

Effect of Ethanolic and Aqueous Extracts of Purslane on Probiotic Bacteria (*Lactobacillus acidophilus* and *Lactobacillus casei*)

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Received: October 29, 2014

Accepted: December 31, 2014

ABSTRACT

The use of natural ingredients such as herbs had a tremendous growth in food preservation. These compounds, in addition to antimicrobial properties, have flavoring and antioxidant properties as well. Recent research suggests that certain live microorganisms may play a role in regulating the immune system and cancer prevention that of among them to the probiotic bacteria can be noted. On the other hand, probiotic products by improving the intestinal microbial flora and digestive tract, and boost the immune system have significant impact on consumers' health. This study was conducted to evaluate the effect of aqueous and ethanolic extracts of Purslane on activities of probiotic bacteria *Lactobacillus casei* strain T4 and *Lactobacillus acidophilus* strain LA-5. The purpose of this study was to evaluate the effect of Purslane on the growth of *Lactobacillus* bacteria hence was prepared the aqueous and ethanolic extracts of Purslane and probiotic bacteria were exposed to various concentrations (2.5, 5 and 7%) of aqueous and ethanolic extracts. The results were evaluated after 24 hours by spectrophotometry and optical density (OD) methods. The results showed that some concentrations (2.5 and 5 %) of ethanol extract of Purslane and only the 7% concentration of aqueous extracts of purslane increase the growth of LA-5, whereas, the aqueous extract and also ethanolic extract of purslane at certain concentrations, can reduce the growth of T4.

KEYWORDS: *Lactobacillus acidophilus*, *Lactobacillus casei*, probiotics, Purslane extract

INTRODUCTION

Functional foods are the foods that at least contain a clear and proven health benefits, in addition to the basic nutritional properties, and the functional form are recommended by the manufacturer or nutritional scientists and are used by consumers. Milk and dairy products, especially fermented milk products, are important beneficial foods. One of the important issues associated with the selection and production of beneficial food is the safety and safe to use. Nowadays, because of the practically proved unsatisfactory consequences resulted from an imbalanced and bad eating in human societies, the tendency to produce and consume functional foods is increased.

Probiotics are emerging as one of the most popular functional products which are of particular importance in this connection. The benefits of probiotics for humans, has long been debated [9]. Probiotics are widely known as healthy food. The use of probiotics helps to maintain the health, and power of body, and to deal with intestinal diseases and other diseases [1]. Dairy products are one of the most functional products. On the other hand, the fermented dairy products and probiotic microorganisms have many positive effects in terms of health care that can be pointed to reducing the risk of heart attacks and improving the microbial flora of the digestive tract, and etc. One of the most important products is the yogurt enriched with *Lactobacillus acidophilus* [5].

Essential oils and plant extracts and their ingredients are known to have antibacterial effects. They have many applications in controlling the growth of pathogenic bacteria or bacterial spoilage of food origin which has led to its usage as food preservative. Because of the public's new approach on one hand and the national and international organizations in charge of food hygiene on the other hand, the use of various natural food preservative welcomed rather than the chemical [8].

Purslane (*Portulaca oleracea*) is of Portulacaceae family. It is the native plant of India and the Middle East, but in other places and in some areas it is considered as invasive weeds. This herb has antipyretic, anti-bacterial, anti-diabetes, anti-lust (the aphrodisiac), softening, housing, diuretic, refreshing agent [7]. Extensive review of the literature showed that purslane, an important medicinal plant with a diverse range of pharmaceuticals. Due to its nutrient content, especially alpha-tocopherol, antioxidants, vitamins C and A, beta-carotene, alpha-glutathione and omega 3 fatty acids, this plant has an important role in wound healing. This plant also has anti-microbial effects and its traditional use it topically, effective in the treatment of inflammation. Purslane is a very suitable candidate as a useful cosmetic ingredient. Since most of the reported effects of purslane are from fresh or boiled water, the

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extractive juice, would be the most appropriate material [6]. The new issue of probiotics and their related aspects on one hand and the benefits of plant extracts on the other hand, have placed a wide range of research fields against of researchers. Hence, in this study evaluating the effect of *Lactobacillus acidophilus* and *Lactobacillus casei* purslane bacterial growth was studied.

MATERIALS AND METHODS

Plant material and plant extraction

Purslane were collected in North West region of Kashan and then washed with tap water and dried in the shadow. Purslane dry, income to a powder and extraction was performed by percolation method on two separate stages, once with water and once with ethanol solvent. The extraction was measured as 10 grams of plant powder in 100 ml of solvent, so extracts of purslane was achieved 10%. After extraction, the extracts were concentrated in the rotary evaporator until the solvent is evaporated and condensed. This method was for better durability of extracts in the long time.

Bacterial strains tested

Strains tested, is including commercial strains of *Lactobacillus acidophilus* (LA-5) and native strain *Lactobacillus casei* (T₄) for the purposes of this experiment, after culturing the bacteria in MRS Broth, OD of bacteria adjusted to with the OD obtained from the kinetics of bacterial growth curve (OD₆₀₀, LA-5 = 4.2 and OD₆₀₀, T₄ = 3.9).

Methods

Different concentrations of aqueous and alcoholic extracts of purslane (2.5%, 5% and 7%) were added separately to MRS Broth. The new culture of each strain of bacteria *Lactobacillus acidophilus* LA-5 and *Lactobacillus casei* strain T₄ that OD was adjusted to the OD desired (OD₆₀₀, LA-5 = 4.2 and OD₆₀₀, T₄ = 3.9) and a 5% inoculum was added to the medium, after a 24 hour incubation period were incubated at 38 ° in CO₂ incubator, OD of the samples was read at 600 nm by spectrophotometer and the results were evaluated. All treatments were replicated three times.

RESULTS

In this study the effect of aqueous and alcoholic extracts purslane compared with probiotic bacteria *Lactobacillus*. All data were analyzed using Prism software, at the 5% level by ANOVA analysis of variance and Tukey's post -test. As shown in Figure 1, a seen, compared between the control (medium lacking Plant Extract) with various concentrations of the plant extract showed purslane extract concentrations of 2.5 and 5%, the growth of the bacteria *Lactobacillus acidophilus* strain LA-5 was significantly increased (P <0.05). However, 7% ethanol extract of purslane had no effect on the growth of LA-5 (P > 0.05). Data analysis and comparison between different concentrations of the extract also showed purslane, between 2.5% and 5% concentrations, there was no significant difference in growth increase of LA-5. Also, according to Figure 1, b results of the OD of purslane aqueous extract shows, concentrations of 2.5% and 5% aqueous extracts of purslane had no effect on the growth of LA-5. While, 7% aqueous extract of purslane somewhat increased the growth of LA-5 in compared with control. Also according to the results of the effect of aqueous and ethanolic extracts of purslane the bacterium *Lactobacillus casei* strain T₄ as shown in Figure 2, a seen purslane can be said ethanolic extract of purslane at concentrations of 2.5 and 5% decrease T₄ the greatest decrease was related to the concentration of 5%. However, according to Figure 2, b purslane aqueous extracts at all concentrations significantly reduced the growth of T₄ (P <0.05).

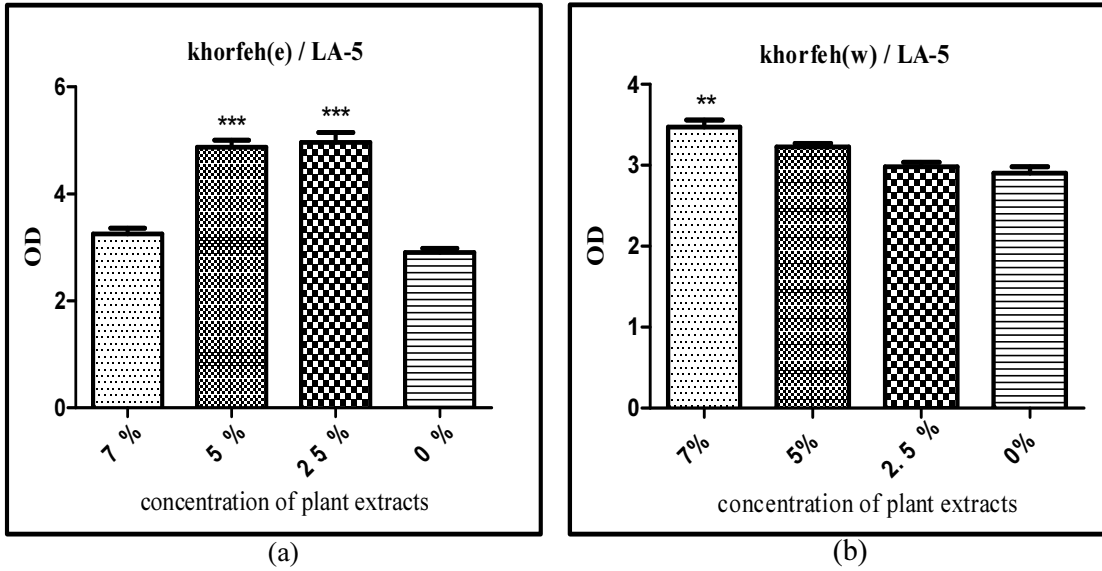


Fig.1. (a):Ethanolic extract of Purslane /LA-5(b): Aqueous extract of Purslane /LA-5
 Concentrations of 2.5 and 5 % ethanol extract of purslane, increased the growth of LA-5 (a)
 Concentrations of 7 % aqueous extract of purslane, increased the growth of LA-5 (b)
 The data show the Mean± SEM.(**P<0.01, ***P < 0.001,Difference with the control group)

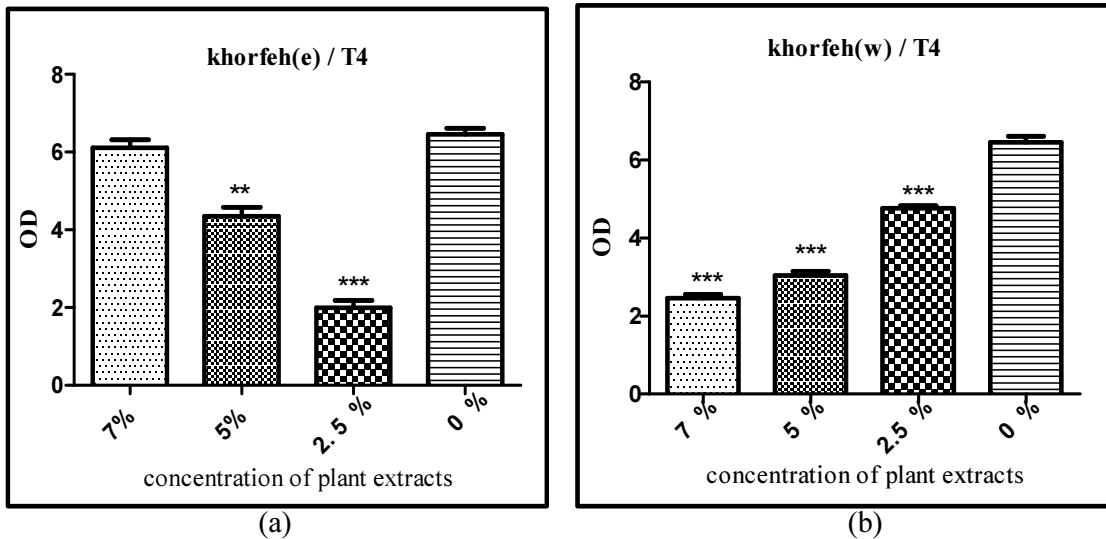


Fig.2. (a): Ethanolic extract of Purslane /T4 (b):Aqueous extract of Purslane /T4
 Concentrations of 2.5 and 5 % ethanol extract of purslane decreased the growth of T4 (a)
 All concentrations of the aqueous extract of Purslane reduced the growth of T4 (b)
 The data show the Mean± SEM. (**P<0.01, ***P < 0.001, Difference with the control group)

DISCUSSION

Probiotics are kind of dietary supplements that are formed potentially from beneficial bacteria and fungi. Difference of probiotics with other fine living that may be a health benefit for the host, are the property lies that health effects of probiotics will be created through its biological activity in vivo, after the establishment in various departments. Therefore this food product is classified as functional food ingredient or food component targeted. Probiotic products consumption in comparison with other methods such as oral consumption of probiotics through supplements (drugs) is more popular at least for two reasons, first, people are less inclined to use drugs and second, sensory aspects of food products consumption have supportive effects on probiotics. Hence, in recent years the desire to study them is increased. Many studies there have been about the effect of essential oils and plant extracts

on different probiotic bacteria; some of them are mentioned in the following. A study has been done by Halah and Mehanna investigating the effect of green peppers on *L. rhamnosus* as probiotic bacteria of Lactobacillus genus alone or with yogurt starter. The results showed that effects of pepper extracts on growth of probiotics were dependent on the concentration of pepper in growth medium. Pepper juice was effective in rising the growth rate of strain (*L.rhamnosus*) compared with the control, and this may be due to the combination of fruit juices that contain complex carbohydrates and fiber [4]. In another study, was investigated effect of olive leaf extract on the growth and metabolism of probiotic bacteria *Bifidobacterium infantis* and *Lactobacillus acidophilus*, in this study, were used three olive leaf extract (water, ethanol and methanol extracts). Each of three olive leaf extract increased the cell number over than control. Olive leaf extract also indicated the greatest effect on the cell number and production of SCFA (Short-Chain Fatty Acids) for both of the bacterial species. The results shows that the polyphenolic compounds in olive leaf extract are responsible for stimulate growth and metabolism of probiotic bacteria [3].

In a study, which was conducted in 2008 by Alexander et al. Effects of antioxidants (both pure herbal extracts and antioxidants) was evaluated on cultures of Lactobacillus casei, as a sample of probiotic microorganisms. The results of this study showed that natural antioxidants such as catechins at a concentrations of 100 to 400 micromoles and chlorogenic acid (400 micromol) had a stimulatory effect on the growth of Lactobacillus casei, but coffee extract, chokeberry, and dog rose, inhibits the growth of Lactobacillus [2]. In a study conducted by Amal Bakr Shoriet alin 2012 to evaluate the effects of extracts prepared from garlic and cinnamon over 21 days storage in a refrigerator, on survival of lactic acid bacteria (*Lactobacillus* species and *Streptococcus thermophilus*) in yogurts made from cow's milk and camel milk and the results showed that the number of Lactobacillus species in fresh camel milk yogurt was 13.2×10^6 . While fresh yogurt of camel milk with cinnamon and *Allium sativum* extract, was increased number of Lactobacillus species (respectively 19.2×10^6 and 26.9×10^6 cfu / ml) [10]. It should be noted, which has not been evaluated so far the effect of Purslane on probiotic bacteria, especially Lactobacillus. Since the viability of probiotics has special importance and is considered as their basic value, in this study because of the beneficial properties and health benefits of PO was evaluated the effect of aqueous and alcoholic extracts of this plant on Lactobacillus. Due to the positive effect of the alcoholic extract of purslane on probiotic bacteria, in particular LA-5 this increase of growth may be due to the phenolic compounds present in Purslane. On the other hand, according to the results, in some concentrations of aqueous and ethanol extracts of Purslane reduced growth of T4 which may be due to the antioxidant properties of these plant and these findings confirms a study conducted by Alexander et al which examined the effects of herbal antioxidants and pure antioxidants on *L. casei*. Thus it can be said that, on the one hand plant additives can enhance the health and nutritional value (Vitamins, Antioxidants), on the other hand can decrease the number of probiotic bacteria significantly Hence, further studies should be conducted, about effect of plant material on probiotic bacteria in probiotic products containing plant material.

Acknowledgment

This research was supported by Islamic Azad University, Science and Research branch of Tehran and Tak Gene Zist Company. The laboratory facilities provided by the Islamic Azad University of Central Tehran Branch, Thank all of them.

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