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Antimicrobial Activity of Clove Plant Extract (*Syzygium aromaticum*) on *Staphylococcus aureus*

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Abstract **Original Research Article**

The continuous use of antibiotics for the treatment of bacterial infections has led to the increase in bacterial resistance to these antibiotics. Many bacteria, including *Staphylococcus aureus* are currently multi-drug resistant, which poses a great problem to the medical and pharmaceutical industry [1]. This research was conducted to investigate the antimicrobial properties of *Syzygium aromaticum* against the above-mentioned organism. The plant extracts were Hot water, Cold water, and Ethanol, and the susceptibility of the organism was tested using agar well diffusion and disk diffusion methods. The plant extracts showed antimicrobial activity on the isolate used i.e. *Staphylococcus aureus*. From the result, the minimum inhibitory concentration (MIC) was 0.24mg/ml, and the Minimum bactericidal concentration (MBC) was 3.91mg/ml. The Diameter Zone of Inhibition in (mm) against the plant concentration in (mg/ml) parameters were 500mg/ml and 250mg/ml. The values of the diameter zone of inhibition against the plant concentrations ranged from 16mm to 0mm while control ranged from 38mm to 10mm. The Phytochemical components determined in Cloves were Alkaloids, Saponin, Phenols, Tannins, Flavonoids, Steroids, Soluble Carbohydrate, Glycosides, and Terpenes.

Keywords: Cloves, Plant extracts, Multi-drug resistant, antimicrobial propertie.

INTRODUCTION

Antibiotic resistance is an increasing threat to global health. Therefore, in this study, extracts of organic solvents of a medicinal plant *Syzygium aromaticum* was evaluated for their antibacterial activities against *Staphylococcus aureus.* This was carried out by taking the organic extracts of both the leaf and stem parts of the plants natural sources. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [1]. This study contains the evaluation of the antibacterial activity of aqueous extracts isolated from Clove (*Syzygium aromaticum*) against gram-positive *Staphylococcus aureus*. Due to limited choice of antibiotics, medicinal plant extracts have gained interest because of their known antimicrobial nature. Medicinal plants are the richest bio resource of drugs for traditional systems of medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. Many spices around the world have been used for several medicinal purposes and as food preservatives, and out of those *Syzygium aromaticum* (clove) is widely used as it has got anti-inflammatory, antimicrobial, antithrombotic, antioxidant, ant mutagenic, and anti-ulcer genic properties [2].

This research is concerned with investigating the antimicrobial properties of the plant *Syzygium aromaticum* against *Staphylococcus aureus* and reducing the problem of wound infections and microbial resistance to antibiotics by proffering a cheaper and more accessible alternative to antibiotics, which was successful and was of tremendous advantage and impact to the medical and scientific field [2].

LITERATURE RIVIEW

Antimicrobial Activity

Antimicrobial activity of a plant or a substance can be defined as its ability to kill or inhibit the growth, metabolism, and replication of a microorganism, ultimately leading to its death. The discovery of the antimicrobial effects of certain plants, chemicals, and substances (synthesized or found in nature) has been of great impact and importance in microbiology and medicine. It has led to the development of antibiotics, and alternative herbal remedies for the treatment and control of contamination and infection caused by microorganisms [3].

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen [4]. Although *S. aureus* usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for *S. aureus* has been approved. *Staphylococcus aureus* on basic cultivation media.

Description of Cloves

Cloves are the aromatic dried buds of a tree (*Syzygium aromaticum*) used as a spice in virtually all the world's cuisine. The dried clove bud contains carbohydrates, fixed oil, steam-volatile oil, resins, tannins, proteins, cellulose, pentosans and mineral elements. A few non-volatiles have been isolated from clove, which include tannins, sterols, triterpenes and flavonoids. (Gopalakrishnan *et al.*, 1988). Volatile constituents yields different types of volatile oil [oil extracted from i. leaves, ii. the stem, iii. the buds and iv. the fruit, which are Eugenol (70- 85%), eugenyl acetate (15%) and p-caryophyllene (5-12%), together make up 99% of the oil [5].

SAMPLE COLLECTION

The plant material cloves was collected from Maraba Central market Karu, Nasarawa state by observing its physical characters like shape, smell, odour and colour of the plant. The sample was then pounded to powder with help of mechanical grinders i.e. mortar and pestle and then stored in a sterilized container. The antibacterial activity of the clove extract on *Staphylococcus aureus* was determined using agar well diffusion method. For extraction of phytochemicals, pulverized plant material, was soaked in polar solvent such as ethanol. The extraction process was completed with the application of heat. The pure isolate of *Staphylococcus aureus* was sub cultured using nutrient agar and then inoculated using streaking plate method. The plates were labelled respectively and then incubated at 37℃ to obtain a viable culture of the susceptibilities of the test organism.

METHOD

Agar plates were inoculated with standardized inoculum (1.5 x108 CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Five wells of 8 mm size were made with sterile borer into the agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. 0.04ml of each of the plant extracts were poured into a well of inoculated plates. Sterilized distilled water was used as a negative control which was introduced into a well instead of *Syzygium aromaticum* plant extract. Ciprofloxacin was used as a control. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extracts into the agar. After incubation for 24 hrs. At 37^{o}C, the plates were observed. Antibacterial activity was present on the plates, indicated by an inhibition zone surrounding the well containing the plant extract. The Diameter of Inhibition Zone (DIZ) was measured and expressed in millimetres. The mean values of the diameter of inhibition zones were calculated according to the method of Mohammed *et al.*, [7].

Determination of the Minimum Inhibitory Concentration (MIC)

Since the *Syzygium aromaticum* extracts exhibited antimicrobial activity against the pathogenic *Staphylococcus aureus* isolate, it was further assayed for its minimum inhibitory concentration (MIC). This was carried out by the two-fold serial dilution of the tested extracts in Mueller Hilton broth (2 ml volume), then inoculated with 100µl inoculum size with the test organisms. The aqueous extracts of the plant were prepared at concentrations of 500,250,125,62.5,31.25,15.63,7.71,3.91,1.95,0.98,0.49, and 0.24(mg/ml). The MIC was determined by the broth dilution method. Mueller Hilton broth samples (10 ml) were inoculated with different concentrations of the plant extracts and with active inoculum of bacterial isolates (1.5 x 108 CFU/ml) in tubes and incubated for 24 hrs. at 37^{0}C. The MIC was determined as the lowest concentration of the extract which inhibited the organisms.

RESULTS

Results of Phytochemical Analysis of *Syzygium aromaticum* Extracts

Phytochemical analysis was conducted on the various aqueous *Syzygium aromaticum* extracts, and the

following results were obtained and recorded. The plants extract showed the presence of flavonoids, tannins, alkaloids, and terpenoids. Glycosides were absent in all plant extracts.

Diameter of Inhibition Zones (DIZ) shown by the plant extracts against *Staphylococcus aureus* using the Agar Well Diffusion Method.

The selected organisms were tested for susceptibility to the plant extracts, and the results were observed and recorded. The plant extracts exhibited strong antimicrobial activity against *Staphylococcus aureus.*

Minimum Inhibitory Concentration

The *Syzygium aromaticum* showed antimicrobial activity against *Staphylococcus aureus* hence the plant extract was assayed for its minimum inhibitory concentration by conducting a fourfold serial dilution of the extract and inoculating them with the test organisms. The minimum inhibitory concentration of the plant extracts were observed and recorded, as shown in table 3.

Minimum Bactericidal Concentration

Minimum bactericidal concentration (MBC) was specified as the least clove concentration resulting in removal of bacterial growth. Inocula were collected from the suppressive zones of MIC concentration and the two other sequential concentrations and plated onto Mueller-Hinton agar plates. The minimum Bactericidal concentration of the plant extracts were observed and recorded, as shown in table 4.

Table-2: ANTIBACTERIAL ACTIVITY OF CLOVE PLANT AGAINST *Staphylococcus aureus*

ISOLATES	EXTRACTS	DIAMETER ZONE OF INHIBITION (mm)					
		PLANTS CONCENTRATIONS (mg/ml)					
		500	250	125	62.50	31.25	15.63
Staphylococcus aureus	Hot water	15	10	9	7	4	2
	Cold water	16	14	12	9	7	4
	Ethanol	12	8	6	5	4	0
	Positive control	38	35	32	26	21	15

Table-3: Minimum inhibitory concentration (mic) of clove plant in mg/ml.

	Staphylococcus aureus
Hot water	62.5
Cold water	31.25
Ethanol	15.63
Positive control (Ciprofloxacin)	3.91
Negative control (DMSO)	-

The results observed in Table 3 above show the MIC values of the extracts which showed antimicrobial activity. The activities recorded were that of the *Syzygium aromaticum* extracts (hot, cold, and ethanol) against *all isolates*. The MIC value of the hot water extract of the plant against *S.aureus* was 62.5 mg/ml, that of the cold water extract was 31.25mg/ml, and that of the ethanol plant extract was 15.63mg/ml.

This shows that higher concentrations of the plant extracts are needed to show antimicrobial activity for *S.aureus*.

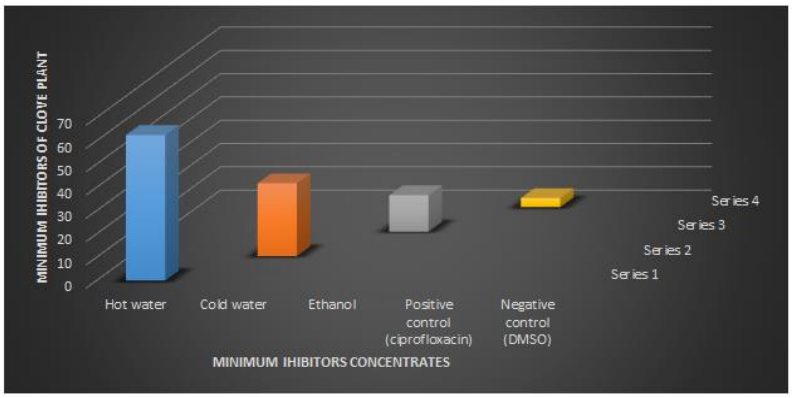

Chart showing the minimum ihibitors concentration (mbc) of clove plant

Table-4: Minimum bactericidal concentration (MBC) OF CLOVE PLANT IN mg/ml.

	Staphylococcus aureus
Hot water	500
Cold water	-
Ethanol	125
Positive control (ciprofloxacin)	15.63
Negative control (DMSO)	-

The results observed in Table 4 above show the MBC values of the extracts which showed antimicrobial activity. The activities recorded were that of the *Syzygium aromaticum* extracts (hot, cold, and ethanol) against *all isolates*. The MBC value of the extracts only showed on Hot, ethanol and positive control of the plant against *S.aureus*.

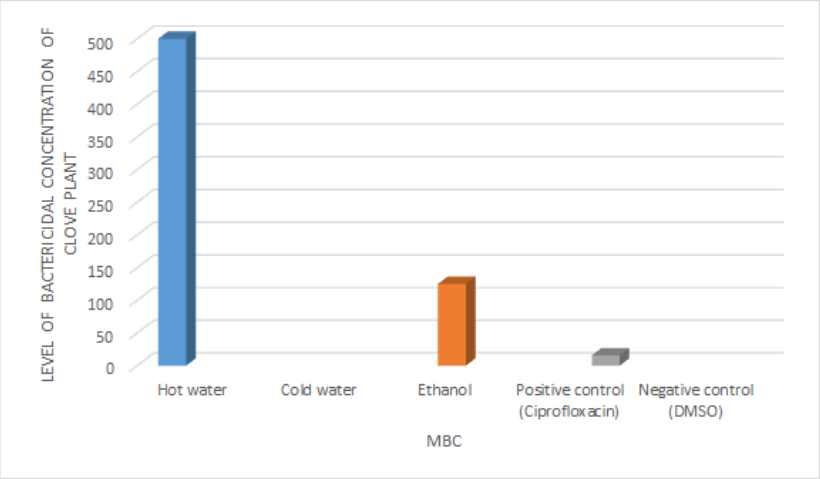

Chart Showing Minimum Bactericidal Concentrations of Clove plant (*Syzygium aromaticum*) against *Staphylococcus aureus*

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

The plant extracts were analysed to determine their phytochemical contents, and the results are shown in Table 1. The Hot extracts of *Syzygium aromaticum* showed the presence of Alkaloids, Soluble Carbohydrate, Phenols and Saponins, but the absence of Glycosides, Terpenes, Steroids, Tannins and Flavonoids. The Cold extracts showed the presence of Alkaloids, Saponins, Phenols and Terpenes, but Glycosides, steroids, flavonoids, tannins, were absent. The Ethanol extracts contained Flavonoids, Phenols, and Terpenes, but showed the absence of soluble carbohydrate, steroids, Glycoside, Saponins, Tannins and Alkaloids [8].

The results in Table 2 show the values of the Diameter if Inhibition Zone (DIZ) which indicated antimicrobial activity of the *Syzygium aromaticum* extracts on the selected microorganisms, using the agar well diffusion method. The hot, cold, and ethanol plant extracts showed various Diameter of Inhibition Zones of 15mm, 10mm, 9mm, 7mm, 4mm, and 2mm against *Staphylococcus aureus*, and the positive control antibiotic (chloramphenicol) showed DIZ of 38mm, 35mm, 32mm, 26mm, 21mm, 15mm. This result shows that in comparison to the control, the *Syzygium aromaticum* extracts exhibited a relatively low antimicrobial activity against *Staphylococcus aureus*. The ethanol extract of the plant proved to be the most effective of the three plant extracts.

It is shown that the activity of the plant extracts is concentration dependent, as the DIZs recorded are at high concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.50, 31.25 and 15.63mg/ml.

The results observed in table 3 show the MIC values of the extracts which showed antimicrobial activity. All activities of *Syzygium aromaticum* extracts (hot, cold and ethanol) were recorded against *all the isolates*, and the MIC values were determined as the lowest concentration of the plant extract that showed inhibition against the microorganisms. The MIC value of the hot water extract of the plant against *S. aureus* was 62.5 mg/ml, that of the cold-water extract was 31.25mg/ml, and that of the ethanol plant extract was 15.63mg/ml. This shows that higher concentrations of the plant extracts are needed to show antimicrobial activity for *S. aureus.*

Syzygium aromaticum has been by this research, seen to have no activity on *S. aureus* which is known as multi-drug resistant organisms. The results

observed in table 3 show the MBC values of the extracts which showed antimicrobial activity. All activities of *Syzygium aromaticum* extracts (hot, cold and ethanol) were recorded against *all the isolates*, and the MBC values were determined as the Minimum Bactericidal concentration of the plant extract that showed inhibition against the microorganisms. The MBC value of the extracts only showed on Hot, ethanol and positive control of the plant against *S.aureus*.

CONCLUSION

Syzygium aromaticum has been used for a long time traditionally for the remedy of various illnesses and infections, and has been assumed to replace antibiotics in the treatment of ailments. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases for example alkaloid protect against chronic diseases, saponins protect against hypercholesterolemia and other antibiotic properties. Phytochemical screening of clove extract used to study the presence of flavonoids, tannin, saponin, glycoside, phenol, and terpenoids have various medicinal values such as anti-inflammatory, antidiabetic, and analgesic activities and for central nervous system activity. From the aim of this research, Clove is expected to be active against *Staphylococcus aureus* and also resistant to the antibiotic. Phytochemical components determined are Alkaloids, Terpenes, Steroids, Soluble Carbohydrate, Flavonoids, Saponins, Phenols, Tannins, Glycoside. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

RECOMMENDATION

1. We recommend that, cloves should be used at times instead of consumption of a lot of antibiotic drugs which may lead to resistance if taken excessively. The fact that the extract of these medicinal plants inhibited some medically important bacteria proves that the plant might have some potential as an alternative source of antibacterial substances.
2. The public should be enlightened on the use of herbs for the treatment of ailments and infections. The use of plants should not be indiscriminate, or based on rumours.

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