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Antibiotic Activity Of Common Spices Against Staphylococcus Aureus

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Abstract
The primary objective of the study is to show antibiotic activity of plant which we use as spices. We aim to find the vitro antibacterial activity of four different medicinal spices against staphylococcus aureus. In this present study was conducted to isolate identify and characterize the staphylococcus aureus from pus and check its antibiotic activity against Clove (syzygium aromaticum), Cinnamon (cinnamomum zeylanium), Black pepper (piper nigrum) & Methi (leaf) (Trigonella foenum – graecum). For this prepare fine powder of such ingredients and make antibiotic disc and observe the zone of inhibition against staphylococcus aureus. The maximum growth of inhibition was seen under the Clove.

Keywords: Staphylococcus aureus; Spice; Clove; Cinnamon; Black pepper; Methi; Antibacterial Activity.

1. Introduction

Antimicrobial agent is essentially important in reducing the global burden of infection diseases [i]. *Staphylococcus aureus* is major bacterial human pathogen that causes a wide variety of clinical manifestation [ii]. *Staphylococcus aureus* does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstreams or internal tissues, these bacteria may cause a variety of potentially serious infection [i].We used Clove, cinnamon, Black pepper and methi for this work. Spice has been used for many centuries by various cultures to enhance flavour and aroma of as food as our ancestors have recognized the usage of spice in food. Preservation and in treatment of clinical ailments and there are several report on development of antibiotic resistance in diverse bacterial pathogen [iii].

Clove oil has biological activities such as antibacterial, antifungal, insecticidal and antioxidant properties and is used traditionally as a savouring agent and antimicrobial material in food [iv,v ,vi]. Cinnamon posse's potent antibacterial and also antifungal effect. Black pepper is known to inhibit the growth of various microbes such as *staphylococcus aureus* .Meth is also show the reaction against staphylococcus aureus.The primary purpose of spics to impact the flavour and piquancy to food, the medicine, antimicrobial and antioxidant properties of spices have also been exploited. Despite the fact that the basic role of flavours is to improve the taste and fragrance of the nourishment properties [vii, viii].arrangements, they are significant because of its therapeutic, antimicrobial and cancer prevention agent.

2. Materials and Methods

Collection of bacteria: - We isolate microscopic organisms from pus sample in microorganism free container and then inoculate on nutrient agar plate. Which we isolate a bacterial colony. Then this isolated bacteria inoculated in Manito salt agar (MSA) which is their selective media and incubates 37°c for 24 hours. After isolate the pure microorganism colony.

Bacterial colony identification, morphology study: - The usage of spread plate method the bacterial colony identifies and external morphology becomes studied for which Mannitol salt agar media transformed into composed. Consequently one hundred ml of Mannitol salt agar media was prepared for five petri plates. The Mannitol salt agar media become autoclaved and then poured in five petri dishes which have been additionally sterilized via autoclave. Then the serial dilutions of 10 to the power -2,-3,-4,-6 and -8 were selected and from that 0.1 ml of way of life turned into transferred from each serially diluted test tubes and spread at the petri plates with the help of the spreader. Then the petri dishes were stored within the incubator for 37℃ for 24 hrs for the incubation for optimum growth of bacteria. After 24hrs of the incubation the petri dishes were taken out of the incubators and the subsequent bacterial outside morphology have been studied.

Pure culture isolation of bacteria: - Well developed and separated colonies which were perceived on the Mannitol salt agar plates had been set apart after which those isolated provinces had been picked and through the help of inoculating needle the states had been moved and streaked independently on test tube having Mannitol salt agar slant for the development of the single states of bacterial colony from the blended culture of microorganism. That transformed into developed inside the petri plates. The step through an exam test tube had been set apart after the strains of picked states from petri plates and had been left inside the incubator at 37℃ over night for development and incubation. After incubation of the pure culture in a one day unique single types of bacterial culture slant developed in the step through a test tubes which have been likewise picked and purified.

Microscopic study: - The single type of bacterial culture of different colonies that were gotten in the test tube was put for gram staning for progressively one of a kind recognizable proof of the bacteria. The gram staining performs into laminar air flow hood. For this purpose slide were taken from slide rack. The slides have been washed with ethanol. Then each colony changed into marked on the slides. At that point with the help of inoculating needle the loopful strains had been picked from each step through a test tube and make a smear at the slides and warmth constant. The slides were then taken in the recoloring space for recoloring the smears. At that point smears were recolor in following stages a) first applied crystal violet on every slide and put away for 30sec. b) Distilled water wash. c) Iodine on the slides as Mordant (1min) at that point 95% alcohol washes and afterward washed with distilled water. d) safranin ends up applied at the slides after which washed with distill water. f) The slides air dried. The entire gram staining technique was done following the Christian Gram technique [ix].

Identification of Bacterial Isolates through Biochemical Test:- The collected bacteria from pus transformed into perceived up to familiar degree principally dependent on morphological cultural and Biochemical tests as laid to in Berger's guide of Determinative Bacteriology [x]. Biochemical step through an examination at ended up completed as guided with the guide of [xi] which performed tests as like Gram's stain, IMViC reaction , catalase test , starch hydrolysis test oxidation fermentation test, Nitrate reduction, Gelatin hydrolysis test, Urea hydrolysis test, Dehydrogenase test, citrate utilization test , Indol production test , Triple sugar Irons (TSI) test, carbohydrate fermentation test. For isolated bacterial identification as per to Bergey's manual we performed test. For disengaged bacterial recognizable proof according to Bergey's manual we performed test. IMViC test shows Methyl red, Voges-proskauer (VP). Then catalase and coagulase.

Collection of plant: - There are four types of spices are used for the study as shown in the table. This is easily available in environment.

Table 1: Collected plant

Sr.no	Name	Scientific name
1.	Clove	Syzygium aromaticum
2.	Cinnamon	Cinnamomum verum
3.	Black pepper	Piper nigrum
4.	Methi	Trigonella foenum graecum

Preparations

- **Preparation of fine powder**: - First we take four flavors and will dry it and made a fine powder. Leading we take a four flavors Clove, Cinnamon, Black pepper, Methi. At that point these flavors washed with D.W. To discard the undesirable materials at that point air-dried and dry in underneath sunlight. The temperature of sunlight is 42-43℃ for 48 hrs after which we scour or crush this spices. At that point we found a fine powder. At that point we disinfect this powder in warm air oven and store in sterile container.

- **Preparation of antibiotic tablet**: - We use the sterile fine powder of Spices and distilled water. Make the tablet like structured disc for the antibiotic test.

Assay of antimicrobial activity using agar disc diffusion method: - The sensitivity of various bacterial strains to the watery plant extracts was estimated utilizing a standard agar diffusion assay [xii]. The 40 ml of nutrient agar became poured in to sterile petriplate, after solidification 100 micro ml of fresh culture of s.aureus had been poured on the petriplates. After some time culture diverted into dispose of from agar plate. At that point take cleaned forceps and pursue the tablet cautiously in center point of agar plate. The plates had been incubated for 24-48 hrs at 37℃. After incubation the diameter of inhibitory zone shaped around each tablet were measured in cm and recorded.

3. Result and Discussion

The aim of this study was initial upon assortment of *S.aureus* and compare the antimicrobial activity of clone, cinnamon ,black pepper and methi. Based on cultural character, Morphological character (colony color, Shape and size) and biochemical character isolated bacteria was distinguishing. Following character was compare with 'BERGEY'S. Isolated bacterial result was given in following tables. An isolated bacterium was found cocci in cluster shape and show violate color in gram staining.

Table 3.1: Microscopic study.

Sr.No.	Test	Result
1)	Gram	Positive (+ve)
2)	Spore	Non- spore forming
3)	Motility	Non-motile

After performing microscopic character then we performed biochemical test such as IMViC, Gelanitase, Nitrate, Catalase, Oxidase, Coagulase, Mannitol, and Urea. And their results given in following table no 3.2.

Table 3.2: Biochemical test.

Sr.No.	Test	Result
1)	Indol	Negative(-ve)
2)	Methyl Red	Positive (+ve)
3)	Voges-proskauer (VP)	Positive(+ve)
4)	Citrate	Negative (-ve)
5)	Gelatinase	Positive(+ve
6)	Nitrate	Positive(+ve)
7)	Catalase	Positive(+ve)
8)	Oxidase	Negative(-ve)
9)	Coagulase	Positive(+ve
10)	Mannitol	Positive(+ve)
11)	Urea	Positive(+ve)

After conforming selected bacterial identification then isolated bacterial activity check against 4 spices selected that is Clove, cinnamon, black pepper and methi. It was collected for the test of Antimicrobial activity of *S.aureas.*

Table 3.3: Zone Of inhibition

Sr.no.	Spices	Zone of inhibition
1.	Clove	3.1 cm
2.	Cinnamon	1.1cm
3.	Black pepper	1.9cm
4.	Methi	2.6 cm

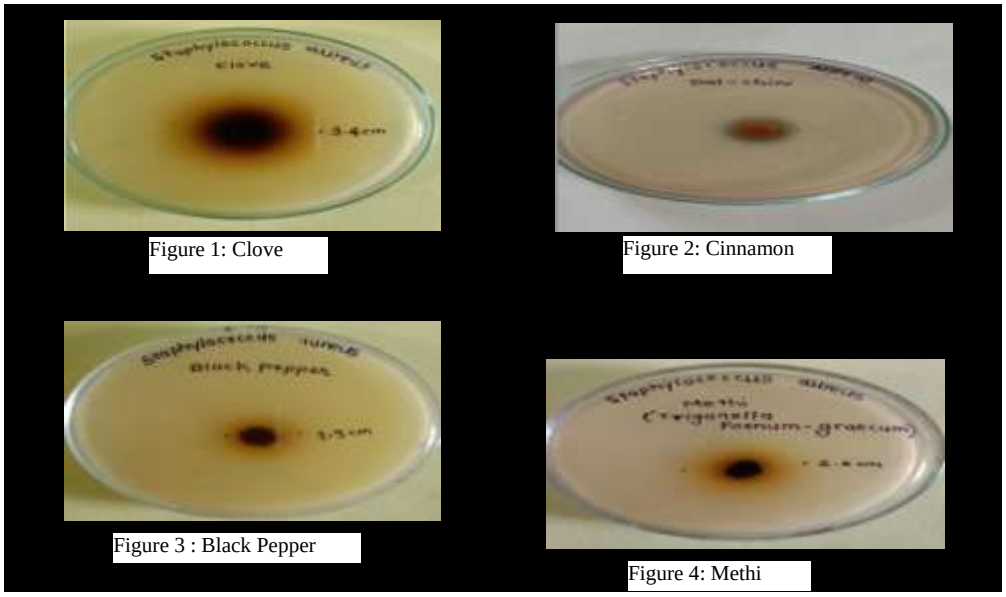

Figure 1: Clove

Figure 2: Cinnamon

Figure 3 : Black Pepper

Figure 4: Methi

Fig. 3.1.: zone of inhibition

In the present study the, Clove show 3.1 cm of inhibition of zone, Cinnamon show 1.1cm of zone of inhibition, Black pepper show 1.9cm of zone of inhibition, Methi show 2.6 cm of zone of inhibition. Various researchers have work in exploring the antimicrobial potency of extract against infection bacteria. In the study, *S.aureas* was found to be sensitive to the four plants sensitive. The highest sensitivity of *s.aureas* may be due to its cell wall structure may be due to its cell well structure and outer member [xiii]. Our result suggest that 'Gram positive' bacteria are show sensitivity on spices. This was consistent with the previous studied on other Spices and herb [xiv].The result obtained from the present study provide evidence that Clove, Cinnamon, Black pepper and methi and exhibit antibacterial activity against isolated *S.aureus* stain , which suggest that may be clinically useful. One of the effort in this research is focused on the use of medicinal plant, which are widely available resources, less if no side effects ,less expensive and have shown antimicrobial properties [xv].

4. Conclusion

The current study has identify has S.aureus bacteria from pus and check antibacterial activity against Clove, cinnamon, black pepper and methi. It was investigated that clove spices and methi spices showed maximum antibiotic activity against *S.aureus*. Hence here we concluded that for controlling *S.aureus* infection use clove spices.

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