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## The Effects of *Thymus daenensis* extract on Maintenance and Growth of Yogurt Starter Bacteria

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### ABSTRACT

Global interest in biopreservation of food systems has recently been increased because of high economic costs of deterioration of food products by food pathogens as well as side effects of chemical preservatives. This study aims to evaluate the antibacterial activity of *Thymus daenensis* extract against two yogurt starter bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. was investigated by pour plate method and serial dilution assays. It was shown that *Thymus daenensis* extract reduces the number of two examined bacteria dose dependently. The highest effect was observed at 0.04% concentration of the extract but higher doses are not recommended because it will cause a change in taste of yogurt. However the main point is that *Thymus daenensis* extract does not diminish all bacteria, which are biologically active components of yogurt, and this feature makes it a good candidate for biopreservation of proper foods.

**Keywords:** antibacterial, functional foods, *Thymus daenensis*, yogurt starter bacteria.

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## INTRODUCTION

In recent years, nutraceuticals and functional foods have been subject to many researches' attention and the link between nutrition and healthful living has been properly elucidated. Linking food and natural ingredients to health impacts, often with success, goes back to traditional experiential knowledge of ancient people. Nowadays conservative chemicals are used to protect industrial food, as regard to scientific researches and public awareness of harmful effects of these chemicals plants natural components are increasingly applied for preventing microbe growth and food maintenance. One of popular favorites among Iranian people, particularly in western and central parts of the country, is taking *Thymus* - leaf - rich yogurt. This food is served as a nutraceuticals food. Milk and its products including yogurt are among functional foods in which microorganisms act as biologically active ingredients and facilitate digesting process. In addition *Thymus* species with lots of biological and pharmacological properties including anti microbial antiviral, antiparasitic, spasmolytic and antioxidant activities [1-5] may enrich the food to make it a worthy functional food.

*Thymus* with about 215 species in the world is one of the largest genera belonging to the family Lamiaceae [6, 7]. Some 14 species of the genus are distributed in Iran of which four species are endemic, including *Thymus daenensis* [8]. In traditional medicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, antiseptic, antitussive and carminative as well as treating colds [2,4]. *Thymus* oils and extracts are widely used in pharmaceutical, cosmetic and perfume industry also for flavoring and preservation of several food products [9].

In this study in order to elucidate the interactions between active ingredients of *Thymus daenensis* and yogurt microorganisms, antimicrobial activity of *Thymus daenensis* extract against two sanitarly important gram positive yogurt starters, namely, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was investigated.

## MATERIAL AND METHODS

### Plant Material

Flower bearing shoots of *Thymus daenensis* was collected from forenamed locality in Kohkiluyeh va Buyerahmad province (Iran) in June 2011 and authenticated by the fourth author. The shoots were dried under shade for seven days at room temperature and then were ground into powder.

### Preparation of *Thymus daenensis* Extract

The extract of the sample was isolated using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia [10]. 10 ml of the sampled yogurt was diluted by the same amount of sterile distilled water (v/v=1:1) then using serial dilution method, one ml of  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  concentrations were obtained and were plated (using the

pour plate method) in nutrient agar media. After incubation at 37°C for 24 hours in an anaerobic condition, colonies of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were observed and ensured us that our sampled yogurt contained starter bacteria.

### Antibacterial Test

In order to do the test twelve pipes divided into four groups of three each. The first group serving as control contained 100 ml distilled water plus 10 ml diluted yogurt. Other groups serving as experimental contained 10 ml diluted yogurt and 100 ml 0.01%, 0.02% and 0.04% concentrations of *Thymus daenensis* extract respectively. One ml of each pipe was plated in nutrient agar medium and incubated for 48 hours at 37°C in an anaerobic condition. Then the number of colonies per plate was recorded and the number of bacteria per ml diluted yogurt was calculated.

### RESULTS

Our observation revealed that each milliliter of the sampled yogurt contained  $4 \times 10^8$  bacteria. The result of bacterial counting in control and experimental groups of this study is summarized in table 1. It has been shown that the extract of *Thymus daenensis* reduces the number of bacteria grown in the yogurt in an interesting way. While the effect of *Thymus daenensis* on yogurt starters increases dose dependently the numbers of bacteria are still significant after 46 hours in pipes treated with the highest concentration. Yogurt starter bacteria are highly useful and are the biologically active ingredients of yogurt but vast amount of them make the yogurt rotten hence using *Thymus daenensis* leaves in yogurt not only provide the consumers worthy plant ingredients such as vitamins and fibers but also keep the yogurt safe for longer time. As is the case in most of the time, once more the traditional use of plants by native people coincides with scientific studies.

**Table 1: the number of bacteria in control and experimental groups treated with different concentrations of *Thymus daenensis* extracts.**

groups	Control	experimental		
concentrations	0.00%	0.01%	0.02%	0.04%
Number of bacteria per 1 ml yogurt	$10^7$	$5.4 \times 10^6$	$9.1 \times 10^5$	$2.1 \times 10^3$

### DISCUSSION

Plant extracts are composed of natural compounds with ample biological activities that make them possible natural additives and condiments in many foods. Numerous studies have shown antimicrobial properties of *Thymus* extracts of which the most potent components are Thymol and carvacrol [11-13]. These two ingredients may account for bactericide effects of the plant as have been shown in many studies [14-18] especially carvacrol which is the main component of the extract [5, 19]. Anyway the main point is that the concentrations used in this study reduces the number of bacteria but still let them be exist in the yogurt since they are

biologically active microorganisms in this functional food. So the most effective concentration used in our study (0.4%) not only helps conserving of yogurt for longer time through reducing the number of micro-organisms but also retains the number of starter bacteria in a coherent level to do what they are expected to do in digestive tract. However Using higher concentration of *Thymus daenensis* is not logical since it changes the taste of the yogurt. It was concluded that low concentration of *Thymus daenensis* can be regarded as a good candidate for natural conservator in food industries.

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