

## The Antibacterial Efficiency of Some Herbs Used in Herby Cheese

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### SUMMARY

In this study, the inhibitory effects of some herbs such as sirmo, mendo, heliz, sov and siyabu which are added to herby cheese on some of pathogenic bacterial strains were studied. The diethyl ether extract of the samples was tested on *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* with disk diffusion method as in vitro. Sirmo was found to have different levels of effect on all of the test strains in terms of the inhibitory effect, whereas mendo was effective on *K. pneumoniae*, *P. aeruginosa* and *S. aureus*, and heliz was effective on *P. aeruginosa*, *E. faecalis* and *S. aureus* in terms of such inhibitory effects. The poorest inhibitory activity was found in sov and siyabu.

**Keywords:** Sirmo, Mendo, Heliz, Sov, Siyabu, Antibacterial efficiency.

### Otlu Peynirde Kullanılan Bazı Otların Antibakteriyal Etkinliği

#### ÖZET

Bu çalışmada, otlu peynire katılan otlardan sirmo, mendo, heliz, sov ve siyabu'nun bazı patojen bakteriler üzerindeki inhibitör etkinliği araştırıldı. Örneklerin diyetel eter ekstraktları *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* ve *Staphylococcus aureus* üzerinde disk difüzyon yöntemi ile in vitro olarak denendi. Sonuç olarak; sirmo'nun test suşlarının tümü üzerinde değişik düzeylerde olmak üzere inhibitör bir etkiye sahip olduğu tespit edildi. Mendo *K. pneumoniae*, *P. aeruginosa* ve *S. aureus* üzerinde etkili olurken, heliz'in *P. aeruginosa*, *E. faecalis* ve *S. aureus* üzerinde inhibitör etki gösterdiği belirlendi. En zayıf inhibitör aktivite sov ve siyabu'da gözlemlendi.

**Anahtar kelimeler:** Sirmo, Mendo, Heliz, Sov, Siyabu, Antibakteriyal aktivite.

### INTRODUCTION

Herby cheese is a type of cheese which is largely consumed in some areas of Eastern and Southeastern Turkey (3,10). The milk products such as herby cheese are produced with different names in many countries worldwide (10). The typical character of this cheese is to include various types of herbs after coagulation with the addition of the cheese ferment into the milk (12).

Approximately 30 plant species planted in the nature including *Liliaceae*, *Labiatae*, *Umbelliferae*, *Caryophyllaceae* and *Ranunculaceae* are used in herby cheese production. The plant species vary from region to region (0.1-15 %) (11). Most of the herbs are in quality of spice and they are added to cheese in order to provide a special flavour and aroma. It has been reported that herbs play an important role in modulating  $\beta$ -carotene, vitamin C and E levels in the cheese (2,13). Herbs have been traditionally used to treat some diseases such as urinary tract infections (11).

In this study, the antibacterial activities of some herbs which are added to herby cheese, on some of the pathogenic bacteria were investigated.

### MATERIALS and METHODS

The investigation material consists of five different herbs such as sirmo (*Allium atroviolaceum* Boiss.), mendo (*Chaerophyllum crinitum* Boiss.), heliz (*Ferula orientalis* L.), sov (*Heracleum crenatifolium* Boiss.) and

siyabu (*Ferula rigidula* DC.) which are grown in Turkey and widely used in the manufacturing of herby cheese. Being collected from regional flora, herb samples were botanically identified (7). Samples were first cleared from weed and then washed with distilled water, dried in aseptic conditions and frozen with nitrogen gas. Samples were crumbled in the laboratory mill whose size was 1 mm and kept in closed containers (8).

#### Bacterial culture

Test organisms used in this study are known to be the cause of disease in human and some are known to be involved in food poisoning. The bacterial strains of *Klebsiella pneumoniae* FML 5, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 15753 and *Staphylococcus aureus* ATCC 25923 were obtained from culture collection of Department of Microbiology Laboratory, Medical Faculty of Yuzuncu Yil University.

#### Preparation of extract samples

Two hundred grams of ground samples of herbs were soaked in 500 ml of diethyl ether for 6 h, and filtered then ether was evaporated at evaporator (60 °C). The obtained dark brown or green oily liquid extracts were used without diluting it in the analyses. The sample extracts were kept in freezer (+4 °C) till the analyses were resulted (8).

#### Antibacterial activity test

Ether extractions of herb samples were transferred to sterile bottles containing discs (6 mm in diameter) of filter paper (Whatman No:1). Bottles were put in a water bath (50 °C) and occasional shaken to allow an even

distribution of the extract between discs until complete evaporation of ether had been achieved. Disc diffusion techniques were used for this study as described by Hanafy & Hatem (8). In the reproduction of bacterial cultures, Trypticase Soy Broth (TSB, DIFCO 0369-01-4) and in the antibacterial activity trials, Mueller-Hinton Agar (MHA, OXOID CM337) were used as media. Having been taken from stock culture, the strains were inoculated into TSB and incubated at  $35\pm 0.1$  °C for 24 h. At the end of incubation, bacterial suspension, containing approximately was  $10^8$ - $10^9$  cfu/ml levels of organisms, was mixed with a sterile loop and then, 0.1 ml of the mixture was inoculated on MHA petri plates. The inoculum was completely spread on surface of plates and the inoculated petri plates were dried in room

temperature. Then, paper dicks penetrated from plant extract were placed on petri plates which were inoculated previously and they were incubated at  $35\pm 0.1$  °C for 48 h. Diameter of inhibition zone around dicks was measured and expressed as mm. Antibacterial activity studies were carried out for each test strains in duplicate and average measurement were calculated (4).

## RESULTS

In this study, the herbs used for manufacturing the herby cheese were investigated in terms of their antibacterial activity on some pathogenic microorganisms. The related findings are shown in Table 1 and Figure 1.

Table 1. Results of antibacterial activity of herbs

| Bacterial strains               | Inhibition zone (mm) |       |       |     |        |
|---------------------------------|----------------------|-------|-------|-----|--------|
|                                 | Sirmo                | Mendo | Heliz | Sov | Siyabu |
| <i>K. pneumoniae</i> FML 5      | 21                   | 24    | -     | -   | -      |
| <i>P. aeruginosa</i> ATCC 27853 | 20                   | 18    | 26    | -   | -      |
| <i>E. faecalis</i> ATCC 15753   | 15                   | -     | 21    | 10  | -      |
| <i>S. aureus</i> ATCC 25923     | 20                   | 38    | 30    | 12  | 11     |

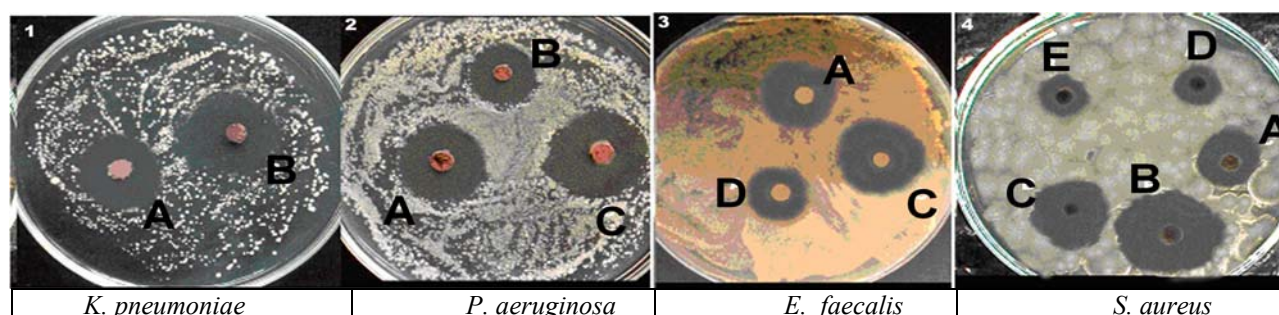


Figure 1. The inhibition zones of herb extracts, which were determined with standard bacterial strains.

A: Sirmo, B: Mendo, C: Heliz, D: Sov, E: Siyabu

After microbiological analyses had been conducted, sirmo was found to have an inhibitory effect on all of the bacterial strains tested. Whereas mendo was determined to be effective on some of bacterial strains like *K. pneumoniae*, *P. aeruginosa* and *S. aureus*, it exerted no inhibitory activity on *E. faecalis*. Similarly, heliz exerted an inhibitory effect on three bacterial strains (*P. aeruginosa*, *E. faecalis* and *S. aureus*) but was ineffective on *K. pneumoniae*. Of the tested herbs, sov was found to exert an inhibitory effect on *E. faecalis* and *S. aureus*, whereas siyabu was effective only on *S. aureus*.

## DISCUSSION

Nourishment is still an important problem in Turkey as it is in other developing countries. This is due to economical reasons and the lack of supplements consisting proteins whose biological value is high. This

type of cheese has an important role on the nutrition of people living in this area. Herby cheese, obtained from untreated sheep and cow milk or from a mixture of these two types of milk, is manufactured with traditional method (3,10). Many pathogenic organisms may be existing in raw milk (12,15). They may be derived from an infected udder (mastitis), from the faeces and other excreta of infected or asymptomatic carriage cows, from human sources, from a contaminated environment or dairy equipment. If pathogens are existing in such milk and survive during cheese making process, this may result in incidents of food poisoning due to ingestion of cheese contaminated with pathogenic organisms or with their enterotoxins. Because of the frequent contamination of milk from dairy personnel, and the high incidence of staphylococcal mastitis in dairy herds, the most commonly occurring type of food poisoning is due to enterotoxin producing strains of *S. aureus*. As for the other pathogens such as *P. aeruginosa* and *K.*

*pneumoniae*, they can cause lung, respiratory and urinary tract infections in humans (15).

The antibacterial activities of some spices have been investigated in Turkey (1,5,9,14) However; the studies on the inhibitory efficiency of the herbs used in herby cheese were limited.

When the findings of our investigation are interpreted, all of the herbs could be considered to have exerted inhibitory effect on *S. aureus* strains which play an important role in food intoxication. Whereas the strongest effect was exerted by mendo on *S. aureus*, the least inhibitory effect was found to be by sov and siyabu. Sirmo, mendo, heliz, sov and siyabu inhibition zones on *S. aureus* are measured as 20, 38, 30, 12 and 11 mm respectively. Sirmo was the most effective on *K. pneumoniae* and heliz was the most effective on *S. aureus* and *P. aeruginosa* (Table 1, Figure 1).

Çetin and Gürler (5) noted that sensitivity of microorganisms to chemotherapeutic substances differs according to type of strain. According to the results, it was observed that the susceptibility of the bacteria used as the test strains against the extracts prepared from the herbal samples were differed. This finding is similar to the findings of Çetin and Gürler (5).

In a study performed by Çon et al. (6) related with volatile oils in spices was found to have no inhibitory effect of sirmo on *S. aureus*. This result is not parallel with our findings. This result may be due to the difference of methods applied in extraction of the herbs grown under various climate conductions (6).

Within the results of our investigation, the examined herbs; particularly sirmo, mendo and heliz were found to have an important inhibitory effect. Such properties of these herbs in cheese technology are appreciated. In conclusion, these herbs could improve the microbiological safety of herby cheese if coupled with greater hygiene.

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