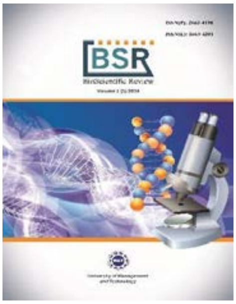

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Article: **Antibacterial Effects of Common Spices against *Staphylococcus aureus* under Laboratory Conditions**

Author(s): **Barera Rani[1], Saima Naz[1], Saba Saeed[1], Ahmad Manan Mustafa Chatha[2]**

Affiliation: [1]Department of Zoology, The Government Sadiq College Women University Bahawalpur, Pakistan
[2]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan

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Research Article

Antibacterial Effects of Common Spices against *Staphylococcus aureus* under Laboratory Conditions

Barera Rani[1], Saima Naz[1], Saba Saeed[1*], Ahmad Manan Mustafa Chatha[2]
[1]Department of Zoology, The Government Sadiq College Women University Bahawalpur, Pakistan
[2]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan
*Corresponding Author: ssaba5306@gmail.com

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Abstract

A variety of botanical extracts are currently being used for remedial purposes as they are inexpensive, safe and effective. The current study was designed to assess the antimicrobial effects of different spices such as black pepper (*Piper nigrum*), fennel seed (*Foeniculum vulgare*), carom (*Trachyspermum ammi*), cinnamon (*Cinnamomum verum*), and turmeric (*Curcuma longa*) against *Staphylococcus aureus*. The effectiveness of different spices against *S. aureus* was evaluated using the Minimum Inhibitory Concentration (MIC) method. It was found through statistical analysis carried out using the regression method that *C. verum* has a significantly higher ($p < 0.00$) antimicrobial effect against *S. aureus* followed by *C. longa* ($p = 0.005$), while *F. vulgare* ($p = 0.90$) and *T. ammi* ($p = 0.78$) have a non-significant effect against *S. aureus*. Furthermore, *P. nigrum* (*p* = N.A) has no effect against the bacteria. The purpose of this research is to ascertain the antibacterial action of easily cultivated spices against *S. aureus* and the findings will be helpful in treating gastrointestinal infections using common spices instead of antibiotics.

1. Introduction

Plants are an important functional part of the food web and are used widely as food source, directly or indirectly. They are used as medicine and cure for different diseases and ailments. [1] Different additive compounds are currently manufactured and used to avoid food spoilage caused by a number of microorganisms including food spoiling bacteria. However, a major concern with these food additives is their adverse effects on non-target organisms. [2] Such concerns created the need to discover more secure and regulated elective additives. Various studies concluded that plant extracts can be utilized to control food contamination and spoilage. For example, the antimicrobial activity of plants against bacteria using the agar plate method was determined in the previous study. [3]

Plants are considered as a major food source for human consumption, providing around 70-80% of the overall energy requirements. [4] Plant based food sources are economically cheap and function as sources of different flavors, antibiotics, antimicrobials, dyes, analgesics, perfumes and poisons. [5] Different plant species have been used widely to treat microbial diseases. Currently, only a small number of around 400,000 plant species have been investigated thoroughly as the source of antibacterial medicines used to cure and treat bacterial diseases. [6]

The antibacterial impact of twenty different types of spices and herbs against *Staphylococcus epidermidis* 1878 and *Pseudomonas aeruginosa* 1471using agar and broth weakening strategy was demonstrated previously. [7] Spices have antimicrobial or antibacterial characteristics. [8] For drinks and foods, spices are also used as piquancy agents. [9] Generally, spices possess the most of the antimicrobial compounds present in natural sources. [10] Phytochemicals containing antibacterial characteristics extracted from these plants can be used to cure different bacterial diseases. [11] The main object of spices is to give taste, aroma and flavor to the food. However, these spices also have antimicrobial, antioxidant and antibacterial properties. The antibacterial properties of spices have been acknowledged recently. A number of scientists have confirmed and explained antimicrobial properties of different plant species. In order to cure bacterial diseases, plant extracts function as antimicrobial agents against the source of the disease itself or its contributory mediator. [12]

The extracts of plants and spices are beneficial as antimicrobial source. In vitro antibacterial properties are present in the secondary metabolites of plants containing alkaloids, tannins and flavonoids. [13] Plants are used to cure illness in different cultures and different parts of plants such as roots, peels, bulb, leaves and gel are used against different diseases. For a long time, spices have been used traditionally by peoples of different regions to save, store and preserve food and as food additives to increase the taste, aroma and flavor of the food. [14] Recently, antimicrobial compounds were developed from natural sources such as herbs and spices to cure the diseases caused by the spoilage bacteria and food borne pathogens. Five common spices namely fennel seeds, cinnamon, carom , turmeric, and black pepper were studied to test their antimicrobial properties against different bacterial strains. [15]

Black pepper or *Piper nigrum* (Piperaceae), also known as the king of spices, has a specific spiciness to it. [12] It is used in cooking due to its pleasant aroma and flavor as well as in different medicines because of its antimicrobial properties. [16] Cinnamon or *Cinnamum verum* (Lauraceae) is among the most studied herbs. [17] In ancient times, it was used as a traditional medicine to cure common ailments. It was later discovered that it has antimicrobial and antibacterial properties against different microbes, especially against those pathogens which cause periodontal disorder. This herb has antiseptic, pharmacological, and antibacterial properties. [18]

Turmeric or *Curcuma longa* (Zingiberaceae) has been used as medicine due to its antibacterial effects since long. [9] Although it is primarily used to give taste, aroma, and coloring to the cooked food, it has known medicinal properties as well. [19] Fennel or *Foeniculum vulgare* (Apiaceae) is a common herb found in the temperate areas of the northern hemisphere. This herb is used as medicine and for flavoring purposes. The dry seeds or powder of this herb is used as an anti-inflammatory, diuretic and analgesic medicine. [20]

The core objective of the current study was to determine the antimicrobial effect of some common spices against the pathogenic bacterial strain of the human gut. Five common spices including *Trachyspermum ammi* (Apiaceae), *C. longa*, *C. verum*, *F. vulgare*, and *P. nigrum* were tested for their antibacterial properties against *S. aureus* in this study.

Table 1. Details of Different Spices Tested for their Antibacterial Activity against *Staphylococcus aureus* under Laboratory Conditions

Common Name	Scientific Name	Family	Parts used
Black pepper	*Piper nigrum*	Piperaceae	Seeds
Fennel	*Foeniculum vulgare*	Apiaceae	Seeds
Carom	*Trachyspermum ammi*	Apiaceae	Seeds
Cinnamon	*Cinnamomum verum*	Lauraceae	Bark
Turmeric	*Curcuma longa*	Zingiberaceae	Tuber

2. Material and Method

2.1 Sample Collection

The seeds of *P. nigrum*, *F. vulgare*, and *T. ammi*, the tubers of *C. longa* and the bark of *C. verum* were used in the current study. These were purchased from the local market of Pattoki, Punjab, Pakistan (see Table 1).

2.2 Bacterial Strain

Bacterial strain *S. aureus* was obtained from the Microbial Biotechnology Laboratory, Department of Zoology, University of the Punjab, Lahore, Pakistan. Bacterial growth was revived in the nutrient agar for all further experiments.

2.3 Preparation and Storage of Extracts

Spices viz., *P. nigrum*, *F. vulgare*, *T. ammi*, *C. verum*, and *C. longa* were dried in a hot air oven (Bionics Scientific Technologies (P) Ltd. Model # BST/HAO-1122) for 3 days at 70°C in the Microbial Biotechnology Laboratory, Department of Wildlife and Ecology, UVAS. Oven dried samples were ground individually in an electric blender (Vitamix 5200) at 6000 rpm for 15 minutes. Twenty grams of the ground powder of each spice (*P. nigrum*, *F. vulgare*, *T. ammi*, *C. verum*, and *C. longa*) was measured in an electronic analytical balance (Bio base, Model: BA1004B), soaked in 100 ml of hot sterile water and allowed to stand for 72 h at 25º C and 65% R. H. After 72 h, these solutions were filtered using Whatman filter paper # 41. The resulting aqueous solutions of the extracted spices were then stored in a refrigerator (Dawlance Model: DW-600) at 4°C for later usage.

2.4 Preparation of the Bacterial Culture

The startup culture of the bacterial strain was obtained from the Microbial Biotechnology Laboratory, Department of Zoology, University of the Punjab, Lahore, Pakistan. This culture was sub-cultured in the Microbial Biotechnology Laboratory, Department of Wildlife and Ecology, UVAS for 24 h at 37°C in an incubator (BioTek Instruments, Model: BioSpa8 Automated).

2.5 Experimental Design

The effectiveness of different spices against *S. aureus* was evaluated using the Minimum Inhibitory Concentration (MIC) method. For this purpose, the agar media was spread over borosilicate glass petri dishes (Pyrex; O.D. × H: 100 mm × 17 mm). Then, 2 ml of each aqueous extract concentration (5%, 10%, and 15%) of the five spices was added to the growth media and waited for 10 minutes to let the media settle down at room temperature. Afterwards, 5 µl of the tested bacteria was inoculated over the agar media and spread on the dish surface using a sterilized spatula. Finally, dishes were incubated at 37°C for 24 h in an incubator.

2.6 Assessment of Antibacterial Activity

The death or inhibition of microorganisms is caused by antimicrobial activities of an active agent. To check the antibacterial activity of the spices, the MIC was determined for all the extracted samples after 24 h of incubation by counting the number of colonies (CFU) developed for each spice at different concentrations (5%, 10%, 15%). At the end of the experiment, the inhibition of bacterial growth was designated as "Complete Inhibition" and expressed with a "+++" sign when there was zero development of the bacterial colonies. Whereas, if the bacterial colonies were developed in a range of 1-60 CFU per treatment, it was designated as "Relative Inhibition" and expressed with a "++" sign. However, if the number of colonies (CFU) formed was greater than 60, then the bacterial growth was designated as "No Inhibition" and expressed with a "-" sign. There were three replications for each treatment and a control treatment with no added concentration of any of the spices was tested.

2.7 Data Analysis

The data was presented both in a tabular form and figuratively using Microsoft Excel 2016 (version: 16.0.12527.20260). Data was statistically analyzed using the regression curves fitted to find the correlation between the different concentrations of each spice and their inhibition activity for *S. aureus* with RStudio (version: 1.2.5033).

3. Results

The results demonstrate the antibacterial effects of the five selected spices against *S. aureus* by assessing the growth inhibition of the bacterial strain under laboratory conditions against the control treatment (Figure. 1). We observed that among the five selected spices, the spice that inhibited the bacterial growth most effectively against *S. aureus* was *C. verum.* It completely inhibited the growth of the bacterial strain at 15% concentration, while *P. nigrum* failed to inhibit the growth of the bacterial strain even at the maximum concentration (15%). The overall performance of all spices is tabulated and represented by appropriate symbols (Table 2). The results showed that at 5% concentration, the inhibitory effect of all the spices was in the order of *T. ammi* > *C. verum* > *F. vulgare* > *C. longa*, > *P. nigrum*, at 10% concentration the inhibition effect was in the order of *T. ammi* > *C. longa* > *C. verum* > *P. nigrum*, > *F. vulgare*, and finally, at 15% concentration the spices inhibited the bacterial growth in the order of *C. verum* > *T. ammi* > *C. longa* > *F. vulgare* > *P. nigrum* (Figure. 2). *T. ammi* had a significant effect at all concentrations, whereas *F. vulgare* was significantly effective at 5% and 15% concentrations. *C. longa* showed a relative inhibiting effect at 10% and 15% concentrations (Figure. 3). Only *C. verum* showed complete inhibition at 15% concentration (Figure. 4).

Regression analysis and regression curve of different spices showed that *C. longa* (p= 0.005) and *C. verum* (p < 0.00) have a significant correlation with bacterial growth inhibition at different concentrations. While *F. vulgare* (p =0.90) and *T. ammi* (p =0.78) showed a non-significant inhibition of bacterial growth at different concentrations. Only *P. nigrum* had no effect on bacterial growth inhibition. This could not be statistically tested, as in all replications and in all concentrations it did not show any inhibition of bacterial growth (Figure. 5).

Figure 1. *Staphylococcus aureus* colonies formed in the control treatment

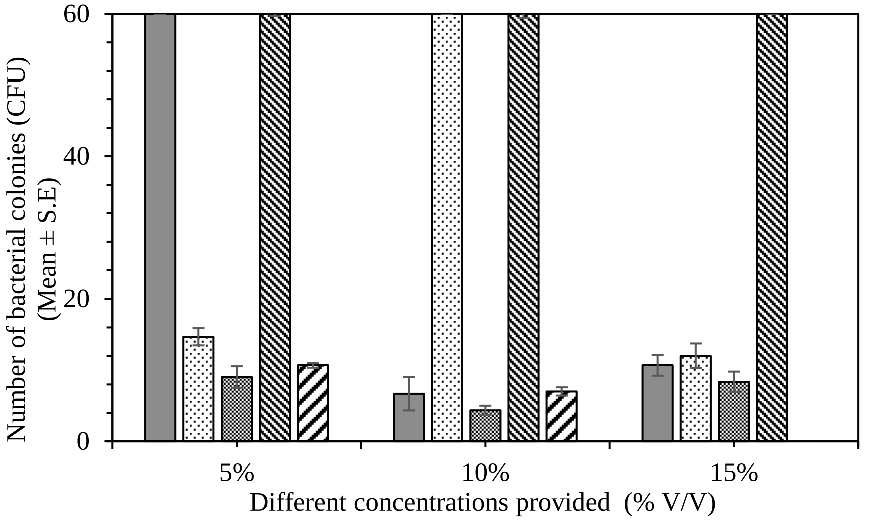

Figure 2. Number of *Staphylococcus aureus* colonies (CFU) formed of at various concentrations of five spices (■:*Curcuma longa,* ░:*Foeniculum vulgare,* ▒:*Trachyspermum ammi,* ▧:*Cinnamomum verum,* and ▨:*Piper nigrum*). Mean values reaching 60 CFU represent null inhibiting effects of a particular dose of a spice

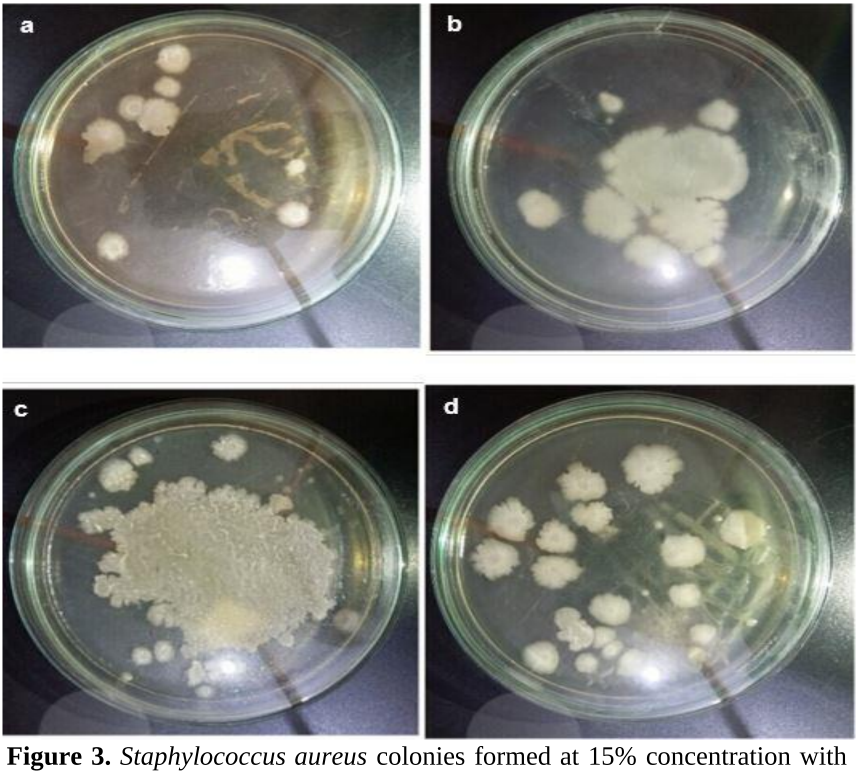

Figure 3. *Staphylococcus aureus* colonies formed at 15% concentration with different spices used a) *Trachyspermum ammi;* b) *Curcuma longa;* c) *Piper nigrum;* d) *Foeniculum vulgare*

Table. 2. Antibacterial Effect of Different Spices on *Staphylococcus aureus* at Different Concentrations (5%, 10%, And 15%) by Agar Colony Counting Method

Species of Spices	**Concentration Used (% V/V)**		
	5%	**10%**	**15%**
Piper nigrum	--	--	--
Foeniculum vulgare	++	--	++
Trachyspermum ammi	++	++	++
Cinnamomum verum	++	++	+++
Curcuma longa	--	++	++

+++: Complete Inhibition, ++: Relative Inhibition, --: No-Inhibition

Figure 4. Complete Growth Inhibition of *Staphylococcus aureus* Colonies Formation at 15% Concentration of *Cinnamomum Verum*

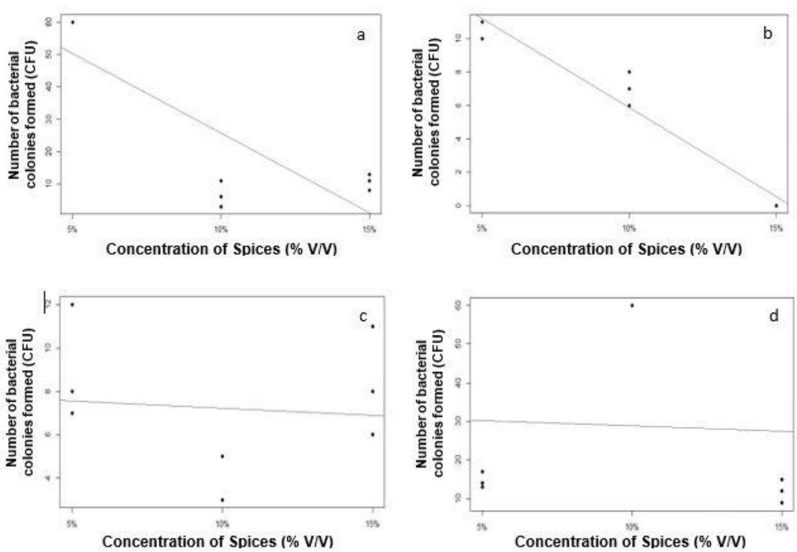

Figure 5. Regression curve between different concentrations of each spice and its growth inhibiting effect on *Staphylococcus aureus*. a) *Curcuma longa;* b) *Cinnamomum verum;* c) *Trachyspermum ammi;* d) *Foeniculum vulgare. Piper nigrum* is not included in this analysis due to its null effect of bacterial growth inhibition at all concentrations

4. Discussion

In the current study, the antibacterial activity of *T. ammi*, *C. verum*, *F. vulgare*, *C. longa*, and *P. nigrum* was tested against *S. aureus* under laboratory conditions. Our results showed that the aqueous extracts of all the tested spices except *P. nigrum* worked well against *S. aureus* and exhibited an inhibiting effect at a given concentration. We found that *C. verum* is more effective than other spices at all concentrations and it exhibited maximum bacterial growth inhibition. These finding are consistent with the study that showed that at 250 ml/l concentration in methanol, *C. verum* has a maximum inhibition zone of 12mm against *S. aureus.* [19] However, in our study we found more inhibiting activity of this spice against *S. aureus* even at lower concentrations.

Similarly, it was found that by using agar disc diffusion technique, *Vanilla planifolia*, *Rosmarinus officinalis* and *Engenia caryophyllata* were found to have an antibacterial impact on the development of two microscopic organisms. Moreover, *P. nigrum* has an antibacterial impact on the development of *S. epidermidi* when used in combination with other spices. [3] However, in our study we did not find any inhibiting properties of *P. nigrum* against the bacterial strain at any given concentration. It is plausible that *P. nigrum* is not effective against the bacteria at concentrations as low as 15% used in this experiment.

Our results showed that *F. vulgare* is significantly effective against the tested bacterial strain at 5% and 15% concentration. Our results are aligned with the findings of other studies. For instance, it was found that the oil extracts of *F. vulgare* are effective against the different strains of gram-positive and gram-negative food spoiling bacteria. [21] Similarly, it was found that *F. vulgare* is an effective antibacterial agent against different bacteria in the form of aqueous extract. [22] Antibacterial activity of oils is dependent on the principle constituents and also on their intensity. Suppressive effects of vital oils are primarily due to the antimicrobial properties of their biochemical components with bacterial susceptibility. [23] Small quantities of minor components can also make a significant contribution to the antibacterial activity of the oils. MIC measurement of gram-positive and gram-negative bacteria indicated varied sensitivity towards oils and Surinam cherry indicated maximum antibacterial activity [24] Plant extracts act as natural antimicrobials and are used as food preservatives to control the growth of pathogenic microbes. [25]

Most antibacterial constituents of spices are phenolic substances with a hydroxyl group (-OH). The existence of a scented center together with the/a polar functional group defines the inhibitory characteristics of important oils. A hydroxyl group is much more efficient in comparison with a carbonyl group. It can readily attach to the active places of the enzymes and modify their metabolic rate. Necessary oils or their active composites comprising hydroxyl group (-OH) are extreme antibacterial agents and the antibacterial activity of phenolic acids is more effective for gram-positive than gram-negative bacteria. [26, 27] Although phenolic acids are active against gram-negative bacteria, their antimicrobial effect is strain dependent. Many plant extracts having saponin revealed antimicrobial activities at different concentrations. [28] Antibacterial properties of *C. longa* against *S. aureus* found in our study were also reported previously. For example, it was documented that *C. longa* has growth inhibiting properties against different bacteria such as *S. typhi* and *S. aureus*. [29] We found that *T. ammi* possessed antibacterial properties against *S. aureus* at all provided concentrations (5%, 10% and 15%). These results are consistent with the findings of the study that described the antimicrobial activity of this spice against *S. aureus* and *E. coli.* [30]

5. Conclusion

Most of the herbs and spices commonly used in our daily diet and in the cooking process for the purpose of introducing aroma and flavor have very effective antibacterial and antimicrobial properties against different bacteria. Thus, these spices can be used to reduce the chances of food-poisoning and loss to raise food security and shelf-life expectancy and to treat certain infectious illnesses. Possible combinations of numerous spices prove that they have advance inhibitory properties against specific bacteria as compare to individual spices. The use of these spices as food additives to preserve food for a long time and in herbal medicine can be a very cost effective and risk-free approach in the future. Spices that indicate antibacterial activity can be a source of antimicrobial agents against foodborne pathogens and are suitable as food preservatives. Since antimicrobial compounds are unable to compete with antibiotics, so the investigation of antimicrobial plant agents still continues.

Spice extracts have antibacterial properties that can be used to treat diseases. Indeed, the nano-formulates of spices can be used against multi-drug resistant microbes. Various approaches for nano-encapsulation and synergistic studies can offer a platform for research in the near future. Moreover, Spice combinations, natural compounds and novel technologies can develop molecules against spoiled microbes. In the future, the mechanism of action of individual biochemical components of oils as well as the combined effect of different components need to be explored.

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Declarations

- **Funding**

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- **Conflict of interest / Competing interests**

The authors declare no conflict of interest.

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