

The effects of essential oils, extracts and powder of *Satureja bachtiarica* on the bacterial growth of *Staphylococcus aureus* in cream cheese

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ABSTRACT

Background and aims: *Staphylococcus aureus* is an important contaminant of milk and dairy products. It can cause a wide range of illnesses including, food poisonings and nosocomial infections. The aim of this study was to compare the antimicrobial effects of the essential oil, aqueous extract and powder of the *Satureja bachtiarica* on *staphylococcus aureus*.

Methods: *Satureja bachtiarica* was collected from the heights of the province of Chaharmahal va Bakhtiari and its extract and essential oil were collected. The bacteria *staphylococcus aureus* was obtained from the Iranian Research Organization for Science and Technology. The bacterium was inoculated in equal amounts to specimens of cream cheese; afterward different concentrations of the essential oil, aqueous extract, and herbal powder were produced and inoculated in equal amounts to the cream cheese specimens; subsequently, the specimens were checked 20, 40 and 60 days after inoculation. In the end, the microbial activity of the essential oil, aqueous extract and herbal powder were reported according to mg/ml.

Results: The minimum growth of the bacteria *staphylococcus aureus* was observed in the 1000 milligram concentrations of essential oils and 125 mg of the aqueous extract and 125 mg of powder of the plant. At all the concentrations tested the pH changes in the fortieth day, and the changes in the rigidity of the cream cheese specimens were significant as well. At some of the concentrations of the aqueous extract and some concentrations of the herbal powder the changes in the texture of the cream cheeses, comparing to the controls, were significant.

Conclusion: The essential oil, aqueous extract and powder of the *Satureja bachtiarica* have a great inhibiting effect on the growth of the bacterium *staphylococcus aureus*. Thus, it can be used as a natural preservative in foods with high lipid content.

Keywords: Essential oils, *Satureja bachtiarica*, *Staphylococcus aureus*, Extracts, Powder.

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INTRODUCTION

Milk is a complete and adequate food that can provide a main part of a humans nutritional needs in any age, especially in childhood. Because milk contains organic nutrition's it can provide a suitable media for the growth of different microorganisms.¹ Microorganisms have a crucial role in causing diseases in humans. Because of the high death toll of these humans with this disease has always been searching for methods to fight against these microorganisms, and there has been a great amount of progress in the food industry, microbial contamination of foods is still a major challenge.² So, in modern countries 30% of the people contract diseases due to eating contaminated food at least once a year.³ Recent reports show that *Staphylococcus aureus* along with *Listeria monocytogenes* are amongst the most important food poisoning pathogens.⁴ *Staphylococcus aureus* is one of the main contaminators of milk and dairy products and it can cause a wide range of diseases, food poisonings, and hospital infections.⁵ Though antibiotics have been used for treating diseases for a long time, because of the side effects of antibiotics and bacterial resistance to them focus has shifted towards using herbal antimicrobial compounds.⁶ One of these herbs is Bakhtiari Savory (*Satureja bachtiarica*). *Satureja bachtiarica* is from the Lamiaceae family. It is a wild plant that grows in mild climates.⁷ Nowadays *Satureja bachtiarica* is used as an antiseptic, discharger of mucus, and pain killing drug. Because of the high content of Phenol compounds and different Monoterpenes and sesquiterpenes in the essential oil of *Satureja bachtiarica*, as well as its high antioxidant capacity, this extract has notable antibacterial properties.⁸ In the year, Yosefly showed the extract of the leaf of *Salvia leriifolia* reduced the growth of *Staphylococcus aureus* bacterium on the surfaces which powder was applied.⁹ The aim of the current study was to examine the

antimicrobial effects of the essential oil, aqueous extract and powder of *Satureja bachtiarica* on samples of cream cheeses contaminated with *Staphylococcus aureus*.

METHODS

After identification and collecting the plants from different regions of Chaharmahal Va Bakhtiari province, the aerobic organs of *Satureja bachtiarica* were severed before its flowers bloomed; these organs are were kept under suitable conditions (dark and dry) and were completely dried. The parts that were going to be used were ground and then decocted. After collection and identification of *Satureja bachtiarica* by the Research Center of Natural Resources of Chaharmahal Vabakhtiari as number 3621, 1.5 kilograms of the plant was dried in the shades and ground. 1000 grams of the yielded powder of *Satureja bachtiarica* was mixed in 1.5 liters of 86% ethanol and was left for 24 hours in a room away from sunlight. Then, it was filtered with filter paper. The remaining powder was left in 800 ml ethanol 86% for 24 hours for the second time and then it was filtered two times and again time the remaining powder was left in 0.5 liter ethanol 86% for 24 hours.

In order to concentrate the extract first it was left the yielded solution in a 37 degrees centigrade incubator for 24 hours. Then, with the help of the rotary apparatus the remainder of the ethanol was evaporated. Finally, with the help of an electrical mill, the yielded extract was turned into powder. The mentioned powder was mixed with sterile distilled water in a proportion of 1 to 5 (the unit of the proportion is (g/mL)) and in this manner the 20% extract of *Satureja bachtiarica* was produced. In order to sterilize the final product number, 0.22 microns millipore strainer was used.¹⁰

In order to carry out physical and chemical tests, we referred to Pak Pey Company and with consideration of our treatments (essential oil, extract, powder, and control) in three repeats, 15 kilograms of freshly prepared and packaged cream cheese were randomly sampled from this company and transferred to the laboratory. In the companies, laboratory concentrations of the treatments were prepared and added as follows: plant powder with dilutions of 0.1, 0.2, 0.3 and 0.4%, plant essential oil with dilutions of 125, 250, 500 and 1000 microgram per gram, plant extract with dilutions of 1000, 500, 1500, 2000 micrograms per gram. Afterwards, they were packaged, labeled and transferred to the quality control laboratory of the Islamic Azad University of Shahrekord under sterile conditions in order to carry out physical and chemical tests on days 20, 40, and 60 after production. Samples were kept in a 4 degrees centigrade refrigerator.

In order to prepare the first extract, it was weighed 100 grams of the plant *Satureja bachtiarica* with a digital balance and mixed it with distilled water in a proportion of 1 to 5. After keeping the solution at room temperature for 24 hours it was sieved with four layer of gas and funnel. In order to purify it, it was centrifuged the extract in 4 degrees centigrade for 20 minutes with 2500 rpm. Afterward, to extract the extra water, it was transferred the plant extracts to a distillation apparatus and finally it was obtained a quite dense extract of the plant.

For preparing the essential oil, at first, it was weighed 100 grams of the powder of *Satureja bachtiarica* with a digital balance. Then, it was mixed this amount of powder with 1 liter of distilled water and heated it on a heater with a temperature of 100 degrees centigrade for 3 hours. Next, the essential oil producing process was carried out with the help of a Clevenger apparatus.¹¹

The bacteria *Staphylococcus aureus* with PTCC= 1889 were obtained from the Iranian Research Organization for Science and Technology and then they were cultured. They were identified according to the manufacturing companies guidelines (Catalase, Coagulase, and culture on Mannose media). Afterward, a bacterial suspension from these bacteria was prepared according to the half standard of McFarland.¹⁰

In this method paper disks were purchased and soaked in the intended dilutions of plant extract, essential oil, and powder; later on these disks were put on Baird Parker agar media that have been cultured with the bacteria *Staphylococcus aureus* using a swap. The plates were incubated in a 37-degree centigrade incubator for 24 hours and eventually, we measured its non-growth halo.^{11,12}

Via this method, the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of *Satureja bachtiarica* were determined.

This method was done as follows: We added 10 microliters of the aforementioned bacterial suspension to wells containing 50 microliters of different concentrations of *Satureja bachtiarica* essential oil and 50 microliters of Muller Hinton broth media. The first well was used as a positive control and only contained the microbial suspension and Muller Hinton broth media. The second well was used as a negative control and only contained the aforementioned media and the essential oil of *Satureja bachtiarica*. In order to obtain different dilutions of the extract, at first, it was mixed 80 milligrams of the extract in 1 milliliter of 5% dimethyl sulfoxide (DMSO) solution so that it was obtained 80 milligrams per milliliter of each extract. Then, with Muller Hinton broth and by making serial dilutions of 80, 40, 20, and 10 etc. of *Satureja bachtiarica* essential oil. Finally, the samples were incubated for 24 hours in 37 degrees centigrade. According

to the description, MIC is calculated by considering the last concentration (most diluted) of the wells, which had no turbidity, as MIC. In order to calculate MBC all the wells with no turbidity were cultured on blood agar separately and incubated for 24 hours in 37 degrees centigrade; the minimum concentration that did not show any growth is considered as MBC.¹³

RESULTS

In this study, the minimum growth of *Staphylococcus aureus* occurred in the concentration of 1000 ppm and days 20, 40 and 60. The minimum growth was from the essential oil of *Satureja bachtiarica* and the

maximum growth was from its powder and on day 60, 3.63×10^5 respectively. These results show that industries of food production can use the essential oil of *Satureja bachtiarica* as an antibacterial in their products. Amongst the essential oil, extract and powder of this plant and the essential oil had the strongest antibacterial effect, this might be due to its high amount of Thymol. The highest rate in three compounds phenol, phlanoid and phelaonol is showed (169.37, 25.82, 14.98), respectively.

The amount of phenol compounds in the essential oil is noticeably higher than in the others. The results are illustrated as the following Tables (Tables 1, 2, and 3 and Graphs 1, 2, and 3).

Table 1: Mean and standard deviation of *Staphylococcus aureus* colonies (CFU/g) on the cream cheese with the essential oil of *Satureja bachtiarica*

Concentration \ Day	Zero (control)	125 ppm	250 ppm	500 ppm	1000 ppm
20	1.9×10^7	3.15×10^6	6.3×10^5	5.8×10^5	3.4×10^5
40	3.7×10^7	1.57×10^6	3.26×10^5	2.7×10^5	1.45×10^5
60	5.8×10^7	0	0	0	0

The numbers that are not alike show a meaningful difference in the Duncan test with a probability level of ($P < 0.05$).

Table 2: Mean and standard deviation of *Staphylococcus aureus* colonies on the cream cheese with the extract of *Satureja bachtiarica*

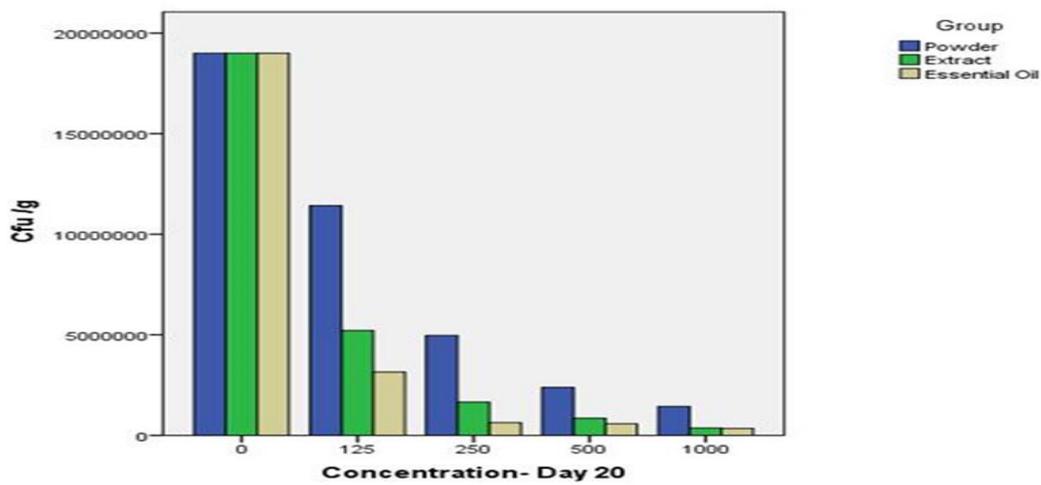
Concentration \ Day	Zero (control)	125 ppm	250 ppm	500 ppm	1000 ppm
20	1.9×10^7	5.22×10^6	16.6×10^5	8.5×10^5	3.7×10^5
40	3.7×10^7	3.08×10^6	7.9×10^5	7.9×10^5	2.23×10^5
60	5.8×10^7	0	0	0	0

The numbers that are not alike show a meaningful difference in the Duncan test with a probability level of 5 ($P < 0.05$).

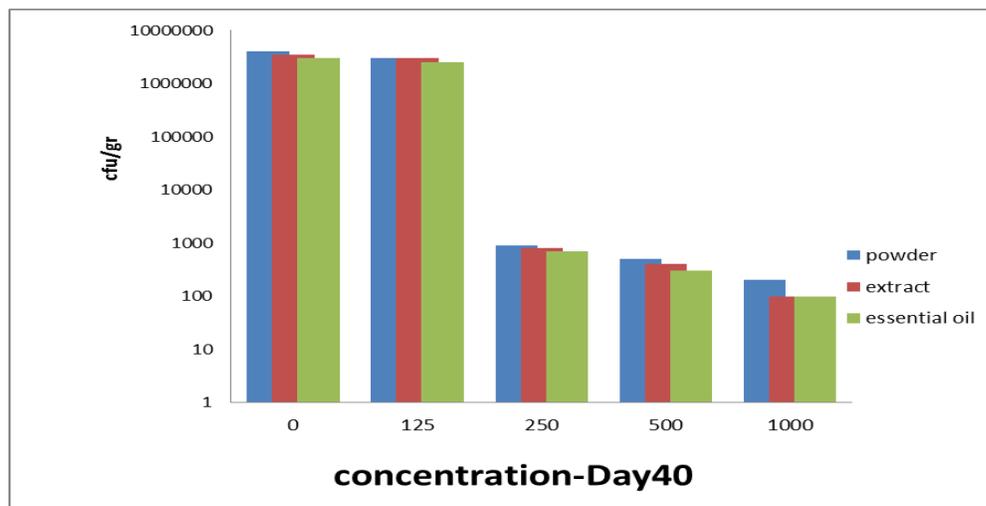
Table 3: Mean and standard deviation of *Staphylococcus aureus* colonies(CFU/g) on cream cheese's with the powder of *Satureja bachtiarica*

Concentration \ Day	Zero (control)	125 ppm	250 ppm	500 ppm	1000 ppm
20	1.9×10^7	11.41×10^6	4.96×10^6	2.38×10^6	1.44×10^6
40	3.7×10^7	5.18×10^6	2.41×10^6	1.2×10^6	7.26×10^5
60	5.8×10^7	1.1×10^6	6.77×10^5	4.77×10^5	3.63×10^5

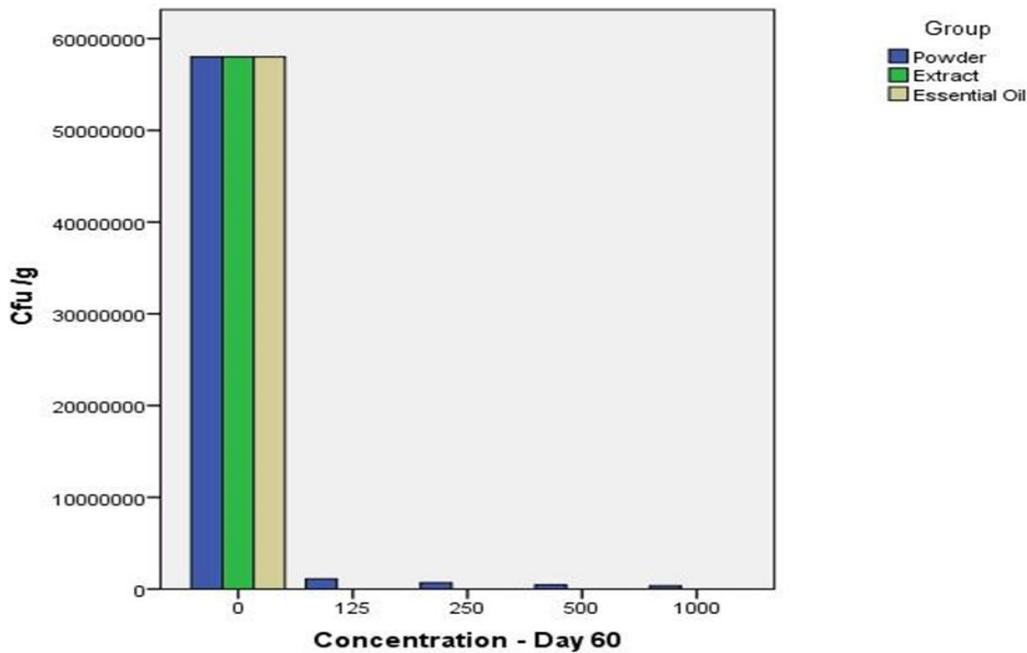
The numbers that are not alike show a meaningful difference in the Duncan test with a probability level of 5 ($P < 0.05$)



Graph 1: Comparison of the growth rate of *Staphylococcus aureus* on day 20 on cream cheese samples with the essential oil, extract and powder of *Satureja bachtiarica* in gram units



Graph 2: Comparison of the growth rate of *Staphylococcus aureus* on day 40 on cream cheese samples with the essential oil, extract and powder of *Satureja bachtiarica* in gram units



Graph 3: Comparison of the growth rate of *Staphylococcus aureus* on day 60 on cream cheese samples with the essential oil, extract and powder of *Satureja bachtiarica* in gram units

After transferring the sample to the GC/MS apparatus in the laboratory of Islamic Azad University, Branch Isfahan by calculating the inhibition coefficient and

mass spectrum of each separate part and comparison with standards the components of the aqueous extract were identified (Table 4).

Table 4: The amounts of the components of the herbal extract, essential oil and the herbal powder

Compound	Thymol percentage of compound	Carvacrol percentage of compound	Parasaymn percentage of compound
Essential oil	28.61	24.98	14.04
Extract	38.3	0.21	21.3
Powder	6.69	4.8	32.7

DISCUSSION

With considering the fact that nowadays there is a lot of emphasis on using herbs for treatment and keeping healthy, examinations on the efficacy of herbs on the bacteria

Staphylococcus aureus have been carried out. Studies carried out in Nigeria on 6 species of native herbs of that area showed that 4 species of the herbs that are usually

used for treating dermal and respiratory infections by herbalists worked against *Staphylococcus aureus* bacteria.¹⁴

With the cream cheese with different concentrations of the aqueous extract alike the ones with different concentrations of essential oil on day 60 of keeping (Graph 3), no growth of *Staphylococcus aureus* was witnessed. Amongst the concentrations used for the aqueous extract of the plant, just like the ones used for the essential oil, the most reduction in bacterial growth was witnessed in the concentration of 1000 ppm and the least reduction in bacterial growth was 125 ppm. Therefore, the aqueous extract influences the growth of the bacteria and it follows a dose-dependent pattern. Of the constituents of aqueous extract, 0.021 from the whole of compounds is carvacrol that this compound exists in the study of effects of *Satureja hortensis* of the mountains and *Mentha pulegium* on *Staphylococcus aureus*. Also, phenolic acid and this compound with the hydrophobic nature caused the death of microorganisms.

This study shows that the powder of *Satureja bachtiarica* has less effect on the bacteria relative to its essential oil and extract. However, its effect is acceptable, because the number of counted bacteria able to grow on day 60 of the high concentrations of powder were less than the pathogenic dose of the bacteria (610 cfu/g).¹⁴ The least reduction in the growth of *Staphylococcus aureus* was witnessed in concentration 125 of the essential oil, aqueous extract, and powder, respectively. A study also showed that the extract of savory and rosemary had antibacterial effects on the bacteria *Staphylococcus aureus*.¹⁵ In this study, the amount of Thymol compounds were more in the extract in comparison to the essential oil and powder. With considering the fact that there was less bacterial growth in the extract in comparison to the essential oil and the powder, we can conclude that this is due to the high amount of Thymol compounds in the

extract. Many studies have demonstrated that Carvacrol and Thymol are amongst the most important compounds that have a strong antibacterial effect in herbal extracts.¹⁶ These two compounds are amongst the most active compounds against pathogenic bacteria that contaminate food.¹⁷ Structurally Thymol and Carvacrol are very similar and they both have a hydroxyl group in their phenolic ring. It has been reported that Thymol and Carvacrol are two of the most important compounds in *Satureja bachtiarica*.¹³ According to a research done, the antimicrobial activity of the essential oil of *Satureja bachtiarica* is due to its phenolic compounds like Thymol, Carvacrol, and Parasaymn. Thymol and Carvacrol increase the permeability of the cell membrane, disrupt the ionic balance on the two sides of the membrane, change the pH, and eventually destroy the cellular membrane of the bacteria. Parasaymn does not have any antimicrobial activity but it intensifies this property in Thymol and Carvacrol.¹⁹ According to the reports of Nikpoyan and colleagues, Thymol and Carvacrol can hydrolyze the outer membrane of gram positive and gram negative bacteria; they can disband the lipopolysaccharides and increase the permeability of the cytoplasmic membrane.²⁰

The components of savory depend on the weather where they grow, Plant varieties, and plant age may vary.²¹ The major components of the essential oil of *S. khuzistanica* are 6.39% Parasaymn and 6.29% Carvacrol.²¹ Meanwhile, the essential oil of *S. bachtiarica* gathered from the province of Chaharmahal va Bakhtiari has 44.5% Thymol and 23.9% Gamma-principally terpenes as the main components. The essential oil of *S. spiciger* also contains 35.1% Thymol, 22.1% Parasaymn, and 13.7% Gamma-principally terpenes.²² The main components of the essential oil of *S. mutica* were 30.9% Carvacrol, 26.5% Thymol, 14.9% Gamma-

principally terpenes, and Parasaymn. The essential oil of *S. macrantha* had more Parasaymn (25.8%) and Limonene (16.3%) and had less Thymol (8.1%).²³ with consideration of these results, we can conclude that amongst all the different species of savory, *Satureja bachtiarica* has the most amount of Thymol. On the other hand, according to the findings of Naghavi and colleagues, Thymol has a strong antimicrobial effect against *Staphylococcus aureus*, while Carvacrol and Parasaymn are better inhibitors of *Escherichia coli*. Therefore, the results of this current study suggest that the extract of *Satureja bachtiarica* has a stronger effect against the *Staphylococcus aureus* bacteria in comparison to the bacteria *Escherichia coli* seem rational.

CONCLUSION

Staphylococcus aureus's resistance to antibiotics is increasing on a daily scale. The results of this study show that although the essential oil of *Satureja bachtiarica*, when it is in direct contact with the bacteria *Staphylococcus aureus*, has a remarkable effect on inhibiting the growth of the bacteria; its powder does not have much effect on the bacteria *Staphylococcus aureus*. However, we can use the essential oil of *Satureja bachtiarica* as an herbal antibiotic in cream cheese.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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