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Review Article

# The Effect of Medicinal Plants on Probiotic Bacteria: A Review

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ABSTRACT

Probiotics are one of the functional and useful microorganisms that protect the host against many diseases and are used as live food supplements in food industry. Isolation of new bacteria with probiotic capabilities has expanded the effectiveness of this beneficial group of microorganisms in human and animals' health. Considering development of pathogenic bacteria and their increasing resistance to antibiotics, the importance of studying probiotics in treatment and prevention of diseases is crucial. Medicinal plants contain natural compounds with low complications, while their combination with probiotics can have more positive and surprising effects on humans and animals' health. Since the simultaneous use of medicinal plants and probiotics in food industry, including probiotic milk and yogurt products, can prevent the growth of pathogenic microorganisms; the present study was conducted to investigate the effect of medicinal plants on the performance and function of the probiotic microorganisms.

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## Introduction

In recent years, the design and production of plant-based probiotic products due to their benefits on people's health and based on variety in their production have attracted great attention (1). Although in the past decades, chemical drugs have been in great demand; their side effects over time have always been an important challenge and issue in human health (2). The benefits of medicinal plants and their few side effects have placed them as a suitable alternative to chemical drugs especially in Iran which has diverse sources of medicinal plants in different climates (1). Therefore, the increasing trend towards plant-based foods in many parts of the world has led to the design, production, and supply of plant-based or plant-based probiotic products (3). Also, over the past few years, superfoods have increasingly attracted the attention of consumers based on several health benefits of probiotics (4).

Today, food consumers pay attention to the pleasant taste, low calorie content and health effectiveness of food products to provide essential nutrients for body growth, and to treat and prevent various types of diseases. In addition, recent advances in food technology, lifestyle changes, and the growing social and economic population around the world reveal the need for foods with increased nutritional value and health benefits (5). So there is a driving force for the development and production of products to be attractive in global market (6). Therefore, the food industry is trying to produce and supply products that can bring better taste and properties. Meanwhile, dairy products processed with probiotics that can have many benefits on health

status, have received great attention (4).

Functional foods that are consumed as part of a regular diet contain biologically active compounds that can reduce the risk of many diseases (7). Fruitful foods should at least contain $10^7$ cfu/g of probiotic. They should be consumed more than 100 grams per day to play a positive role in body functions (8). On the other hand, super-foods are foods that are similar to normal foods and are consumed as part of a normal diet with physiological benefits or reduction of the risk of chronic disease beyond basic nutritional functions. Therefore, healthy foods that have been fortified and improved with various essential nutrients as functional foods such as vitamins and minerals can provide health for their consumers (9). Different probiotics with various microorganisms have been introduced to market to promote health status of consumers such as probiotic milk and yoghurt products (10).

One of the new methods in the treatment and prevention of diseases has been the use of probiotics in combination with food enzymes. The importance, popularity, and consumption of these probiotic products, whether food or pharmaceutical products, has been expanded so much even for pets and farms in addition for human needs (11). Also, interest in identifying new probiotic agents with valuable technologies are increasing (12). On the other hand, for probiotic bacterial role in the consumer's health status, they must be present in food products at least at a dose of $10^7$ grams (8). Various methods including addition of special food supplements or micro-coating techniques have been studied to ensure the survival of lactic acid bacteria (12).

Foods that contain probiotic bacteria have been classified as functional foods with positive effects on human health (8). Many diseases, including obesity, diabetes, inflammatory bowel diseases, and colon cancer were shown to be related to the imbalance in the intestinal microbial composition. So dysbiosis is associated with a decrease in the number of beneficial bacteria, an overgrowth of pathogenic species, or a loss of microbial diversities (13). Considering the importance of probiotics and medicinal plants (14), this review was carried out to determine the beneficial effects of different medicinal plants on physicochemical, textural, and sensory properties and viability of probiotics.

## *Probiotics*

Probiotic is a Greek word that means life. In 1965, the term probiotic was defined by Lilly and Stillwell for substances secreted by microorganisms that can stimulate the growth of other microorganisms, so probiotics play a role that is completely against antibiotics (15). According to the definition of Food and Agriculture Organization (FAO), probiotic microbes are living organisms when consumed have one or more beneficial effects on the host's body (16). They can regulate the immune system, play antimicrobial role, reduce the cholesterol level (17, 18), increase the nutritional value of foods (19), and improve the lactose tolerance (18, 20). These beneficial microorganisms have therapeutic benefits, and their biological activity is partially due to their ability to bind to enterocytes (21). The useful effects of probiotics on human microorganisms may improve metabolic or physiological processes and reduce the risk of many diseases (22). Probiotics can also reduce the antibiotic-related infections and increase general mucosal immunity in the digestive and urinary tracts (23).

According to the joint FAO and World Health Organization (WHO) guidelines for the evaluation of probiotics in foods, appropriate laboratory studies demonstrated the potential health benefits of probiotics before any clinical trial (24). Some tests such as resistance to acid, bile and antimicrobial production are usually utilized as the first step to select beneficial probiotic microorganisms. The prerequisites for probiotics are that they must be recognized as an approved additive, show antimicrobial activity against pathogenic bacteria and have good adhesion to intestinal mucosal layer. One of the most important functions of probiotics is to improve the microbial balance of the host's intestine. Other benefits enroll increasing resistance to diseases and therapeutic effects in chronic diseases such as hypercastrolemia (25).

Preventing growth of pathogenic organisms is considered as one of the main characteristics of probiotics. The antagonistic effects of *Lactobacillus* isolates on *Staphylococcus aureus* and *Escherichia coli* showed that in the spot culture method, *Lactobacillus* isolates illustrated a clear inhibition zone against the two pathogenic bacteria and in this way, the growth inhibitory effects against pathogens related to *Lactobacillus paracasei* with the diameter of the halo of no growth (2.94±0.139 mm) against *Staph. aureus* and 2.59±0.137 mm against *E. coli*. It should be noted that there was no significant difference in the antagonistic effects of *Lactobacillus* isolates against gram positive and negative bacteria and the greatest growth inhibitory effect against the pathogen was related to *L. paracasei* against *Staph. aureus* with the diameter of the non-growth halo of 1.45 mm (25).

## *The Importance of Probiotics*

The issue of the benefits of probiotics for human's

health has been discussed for a long time (26). After consumption of enough probiotics, beneficial effects can appear in the natural microbial population of the digestive system. In this relation, the most well-known probiotic bacteria are *L. acidophilus* and *Bifidobacterium bifidum* (27). However, some other bacteria such as *E. coli* and *Bacillus cereus* and *Saccharomyces cerevisiae* have been used in food industry to improve human health status (28). Beneficial probiotic bacteria must have a great ability to adhere to the intestinal epithelium to improve the intestinal microbiota. It seems that probiotic strains with self-aggregation can significantly affect the binding capacity of bacteria to intestinal epithelial tissue, while aggregation with pathogens can prevent the colonization of pathogens in the intestine (29).

The ability of probiotics to protect humans against the dangerous pathogenic agents is considered a natural pathogen inhibitor (30). *L. casei* can keep the intestine safe from formation of precancerous lesions via destruction of carcinogenic enzymes such as azoductase (31). First the research was conducted to determine the effect of different doses of garlic on the growth of *L. acidophilus* and *B. bifidum* and in the second step by using probiotic yogurt (0.33 grams) added separately to one liter of sterilized low-fat milk, the samples were examined based on pH and microbial count during the warm storage period and shelf life. It was found that by increasing the concentration of garlic extract in the samples containing *L. acidophilus*, a favorable consistency, taste, aroma, and smell were visible in the yogurt; even there were no significant differences in concentration of garlic containing *B. bifidum* bacteria. *B. bifidum* milk was more effective than *L. acidophilus* in reducing serum cholesterol level of experimental rats. Milk with *L. acidophilus* showed a greater effect than *B. bifidum* in reducing serum triglycerides in rats too. Finally, increase in the concentration of garlic in the probiotic milk with the two mentioned bacteria demonstrated a direct relationship with the reduction of cholesterol and triglyceride levels. Figure 1 shows the effect of garlic extract on milk and yogurt containing *L. acidophilus* and *B. bifidum*, as well as its effect on triglycerides and cholesterol level (32).

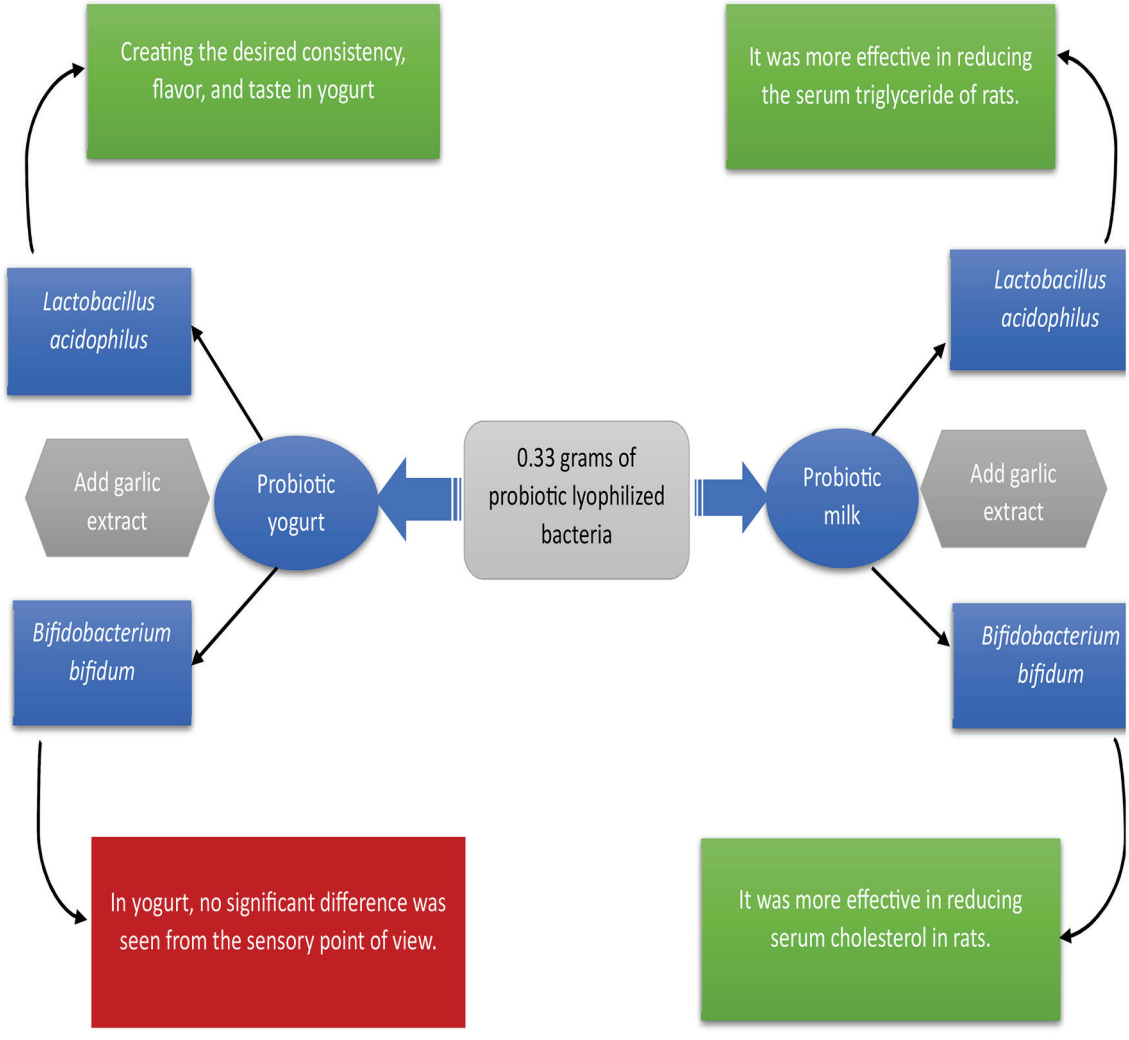


**Figure 1:** The effect of garlic extract on milk and yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, as well as its effect on triglycerides and cholesterol levels in rats.

The effect of garlic extract was an increase in the concentration of dill with a good taste in comparison to yogurt. Yogurt containing 0.6% dill had the best taste; while yogurt containing 0.2% dill and the control that did not contain dill extract were more consistent than other samples. In evaluation of microbial culture, *B. bifidum* grew less than *L. acidophilus* when MRS agar culture medium was utilized. So milk samples containing *B. bifidum* were more effective in reduction of cholesterol and triglyceride levels in comparison to *L. acidophilus* (33). Accordingly, consumption of dairy products containing probiotics was shown to control serum cholesterol level in both animals and humans (34).

### *Use of Probiotics*

In recent years, probiotics among food products have shown positive effects on human health opening a window in marketing of these products (35). Among the beneficial uses of lactic acid bacteria and their metabolites, improvement in quality and increase in shelf life of fermented food products) can be mentioned (36). According to WHO report, more than 40% of pregnant women reported iron deficiency and anemia that can increase the risk of death during pregnancy and infancy, raise the premature births, and lead to neurological and developmental disorders among children. Probiotics were demonstrated to decrease the rate of spontaneous premature births, long-term pregnancies, and eczema in pregnant women. If *L. plantarum* is combined with 4.2 mg of iron, 12 mg of ascorbic acid and 30 mg of folic acid, it can have positive effects on iron deficiency of women (37).

It was shown that probiotics can have favorable impacts on humans and animals health, such as anti-cancer properties, increasing growth efficiency, and improving nutritional properties (11). As drug resistance for some bacteria is increasing day by day, there is a need to prevent the growth of pathogenic bacteria by competitive inhibition of probiotic bacteria such as lactobacilli (38). Probiotics of *Streptococcus thermophiles* were shown to increase ceramide and skin hydration (39). In patients with sensitive skin, *B. longum* could reduce the sensitivity and resistance of the skin against allergens (40). These topical treatments do not cause any secondary skin diseases too (41). Although antibiotics are utilized in treatment of some skin diseases and have short-term effects on disease control, their long-term negative effects on the gut microbiome cannot be denied (42).

If topical probiotics are used in treatment of skin diseases, they can provide a better condition for the recovery of beneficial bacteria by avoiding the destruction of useful bacteria and finally improve the treatment process. Although the effects of topical probiotics on various skin diseases have not yet been fully determined, human and animal models have been successfully employed for topical use of probiotics in various skin diseases. Oral probiotics have also been effectively used in treatment of topical skin diseases (41). The viability and stability of probiotic strains in promoting health status during processing of probiotic has been studied. Use of enteric food formulas was demonstrated to be vital and innovative by applying nanotechnology and food encapsulation methods to improve intestinal function (42). Figure 2 demonstrates the effect of medicinal plants on probiotics in food and dairy products industries.

### *The future perspective of Probiotics*

Probiotics may exert protective effects against drug-resistant infections. In addition, probiotics and their antimicrobial byproducts are being explored as new future alternatives to antibiotics (43). Probiotics can also be added to food products with the aim of increasing the nutritional value (8). In the last decade, many societies have faced with dangerous challenges of swine flu and the dangerous Covid-19 virus, among which probiotics have been proposed as acute and preventive care strategies to deal with Covid-19 (44). In a report on a small group of patients hospitalized for Covid-19, it was found that adding a multi-strain formulation of probiotics could reduce mortality from the disease (45). Therefore, it is expected that in future, probiotics would be increasingly used as additives to stimulate growth and improve health due to their balancing effects on immunity, intestinal microbiota, food consumption and productivity (46).

Probiotics have been evaluated for women with various diseases. Probiotics have been suggested as a complementary treatment in vaginal infections along with estrogen hormone therapy in postmenopausal women (47). The oral supplement use of *L. rhamnus* and *Bifidobacterium* for 6 weeks was shown to significantly improve the mobility and reduction of DNA fragmentation and reactive oxygen in asthenozoospermic men (42). There are also lots of evidences about the role of microbiota in reproductive health and the immune system, as well as, the positive effects of probiotics to improve reproductive disorders (48). Recently, endometriosis with changes in the reproductive system was demonstrated to be correlated with the intestinal microbiota (49). Studies on vaginal dysbiosis revealed the promising effects of *L. rhamnosus* for maintenance of the normal microbiome (50) and introduced probiotics as a new therapeutic method in treatment of endometriosis (42).

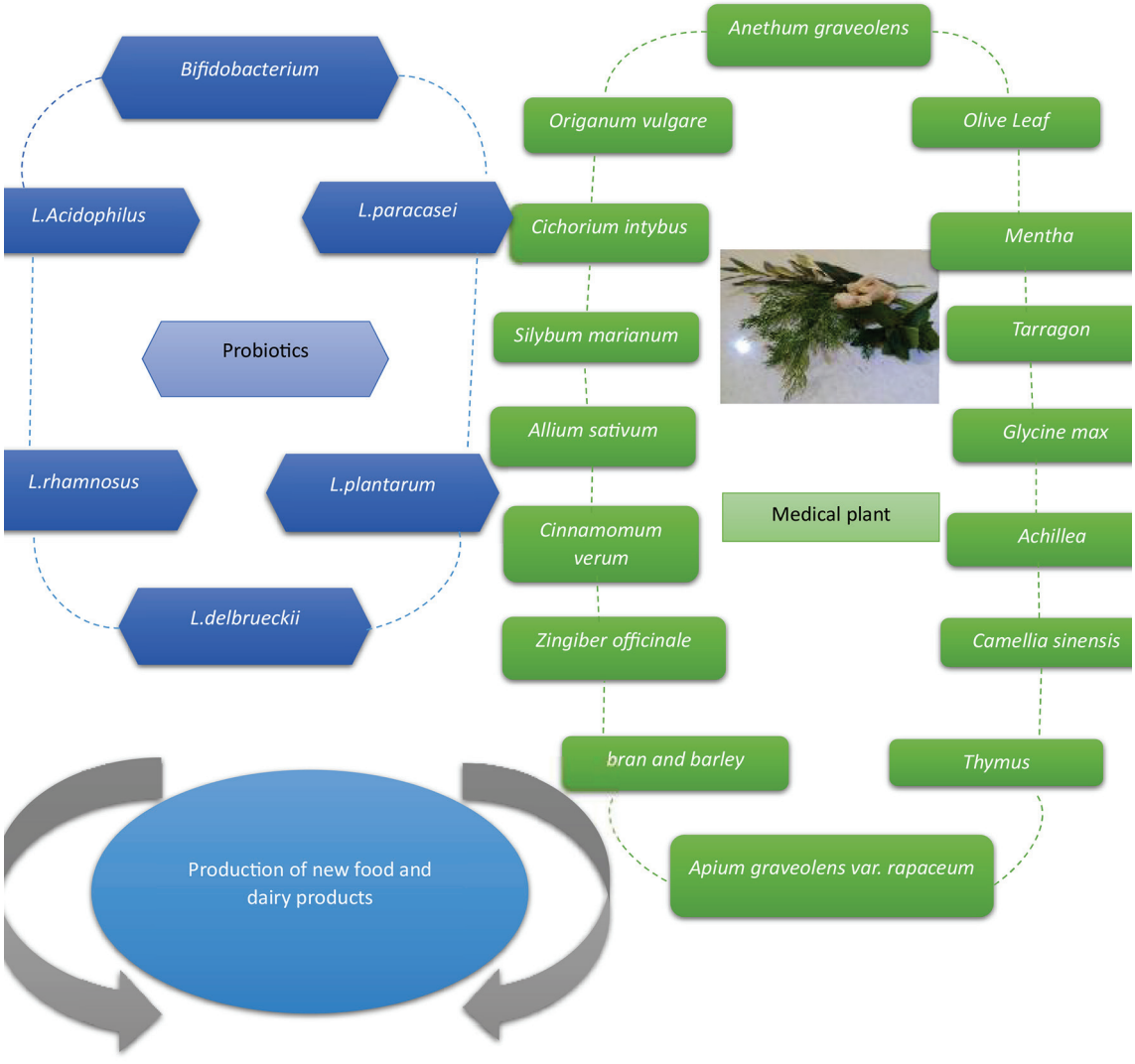


**Figure 2:** The effect of medicinal plants on probiotic bacteria in production of food and dairy products with more nutritional value.

## *Medicinal Plants*

Medicinal plants are used in different ways in prevention and treatment of several diseases (51). The use of natural antimicrobial compounds for food preservation has been of great value. In addition to antimicrobial properties, these compounds can have flavoring and mild antioxidant properties too (3). The polyphenols in plants can have a positive double effect simultaneously to inhibit pathogens and stimulate beneficial bacteria (52). Some probiotic microorganisms, especially *B. bifidum* have a slow grow rate in milk as they lack proteolytic activity. Therefore, it needs essential growth factors such as peptides and amino acids to increase growth rate (53). The design and production of plant-based probiotics have received attention due to their natural health benefits and based on their production diversity (54). It seems that there is a need for appropriate plant compounds to produce probiotic foods (55).

Medicinal plants have been used in traditional medicine to treat many diseases based on their antioxidant properties including infectious diseases (56), infertility (57), and burns (58). But these plants may still have some side effects (59). In many cases, it was shown that medicinal plants can reduce the toxicity of chemical drugs (60, 61). But they are still low-cost treatment choices with fewer side effects in the treatment of many diseases (62).

## *Effects of Medicinal Plants on Probiotics*

The use of medicinal plants in probiotics has been investigated since the ban on the use of antibiotics by the European Union in 2006. They were called a new therapeutic alternative that can solve the gap for presence of vitamins and various phytochemicals such as alkaloids, phenols, flavonoids, etc. In this relation, probiotics were reported to control pathogens, reduce mortality and improve the overall performance of body functions (63). The effect of tarragon on *L. casei* and *L. paracasei* has been investigated revealing that milk containing 3% of tarragon and *L. casei* could provide an acidity of

84-87 degree Dornic; while the greatest effect was on the growth of bacteria during incubation. It was shown that samples containing 2% tarragon essential oil, samples containing 1% essential oil, those containing 0% tarragon essential oil, and finally the samples containing both *L. casei* and *L. paracasei* could provide an acidity of 84-87 degree Dornic. As a result, 0% *L. casei* within milk had the least effect on the growth of bacteria during incubation. Within 21 days of storage, milk samples containing *L. casei* and *L. paracasei* in the refrigerator resulted in a higher acidity level for samples containing 3% tarragon essential oil in comparison to other samples containing 2% essential oil, 1% essential oil and 0% essential oil within 21 days of storage. Milk samples containing *L. casei* and *L. paracasei* kept in the refrigerator caused higher acidity level for samples containing 3% tarragon essential oil in comparison to other samples containing 2% essential oil, 1% essential oil and 0% essential oil of this plant. So during refrigeration, the control samples had the highest stability, while the samples containing 3% tarragon essential oil showed the least stability (8).

In a study that borage flower extract was used as a growth medium for four bacteria, including *L. paracasei*, *L. acidophilus*, *L. plantarum* and *L. delbrueckii*, the results showed these bacteria can grow in borage flower extract without any supplement) 64). The effect of thyme has also demonstrated that samples containing *L. acidophilus* and *B. bifidum* and samples containing both bacteria could not create a favorable taste in yogurt. Also, by increasing the concentration of thyme, no impact on the consistency of yogurt happened, while the samples of yogurt contained 3% thyme were more colorful and with a better smell (3). Also, in a research conducted on green tea, it was found that by increasing the concentration of this plant in probiotic milk and yogurt products, a favorable taste was visible and the extract increased the metabolism of lactic acid bacteria in the milk and yogurt products (65).

On the other hand, one of the most important indicators in the production of probiotic products is to ensure the viability of bacteria to maintain their favorable effect until the end of their life cycle (66), so in the experiments that were conducted to investigate the effect of bran barley and bran-free barley on *L. acidophilus* and *B. bifidum*; there was a direct relationship between the increase in bacterial growth and the amount of barley added revealing that the highest acidity and the highest number of live bacteria were observed in the 15% barley samples. It was shown that the lowest growth and survival of probiotic bacteria was correlated with the samples containing barley bran as an inhibitory factor for bacterial growth due to the phytic acid. For all samples, the effect of the atmosphere on *L. acidophilus* was more noticeable, so the growth rate and survival of probiotic bacteria with bran-free barley extract was $10^{10}$ cfu/mL, which was higher than a minimum probiotic property (67).

The essential oils of cumin and oregano in low concentrations were shown to stimulate the growth rate and acid production of *L. plantarum*, while in high concentrations they prevented the growth of *L. plantarum* )11). However, in another study conducted on oregano, a sample contained *B. bifidum* with increasing concentration, a favorable taste was visible in yogurt for all samples, while 0.1% oregano yogurt demonstrated the best taste even the control samples in absence of oregano had more consistency than other samples. The results showed that increase in the concentration of this plant provided a pleasant taste with a positive effect on the growth of *L. acidophilus* and *B. bifidum* in probiotic milk products (34).

The growth of *L. plantarum* was stimulated by an increase in *Achillea millefolium*. However, by adding plant extracts at different concentrations, *L. rhamnosus* strains were not affected) 68). In the process of yogurt production, by an increase in the concentration of soy extract, the acidity increased and the consistency of the yogurt improved. The consistency of yogurt containing soy extract was better than the consistency of yogurt containing malt extract, even the taste of yogurt with malt extract was better than yogurt containing soy extract. The effect of soybean extract on *L. acidophilus* and *B. bifidum* in the production of probiotic milk and yogurt products was significantly more than that of malt extract, even the duration of heating was less. Soybean extract could shorten the time to reach the desired acidity, which can be due to the presence of nutrients as growth stimulants in soybean extract, which malt extract lacked (69).

In another experiment on soybean extract, similar results were noticed so that increase in the concentration of soybean extract could shorten the time to reach the desired acidity of milk and yogurt products, while the consistency of yogurt improved too. On the other hand, the use of concentrations of 6%, 4%, 2% of soybean extract did not cause a bitter taste in yogurt products (70). When olive leaf extract in samples containing *L. acidophilus* and *B. bifidum* was investigated, it was shown that an increase in the content of olive leaf extract could create a favorable taste in milk and yogurt products. The samples containing 6% olive leaf extract were displayed to have more viscosity than other samples in milk and yogurt products (71). The addition of chicory gel in


**Figure 3:** The combination of medicinal plants and probiotics produces milk and yogurt with a better taste, smell, and consistency.

probiotic bacteria was shown to produce a significant difference with the control sample revealing that the concentration of chicory did not have a significant effect on the initiator bacteria, but could increase the growth of *L. acidophilus* and *B. bifidum* in dairy products (21).

The effect of cinnamon in banana milk on *L. acidophilus* and *B. bifidum* probiotic bacteria showed that *B. bifidum* cinnamon banana milk in 0.6% sample of cinnamon could provide 30 degrees Dornic acidity faster than other products and the contro. In *L. acidophilus* cinnamon banana milk, the samples containing 0.4% and 0.6% cinnamon provided 30 degrees Dornic acidity faster than other products and the controls. In cinnamon banana milk containing a mixture of both types of bacteria, the samples with 0.4% and 0.6% cinnamon provided 30 degrees Dornic acidity faster than the control. It should be noted that in banana yogurt containing only one type of bacteria, the samples reached the desired acidity after about 5 hours, but in banana yogurt containing both types of bacteria, the products reached the desired acidity after 8 hours. It is also important to mention that the pH of *B. bifidum* cinnamon banana milk product did not drop below 5 during 10 days of storage in the refrigerator, which is considered a good advantage for this product. Therefore, cinnamon increased the growth of the mentioned bacteria in dairy products. The effect of mint on growth of *L. acidophilus* and *B. bifidum* was investigated and was shown that the mixture of both mentioned bacteria in dairy products was more than cinnamon due to low growth rate of bacteria in banana milk in comparison to other manufactured products and based on their high fat contents (72). The impact of celery essential oil on pathogenic and probiotic bacteria was studied showing that celery essential oil had a relatively high growth inhibition influence on *L. casei*, *L. acidophilus*, *L. plantarum* and *L. rhamnus* probiotic bacteria with the greatest growth inhibition zone on *L. rhamnus* with MBC and MIC of 2500 ppm and 1250 ppm, respectively (73).

Probiotic milk containing 2% ginger was reported to provide 42 degrees Dornic earlier than other samples, with the most effect on the growth of bacteria during incubation; while samples containing

1%, 3% and the control reached the 42 degrees Dornic. Therefore, the control samples had the least effect on bacterial growth, even during refrigeration of the samples, the control samples were associated with the longest shelf life and the samples containing 3% ginger were with the least shelf life. So control samples, 1% ginger, vegetable yogurt product (without bacteria) and samples containing 2% and 3% ginger were more popular than other samples regarding aroma, smell, color, thickness, and taste, respectively. The control samples and the yogurt sample containing 3% ginger were tested and it was shown that the amount of protein in the control samples and yogurt samples containing 3% ginger was 2.90 and 3.21, respectively. Therefore, the effect of ginger on the amount of probiotic yogurt protein was greater for the samples containing 3% ginger when compared to the control samples and the use of ginger extract in the production of dairy products was found to have a favorable quality (1). Figure 3 illustrates the effect of the combination of medicinal plants and probiotics in milk and yogurt industries to provide a better taste, smell, and consistency.

## Conclusion

It was shown that medicinal plants have been extensively used in food industry and probiotic products can improve food quality by accelerating the growth of probiotic bacteria, even sometimes it may decrease the bacterial growth too. Therefore, since medicinal plants have different medicinal and nutritional properties; they can be utilized as effective compounds in food and pharmaceutical industries to create the desired taste, consistency and aroma. They can reduce blood cholesterol level, improve disorders such as diabetes, anemia, asthenozoospermia, endometriosis and vaginal infections too. The combination of probiotic microorganisms with medicinal plants can promote the health status too. More researches are needed to be undertaken to demonstrate the efficiency of probiotic bacteria, medicinal plants, and their combination in treatment and prevention of many diseases.

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## Authors' Contribution

The responsible author designed and wrote the study and supervised the study. The second author also wrote and revised the article.

## Conflict of Interest

None declared.

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