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 - 63100, Pakistan
[2]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur- 63100, Pakistan.
Corresponding author:ssaba5306@gmail.com

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ABSTRACT

Large variety of botanical extracts are being used for remedial purposes as they are inexpensive, safe and effective. The present study was designed to assess the antimicrobial effects of different spices; 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 Minimum Inhibitory Concentration (MIC) method. It was found by statistical analysis regression method that *C. verum* possessed significantly higher ($p < 0.00$) antimicrobial effects followed by *C. longa* ($p = 0.005$) against *Staphylococcus aureus*, while *F. vulgare* ($p = 0.90$) and *T. ammi* ($p = 0.78$) had non- significant effect against *S. aureus*. Furthermore, *P. nigrum* (*p* = N.A) had no effects against the bacteria. The purpose of this research is to ascertain the antibacterial action of easily cultivated spices against *Staphylococcus aureus* and will be helpful to treat gastrointestinal infections using common spices instead of antibiotics.

Keywords: antimicrobial, bacterial strain, plant extracts, gastrointestinal, spices

INTRODUCTION:

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

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

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

The extracts of plants and spices are beneficial as the antimicrobial source. In vitro antibacterial properties present in secondary metabolites of plants containing alkaloids, tannins and flavonoids.[13] Plants have been used to cure the illness in different cultures and different part of plants like roots, peels, bulb, leaves and gel have been used against different diseases. For a long time, spices use traditionally by different people of different regions to save, store or preserved the food and as a food additive to increase the taste, aroma and flavor of food.[14] Recently, antimicrobial compounds are being the developed from natural sources like herbs and spices to cure the diseases caused by spoilage bacteria and food borne pathogens. For example, five common spices namely, sounf, dalchini, ajwain, turmeric, and black pepper were studied to test their antimicrobial properties against different bacterial strains.[15]

Black pepper, *Piper nigrum* (Piperaceae) also known as the king of spices, has specific spiciness to it.[12] It is used in cooking for its pleasant aroma and flavor as well as in different medicines for its antimicrobial properties.[16] Cinnamon, *Cinnamon verum*

(Lauraceae) is one of the most studied herbs.[17] In ancient times, the cinnamon was used as traditional medicine to cure common ailments. It was later discovered that it has antimicrobial and antibacterial properties against different microbes, especially for those pathogens which cause periodontal disorder. This herb like many medicinal plants have antiseptic, pharmacological, and antibacterial properties.[18]

Turmeric, *Curcuma longa* (Zingiberaceae) have been used as medicine due to its antibacterial effects since long.[9] Although it is primarily used to give taste, aroma, coloring property to the cooked dishes yet it has medicinal properties as well.[19] Fennel, *Foeniculum vulgare* (Apiaceae) is common herb found in temperate area of northern sphere. 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 present study was to determine antimicrobial effect of some common spices against pathogenic bacterial strain of the human gut. Five common spices including carom, *T. ammi* (Apiaceae), *C. longa*, *C. verum*, *F. vulgare*, and *P. nigrum* were tested for their antibacterial properties against *Staphylococcus aureus* were tested in this study.

MATERIAL AND METHOD

Sample collection

Seeds of *Piper nigrum*, *F. vulgare*, and *T. ammi*, tubers of *C. longa* and the bark of *C. verum* used in present study were purchased from the local market of Pattoki, Punjab, Pakistan. (**Table 1**).

Bacterial strain

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

Preparation and storage of extracts

Spices viz, *P. nigrum*, *F. vulgare*, *T. ammi*, *C. verum*, and *C. longa* were dried in hot air oven (Bionics Scientific Technologies (P) Ltd. Model # BST/HAO-1122) for 3 days at 70°C in Microbial Biotechnology Laboratory, Department of Wildlife & Ecology, UVAS. Oven dried samples were ground individually in an Electric blender (Vitamix 5200) at 6000 rpm for 15 minutes. Twenty grams from 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), then 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 of spices were filtered by using Whatman filter paper # 41. The resulted aqueous solutions of extracted spices were then stored in a refrigerator (Dawlance Model: DW-600) at 4°C for later use.

Preparation of bacterial culture

The start-up 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 Microbial Biotechnology Laboratory, Department of Wildlife & Ecology, UVAS for 24 h at 37°C in an incubator (BioTek Instruments, Model: BioSpa8 Automated).

Experimental design

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

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 concentration of minimum inhibition was determined for all extracted samples after 24 h of incubation period, 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 is designated as "Complete Inhibition" and expressed with "+++" sign, when there was zero development of 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 "++" sign. However, if the number of colonies (CFU) formed were greater than 60 the bacterial growth is designated as "No Inhibition" as expressed with "-" sign. There were three replications for each treatment and a control treatment with no added concentration of any of the spices tested.

Data Analysis

The resulted data was tabulated in a table form as well as presented in a figure with Microsoft Excel 2016 (version: 16.0.12527.20260). Data was statistically analyzed by using Regression curves, fitted to find the correlation between different concentrations of each spice and its inhibition activity for *S. aureus* with RStudio (version: 1.2.5033).

RESULTS

Our results demonstrated the antibacterial effects of five spices against *S. aureus* by assessing growth inhibition of bacterial strain under laboratory conditions against control treatment (**Fig. 1**). We observed that among the five species of spices, the most effective spice to inhibit bacterial growth was *C. verum* against *S. aureus* by completely inhibiting the growth of the bacterial strain at 15% concentration, while *P. nigrum* failed to inhibit the growth of bacterial strain even at maximum used 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 inhibition activity of all the spices was in 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*, while, at 15% concentration the spices inhibited the bacterial growth in the order of *C. verum* > *T. ammi* > *C. longa* > *F. vulgare* > *P. nigrum* (**Fig. 2**). *Trachyspermum ammi* had significant effects at all concentrations provided. Whereas, *F. vulgare* was significantly effective at 5% and 15% concentrations and *C. longa* showed relative inhibiting effects at 10% and 15% concentrations (**Fig. 3**). Only *C. verum* showed complete inhibition at 15% concentration (**Fig. 4**).

The regression analysis and regression curve of different spices showed that *C. longa* (p= 0.005) and *C. verum* ($p < 0.00$) had significant correlation of bacterial growth inhibition at different concentrations. While, *F. vulgare* (p =0.90) and *T. ammi* (p =0.78) had non-significant inhibition activity of bacterial growth at different concentrations. Only *P. nigrum* had null effects of bacterial growth inhibition could not be statistically tested as in all replications and in all the concentration it did not show any inhibition of bacterial growth (**Fig. 5**).

DISCUSSION

In present study 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 aqueous extracts of tested spices except *P. nigrum* worked well against *S. aureus* and exhibited inhibiting effects at some concentration. We explored that *C. verum* is more effective than other spices at all concentration tested and exhibited the maximum bacterial growth inhibition. These finding are coherent with the study that showed that at 250 ml/l concentration in methanol, *C. verum* had maximum inhibition zone of 12mm against *S. aureus.*[21] However, in our study we found more inhibiting activity of this spice even at lower concentrations against *S. aureus*.

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

Our results showed that *F. vulgare* was significantly effective against tested bacterial strain at 5% and 15% concentration. Our results are aligned with others. For instance, it is found that oil extracts of *F. vulgare* are effective against different strains of gram positive and gram negative food spoiling bacteria.[22] Similarly, it was concluded that *F. vulgare* is an effective antibacterial agent against different bacteria in the form of aqueous extract.[23] Antibacterial activity of oils is dependent on the principal constituents and also their intensity. Suppressive effects of vital oils are primarily due to the main component antimicrobial properties are associated with bacterial susceptibility.[24] The small quantities of minor components could also make a significant contribution to the antibacterial activity of the oils. MIC measurement of Gram-positive and Gram-negative bacteria indicated diverse sensitivity towards oils and surinam cherry indicated maximum antibacterial activity[25] Plant extracts acts as natural antimicrobials and are used as food preservatives to control the growth of pathogenic microbes.[26]

Most of the antibacterial constituents of spices are phenolic substances with a hydroxyl group (-OH). The existence of scented center together with the polar functional group defines the inhibitory characteristics of the important oils. A hydroxyl group is much more efficient in comparison with a carbonyl group. The hydroxyl group can readily attach the active place of enzyme and modify their metabolic rate. Necessary oils or its active composites comprising hydroxyl group (-OH) are extremely antibacterial agents and antibacterial activity of phenolic acids is more effective for Gram-positive bacteria than Gram-negative bacteria.[27,28] Though, phenolic acids are active against Gram-negative bacteria, their antimicrobial effect is strain dependent. Many plant extracts having saponin revealed antimicrobial activities using different concentrations[29]. Antibacterial properties of *C. longa* against *S. aureus* found in our studies are also been reported previously. For example, it is documented that *C. longa* has growth inhibiting properties against different bacteria like, *Salmonella typhi* and *S. aurous*.[30] We found that *T. ammi* possessed antibacterial properties against *S. aureus* at all provided concentrations (5%, 10% and 15%). These results are coherent with the study that described the antimicrobial activity of this spice against *S. aureus* and *E. coli*.[31]

CONCLUSION

Most of the herbs and spices that are commonly used in our daily diet and cooking process for the purpose of aroma and flavor, have a very effective antibacterial and antimicrobial properties against different bacteria. Thus, these spices can be used to reduce the chance of food-poisoning and loss, to rise the food security and shelf-life expectancy of goods, and to treat certain infectious illnesses. As the possible combinations of numerous spices proves that they have advanced inhibitory properties on definite bacteria than those of specific spices. Use of these spices as food additive to preserve foods for longer time and in the herbal medicine could be very cost effective and risk-free approach in future. Spices that indicate antibacterial activity could be a source for antimicrobial agent against foodborne pathogens and are suitable as food preservatives. As antimicrobial compounds are unable to compete with antibiotics so, antimicrobial plant agents' exploration still continues.

Recommendations

- Spices extracts possesses antibacterial activities that can be used to treat diseases instead of their usage as flavor/nutrient supplement.
- The nano-formulates of spices can indicate antimicrobial properties against multi-drug resistant microbes.
- Spices' combination, natural compounds and novel technologies can develop molecules against spoiled microbes.
- In future, mechanism of individual oil component as well as combined effect of different components need to be explored. Hence, various approaches for nano-encapsulation and synergistic studies can offer platform for the research in near future.

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DECLARATIONS

- **Funding**

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- **Conflicts of interest / Competing interests**

  Authors declare no conflict of interest.

REFERENCES

1. Nascimento G.G.F., Locatelli J., Freitas P.C. and Silva G.L., Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, Braz. J. Microbiol., 31, 247-256 (2000)

2. Odugbemi T., Medicinal plants as antimicrobials In: Outline and pictures of medicinal plants from Nigeria, In. University of Lagos press, 53-64 (2006)

3. Mostafa A.A., Al-Askar A.A., Almaary K.S., Dawoud T.M., Sholkamy E.N. and Bakri M.M., Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, Saudi J. Biol. Sci., 25, 361-366 (2018)

4. Zamin I., Khan I., Hyder H. and Naz B., In-vitro efficacy of crude extract of Zizipus Jujuba against selected bacterial strains, In, (2014)

5. Zamin I., Majid A., Ali I., Tahir A., Khan M., Ibrar J., Ali, Ullah Z., Aman I., Naveed M., Khan I., Mujaddad M. and Rahman U., In-vitro antibacterial activity of Mentha Piperita leaf extracts to some selective pathogenic bacterial strains, Int. J. Med. Pharm. Sci. Res. and Rev., 103, 1- 130 (2013)

6. Varahalarao V. and Chandrashekar N., In Vitro bioactivity of Indian medicinal plant Calotropis procera (Ait.), J. Glob.Pharma technol., 2, 43-45 (2010)

7. Alireza G., Mehdi R.R.S, Razzagh M., Ata K. and Masoud K., Antimicrobial effects of some herbal plants and spices on Staphylococcus epidermidis and Pseudomonas aeruginosa, Int. J.Food Nutr. Saf., 9, 40-48 (2018)

8. Nanasombat S., Prasertsin V., Graisin K., Shain H. and Thanaboripat D., Efficacy of new enzyme-linked immunosorbent assay for rapid detection of Salmonella in foods, Government Pharmaceutical Organization Report, Bangkok, 51, 53-57 (2002)

9. Chaudhry N.M. and Tariq P., Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates, Pak. J. Pharm. Sci., 19, 214-218 (2006)

10. Indu M.N., Hatha A.A.M., Abirosh C., Harsha U. and Vivekanandan G., Antimicrobial activity of some of the south-Indian spices against serotypes of Escherichia coli, Salmonella, Listeria monocytogenes and Aeromonas hydrophila, Braz. J. Microbiol., 37, 153-158 (2006)

11. Ağaoğlu S., Dostbil N. and Alemdar S., Antimicrobial activity of some spices used in the meat industry, Bull. Vet. Inst. Pulawy., 51, 53-57 (2007)

12. Ghori I. and Ahmad S.S., Antibacterial activities of honey, sandal oil and black pepper, Pak. J. Bot., 41:461-466 (2009)

13. Dahanukar S., Kulkarni R. and Rege N., Pharmacology of medicinal plants and natural products. Indian J. Pharmacol., 32, 81-118 (2000)

14. Snyder O.P ., Antimicrobial effects of spices and herbs. In, (2013)

15. Pandey B., Khan S. and Singh S., A study of antimicrobial activity of some spices, Int. J. of Curr.Microbiol. Appl.Sci., 3, 643-650 (2014)

16. Meghwal M. and Goswami T., Nutritional constituent of black pepper as medicinal molecules: a review, Sci. Rep., 1, 129 (2012)

17. Unlu M., Ergene E., Unlu G.V., Zeytinoglu H.S. and Vural N., Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae), Food Chem. Toxicol., 48, 3274-3280 (2010)

18. Ushimaru P.I, Silva M.T.N., Di Stasi L.C., Barbosa L. and Fernandes J.A., Antibacterial activity of medicinal plant extracts, Braz. J. Microbiol., 38, 717-719 (2007)

19. Luthra P.M, Singh R. and Chandra R., Therapeutic uses of Curcuma longa (turmeric), Indian J. Clin. Biochem., 16, 153-160 (2001)

20. Crellin J., Philport J. and Tommie B., A reference guide to medicinal plants: Herbal medicine past present. Duke University Press, (1989)

21. Pandey B., Khan S. and Singh S., A study of antimicrobial activity of some spices, Int. J. of Curr.Microbiol. Appl.Sci., 3, 643-650 (2014)

22. Akhbari M., Kord R., Nodooshan S.J. and Hamedi S., Analysis and evaluation of the antimicrobial and anticancer activities of the essential oil isolated from Foeniculum vulgare from Hamedan, Iran, Nat. Prod. Res., 33, 1629-1632 (2019)

23. Maghami M., Motalebi A.A. and Anvar S.A.A., Influence of chitosan nanoparticles and fennel essential oils (Foeniculum vulgare) on the shelf life of Huso huso fish fillets during the storage, Food Sci. Nutr.,7, 3030-3041 (2019)

24. Chouhan S., Sharma K. and Guleria S., Antimicrobial activity of some essential oils—present status and future perspectives. Medicines, 4, 58 (2017)

25. Soetjipto H., Antibacterial Properties of Essential Oil in Some Indonesian Herbs. Potential of Essential Oils, 41 (2018)

26. Gonelimali F.D., Lin J., Miao W., Xuan J., Charles F., Chen, M. and Hatab S.R., Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Front Microbiol., 9,1639 (2018)

27. Patra A.K., An overview of antimicrobial properties of different classes of phytochemicals. Dietary Phytochemicals and Microbes, 1-32 (2012)

28. Cueva C., Moreno-Arribas MV., Martín-Alvarez P.J., Bills G., Vicente M.F., Basilio A., Rivas C.L., Requena T., Rodríguez J.M., Bartolomé B., Antimicrobial activity

of phenolic acids against commensal, probiotic and pathogenic bacteria. Res. Microbiol., 161, 372–382 (2010)

29. Avato P., Bucci R., Tava A., Vitali C., Rosato A., Bialy Z., Jurzysta M., Antimicrobial activity of saponins from *Medicago sp*.: structure-activity relationship. Phytother. Res., 20, 454–457 (2006)

30. Akbar A., Ali I., Samiullah N., Khan S.A., Rehman Z. and Rehman S.U., Functional, antioxidant, antimicrobial potential and food safety applications of curcuma longa and Cuminum cyminum, Pak. J. Bot., 51, 1129-1135 (2019)

31. Azarifar M., Ghanbarzadeh B., Khiabani M.S, Basti A.A., Abdulkhani A., Noshirvani N. and Hosseini M., The optimization of gelatin-CMC based active films containing chitin nanofiber and Trachyspermum ammi essential oil by response surface methodology, Carbohydr. Polym., 208, 457-468 (2019)

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

Table. 2: Antibacterial effect of different spices on *staphylococcus aureus* with 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, --: Non-Inhibition

FIGURE LEGENDS

Fig. 1: *Staphylococcus aureus* colonies formed in control treatment.

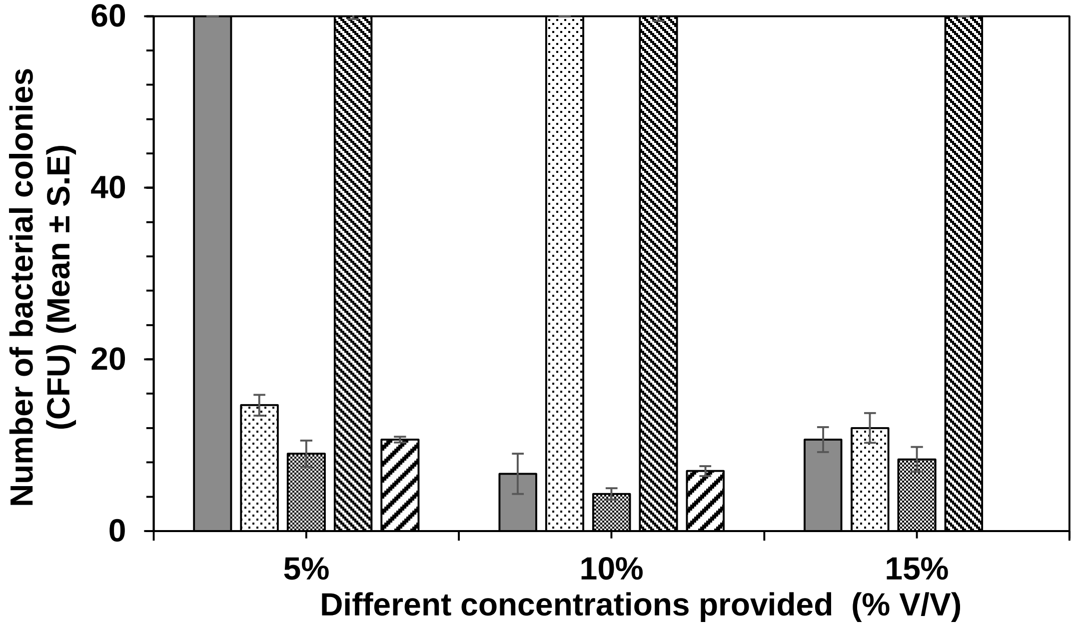

Fig. 2: Number of *Staphylococcus aureus* colonies (CFU) formed of at various concentrations of five spices (■:*Curcuma longa,* ░:*Foeniculum vulgare,* ▒:*Trachyspermum ammi,* ▧:*Cinnamomum verum,* and ▨:*Piper nigrum*). Mean values reaching the 60 CFU represents null inhibiting effects of particular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

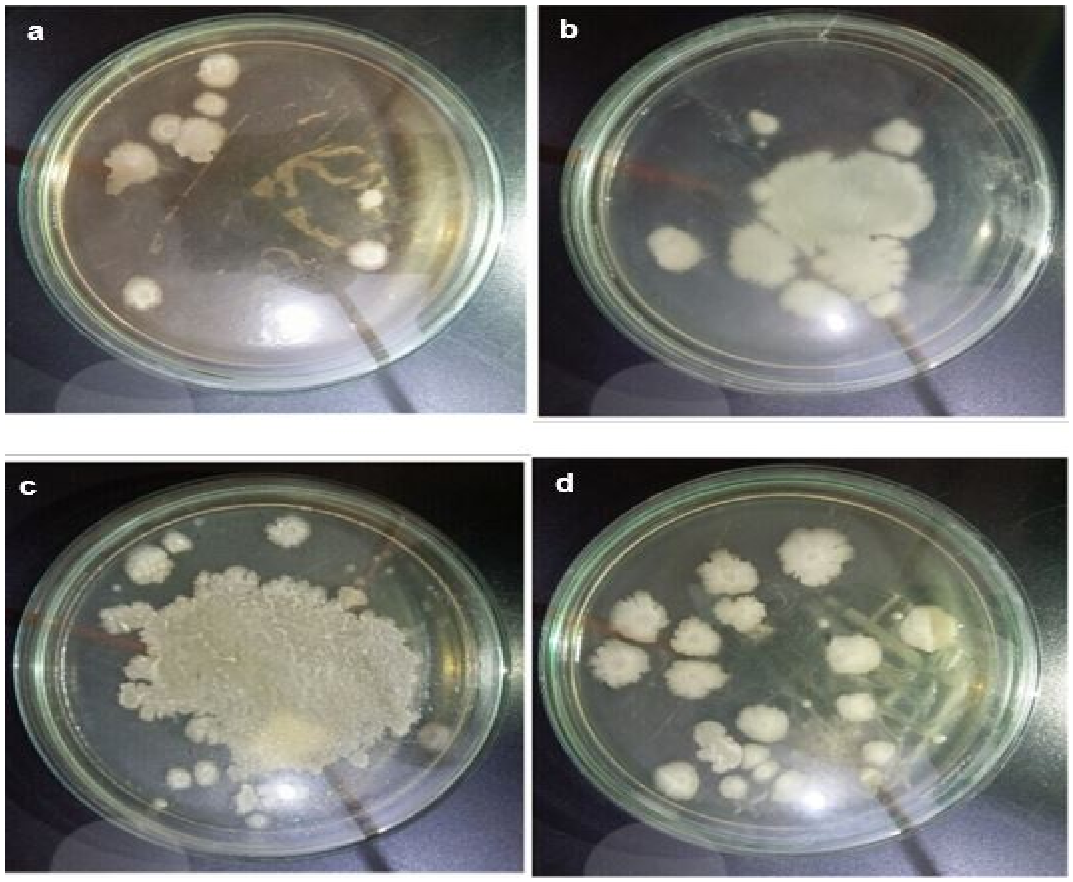

Fig. 3: ***Staphylococcus aureus*** **colonies formed at 15% concentration with different spices used. a)** *Trachyspermum ammi;* **b)** *Curcuma longa;* **c)** *Piper nigrum;* **d)** *Foeniculum vulgare.*

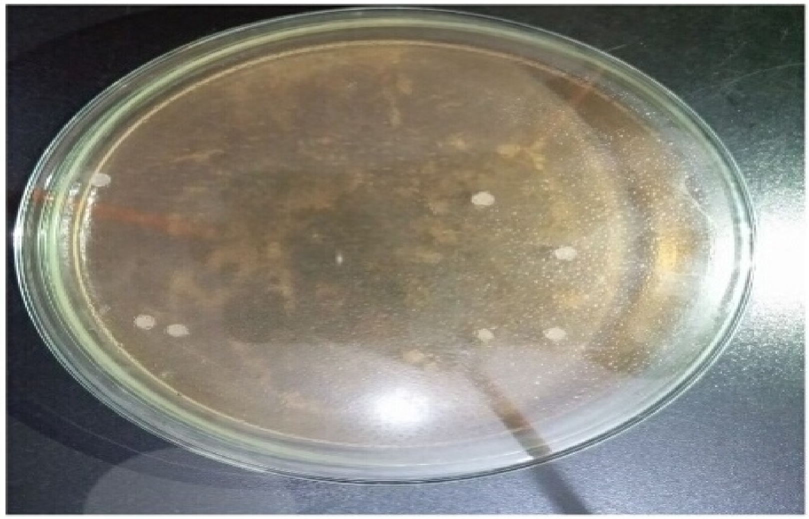

Fig. 4: Complete growth inhibition of *Staphylococcus aureus* colonies formation at 15% concentration of *Cinnamomum verum.*

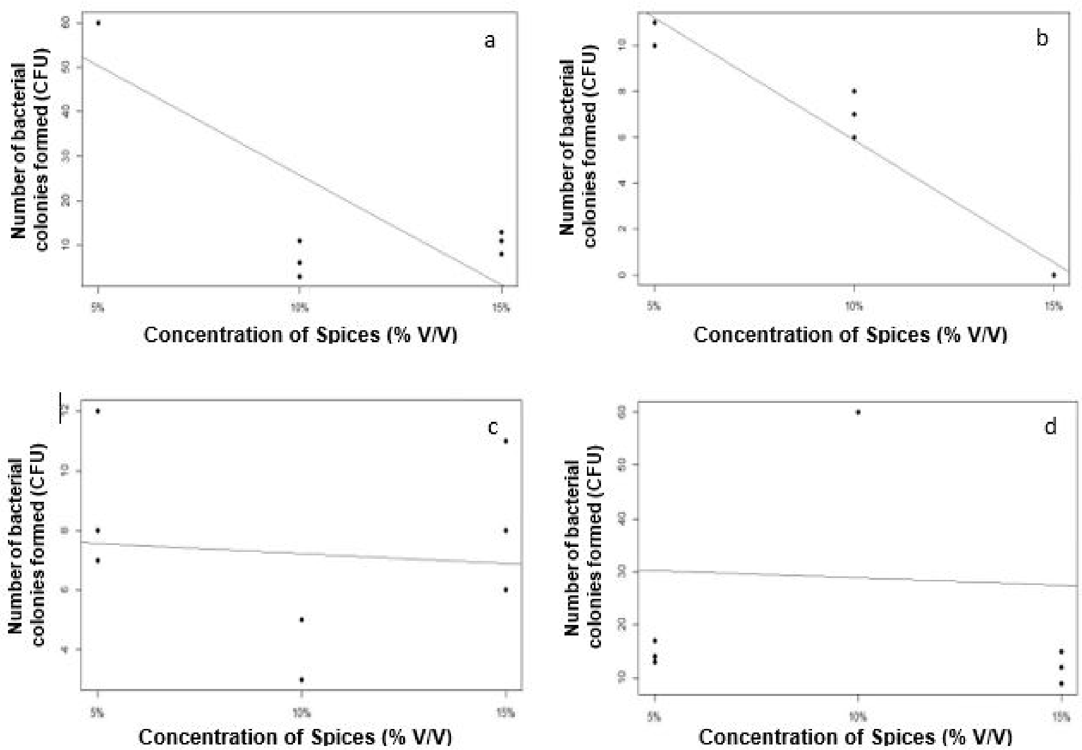

Fig. 5: Regression curve between different concentrations of each spice and its growth inhibiting effects on *Staphylococcus aureus*. a) *Curcuma longa;* **b)** *Cinnamomum verum;* **c)** *Trachyspermum ammi;* **d)** *Foeniculum vulgare.* ***Piper nigrum*** **is not included in this analysis due to its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