The Effect of Some Medicinal Plant Extracts on Biochemical, Physicochemical, and Antimicrobial Activity of Exract Added Yogurt

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Abstract: In this study some plant extracts added in yogurt was aimed more useful products for people. Some physical, chemical and antibacterial activities of additive plant extracts yogurts were analyzed in the yogurt samples. The pH, yeast and mold counts were determined in yogurt samples at the 1st, 7th and 14th day's interval. There were significant differences in the fat, pH and total solids content in the sample amounts at the 1st storage day. There were marked differences in the antibacterial, fat and dry matter due to different flavor additives. The pH was increased in the yogurt samples throughout the storage period. Yeast and mold counts were not observed with naked eyes on the surface of yogurt samples throughout the 1st, 7th and the 14th day's interval. While only the C samples showed a weak antibacterial activity against the *Staphylococcus aureus,* the other samples did not show any antibacterial activity against the *S. aureus.* The samples of B and C showed the highest antibacterial activity against the *Pseudomonas aeruginosa* and *Escherichia coli*. The antibacterial activity against the Gram-positive bacteria was pronounced more than the Gram-negative.
Keywords: *Antibacterial activity, medicinal plant extract, physicochemical properties, yogurt*

Bazı Tıbbi Bitki Özütlerinin Yoğurdun Biyokimyasal ve Fizikokimyasal Özellikleri Üzerine Etkisi ve Ekstrakt İlave Edilen Yoğurtların Antimikrobiyal Aktivitesi

Özet: Bu çalışmada yoğurda bazı bitki ekstraktları ilave edilerek insanlar için daha yararlı ürün elde edilebileceği amaçlandı**.** Yoğurt örneklerinde, bitki özütü ilaveli yoğurtların bazı fiziksel, kimyasal ve antibakteriyel etkileri analiz edildi. Örneklerde 1., 7. ve 14. günlerde pH, maya ve küf varlıkları tespit edildi. 1. depolama günündeki örnek miktarlarında yağ, pH ve toplam katı içeriğinde önemli farklılıklar tespit edildi. Farklı tatlardaki katkılardan dolayı yoğurtların kuru madde içeriğinde, antibakteriyel ve yağ oranlarında iz bırakacak farklılıklar gözlemlendi. Depolama periyodunda yoğurt örneklerinin pH'sı arttı. Yoğurt örneklerinde 1., 7. ve 14. gün boyunca mantar ve küf gözlenmedi. Sadece C numunesi *S. aureus*'a karşı zayıf bir antibakteriyel etki gösterirken diğer numuneler böyle bir etki göstermedi. *P. aeruginosa* ve *E. coli'*ye karşı en yüksek antibakteriyel etkiyi B ve C numunesi gösterdi. Antibakteriyel etki Gram pozitiflerden daha çok Gram negatiflere karşı oldu.
Anahtar Kelimeler*: Antibakteriyel etki, fizikokimyasal özellikler, tıbbi bitki özütü, yoğurt*

Introduction

One of the most popular and oldest fermented milk products in Turkey is yogurt which results from lactic fermentation of milk. Yogurt is derived from Turkish word "Jugurt" reserved for any fermented food with acidic taste. It involves the use of specific symbiotic/mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Kon, 1959; Meydani and Ha, 2000). Yogurt is defined by the Codex Alimentarius of 1992 as a coagulated milk product that results from fermentation of lactic acid in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Meydani and Ha, 2000). It is a very healthy and nutritious dairy product. It is valued for controlling the growth of bacteria and incuring intestinal disease like constipation, diarrhea and dysentery (Shahani and Chanden, 1974).

Yogurt provides a dietary source of calcium and protein, as well as folic acid, magnesium, and zinc. There is limited clinical information regarding its benefits in lipid regulation and cardiovascular disease. Despite debate regarding the role of exogenous calcium in the prevention of osteoporosis, yogurt remains a recommended source of calcium. It is commonly used as a source of probiotics. In addition to it is widespread use as a food, yogurt has been studied in clinical trials in amounts of 100 to 200 g/day **(**Forssén et al., 2000; Shermak et al., 1995; Arrigoni et al., 1994; Murray, 1996; Lin et al., 1998). Yogurt is also effective in lowering the blood cholesterol (Masud et al., 1991; Annonymus, 1997).

Generally, yogurt is soured by the other microorganism so it changed pH and taste. This information is very important for the people who are involved in milk products (like yogurt) business and sell their products. Since plants have a variety of chemical compounds in their leaves, roots and

flowers, they have been used in the treatment of various human diseases for thousands of years in all over the world. Similarly, a lot of plants have been used by rural people in Turkey for the purpose of the treatment of several diseases, including microbial infections for emetic and strengthening effects, and for increasing urine and decreasing tension (Baytop, 1984). *Nigella sativa* (black cumin; kalonji) is an annual herbaceous plant growing in Western Asia and the Mediterranean region for its seeds. The seeds contain 40% fixed oil, a saponin (melantin) and up to 1.4% volatile oil. The seeds of *Nigella sativa* have been used traditionally for centuries in the Middle East, Northern Africa and South Asia for the treatment of various diseases (Brutis and Bucar, 2000; Gilani et al., 2004).

Tea is an infusion of flavorful leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols (Sofowara, 1984). *Laurus nobilis* has a long history of folk use in the treatment of many ailments, particularly as an aid of digestion and in the treatment of bronchitis and influenza. The leaves and fruit are antiseptic, aromatic, astringent, carminative, diaphoretic, digestive, diuretic, emetic in large doses, emmenagogue, narcotic, parasiticide, stimulant and stomachic (Grieve, 1984). *Urtica dioica* L. and *Urtica urens* L., (stinging nettles) have a long history of use in folkloric and science based herbal medicine (Treasure, 2003).

In the present paper, we analyze the use of different plant extracts in yogurt manufacture that has been attempted increasingly. The aims of this study were to utilize some crude plant extracts in developing yogurt of high acceptability. Another objective of this study was to evaluate the effect of crude plant extracts additives on physical, chemical, and microbiological properties of crude plant extracts yogurt on some bacteria.

Material and Methods

Plant Material: The plants of *Urtica diocia* and *Laurus nobilis* were collected from different parts of the Black Sea Region in Turkey during March, April and May, 2005. The identification of these specimens was carried out using Flora of Turkey (Davis, 1966; 1988). These plants have been identified by the Ordu University Faculty of Arts and botanist teacher in the biology department. The plant samples of *Nigella sativa* and *Camellia sinensis* were obtained from local markets.

Preparation of Extract: Fresh leaves and shoots twigs of the plants were dried at 45°C for 5-6 hours. The extract of the plants were prepared according to the methods described by Holopainen et al. (1988), with slight modification. Dried leaves and twigs of the plants were extracted with 95% ethanol, at a ratio of 10gr plant: 50ml solvent (exract/ethanol), at room temperature. The extracts were kept at 4°C for 5 days and they were filtered through 45 µm membrane filters. And then the solution was dried with an evaporator. The crude extracts were stored at -20°C until used.

Incubation of Yogurt: Cow's milk (fat; 4. 00%, 6.83 pH) was used (5 kg) for yogurt production. The Cow's milk and plant extract was heated to 100°C, homogenized and then rapidly cooled to 45°C. 2% of yogurt (a small amount of yogurt containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was added as a starter culture. The inoculated yogurt mixes were filled into 200 mg plastic cups and the additives (the plant samples) were added at a ratio of 1 g/100 ml and then incubated at 37°C, 12-24 hours. The control yogurt was made without additive plant samples. Incubation was terminated at pH 4.5. The yogurt samples were stored in a refrigerator (5°C) and room temperature (18-22°C) for 14 days. The pH of yogurt samples was analyzed at the 1st, 7th, and 14th day's interval. The other of analysis was made at the first and second days (Kon, 1959; Meydani and Ha, 2000).

Preparation of Yogurt Extracts: The yogurt samples (addition plant extracts and control) were filtered through 45 µm membrane filter. The yogurt samples were stored at -20°C until used.

Microorganisms Tested and Culture Media: Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, Maryland). Antimicrobial activities of non addition plant extract and addition plant extract samples were assayed against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 12228, *Salmonella Typhimurium* CCM5445, *Bacillus subtillis* ATCC 6633 and *Enterobacter cloacae* ATCC 13047. The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck). The concentrations of bacterial suspensions were adjusted suspension to 10^8 cells/ml (Ronald, 1990).

Antibacterial Assay (The Diffusion Disc Plates Method): Antibacterial activity was measured using methods of diffusion disc plates on agar.

Mueller Hinton Agar medium (Merck) (20 ml) was poured into each 15 cm Petri dish. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h, at 37°C. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck). Suspension (100 µl) with approximately 10^8 bacteria per milliliter was placed in Petri dishes, over agar and dispersed. Then, sterile paper discs (Oxoid, CT09988, 6 mm diameter) were placed on agar to load 15 µl of each plant-yogurt samples (%1 w/v). For bacteria, as positive control yogurt (no added extract and ethyl alcohol) of 15 µl (%1 w/v) and as negative control addition of 70% ethyl alcohol-yogurt was used. For bacteria, Amphicillin and Cephazolin were used as positive control. Inhibition diameters were determined after incubation at 37°C for 24 h. All tests were made in means ± SD (standard deviation) of triplicate.

Product Analysis

Physico-chemical Analysis: Fat was determined by Gerber method as described alfresco-vending, loose unpacked availability and hence by Pearson, (1976). Total solids were determined by AOAC (1990) method (No.925.23). The pH was measured by Electronic digital type Hana pH meter No. H 8416 according to method No. 981.12 of AOAC (1990).

Results and Discussion

The fat content of control yogurt and four of addition plant extracts-yogurt i.e., A (*L. nobilis* extract - yogurt), B (*U. diocia* extract –yogurt), C (*C. sinensis* extract–yogurt) and D (*N. sativa* extract-yogurt) are shown in Table 1. The average fat content of control yogurt was. 5 The average fat content of yogurt A was 6.0. These results are in accordance with the findings of (Athar, 1986; Shaid et al., 2002) who reported 3.5 percent fat in typical plain yogurt. The average fat content of yogurt B and C were 5.4. The average fat content of yogurt D was 7.0. The results observed confirmed the findings of Hofi et al. (1978). There was hardly any variation in fat content of different samples of plant made yogurt. That is probably because of some plant extracts for example *N. sativa* seed contain essential oil and standardization of raw milk.

The total solids content of control-yogurt and four of addition plant extracts-yogurt i.e., A (*L. nobilis* extract-yogurt), B (*U. diocia* extract–yogurt), C (*C. sinensis* extract–yogurt) and D (*N. sativa* extract-yogurt) are shown in Table 1. The average total solids content of control-yogurt was 14.13. These results are in line with the findings of Hofi et al. (1978). The average total solids content of yogurt A, B, C and D were 14.74, 14.27, 15.16 and 16.74 respectively. The results are in accordance with the findings of (Athar, 1986; Shahid, 2002). These results are totally different from those reported by Sarkar et al. (1996). There was hardly any variation in total solids of different samples of plant extracts made yogurt. That is most probably because of standardization of raw milk and quality control measures taken to ensure the consistency of end product. The pH of control yogurt and four of addition plant extracts yogurt i.e., A (*L. nobilis* extract yogurt), B (*U. diocia* extract–yogurt), C (*C. sinensis* extract–yogurt) and D (*N. sativa* extract-yogurt) are summarized in Table 1. The average pH of control yogurt (1, 7 and 14 days) was 4.07, 4.18 and 4.23 respectively. Whereas the mean pH values of A, B, C and D were 4.06, 4.17 and 4.24 (A), 4.07, 4.20 and 4.30 (B), 4.10, 4.24 and 4.25 (C), 4.01, 4.21 and 4.22 (D), respectively. These results are in line with the findings of Salji et al. (1985) and Varnam and Sutherland, (1994), Shahid et al. (2002). There was no significant variation found in pH of different samples of plant extracts made yogurt as compared to control-yogurt because yogurt is incubated for specific time and temperature to attain desired pH, which is about 4.6 i.e. isoelectric point of casein. A decrease in pH with time interval of storage is naturally expected (Ahamd, 1994).

Table 1. Physico-chemical analysis of plant extracts addition yogurt sam.

Product (Plants species-yogurt)	Fat	Total Solids %	pH		
			1st day	7th day	14th day
A (*L. nobilis* extract-yogurt)	6.0	14.74	4.06	4.17	4.24
B (*U. diocia* extract –yogurt)	5.4	14.27	4.07	4.20	4.30
C (*C. sinensis* extract–yogurt)	5.4	15.16	4.10	4.24	4.25
D (*N. sativa* extract-yogurt)	7.0	16.14	4.01	4.21	4.22
Control- yogurt	5	14.13	4.07	4.18	4.23

The antibacterial activity of control-yogurt and four of addition plant extracts yogurt i.e., A (*L. nobilis* extract-yogurt), B (*U. diocia* extract – yogurt), C (*C. sinensis* extract-yogurt) and D (*N. sativa* extract-yogurt) are shown in Table 2. The antimicrobial activity of obtained from plant extract yogurt against various pathogenic bacteria was investigated. The samples of yogurt A showed antibacterial activity (10-15 mm/15 µl inhibition zone) against the test organisms. The samples of B showed antibacterial activity (11-17 mm/15 µl inhibition zone) against the test organisms. The samples of C showed antibacterial activity (8-16 mm/15 µl inhibition zone) against the test organisms. The samples of D showed antibacterial activity (8-14 mm/15 µl inhibition zone) against the test organisms. The samples of control yogurt showed antibacterial activity (8-10 mm/15 µl inhibition zone) against the test organisms. The samples of B and C showed the highest antibacterial activity against *P. aeruginosa* and *E. coli* (17 and 16 mm/15 µl inhibition zones, respectively) (CLSI, 2006). While only the samples of C showed weak antibacterial activity (9 mm/15 µl inhibition zone) against S*. aureus,* the other samples did not show antibacterial activity against *S. aureus* (CLSI, 2006). Yeast and mold counts were not observed with naked eyes on surfaces of yogurt samples.

This study was a pilot study so sensory testing wasn't done. Such studies must be made sure of these tests. In this study, the antimicrobial activity against bacteria of the samples of A, B, C and D obtained from four plant extracts addition yogurt have been determined. The plants are known to have healing properties and are used for treating various diseases affecting people. The antibacterial activity against Gram-positive bacteria was more pronounced than against the Gram-negative ones, which is in accordance with the results reported (Gonzalez et al., 1994; Grosnevor et al., 1995). The marketing strategy of yogurt has been partially based on stated nutritional benefits and the production of fruit yogurt increases marketing options especially among young people (Yaman et al., 2006).

Oral et al. (2008) suggest that the use of some plant hydrosols as antimicrobial agents may be exploitable to prevent deterioration of stored foods by bacteria, as long as the taste impact is acceptable in targeted foods. In addition, Ertürk and Taş (2011), suggest that some marine algae have antimicrobial effect. So, algae might be used for food protect. In our study shown that some plant extracts addition yogurts have antimicrobial activity. So, they can use as protective against bacteria for foods.

The isolation of the compounds with antimicrobial and antifungal activity will lower the required doses compared to the crude extracts. In addition, it is noteworthy that these plants are used best in lukewarm meals with yogurt, since the extraction yields will be lower in cold and the active compounds will be transformed into less active or inactive products when heated.

Table 2. Results of antibacterial screening of plant extract addition yogurt samples determined by the agar diffusion method (Inhibition zone in mm).

Plant species-yogurt	**Part used**	**Local name**	**Collection site**	**Inh. Zone (mm) Microorganisms**								
				Ec	**Bs**	**Sa**	**Se**	**Pa**	**Sm**	**St**	**Eclo**	**Ss**
Urtica dioica extract-yogurt	Lf, Sd	Isırgan	Trabzon	12	11	-	15	17	11	12	12	13
Laurus nobilis extract-yogurt	Lf	Defne	Ordu	10	11	-	12	12	15	10	10	11
Nigella sativum extract-yogurt	Sd	Çörekotu	Market	14	8	-	8	13	13	12	11	13
Camelia sinensis extract- yogurt	Lf	Yeşil çay	Market	13	8	9	9	16	11	13	12	13
Control (without addition)				7	6	-	8	8	9	8	7	10
Amphicilin				15	36	36	27	10	24	23	-	10
Cephazolin				15	38	38	30	27	26	24	20	-

-: No inhibition; NT: Not tested; Part used: Lf: Leaf, Sd: Seed; Microorganisms: Ec*: E. coli*, Bs: *B. subtillis,* Sa*: S. aureus,* Se: *S.epidermidis,* Pa: *P. aeruginosa,* Sm: *S mutans,* St: *S.thyphyminium,* Eclo: *E. cloaceae,* Ss: *S.salivarius.*

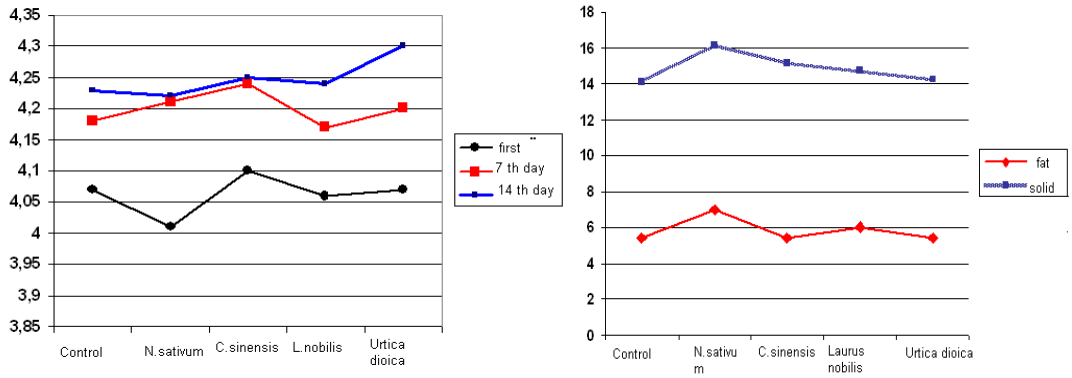

Figure 1. Fat, solid and pH analysis of plant extract addition yogurt samples.

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