

# *Staphylococcus aureus* ATCC 25923 inhibition with propolis in pasteurized and UHT milks

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## Abstract

In this research, *Staphylococcus aureus* ATCC 25923 inhibition with propolis in pasteurized and UHT milks was investigated. Milk samples were divided into five groups. First group was the control samples (has *Staphylococcus aureus* without ethanolic extracts), the second group was inoculated with 70% ethanol (EA); the third, fourth and fifth group were inoculated with 5% EEP, 10% EEP and 15% EEP separately. Samples were then contaminated with *S.aureus* (12.0 log cfu/ml). Milks were incubated at 37°C for 24 hours. Ethanol decreased the pathogen to 9.11 log cfu/ml and 8.6 log cfu/ml in pasteurized and UHT milks, respectively. *S.aureus* was determined as 5.4 log cfu/ml and 7.12 log cfu/ml in pasteurized and UHT milks with 15% EEP, respectively. The most effective extract on the pathogen was the 15% EEP. These results demonstrated that propolis can be used for decrease *S. aureus* in pasteurized and UHT milks at 25°C.

**Keywords:** Ethanol, pathogen, high concentration

## 1. Introduction

Conscious consumers want foods don't contain any chemical additives [1]. For this reason, researchers have sought new natural materials that can be used instead of chemical additives.

Propolis is collected by bees from buds, leaves and similar parts of trees such as pine, oak, birch, eucalyptus, poplar, chestnut and some herbaceous plants. It is a sticky, resin-like odor and color ranging from dark yellow to brown. Bees use it for many purposes in the hive by mixing with wax [2]. Propolis is hard and brittle below 10 °C, waxy elastic at 15-25°C, softens and becomes sticky at 30-40°C. It make beekeepers difficult to work, especially in summer. It partially melts at 80°C. It has a sticky and distinctive odor when taken from the hive. It solidifies immediately when placed in the freezer [3]. While propolis is partially soluble in organic solvents such as acetone and ether, it is largely soluble in 95% ethanol. It is slightly or not soluble in water [4-5].

Flavonoids, aromatic acids and esters are thought to be responsible for the antimicrobial activity of propolis. In addition, pinosembrin, galangin and pinobanksin are also thought to be effective on antimicrobial activity. Pinosembrin also shows antifungal properties.

Other active compounds related to the antimicrobial effect are coumaric and caffeic acid esters [6].

*Staphylococcus aureus* is pathogenic to humans and animals. It is resistant to adverse environmental conditions [7]. They are naturally mostly found in the nasal and throat cavity, human and animal feces. They are widely found in food and food businesses [8]. Food safety is a major global concern in food industry. *Staphylococcus aureus* is, among the reported foodborne pathogen, significant source of foodborne diseases [9].

In this research, it is aimed to inhibit a strong pathogen *S.aureus* ATCC 25923 with different concentration of propolis in pasteurized and UHT milks at 25°C.

## 2. Materials and Methods

### 2.1. Materials

Pasteurized and UHT milks were obtained from the local markets in Karaman, Turkey. Propolis was collected from Pertek district of Tunceli. *Staphylococcus aureus* ATCC 25923 were obtained from Selçuk University and all materials were brought to laboratory under aseptic conditions.

## 2.2. Methods

### 2.2.1. Production of ethanolic propolis extracts

For 5% ethanolic propolis extract, 5 g of propolis was dissolved in 95 ml of ethanol; For 10% ethanolic propolis extract, 10 g of propolis was dissolved in 90 ml of ethanol; For 15% ethanolic propolis extract, 15 g of propolis was dissolved in 85 ml of ethanol. Dissolution was carried out in a closed container in a light-free environment for one week. The solution was shaken twice a day, and the solution obtained at the end of the period was filtered through Whatman no: 1 filter paper and placed in sterile bottles and stored at +4°C until used for analysis [10].

### 2.2.2. *Staphylococcus aureus* ATCC 25923 inhibition

*Staphylococcus aureus* ATCC 25923 was used in the study. Bacterial strains from stock cultures were activated in Nutrient Broth (Merck, Darmstadt, Germany) at 37°C for 24 h. Milk samples were divided into five groups. First group was the control samples (has *Staphylococcus aureus* without ethanolic extracts), the second group was inoculated with 70% ethanol (EA); the third, fourth and fifth group were inoculated with 5% EEP, 10% EEP and 15% EEP separately. All treatments were incubated at 25°C for 20 minutes. Pasteurized and UHT milks are then contaminated with activated pathogen, separately. All treatments were incubated at 25°C for 20 minutes, again. After incubation, Baird Parker (BPA) agar was used. Samples were inoculated by spreading plate method, then they were incubated for 24 hours at 37°C [11].

In order to determine the total mesophilic aerophilic bacteria of pasteurized and UHT milks obtained from local markets, were inoculated in Nutrient Agar (Merck, Darmstadt, Germany). Petri dishes left 37°C for 24 hours. Results were calculated as log cfu/ml.

## 3. Results and Discussion

As can be seen in Figure 1, all extracts significantly reduced *Staphylococcus aureus* ATCC 25923 numbers in milks compared to ethanol and control samples.

Total mesophilic aerophilic bacteria hasn't been calculated in both milk samples. This shows that the producer is producing in accordance with hygiene and sanitation rules.

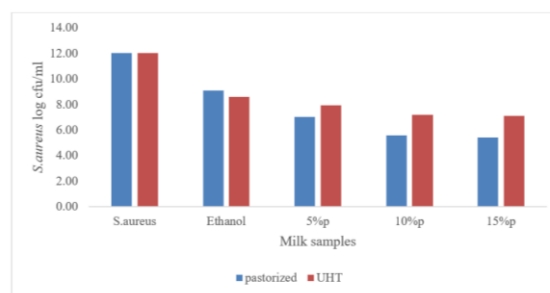


Figure 1. Inhibition of *Staphylococcus aureus* ATCC 25923 in different milks

Pathogen concentration is reached to 12.00 log cfu/ml in both milk samples. Ethanol decreased the pathogen number to 9.11 log cfu/ml in pasteurized milk; 8.6 log cfu/ml in UHT milks. Pathogen was detected as 7.04 log cfu/ml in pasteurized milk; 7.94 log cfu/ml in UHT milk with 5% EEP. *S.aureus* was determined as 5.4 log cfu/ml in pasteurized milk; 7.12 log cfu/ml in UHT milk with 15% EEP. The most effective extract on the pathogen was the 15% EEP. 10% ethanolic propolis extract is more inhibitory effect than 5% EEP. Ethanol shows more inhibitory effect in UHT milk than pasteurized. But ethanolic propolis extracts show more antimicrobial effect in pasteurized milks. The higher protein value of pasteurized milk can be shown as the reason for this situation. The proteins may have helped to propolis on inhibiting *S.aureus*.

In the study conducted by Aly et al., [11], propolis has antimicrobial effect Gram (+) and Gram (-) bacteria; It has also been determined that it has antifungal activity against yeasts. Sarıçoban and Yerlikaya [13] determined 15% propolis were more effected on *S.aureus* in minced beef. They reported the inhibitory effect can be related the presence of various content as flavones, flavonol in propolis. The antimicrobial effect of propolis against *S.aureus* were also reported in different studies [14-16] (Kılıç et. al., 2005; Kim and Chung, 2011; Silva et. al., 2012).

## 4. Conclusions

The use of different concentration of ethanolic propolis extracts is a good strategy to combat *Staphylococcus aureus* ATCC 25923, one of the most dangerous microorganism in milks. Ethanolic propolis extracts (5%, 10% and 15%) has more antimicrobial effect than ethanol (70%) in both milk samples. Three different extracts of propolis could not show completely inhibitory effect on the pathogen.

The very high concentration of *S.aureus* (12.0 log cfu/ml) can be shown as a reason for this situation. If the inoculation concentration had been chosen lower (<12.0 log cfu/ml), the extracts could have completely eliminated the pathogen. The results of this study show that as a natural food preservative propolis can be used to effectively reduce the microbial population.

**Compliance with Ethics Requirements:** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

**Disclosure statement:** No potential conflict of interest was reported by the authors.

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