



PHYTOCHEMICAL SCREENING OF *Syzygium aromaticum* IN DIFFERENT EXTRACTS AND ITS ANTIBACTERIAL PROPERTIES AGAINST DISEASE CAUSING PATHOGENS.

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Abstract

Since ancient times, spices have played an important part in Indian food and people's daily lives. Spices include active phytochemicals that have a comprehensive influence on human health. Spices are also high in antibacterial and antioxidant compounds that have bioactive properties. Medicinal herbs and spices continue to play an essential role in the treatment of human ailments. The goal of this investigation was to see if clove has any antibacterial effect against gram-positive and gram-negative bacteria, specifically *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, using aqueous, 70% ethanol, and 70% chloroform extracts. The antibacterial activity of clove extract was investigated using the agar well diffusion method, with amoxicillin serving as a positive control. When tested against pathogenic bacterial strains, aqueous extract showed the best inhibitory action against *Pseudomonas aeruginosa*, whereas ethanol extract had the best inhibitory activity against *Staphylococcus epidermidis*. Clove extracts in distilled water, ethanol, and chloroform were also tested for phytochemical content. Qualitative phytochemical examination of both spice extracts confirms the presence of many phytochemicals such as alkaloids, steroids, phlobatannins, saponins, tannins, cardiac glycosides, phenolic compounds, carbohydrates, terpenoids, resins, and anthraquinones. Clove has antibacterial action against microorganisms and can be used to prevent drug-resistant microbial illness. Because the spices contain many components that are essential for good health, they were tested for phytochemical ingredients and appeared to have the potential to function as a source of beneficial pharmaceuticals as well as to improve the health status of customers. The present study's findings are encouraging, since clove showed antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* infections.

Keywords: Phytochemicals, antimicrobial, Clove, *P. aeruginosa*, *S. epidermidis*, etc.

Introduction

India is the most well-known country for spices and traditional medicine, producing more than 50 of the world's 86 spices, each having distinct physiological and pharmacological properties. Spices are dried plant seeds, roots, bark, flowers, or fruits, or spices used in tiny amounts for flavour, colour, aroma, additive, preservative, beauty care goods, perfumery, bread kitchen trade, food additives, and medical purposes. [1]. India is a prominent spice grower, user, and exporter. India produces more than fifty of the eighty-six spices grown worldwide. They have a big influence on our national economy as well. On the worldwide market, around twenty major spices are traded. Spices are considered to contain bioactive antibacterial properties. Spices are also used to keep a range of food goods from spoiling [2]. Spices include active phytochemicals such as Alkaloids, Flavonoids, Tannins, Saponins, Terpenoids, Anthraquinones, Steroids, Anthocyanin, Phenols, Glycosides, Phlobatannins, Coumarins, and others that have a comprehensive influence on human health. Spices are also a good source of antibacterial and antioxidant compounds. Many spices are used in the kitchen and have therapeutic properties such as laxative, purgative, expectorant, carminative, and diuretic. Spices have an important role in the treatment of critical bodily ailments, particularly in Ayurveda. Spices have been used as one of the primary components in the majority of homoeopathic arrangements and treatments. Its involvement in anti-hypercholesterolemia, anti-inflammatory, anti-diabetic, and anti-proliferative effects on human health has also been identified in the medical sector. Spices can also aid in the treatment of diabetes, cardiovascular disease, cancer, arthritis, and AIDS. Infectious illnesses continue to be a major source of morbidity and death in both developing and developed countries. [3]. Antibiotic discovery and development have resulted in a significant boost in the capacity to treat infectious diseases, and is regarded as one of the twentieth century's major achievements. Unfortunately, the growth of drug-resistant organisms has been accompanied by antibiotic abuse and overuse, inability to complete a course of treatment, microorganism genetic flexibility, and horizontal transfer of resistance genes between bacterial species. All of the aforementioned factors diminish the clinical

effectiveness of antibiotics. [3]. Phytochemicals are plant-derived bioactive molecules that are classified as secondary metabolites since the plant that produces them may not require them. They are naturally generated in all sections of the plant body, including the flower, bark, stem, seeds, root, leaves, fruits, and so on. [4]. Some common phytochemicals found in various Medicinal plants and spices are Alkaloids, Flavonoids, Tannins, Phlobatannins, Saponins, Terpenoids, Quinones, Anthraquinones, Steroids, Phytosterols, Anthocyanin, Leucoanthocyanins Phenols, Glycosides, Coumarins, Carbohydrates, Proteins and Amino acids, Carboxylic Acid, Diterpenes, Lignins, Carotenoids, Cholesterols, Emodins, Gums and Mucilages, Resins, Volatile oils, etc. Spices' natural antioxidants assist in the decrease of oxidative stress. A high concentration of free radicals in cells and tissues causes oxidative stress, which can be induced by a number of unfavourable circumstances, including UV, gamma and X-ray radiation, polluted food, psycho-emotional stress, smoking, intense physical exertion, adverse environmental conditions, drug addiction, and alcoholism. Chronic oxidative stress has been related to a variety of ailments, including the acceleration of ageing, heart disease, and cancer. Two following products of 4-hydroxynonenal, malondialdehyde and lipid oxidation, can react with biological components such as DNA, amino acids, and proteins. Malondialdehyde has been related to a range of health concerns, including cancer and mutagenesis, and has been discovered to be produced both enzymatically and non-enzymatically. Antioxidants are abundant in spices and culinary plants. As a result, spices may be used to cure or prevent certain health issues. [5]. In tryptone soya broth (TSB) and cheese, clove oil proved efficient against *S. enteritidis* and *L. monocytogenes*. The high quantities of eugenol in clove fundamental oil provide it with excellent biological and antibacterial properties. Clove oil at 2% concentration in potato-dextrose agar (PDA) completely inhibited the growth of seven mycotoxigenic moulds (*A. ochraceus*, *A. parasiticus*, *A. flavus*, *P. patulum*, *P. roqueforti*, *Penicillium sp. M46* and *P. citrinum*) for up to 21 days, as well as other microbes such as *Salmonella sp.*, *Bacillus thermoacidurans*, *Lactobacillus sp.*, *Clostridium botulinum*, *Pseudomonas striafaciens*, *Corynebacterium michiganense*, *Cunninghamella sp.*, *Aspergillus sp.*, *Alternaria sp.*, *Fusarium sp.*, *Penicillium sp.*, and *Mucor sp.* [6].

Material and Methods

Clove was obtained from the Agricultural Produce Market Committee (APMC) market in Mumbai for being used in the study.

Bacterial strains: The microorganisms employed in the investigation are :

1. *Pseudomonas aeruginosa*
2. *Staphylococcus epidermidis*

Antibiotic: Amoxicillin

Solvents: Distilled water, 75% Ethanol and 75% Chloroform.

PREPARATION OF SPICE EXTRACT

To extract phytochemicals from clove, three solvents were used: distilled water, 75% ethanol, and 75% chloroform.

Distilled water-based extraction: To prepare 40 ml of aqueous extract (25 percent w/v), 10 g of powdered plant material was dissolved in sterile distilled water. The combination was kept undisturbed at room temperature for 24 hours in a sterile flask before being filtered using sterile Whatman no.1 filter paper. The extract was filtered and then evaporated in a water bath until just 25 ml remained in the container [7].

Ethanol-based extraction (75%): To prepare 40 ml of ethanolic extract (25 percent w/v), 10 g of powdered plant material was dissolved in ethanol. The extraction method was similar to that of aqueous extract [7].

Chloroform-based extraction (75%): Chloroform was used to extract 40 ml of chloroform extract (25 percent w/v) from 10 g of powdered plant material. The extraction procedure was similar to aqueous extract. The extracts' phytochemical and antibacterial properties were evaluated [7].

Phytochemicals Tests

1. Detection of Alkaloids

Test :- Bouchardat's test

Procedure :-

- Take 500µl of plant extract in a test tube using a micro pipette.
- Add 500µl of ethanol (@60 °C)
- Add a few drops of Bouchardat's reagent (dilute iodine solution).

Observation (Indicating positive test) :- A reddish brown colour

2. Detection of Flavonoids

Test :- Alkaline reagent test

Procedure :-

- Take 1ml of extract in a test tube.
- Add 2ml of 2% NaOH solution.
- Then add a few drops dil. HCl to the test tube.

Observation (Indicating positive test) :- When diluted acid is added to a bright yellow colour, it turns colourless.

3. Detection of Steroid

Test :- Salkowski's test

Procedure :-

- Take 500µl of spice extract in a test tube
- Add 500µl chloroform.
- Add 500µl conc. H₂SO₄.

Observation (Indicating positive test) :- The layer of chloroform shows the greenish yellow fluorescence

4. Detection of Tannins

Test :- Braymer's test

Procedure :-

- Take 500µl of spice extract in a test tube.
- Add few drops 5% Ferric chloride solution.

Observation (Indicating positive test) :- Blue-green colour

5. Detection of Saponins

Test :- NaHCO₃ test

Procedure :-

- Take the spice extract in a test tube.
- Add few ml sodium bicarbonate solution.
- Then add distilled water in the test tube and shake vigorously.

Observation (Indicating positive test) :- Stable honeycomb like froth

6. Detection of Phlobatannins

Test :- HCl test

Procedure :-

- Take 500µl of spice extract in a test tube.
- Add 500µl of 1% HCl (boiled).

Observation (Indicating positive test) :- A red precipitate

7. Detection of Phenolic compounds

Test :- Ferric chloride test

Procedure :-

- Take spice extract in a test tube.
- Add few drops 5% ferric chloride solution in a test tube.

Observation (Indicating positive test) :- Dark green/bluish black colour

8. Detection of Cardiac Glycosides

Test :- Keller-Killani test

Procedure :-

- Take 1ml of spice extract in a test tube.
- Add 1.5ml glacial acetic acid.
- Add 1 drop of 5% ferric chloride after that.
- Along the side of the test tube, add conc. H_2SO_4 .

Observation (Indicating positive test) :- A solution of blue colour (in acetic acid layer)

9. Detection of Carbohydrates

Test :- Fehling's test

Procedure :-

- Take 1ml of spice extract in a test tube.
- Add 1ml each of Fehling's solution A & B.
- Boil in water bath.

(**Solution A:** To generate a final volume of 100ml, mix 34.66gm copper sulphate in distilled water (100 ml). **Solution B:** To prepare 100ml, mix 173g potassium sodium tartrate with 50g sodium hydroxide in distilled water (100 ml)).

Observation (Indicating positive test) :- A red precipitate

10. Detection of Proteins

Test :- Biuret test

Procedure :-

- Take 2ml of spice extract in a test tube.
- Add 1 drop of 2% Copper sulphate solution.
- Then add 1ml of 95% ethanol.
- Add KOH pellets in the test tube.

Observation (Indicating positive test) :- A pink coloured sol. (in ethanolic layer)

11. Detection of Terpenoids

Procedure :-

- Take 500 μ l of spice extract.
- Add 200 μ l chloroform in a test tube.
- Then add 300 μ l conc. H_2SO_4 .

Observation (Indicating positive test) :- At the intersection of two solutions, a reddish brown film forms.

12. Detection of Anthocyanins

Test :- HCl test

Procedure :-

- Take 2ml of spice extract in a test tube.
- Add 2ml of 2N HCl.
- Then add few ml of ammonia.

Observation (Indicating positive test) :- After adding ammonia, the pink-red solution becomes blue-violet.

13. Detection of Anthraquinones**Test :-** Borntrager's test**Procedure :-**

- Take 2ml of 10% ammonia solution in a test tube.
- Add few ml of spice extract and shake vigorously for 30 sec.

Observation (Indicating positive test) :- A pink, violet, or red coloured solution**14. Detection of Coumarins****Test :-** NaOH paper test**Procedure :-**

- Take 0.5 gm moistened extract in a test tube.
- Place 1N NaOH-treated filter paper over the mouth of the test tube.
- Then heat the test tube for few min. in water bath.

Observation (Indicating positive test) :- Under UV light, paper fluoresces yellow.**15. Detection of Resins****Test :-** Turbidity test**Procedure :-**

- Take 0.5 ml of spice extract in a test tube.
- Add 0.5 ml Acetone and Distilled water to it.

Observation (Indicating positive test) :- Turbidity in the solution indicated the presence of resin.**Phytochemicals Quantitative Analysis.****Carbohydrates****Theroy :-** The DNS method is used to calculate reducing sugar. Many reagents can be reduced by reducing sugars, including 3,5-dinitro salicylic acid, which in alkaline solution is reduced to 3 amino 5 nitro salicylic acid.**Reagents required :-**

- Sodium potassium tartrate - 45g sodium potassium tartrate dissolved in 75ml distilled water
- 3,5-dinitro salicylic acid - dissolve 1.5g of DNS reagent in 30ml of 2M NaOH.
- 2M NaOH - 80g of NaOH dissolved in a litre of water.
- DNS reagent – Prepare fresh by mixing the reagent 1 and 2, make up the volume to 150ml of water.
- Stock standard solution- 1mg/ml

- **Stock standard sugar solution:** 250mg of glucose in water and make up to the volume to 100ml- **Working standard solution:** Take 10ml from this stock solution and make up the volume to 100ml**Table 1: Quantitative Carbohydrates Test**

Tube	Distilled water (ml)	Sample (ml)	DNS reagent (ml)	Incubation in boiling water for 5 minutes and allow to cool Take OD at 540 nm
Blank	3	0	2	
T1	2.9	0.1	2	
T2	2.5	0.5	2	
T3	2	1	2	
T4	1.5	1.5	2	
T5	1	2	2	
Clove (Aqueous Extract)	2.5	0.5	2	
Clove (Ethanol Extract)	2.5	0.5	2	
Clove (Chloroform Extract)	2.5	0.5	2	

Phenolic Compounds :

Principle :- Phenolics have a diverse set of biochemical characteristics, including antioxidant, antimutagenic, and anticarcinogenic qualities, as well as the ability to alter gene expression. The majority of antioxidant activity in plant products is attributed to the biggest category of phytochemicals, phenolics. The Folin Ciocalteu reagent, also known as the gallic equivalency procedure, is a phosphomolybdate and phosphor tungstate mixture used in colorimetric phenolic in vitro experiments.

Reagents required

Gallic acid solution (1mg/ml) – 100 mg of gallic acid was dissolved in 100ml of distilled water in volumetric flask.

FC reagent :- 0.2N, 1ml in 10 ml

Sodium carbonate: - 15g of sodium carbonate in 200ml of distilled water.

Table 2: Quantitative Phenolic Compounds Test

Tube	Distilled water (µl.)	Gallic acid/Sample (µl.)	FC reagent (ml.)	Incubation at room temperature for 10 minutes.	7.5% Na ₂ CO ₃ (ml)	Incubation in dark for 1 hour.	Take OD at 765 nm
Blank	500	0	2.5		2		
T1	499	1	2.5		2		
T2	490	10	2.5		2		
T3	480	20	2.5		2		
T4	470	30	2.5		2		
T5	460	40	2.5		2		
Clove (Aqueous Extract)	450	50	2.5		2		
Clove (Ethanol Extract)	450	50	2.5		2		
Clove (Chloroform Extract)	450	50	2.5		2		

Agar well diffusion method.

The antibacterial characteristics of the extracts were tested using the well diffusion technique. The bacterial suspension was evenly distributed on Muller Hinton Agar (MHA) medium. A sterile 8 mm cork borer was used to form a well in the nutritional agar. [7]. Clove extracts at 35µl, 45µl, and 55µl concentrations were put into each well. One standard antibiotic, Amoxicillin, was placed into one well as a positive control and examined against these microorganisms. [8]. After incubating the infected plates at 37°C for 24 hours, the clear zone of growth inhibition surrounding the well was evaluated. [7].

Procedure

- NA medium is prepared, sterilized, and put into petri plates under aseptic conditions under a Laminar air flow.
- The petri plates were kept for a time to allow the medium to settle.
- The petri plates were placed aside for a short period of time.
- The test organisms were now swabbed on the petri plates using a sterile swab.
- Wells were now built in specific places of petri plates.
- A micropipette is used to put the antibiotic and extracts into the wells.
- Each well receives a volume of 35 to 55 microlitres.
- The petri plates were now incubated for 24 hours at 37°C.
- After growth, the zone of inhibition (ZOI) around the well was measured with a ruler. The MIC and MBC were calculated using the extracts or antibiotics that inhibited the most.

Antibacterial Test :-

Antibacterial tests were performed to assess whether a material was capable of killing pathogenic microbes. The well diffusion method was used to conduct this test on petri plates.

Well diffusion method.

In this approach, 5mm wells were made on the media, the plate was spread by bacteria, and a 35 µl, 45 µl, and 55 µl sample was placed or seeded into the wells. NA media is widely utilised for testing in this approach.

Media used :-

In the well diffusion technique, NA medium was employed. After putting the media into a sterile plate, the plates with the media were sterilised by UV light. Before pouring, the plates' surfaces were sanitised with ethanol and then subjected to UV light.

Table 3: Composition of antibacterial media.

Reagents	Concentration(g/l)
Peptone	5
Beef extract	3
Sodium chloride	5
Agar	20
pH	6.8

Procedure :-

1. A 250 mL beaker was filled with 100 mL of distilled water.
2. The reagents to be used in NA medium were weighed and placed in a beaker with 100ml distilled water using a weighing machine.
3. The media was autoclaved, and the plates were cleaned and sterilised under the UV light till then.
4. The autoclaved medium was placed in the laminar air flow and poured into the plates.
5. After pouring the medium, it was sterilised for 5 minutes under UV light before being allowed to set for 20 minutes.
6. The bacteria were distributed throughout the surface of the medium in the plate using a cotton swap. Wells were made and filled each one with 35 µl, 45 µl and 55µl of samples.
7. Amoxillin, an antibiotic, was injected into one of the wells.
8. To guarantee optimal activity and growth, the plate was left undisturbed in the incubator for 24 hours.
9. It was observed on the plates how the samples reacted to the bacteria.

RESULTS**Phytochemicals Screening Test Results**

Sr. No	Phytochemicals	Aqueous	Ethanol	Chloroform
1	Alkaloids	+	+	-
2	Flavonoids	-	+	+
3	Steroid	+	+	+
4	Tannins	+	+	-
5	Saponins	+	-	-
6	Phlobatannins	+	-	-
7	Phenolic compounds	+	+	-
8	Cardiac Glycosides	+	+	-
9	Carbohydrates	+	+	-
10	Proteins	-	-	-
11	Terpenoids	-	+	+
12	Anthocyanins	-	-	-
13	Anthraquinones	+	-	-
14	Coumarins	-	+	-
15	Resins	+	-	-

This study included a phytochemical screening of clove. The results suggest that several of the phytochemicals studied were found in spice extracts. Aqueous extract of clove contained alkaloids, steroids, tannins, saponins, phlobatannins, phenolic compounds, cardiac glycosides, carbohydrates, terpenoids, anthraquinones, and resins; ethanol extract contained alkaloids, flavonoids, steroids, tannins, phenolic compounds, cardiac glycosides, carbohydrates, terpenoids, and coumarins; and chloroform extract contained flavonoids and steroids.

QUANTITATIVE ANALYSIS RESULTS**CARBOHYDRATES**

Tube	Concentration (mg/ml)	Optical Density at 540nm
1	88.33	0.304
2	166.66	0.746
3	250	1.111
4	333.33	1.475
5	416.66	1.642

Clove	Aqueous	Ethanol	Chloroform
Absorbance of Unknown Sample	1.445	1.46	0.803
Concentration of Unknown Sample (mg/ml)	347.80	351.46	191.22

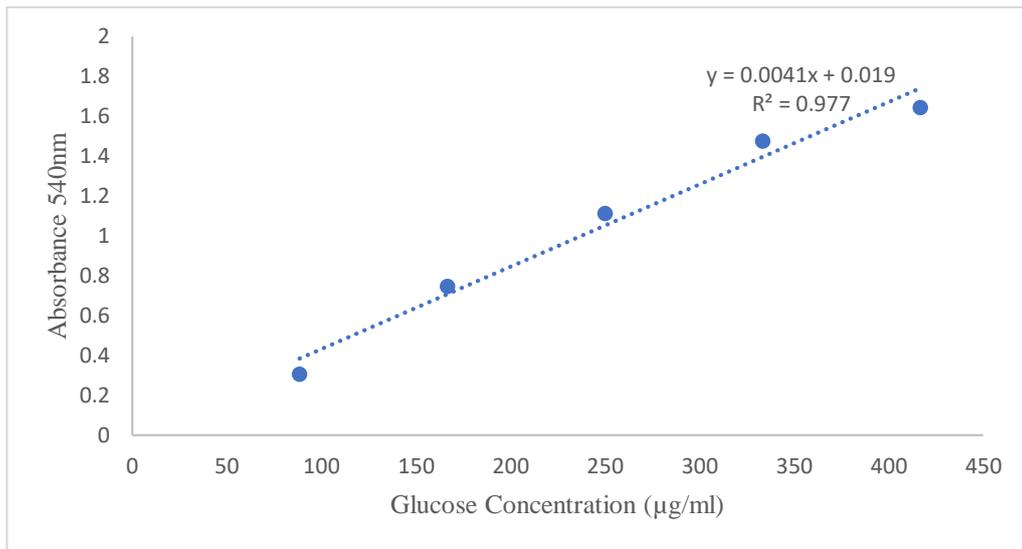


Fig 1. Carbohydrate Standard Curve

PHENOLS

Tube	Concentration(mg/ml)	Optical Density at 765nm
1	1	0.039
2	10	0.197
3	20	0.345
4	30	0.598
5	40	0.671

Clove	Aqueous	Ethanol	Chloroform
Absorbance of Unknown Sample	1.48	1.50	1.49
Concentration of Unknown Sample (mg/ml)	84.28	85.37	84.68

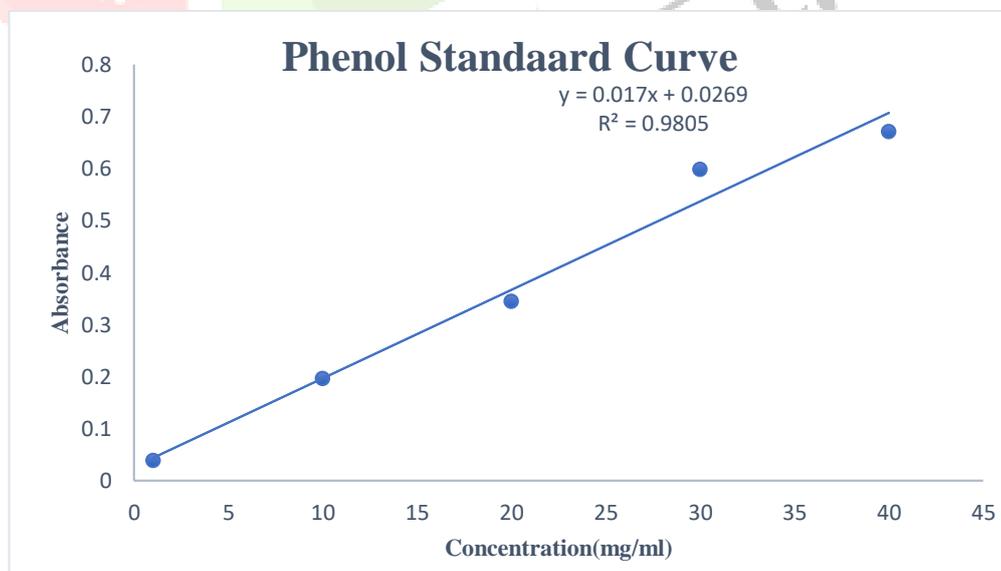


Fig 2. Phenol Standard Curve

Antibacterial Test Results

Sr. No	Micro-organism	Antibiotics	Zone of Inhibition (mm)
1	<i>Pseudomonas aeruginosa</i>	Amoxicillin	20
2	<i>Staphylococcus epidermidis</i>	Amoxicillin	21

1. Clove aqueous extract's antimicrobial efficacy against microorganisms.

Sr. No	Spice	Micro-organisms with zone of inhibition (mm)									
		<i>Pseudomonas aeruginosa</i>					<i>Staphylococcus epidermidis</i>				
		NC	PC	35µL	45µL	55µL	NC	PC	35µL	45µL	55µL
1	Clove (RI)	0	21	20	21	22	0	20	0	12	14
2	Clove (RII)	0	21	21	22	21	0	21	0	13	14
3	Clove (RIII)	0	21	20	20	22	0	20	0	11	13

2. Clove ethanol extract's antimicrobial efficacy against microorganisms.

Sr. No	Spice	Micro-organisms with zone of inhibition (mm)									
		<i>Pseudomonas aeruginosa</i>					<i>Staphylococcus epidermidis</i>				
		NC	PC	35µL	45µL	55µL	NC	PC	35µL	45µL	55µL
1	Clove (RI)	0	20	12	13	15	0	20	14	16	18
2	Clove (RII)	0	21	11	12	15	0	20	14	15	18
3	Clove (RIII)	0	21	13	13	15	0	21	15	17	18

3. Clove chloroform extract's antimicrobial efficacy against microorganisms.

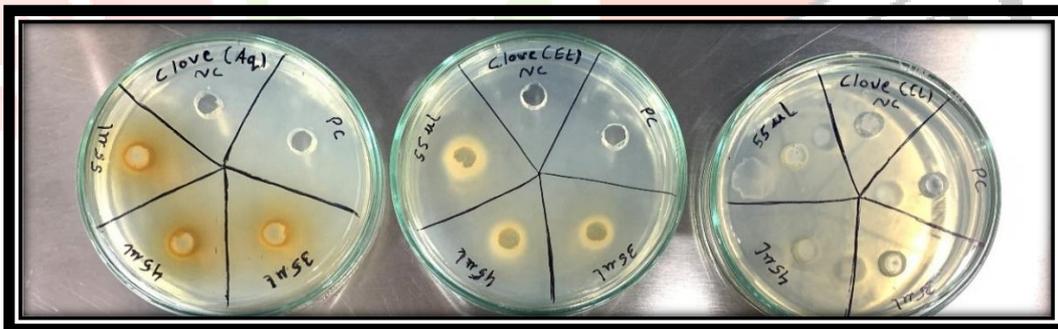
Sr. No	Spice	Micro-organisms with zone of inhibition (mm)									
		<i>Pseudomonas aeruginosa</i>					<i>Staphylococcus epidermidis</i>				
		NC	PC	35µL	45µL	55µL	NC	PC	35µL	45µL	55µL
1	Clove (RI)	0	20	0	0	0	0	20	0	0	0
2	Clove (RII)	0	21	0	0	0	0	20	0	0	0
3	Clove (RIII)	0	20	0	0	0	0	20	0	0	0

R = Replication

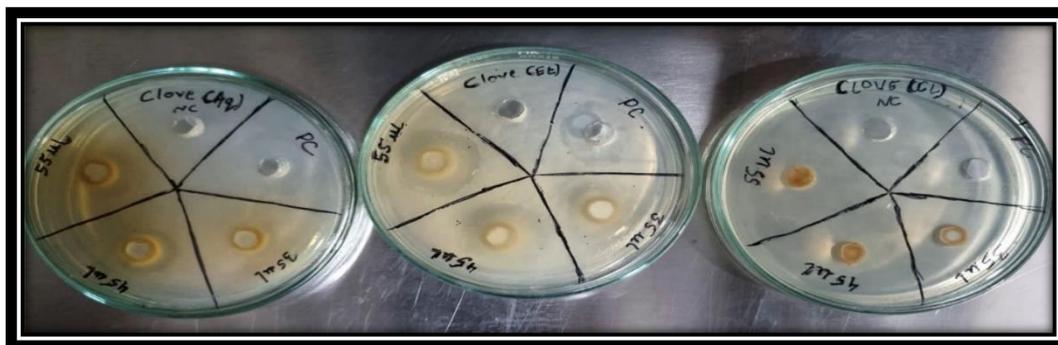
NC = Negative Control

PC = Positive Control (Drug - Amoxicillin)

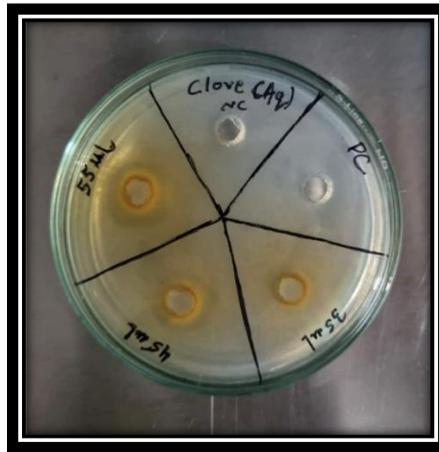
Results Images



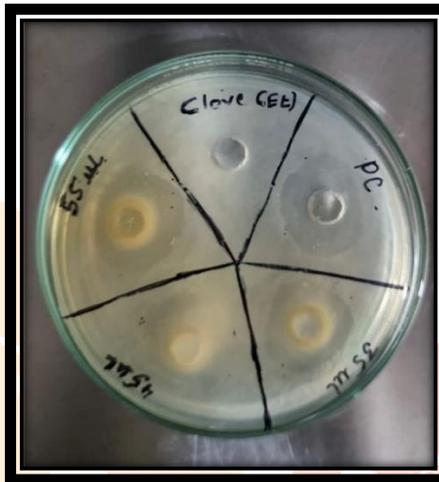
Photoplate 1. Agar Well Diffusion Method



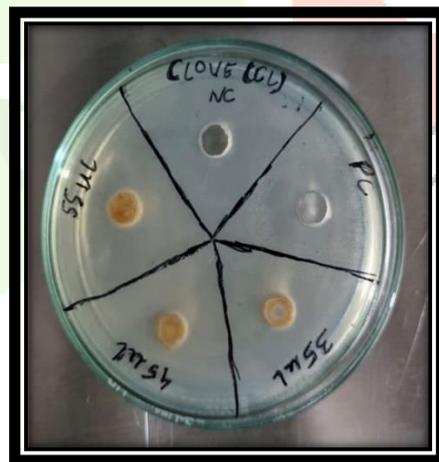
Photoplate 2. Zone of Inhibition after 24 hours



Photoplate 3. Clove aqueous extract's antimicrobial efficacy against microorganisms.



Photoplate 4. Clove ethanol extract's antimicrobial efficacy against microorganisms.



Photoplate 5. Clove chloroform extract's antimicrobial efficacy against microorganisms.

The extracts' antibacterial activity against microorganisms was determined to be extremely strong. The evidence clearly demonstrates the presence of compounds used to treat a range of bacterial diseases, proving that they have been utilised in traditional medicine from ancient times. Furthermore, the broad spectrum action of water, ethanol, and chloroform hints to the development of new antimicrobial formulations in the near future.

DISCUSSION

Plant-based medications have significantly improved human health and serve as a source of inspiration for innovative therapeutic compositions. Based on the findings, it is obvious that clove has significant potential for use in pharmacology and as a source of vital medications. Because it contains a variety of substances that are essential for good health, it may also be used to improve society's health.

Each spice has its own set of bio-functions, as well as additive and synergistic characteristics that help to protect the human body. With a holistic approach, spices have long been a component of the diet. Despite the fact that many spices, particularly those made from seeds, have high fat, protein, and carbohydrate content by weight, they add little calories to meals since they

have powerful scents and are used in small amounts. When used in larger quantities, spices, on the other hand, may give a considerable quantity of minerals and other micronutrients to the diet, including iron, magnesium, calcium, and many more. Antimicrobials originating from plants are significantly more curative than manufactured antimicrobial drugs since they have less side effects. The new study contributes to our awareness of the presence of various phytochemical active components in Clove that have strong antibacterial efficacy throughout a broad range. Further fractionation and purification will disclose the probable ingredient, which is critical given the current antibiotics' oncoming resistance.

Medicinal plants are still a valuable source in the treatment of human ailments. Traditional medicine is gaining popularity again these days, as well as is a rising need for additional plant-based remedies. The current popular belief that "green medicine" is safer and more reliable than pricey synthetic pharmaceuticals, many of which have harmful side effects, has rekindled interest in plant-derived medicinal therapies.

Since ancient times, spices have been employed in meals as flavouring agents, food preservatives, and traditional cures. In general, the phytochemicals found in spices used for medicinal purposes determine their value. Spices, herbs, plant extracts, and their phytochemical components have been shown to have antibacterial, anti-diarrheal, anti-inflammatory, antioxidant, and insecticidal activities. Alkaloids are used in allopathic systems and have important biological features including cytotoxicity. In pharmacy, steroid and sterols are essential because they include molecules that are comparable to sex hormones and may be used to manufacture medications. Saponins have both anti-hypercholesterolemic and antimicrobial properties. Tannins inhibited the growth of a wide range of fungi, yeasts, bacteria, and viruses. Phenols and tannins are antioxidants. The potential of plants as a source of medications has yet to be completely realised. Multiple drug resistance has arisen as a serious concern in pharmacotherapy, with an increasing number of illnesses, notably bacterial infections, exhibiting varying degrees of drug resistance. Herbal medicines and phytochemical screening of diverse plant species for therapeutic leads are currently gaining popularity.

Clove's antibacterial properties have been examined. Water, ethanol, and chloroform were used to create clove extracts. The extracts' antimicrobial properties are seen above. The extracts were phytochemically analysed to identify secondary metabolites produced from both spices that alter antibiotic activity. Plant phytochemicals, or secondary metabolites, are an essential element of our diet. Above is a picture demonstrating the zone of inhibition of extracts against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Saponins have anti-cancerous and cholesterol-lowering properties as a result of the antioxidant capacities supplied by flavonoids and tannins. Alkaloids are used as anti-malarial and analgesic agents.

Using a number of biochemical tests, the isolates were identified, and they were shown to be sensitive to clove extracts. Bactericidal activity was measured using Clove aqueous extracts, ethanol extracts, and chloroform extracts at a minimum inhibitory concentration of 35 µl. The microbiological activity of the spices, Clove was assessed by evaluating the zone of inhibition and determining the anti-microbial activity of the Clove extract.

Conclusion

Clove extracts were discovered to be high in alkaloids, terpenoids, saponins, flavonoids, steroids, and tannins. Because clove include numerous components that are needed for good health, they were analysed for phytochemical elements and looked to have the potential to function as a source of beneficial medicines as well as to improve the health status of consumers. The current study's findings are encouraging because both *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* infections were successfully treated with clove extracts, which also shown anti-bacterial characteristics. Antimicrobial activity, on the other hand, differs significantly depending on the spice, the microbe, and the test medium. This study paves the way for the creation of new antimicrobials as a replacement for antibiotics.

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References

- [1] A. K. Sachan, S. Kumar, K. Kumari, and D. Singh, "Medicinal uses of spices used in our traditional culture : World wide Medicinal uses of spices used in our traditional culture : World wide," *J. Med. Plants Stud.*, vol. 6, no. 3, pp. 116–122, 2018.
- [2] Raja S and Ravindranadh K, "Preliminary phytochemical screening of different solvent extracts of whole plant of *Acrostichum aureum*," *World J. Pharm. Res.*, vol. 2, no. 12, pp. 209–212, 2014, [Online]. Available: <http://www.wjpsonline.org/>.
- [3] T. O. Odugbemi, "Chemical analysis and antimicrobial activity of the essential oil of *Syzygium aromaticum* (clove)," *African J. Microbiol. Res.*, vol. 2, no. 7, pp. 162–166, 2008, doi: 10.5897/AJMR.9000161.
- [4] P. Venkatachalam, "Phytochemical Screening and Antibacterial Activities of Cinnamon against Vancomycin Resistant *Enterococcus*," *Int. J. Sci. Res. ISSN (Online Index Copernicus Value Impact Factor)*, vol. 14, no. 9, pp. 2319–7064, 2013, [Online]. Available: www.ijsr.net.
- [5] A. Yashin, Y. Yashin, X. Xia, and B. Nemzer, "Antioxidant activity of spices and their impact on human health: A review," *Antioxidants*, vol. 6, no. 3, pp. 1–18, 2017, doi: 10.3390/antiox6030070.
- [6] U. Kumar, B. Kumar, A. Bhandari, and Y. Kumar, "PHYTOCHEMICAL INVESTIGATION AND COMPARISON OF ANTIMICROBIAL SCREENING OF CLOVE AND CARDAMOM," vol. 1, no. 12, pp. 138–147, 2010, [Online]. Available: www.ijpsr.com.
- [7] U. Suman, Y. Divya, C. Ram, and A. Naveen, "Evaluation of antibacterial and phytochemical properties of different spice extracts," *African J. Microbiol. Res.*, vol. 12, no. 2, pp. 27–37, 2018, doi: 10.5897/ajmr2017.8731.
- [8] D. Philip, P. K. Kaleena, K. Valivittan, and C. P. Girish Kumar, "Phytochemical Screening and Antimicrobial Activity of," *Middle-East J. Sci. Res.*, vol. 10, no. 4, pp. 512–518, 2011.