bacterial growth will be.

Table 3.6: Prebiotic effect of the extracts relative to the inulin content

Scientific Name	**Plant part tested**	**Inulin content (%)**	***AUC *L. lactis***	***AUC *L. bulgar-icus***	***AUC *L. reuteri***	***AUC *B. longum***	***Median AUC (4 bacteria combined)**
Solanum nigrum	Leaf	2.79	264	2184	n/a	176	6.919
Physalis viscosa	Leaf	2.81	0	0	n/a	1824	0
Momordica balsamina	Leaf	2.80	0	440	n/a	1120	0.381
Amaranthus spinosus	Leaf	2.60	1704	2760	n/a	1760	11.282
Amaranthus hybridus	Leaf	2.77	144	1392	n/a	1520	7.736
Amaranthus dubius	Leaf	2.74	0	768	n/a	832	1.494
Justicia flava	Leaf	2.92	0	672	n/a	248	1.608
Galinsoga parviflora	Leaf	2.77	0	840	n/a	1144	3.083
Ceratotheca triloba	Leaf	3.11	16	2364	n/a	1296	0
Asparagus sprengeri	Tuber	3.55	6588	708	n/a	1944	13.033
Tulbaghia violacea	Leaf	3.29	4896	816	448	224	5.496
	Bulb	3.02	2856	624	n/a	608	4.978
Sonchus oleraceus	Leaf	3.02	5700	312	1504	3436	15.793
	Root	2.94	2472	1512	3044	4832	17.680

Taraxacum officinale	Leaf	2.99	8784	2088	4020	1336	14.700
	Root	2.87	7404	1644	4072	2812	20.975
Cleome monophylla	Leaf	2.48	1128	1492	n/a	1440	8.127

* Compiled using AUC values
n/a: not applicable

Of the four probiotic strains tested, the growth of the three lactobacilli was stimulated by most of the plant extracts. *L. lactis* was only stimulated by 15 of the 25 extracts, whilst *L. bulgaricus* was stimulated by 19 of the 25 extracts. *L. reuteri* was only tested against six extracts and of the six, five extracts stimulated the growth of *L. reuteri*. *B. longum* was only stimulated by two of the 25 extracts that were tested, as can be seen in Table 3.7.

As mentioned earlier, the growth response (rep. by AUC values) was calculated for each organism. All the growth responses of the different bacteria were compared to the positive and negative controls using Wilcoxon's test.

The median growth response (rep. by AUC value) of the four probiotic cultures combined was statistically significantly larger (p<0.05) than the negative control for *A. sprengeri* tuber, *S. oleraceus* root and *T. officinale* leaves and roots as shown in the table below (Table 3.7).

Table 3.7: Overview of the prebiotic potential of the plants studied against probiotic bacteria

Scientific Name	***L. lactis***	***L. bulgaricus***	***L. reuteri***	***B. longum***	**Four probiotics combined**
Solanum nigrum Leaf	+	+	**n/a**	-	+
Physalis viscosa Leaf	-	-	**n/a**	-	-
Momordica balsamina Leaf	-	+	**n/a**	-	-
Amaranthus spinosus Leaf	+	+	**n/a**	-	+
Amaranthus hybridus Leaf	+	+	**n/a**	-	+
Amaranthus dubius Leaf	-	+	**n/a**	-	-
Asystasia gangetica Leaf	+	+	**n/a**	-	-

Justicia flava Leaf	-	+	**n/a**	-	-
Emex australis Leaf	-	-	**n/a**	-	-
Oxygonum sinuatum Leaf	+	-	**n/a**	-	+
Bidens pilosa Leaf	-	-	**n/a**	-	-
Galinsoga parviflora Leaf	-	+	**n/a**	-	-
Portulaca oleracea Leaf	-	-	**n/a**	-	-
Senna occidentalis Leaf	+	+	**n/a**	-	-
Chenopodium album Leaf	-	-	+	-	-
Ceratotheca triloba Leaf	+	+	**n/a**	-	-
Centella asiatica Leaf	-	+	**n/a**	-	+
Asparagus sprengeri Tuber	+	+	**n/a**	-	+ *****
Tulbaghia violacea Leaf	+	+	-	-	+
Tulbaghia violacea Bulb	+	+	**n/a**	-	+
Sonchus oleraceus Leaf	+	+	+	+	+
Sonchus oleraceus Root	+	+	+	+	+ *****
Taraxacum officinale Leaf	+	+	+	-	+ *****
Taraxacum officinale Root	+	+	+	-	+ *****
Cleome monophylla Leaf	+	+	**n/a**	-	+

***** indicates significant difference ($p<0.05$) between the median growth response (rep. by AUC value) of the organisms growing in the plant extract and the median AUC of the negative control.

A plus symbol (+) indicates a prebiotic effect, whilst a negative (-) symbol indicates no prebiotic effect.

n/a: not applicable

Chapter Four:

Discussion

CHAPTER FOUR: DISCUSSION

4.1 Effect of plant extracts on growth of the probiotics

In order to determine if the selected plant extracts had a prebiotic effect, the aqueous plant extracts were added to a standardised number of *Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Lactobacillus reuteri* and *Bifidobacterium longum* growing in MRS broth, and the effect of the extracts on bacterial growth was tested over a 96 hour period. Pure commercial inulin (positive control) stimulated the growth of *L. lactis*, *L. bulgaricus*, *L. reuteri* and *B.longum*. The inulin-free MRS broth (negative control) showed decreased growth with time, indicating that inulin has a long-lasting prebiotic effect, as can be seen in figures 3.26, 3.54, 3.63 and 3.91.

It was noticed in this study that each of the four probiotic strains tested reacted differently to the addition of the plant extracts. For example, certain extracts such as that of *M. balsamina* and *G. parviflora* only stimulated the growth of *L. bulgaricus* but not *L. lactis*, whilst *O. sinuatum* only stimulated the growth of *L. lactis* but not *L. bulgaricus*, as can be seen in Table 3.7.

The inability of the majority of the plant extracts (which contained inulin) in this study to stimulate the growth of *B. longum* was most remarkable as it seemed to be contradictory to its accepted bifidogenic effect *in vitro* but this was not unexpected. The fermentation of fructooligosaccharides in the colon is the result of a complex sequence of metabolic pathways carried out by numerous species. The presence of extracellular hydrolytic enzymes supports mutual metabolic and nutritional dependencies among the mixed population in the intestine (Rossi *et al*., 2005).

Only two (both of the family Asteraceae) of the 25 plant extracts stimulated the growth of *B. longum* as can be seen in fig. 3.92. The inability of certain plant extracts to stimulate the growth of *L. lactis*, *L. bulgaricus*, *L. reuteri* and *B. longum* may be attributed to the degree of polymerisation (DP) of the inulin present in the extract.

The concentration and DP of the inulins stored varies between plant species. As inulin is a polydisperse mixture of oligomers with varying DPs, inulin samples are characterised by their

average degree of polymerisation, DP_n. The differences in DP_n between different inulins account for their unique health and fermentable attributes (Molina *et al*., 2005).

Average degree of polymerisation is the value that corresponds to the total number of saccharide units (G and F units) in a given inulin sample divided by the total number of inulin molecules, without taking into account the monosaccharides glucose (G) and fructose (F) and the disaccharide sucrose (GF) which are already present in the sample (Franck, 2002).

Vergauwen & co-workers (2003) have shown that inulin can be synthesised *in vitro* from sucrose (Suc) by the combined action of Suc:Suc 1-fructosyltransferase (1-SST) and fructan:fructan 1-fructosyltransferase (1-FFT). Although the DP of inulin produced *in vitro* from sucrose can be influenced by the ratio of 1-SST to 1-FFT in the reaction mixture, it can be expected that also the properties of the 1-FFT enzymes involved are responsible for the variation in DP found in different plants.

S. oleraceus (leaf and root) extracts were the only extracts to stimulate the growth of *B. longum* in this study, as can be seen in fig. 3.92. *S. oleraceus* belongs to the family Asteraceae and although the DP of the *S. oleraceus* inulin is unknown, the DP of the inulin from Chicory (also of the family Asteraceae) is known. Inulin from chicory is a mixture of oligomers and polymers of fructose having varying degrees of polymerisation but typically having a DP range from 3 to 60, with a modal DP of 9 (Franck, 2002). Higher DP inulins are found, for example, in Artichoke (*Cynara scolymus*) and Globe thistle (*Echinops ritro*) (Janer *et al*., 2004).

Various responses of bifidobacteria to fructans of different lengths have been described not only in terms of their fermentation capability but also in terms of their ability to induce differently located enzymes (Rossi *et al*., 2005; Van der Meulen *et al*., 2004; 2006). Despite the wide use of fructans as functional food ingredients and their well-studied prebiotic activity, very little is known about the relationship between the chain length of fructans and the ability of bifidobacteria to ferment them.

Van der Meulen & co-workers (2004) reported that large fructan polymers (DP>20) are not metabolised by certain strains of bifidobacteria at all, for which missing uptake systems or the absence of appropriate enzymes for their breakdown may be responsible. They also reported that oligofructose was metabolised preferentially indicating that uptake most probably takes place first, and that the uptake of short chain fructans induces the enzymes necessary for β (2-1) hydrolysis. It is therefore possible that the majority of the plant extracts in this study did not contain short chain fructans which therefore did not induce the necessary enzymes.

Van Laere & colleagues (2000) found similar results with the growth of a *Bifidobacterium adolescentis* strain on galactooligosaccharides and for the growth of a *Bifidobacterium infantis* strain on fructooligosaccharides (Perrin *et al*., 2001).

Biedrzycka & Bielecka (2004) reported that utilisation of inulins by bifidobacteria depends on the DP of fructooligomeric chains and purity. Other studies also mention the inability of some strains of bifidobacteria to metabolise the larger fructan polymers (Bielecka *et al*., 2002; Crittenden *et al*., 2002).

Van der Meulen & co-workers (2006) reported that the ability of bifidobacteria to grow on inulin is related to the production of extracellular enzymes that hydrolyze long-chain fructans. This finding is in agreement with the current paradigm that most bifidobacteria possess only inducible cell-associated β-fructofuranosidases (Van der Meulen *et al*., 2004); strains lacking extracellular hydrolytic enzymes first transport FOS into the cell and then hydrolyze them. Thus, the presence of extracellular β-fructofuranosidases may provide a basis for selection of new probiotic strains.

Rossi & co-workers (2005) screened a collection of 55 strains of bifidobacteria in order to compare their growth on inulin. Perhaps the most striking observation of that study was that 47 of the strains failed to grow on inulin. They also confirmed that bifidobacteria preferred shorter fructan chains as a substrate for growth.

A similar preferential metabolism of the short oligofructose fractions has already been described for *Bifidobacterium animalis* subspecies *lactis* DN-173 010 (Van der Meulen *et al*., 2004).

Hence, this preferential metabolism of shorter oligofructose fractions appears to be a specific characteristic of bifidobacteria. There are several possible explanations why a preferential metabolism of the short oligofructose fractions is observed for the *B. longum* strain and other bifidobacteria (Van der Meulen *et al*., 2004):

- The expression and localisation of the enzyme responsible for oligofructose degradation. This enzyme is inducible and located intracellularly for the *Bifidobacterium* strains. This suggests that the bifidobacteria degrade oligofructose intracellularly and metabolise the released fructose moieties simultaneously.
- A second explanation for the differences in oligofructose metabolism may be ascribed to the oligofructose transport system in *Bifidobacterium* spp.

Janer & co-workers (2004) reported that the carbohydrate source greatly influenced the growth of *B. lactis* cells in MRS broth medium. *B. lactis* was grown in MRS broth which contains neither a carbohydrate source nor meat extract supplemented with inulin. They reported that the β-fructofuranosidase of *B. lactis* was found to be highly selective for oligofructoses and showed a high affinity for β (2-1) bonds between fructosyl monomers. It was observed that the specificity decreased with increasing DP of the substrate. They therefore concluded that only inulin of a low DP supported the growth of *B. lactis*.

A decrease in the rate of hydrolysis of fructans was observed with increasing chain length and with the presence of a glucosyl instead of a fructosyl monomer at the chain end (Avigard & Bauer, 1966).

The inability of *B. longum* to metabolise the larger fructan chains which may have been present in the plant extracts is interesting from a commercial point of view, in creating a product containing a probiotic and prebiotic, in which the probiotic strain does not affect the prebiotic during transport and storage. Once consumed, both the probiotic and prebiotic may then exert their beneficial health effects in the human colon.

Kaplan & Hutkins (2003) reported that inulin, which has the same molecular structure as fructooligosaccharides, only with more fructose monomers had little affinity for the fructooligosaccharide transport system in certain strains of Lactobacilli. They also reported that the uptake of inulin/fructooligosaccharides in *Lactobacillus paracasei* 1195 was inhibited by agents that block substrate level phosphorylation and ATP synthesis.

The common plants which had a prebiotic effect on both *L. lactis* and *L. bulgaricus* were *S. nigrum* (leaves), *A. spinosus* (leaves), *A. hybridus* (leaves), *A. gangetica* (leaves), *S. occidentalis* (leaves), *A. sprengeri* (tuber), *T. violacea* (leaves and bulb), *S. oleraceus* (leaves and root), *T. officinale* (leaves and root), and *C. monophylla* (leaves). *S. oleraceus* (leaves and roots) and *T. officinale* (leaves and roots) also stimulated the growth of *L. reuteri* as can be seen in Table 3.7.

S. nigrum is a member of the family Solanaceae. *S. nigrum* leaf extracts stimulated the growth of both *L. lactis* (Fig. 3.27) and *L. bulgaricus* (Fig. 3.55) however no previous report of a prebiotic effect for this plant exists. The leaves are a good source of carbohydrates, ranging from 7.6-9.03 g/100 g (Hedrick, 1972; Odhav *et al*., 2007).

However, the fibre content is relatively low ranging from 1.6-2.42 g/100 g (Hedrick, 1972; Odhav *et al*., 2007). HPLC analysis of the leaves of this plant in this study revealed an inulin concentration of 2.79% (Table 3.5) which may also have contributed to its prebiotic effect.

A. spinosus and *A. hybridus* are both members of the family Amaranthaceae. *A. spinosus* and *A. hybridus* leaf extracts stimulated the growth of both *L. lactis* (Fig. 3.27) and *L. bulgaricus* (Fig. 3.55). However, no previous report of a prebiotic effect for these plants exists. The leaves of *A. spinosus* are a rich source of carbohydrates (50 g/100 g) and also contain a high amount of fibre (10 g/100 g) (Kruger *et al*., 1998). HPLC analysis of the *A. spinosus* and *A. hybridus* extracts in this study revealed an inulin concentration of 2.60 and 2.77% respectively (Table 3.5), which may have also contributed to its prebiotic effect.

A. gangetica is a member of the family Acanthaceae. *A. gangetica* leaf extracts stimulated the growth of both *L. lactis* (Fig. 3.27) and *L. bulgaricus* (Fig. 3.55) however no previous report of a

prebiotic effect for this plant exists. The leaves contain a relatively high concentration of carbohydrates (8.27 g/100 g) (Odhav *et al*., 2007), which may have contributed to its prebiotic effect. The fibre content is however relatively low (1.63 g/100 g) (Odhav *et al*., 2007).

S. occidentalis is a member of the family Fabaceae. *S. occidentalis* leaf extracts stimulated the growth of both *L. lactis* (Fig. 3.27) and *L. bulgaricus* (Fig. 3.55) however no previous report of a prebiotic effect for this plant exists. The leaves contain a relatively high amount of carbohydrates (9.37 g/100 g) (Langenhoven *et al*., 1991; Odhav *et al*., 2007), which may have contributed to its prebiotic effect.

The fibre content is however higher than that of *A. gangetica* (2.58 g/100 g) (Langenhoven *et al*., 1991; Odhav *et al*., 2007).

A. sprengeri is a member of the family Asparagaceae. *A. sprengeri* tuber extracts stimulated the growth of both *L. lactis* (Fig. 3.27) and *L. bulgaricus* (Fig. 3.55). Although no previous report of a prebiotic effect for this plant exists, the prebiotic effect of this extract on *L. lactis* was higher than that of the positive control (commercial inulin), as can be seen in figure 3.28.

The shoots of Asparagus are a precious vegetable that is cultivated in many countries of the world. In many European countries, it is a delicacy that has been served in spring for centuries. Shiomi (1981) detected 1-ketose, nystose, fructosylnystose and neoketose in Asparagus tubers. The β(2-1) fructans amount to 8% of the sugars (4% fresh weight), which means that Asparagus tubers contain at least 0.3% inulin (fresh weight) (Shiomi & Yamada, 1976; Shiomi *et al*., 1991).

High performance liquid chromatography analysis of the *A. sprengeri* tuber extract in this study revealed an inulin concentration of 3.55%-the highest concentration of all the extracts studied as can be seen in Table 3.5, which may have contributed to its prebiotic effect. Van Loo & co-workers (1995) reported a soluble carbohydrate content of 3.7% (fresh) in the tuber, of which 2.6% was inulin. The inulin content of vegetables is dependant on the time of harvest.

T. violacea is a member of the family Alliaceae. *T. violacea* leaf and bulb extracts stimulated the growth of both *L. lactis* (Fig. 3.27) and *L. bulgaricus* (Fig. 3.55) however no previous report of a prebiotic effect for this plant exists. The non-structural carbohydrates present in members of the family Alliaceae include glucose, fructose, and sucrose together with a series of oligosaccharides, the fructans (Darbyshire & Henry, 1981).

Darbyshire & Henry (1981) also added that DP 12 is the most frequently occurring chain length. High performance liquid chromatography analysis of the leaf and bulb extracts in this study revealed an inulin content of 3.29 and 3.02% respectively (Table 3.5), which may have contributed to its prebiotic effect. *Allium ampeloprasum* (Leek) is also a member of the family Alliaceae. Van Loo & co-workers (1995) reported an inulin content of 2.9% in fresh Leek leaves.

S. oleraceus is a member of the family Asteraceae. *S. oleraceus* leaf and root extracts stimulated the growth of all four probiotic strains tested, including *B. longum* as can be seen in Table 3.7. The *S. oleraceus* leaf is a very rich source of carbohydrates (45 g/100 g) and a good source of fibre (5.9 g/100 g) (Hedrick, 1972; Kunkel, 1984).

High performance liquid chromatography analysis of the *S. oleraceus* leaf and root extracts in this study revealed an inulin concentration of 3.02 and 2.94% respectively (Table 3.5). All these factors may have contributed to its prebiotic effect, and the fact that the prebiotic effect of the *S. oleraceus* leaf was higher than that of commercial inulin (positive control) for *L. lactis* (Fig. 3.28).

T. officinale is also a member of the family Asteraceae. *T. officinale* leaf and root extracts stimulated the growth of *L. lactis*, *L. bulgaricus* and *L. reuteri* but not *B. longum*, as can be seen in Table 3.7. The prebiotic effect of *S. oleraceus* leaf and *T. officinale* (leaf and root) was higher than that of commercial inulin (positive control) for *L. lactis* as can be seen in fig. 3.28. The prebiotic effect of the *T. officinale* root was also higher than that of the positive control for *L. reuteri* (Fig. 3.65).

T. officinale is a nutritious plant, 100 g of the raw leaves contains protein (2.7 g), carbohydrates (9.2 g), calcium (187 mg), phosphorus (66 mg), iron (3.1 mg), sodium (76 mg), potassium (397 mg) and magnesium (36 mg), (Crowe, 1990).

T. officinale (common Dandelion) is regularly eaten in the western world as a Dandelion leaf salad. In 1951, Bacon & Edelman confirmed that this plant contained inulin. Yanovski & Kingsbury (1938) determined an inulin content of 12.8% in fresh plant material.

High performance liquid chromatography analysis of the *T. officinale* leaf and root extracts in this study revealed an inulin concentration of 2.99 and 2.87% respectively (Table 3.5), which may also have contributed to its prebiotic effect.

The high inulin value, against our findings which was reported for *T. officinale* may be due to differences in habitats and environmental factors. It is also important to note that the inulin content of plants fluctuates as a function of time and the average DP of inulin decreases as the weather gets colder. The inulin content of plants is also dependent on times of harvest (Suzuki & Cutcliffe, 1989).

S. oleraceus leaf and root extracts were the only 2 extracts that stimulated the growth of *B. longum* (Fig. 3.92) in this study. There are no previous reports of *S. oleraceus* exhibiting a prebiotic effect, however, a prebiotic effect for other members of the Asteraceae such as *T. officinale* and *Cynara scolymus* has been reported.

Trojanova & co-workers (2004) reported that aqueous root extracts of *T. officinale* contain a high quantity of non-digestible oligofructans which are utilisable by the bifidobacteria and stimulated the growth *in vitro* of 14 strains of bifidobacteria.

Molina & co-workers (2005) found that *C. scolymus* (Artichoke) extracts exhibited a prebiotic effect *in vitro*-the utilisation of the inulin in the artichoke extracts by *Bifidobacterium* spp. and *Lactobacillus* spp. was comparable to Chicory inulin. Their results showed that the growth behaviour of *Bifidobacterium bifidum* was similar with either inulin and bacterial growth was

longer lasting in the presence of the Artichoke extract and Chicory inulins when compared to the control.

Fructosyltransferases present in *T. officinale* are responsible for the production of inulin (van den Ende *et al*., 2000). The Dandelion plant accumulates in its roots nutrients for winter: inulin, rubber, triterpenes, and fatty acids and their glycerides. In *T. officinale* roots, fructans preferentially accumulate near the sites of phloem unloading (Bogacheva *et al*., 1999).

Chicory (*Cichorium intybus*), also a member of the family Asteraceae is currently the primary industrial source of inulin. Fitters & co-workers (1991) followed the evolution of inulin accumulation in Chicory. Van den Ende & Van Laere (1993) purified and characterised the Chicory SST-enzyme (sucrose:sucrose fructosyltransferase), the enzyme that is the basis of the inulin formation in this plant.

The plant, being similar to the Sugar beet root, shares similarities in agronomic practices and inulin production technologies. The inulin production process involves three general steps: extraction of raw inulin with hot water, purification of the raw inulin and spray drying of the purified juice to a pure inulin powder (Franck, 2002).

Chapter Five:

General Conclusions

GENERAL CONCLUSIONS

Oligosaccharides, consisting of a mixture of hexose oligomers with a variable extent of polymerisation, are food products with interesting nutritional properties. They may be naturally present in food, mostly in fruit, vegetables or grains, or produced by biosynthesis from natural sugars or polysaccharides and added to food products because of their nutritional properties or organoleptic characteristics. The dietary intake of oligosaccharides is difficult to estimate, but it may reach 3-13 g/day per person (for fructooligosaccharides), depending on the population. The extent of resistance to enzymatic reactions occurring in the upper part of the gastrointestinal tract allows oligosaccharides to become 'colonic nutrients', as some intestinal bacterial species express specific hydrolases and are able to convert oligosaccharides into short chain fatty acids (acetate, lactate, propionate, butyrate) and/or gases by fermentation. Oligosaccharides that selectively promote some interesting bacterial species (e.g. lactobacilli and bifidobacteria), and thus equilibrate intestinal microflora, are now termed prebiotics.

The pattern of short chain fatty acid production in the caeco-colon, as well as the prebiotic effect, if demonstrated, are dynamic processes that vary with the type of oligosaccharide (e.g. extent of polymerisation, nature of hexose moieties), the duration of the treatment, the initial composition of flora or the diet in which they are incorporated. Experimental data obtained *in vitro* and *in vivo* in animals, and also recent data obtained in human subjects, support the involvement of dietary oligosaccharides in physiological processes in the different intestinal cell types (e.g. mucins production, cell division, immune cells function, ionic transport) and also outside the gastrointestinal tract (e.g. hormone production, lipid and carbohydrate metabolism).

In this study we investigated 22 plants that may have prebiotic effects. The table below (Table 5.1) is a summary of the key findings for each of the plants.

Table 5.1: Summary of overall prebiotic potential of plants studied

Plant	**Combined prebiotic effect (all 4 probiotics?)**	**Stimulated growth of *B. longum*?**	**Exhibited a prebiotic effect higher than inulin?**	**Previously reported prebiotic effect?**
S. nigrum	Yes	No	No	None
P. viscosa	No	No	No	None
M. balsamina	No	No	No	None
A. spinosus	Yes	No	No	None
A. hybridus	Yes	No	No	None
A. dubius	No	No	No	None
A. gangetica	No	No	No	None
J. flava	No	No	No	None
E. australis	No	No	No	None
O. sinuatum	Yes	No	No	None
B. pilosa	No	No	No	None
G. parviflora	No	No	No	None
P. oleracea	No	No	No	None
S. occidentalis	No	No	No	None
C. album	No	No	No	None
C. triloba	No	No	No	None
C. asiatica	Yes	No	No	None
A. sprengeri	Yes	No	Yes	None
T. violacea leaf	Yes	No	No	None
T. violacea bulb	Yes	No	No	None
S. oleraceus leaf	Yes	Yes	Yes	None
S. oleraceus root	Yes	Yes	Yes	None
T. officinale leaf	Yes	No	Yes	None
T. officinale root	Yes	No	Yes	Trojanova *et al.*, 2004
C. monophylla	Yes	No	No	None

Our study has shown that selected plant extracts esp. *A. sprengeri*, *S. oleraceus* and *T. officinale* do contain inulin and can stimulate the growth of the four probiotic strains *L. lactis, L. bulgaricus, L. reuteri* and *B. longum*, exhibiting a prebiotic effect *in vitro*. The effect of the *T. officinale* leaf and root extracts on the growth of *L. bulgaricus* was found to be higher then that of commercial inulin.

Therefore, these plants that grow locally have the potential to be commercialised and with further research be utilised in under-developed areas by local people as a nutritional supplement. The inulin present in *A. sprengeri*, *S. oleraceus* and *T. officinale* has the following properties which also lends itself for improving host health:

- Mouth-no hydrolysis, minimal bacterial breakdown
- Stools-no prebiotic excretion
- Stomach-no acid hydrolysis, no absorption
- Small intestine-no enzymatic hydrolysis, no absorption
- Colon-bacterial fermentation by Lactobacilli and Bifidobacteria.

Since interest in inulin as a functional food ingredient with health promoting properties has been more intense in recent years, further research needs to be completed to fully elucidate the health implications of inulin consumption. Additional data to determine effective levels of intake for specific health purposes also needs to be obtained.

The plants described in this study are expected to draw the interest of scientists and industrialists because of their health promoting properties. Indeed, inulin from these plants could have a major share in the future food ingredients market. Therefore the immediate future challenge lies on the growing of these plants in bulk quantities to extract their inulin at low cost, as well as demonstrating their role in improving the quality of processed foods and human health.

Moreover, it is immensely important to convince/educate the general public on the beneficial health benefits of consuming either these plants as a food source or the inulin obtained from the plants that can be used as a functional food ingredient in order to attract the worldwide market.

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APPENDIX

Appendix 1: MRS Broth

Prepared by suspending 50 g of MRS broth in one litre of distilled water and allowed to stand for 15 minutes to allow the media to dissolve. After the media had dissolved, it was sterilised by autoclaving at 121°C for 15 minutes. After the media had cooled sufficiently, it was used immediately.

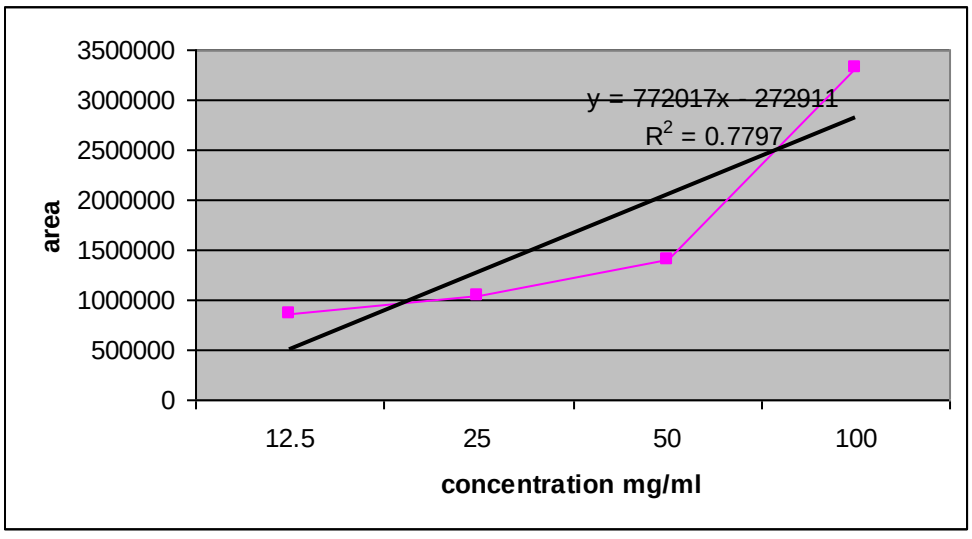
Appendix 2: 1% Inulin solution

Prepared by suspending 1 g of Inulin in 100 ml of sterile distilled water and stirred until completely dissolved.

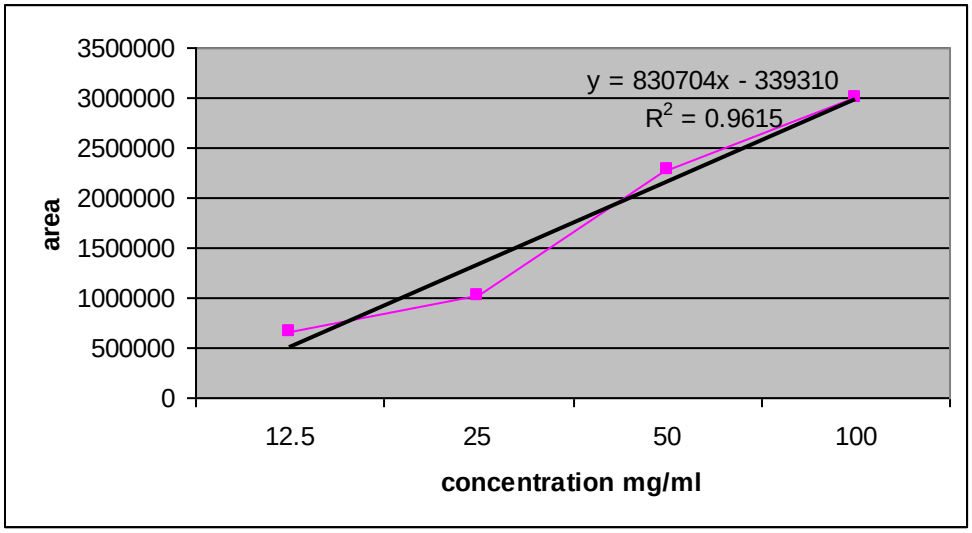
Appendix 3: ROGOSA Agar

Prepared by suspending 74.5 g of Rogosa Agar in one litre of distilled water and boiled until the media had completely dissolved (Do not autoclave). After the media had cooled, it was poured into Petri dishes and allowed to set. The plates were then stored at 4°C.

Appendix 4: Standard curve of fructose standard for HPLC



Appendix 5: Standard curve of sucrose standard for HPLC



Appendix 6: Standard curve of glucose standard for HPLC