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Asian Journal of Plant Science and Research, 2011, 1 (2):69-80

ISSN : 2249 – 7412

Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens

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ABSTRACT

The antibacterial properties of "Syzygium aromaticum" commonly known as "Clove" tested against food borne pathogens (S. aureus, P. aeruginosa, E. coli). Agar diffusion susceptibility test revealed inhibition zone of clove sample. Compare to ethanolic extract, methanolic extract was showing best result against gram positive culture Staphylococcus aureus (MTCC 2940) and two gram negative cultures Pseudomonas aeruginosa (MTCC 2453) and E. coli (MTCC 739). The MIC value was determined by using broth dilution methods. Methanolic extract of clove was subjected to get the MIC against test organisms and it was found to be 2.31 mg/ml for E. coli, 0.385 mg/ml for Stapylococcus aureus and 0.01 mg/ml for Pseudomonas aeruginosa. The addition of metal ions (Zn^{++}, Cu^{++}, Pb^{++}, Ca^{++}, Mg^{++}, Fe^{++}) along with methanolic extract of clove samples gave positive results against test organisms. The metal ions increased antibacterial properties of clove samples but after optimization at various concentrations it could not increase the antibacterial activity of samples compare to 10%, 20%, 30%.

Keywords: Antibacterial properties, metal ions, ethanolic and methanolic plant extract, zone of inhibition.

INTRODUCTION

The most common bacteria causing food-borne illness are *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., *Salmonella typhimurium*, *Listeria monocytogenes*, *Clostridium botulinum*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and others [1][2][3].Now microorganisms have become resistance to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So, it has become necessary to find out new antimicrobial agents. Prevention of pathogenic and spoilage microorganisms in food is usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic

attributes as well as residual toxicity and with growing concern of microbial resistance towards conventional preservatives, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobial for food preservations receives increasing attention [4]. Bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents**.** Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods [5][6].The active ingredients of plants against microorganisms are mostly some of the secondary metabolites (*i.e.* alkaloids, glycosides etc.) that are present in abundance in herbs and spices commonly used in Indian food preparations. Traditional medicines have been used for many centuries by a substantial proportion of the population of India [7]. *Syzygium* species (*Fam. Myrtaceae*) have been reported to possess antibacterial and anti-inflammatory activity [8]. It was reported that the buds of *Syzygium aromaticum* (L.) Merr. & Perry (clove) were used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with carminative and stimulant activity[9]. *Syzygium aromaticum*, commonly called cloves, and locally called ‘kenepeli’ and ‘kanumpari’ by the Igala and Hausa ethnic groups of central and Northern Nigeria, respectively, is a common spice used in local beverages in the middle belt and northern part of the country [10].Clove’s Botanical name is *Caryophyllus aromaticus* which is derived from the Latin "clavus", which means nail due to its resemblance with the shape. The clove tree is an evergreen tropical plant, which flowers twice every year. Cloves are the unopened buds and harvested when the outer green leaves (calyx) have changed from green to a yellow pink.

The objective of this study was to evaluate the invitro antimicrobial activity of crude ethanolic and methanolic extracts of clove and increase the antibacterial activity of clove by introducing metal ions with crude sample in varying ratios against selected three food-associated bacteria *E. coli, Pesudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive).

MATERIALS AND METHODS

Collection of plant:

The clove was purchased from local market of Lucknow, Uttar Pradesh in March 2011.

Preparation of ethanolic and methanolic plant extract:

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant .Clove was dried in an incubator at 37°C for 3-4 days and grinded into fine powder. Now plant material was dissolved in 70% ethanol and 80% methanol (2:15 w/v). Mixtures were kept in the dark for 3 days at room temperature in sterilized beakers wrapped with aluminum foil to avoid evaporation and exposure to sunlight was avoided. After 3 days, mixtures were filtered through Whatman no.1 filter paper and kept it in incubator at 37°C till ethanol or methanol had completely evaporated from mixtures. Now ethanolic and methanolic clove samples were dissolved in tris HCl, pH 8.0 (double amount of remaining mixtures respectively).

Tested microorganisms:

Food associated bacteria were obtained from IMTECH, Chandigarh. Subcultures were maintained by MRD LifeSciences, Lucknow. *Staphylococcus aureus* (MTCC 2940) a gram positive and *Pseudomons aeruginosa* (MTCC 2453), *Escheriachia coli* (MTCC 739), both gram negative were used.

Screening of bioactive compounds:

The antibacterial activity of ethanolic and methanolic extracts of clove against three food-associated gram positive and gram negative bacteria was evaluated by using agar well diffusion method [11]. Nutrient agar plates were prepared for ethanolic and methanolic extract of clove. 50µl inoculum of each selected bacterium was unifomly spreaded on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. The equal volume (50µl) of antibiotic (tetracycline), distilled water and plant extract were poured into the wells. The plates were incubated at 37°C for 24 hrs.

Determination of minimum inhibitory concentration (MIC) of ethanolic and methanolic extracts of clove:

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation [12].MIC of methanolic and ethanolic extracts of cloves were determined by broth dilution method. A two-fold serial dilution of the methanolic and ethanolic extracts of clove and garlic were prepared.

Effect of metal ions on antibacterial properties of methanolic extract:

Antibacterial activity of plant extract against three food associated bacteria were examined in the presence of metal ions. Six metal ions led, iron, copper, zinc, calcium and magnesium (1%) were used. Nutrient agar plates were prepared for each bacterium and plant extract. 50µl inoculum of each selected bacterium was uniformly spreaded on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. Wells were filled by methanolic extract with selected metal ions (50µl) with different concentration *i.e.* first well 10% (45µl methanolic extract + 5µl metal ion), second well 20% (40µl methanolic extract + 10µl metal ion) and third well for 30% (35µl methanolic extract + 15µl metal ion). The plates were incubated at 37°C for 24 hrs.

RESULTS AND DISCUSSION

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne and spoilage microorganisms [13]. Cloves were used in Ayurveda, Chinese medicine and Western herbalism and also as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis The ethanolic and methanolic extracts of clove showed good inhibitory activity in comparison of selected antibiotic (tetracycline) against all three food associated bacteria.

Table1: Antibacterial activity of ethanolic extract of clove against food associated gram positive and gram negative bacteria.

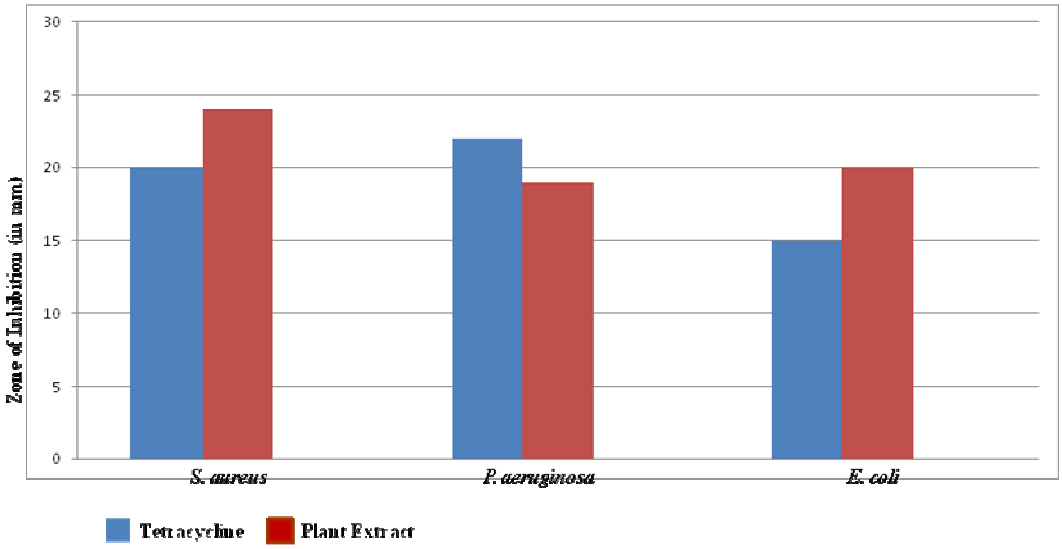
Pathogens	Zone of inhibition (mm.)		
	Tetracycline	Plant extract	Distilled water
Staphylococcus aureus	20	16	0
Pseudomonas aeruginosa	18	20	0
E. coli	0	18	0

Maximum antimicrobial activity of ethanolic extract of *Syzygium aromaticum* was obtained against *Pseudomonas aeruginosa* whereas least in *Staphylococcus aureus.* Distilled water showed negative result.

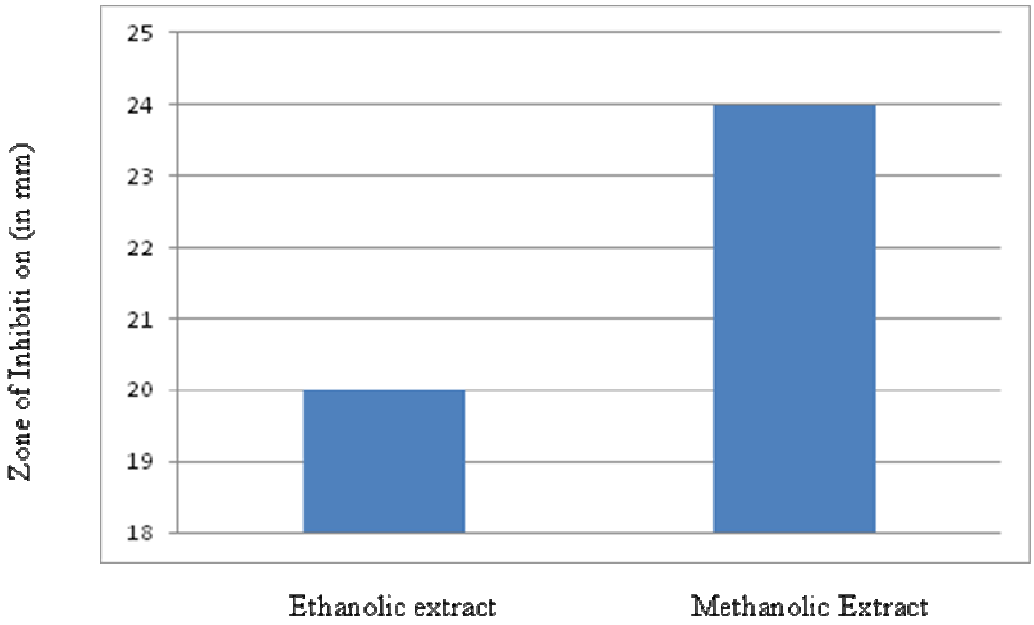
Table 2: Antibacterial activity of methanolic extract of clove against food associated gram positive and gram negative bacteria.

Pathogens	Zone of inhibition (mm.)		
	Tetracycline	Plant extract	Distilled water
Staphylococcus aureus	20	24	0
Pseudomonas aeruginosa	22	19	0
E.coli	15	20	0

Maximum antimicrobial activity of methanolic extract of *Syzygium aromaticum* was obtained against *Staphylococcus aureus* whereas least in *Pseudomonas aeruginosa.* Distilled water showed negative result.



Methanolic extract of clove



Comparative study of ethanolic and methanolic extract

Chart showed that methanolic extract of clove was having maximum antibacterial activity compare to ethanolic extract.

Table 3: MIC of methanolic extracts of clove against food associated gram positive and gram negative bacteria.

Test tube	Conc. of methanolic extracts (mg/ml)	O.D. against *E.coli* (600nm)	O.D. against *Pseudomonas aeruginosa* (600nm)	O.D. against *Staphylococcus aureus* (600nm)
Control	83.33	0.12	0.17	0.15
1	13.89	0.25	0.25	0.20
2	**2.31**	**0.12**	0.32	0.28
3	**0.385**	0.55	0.45	**0.20**
4	0.064	0.56	0.47	0.40
5	**0.010**	0.65	**0.11**	0.50

Table showed that methanolic extract of clove were subjected to get the MIC against test organisms and it was found to be **2.31 mg/ml** for *E.coli*, **0.385 mg/ml** for *Staphylococcus aureus* and **0.01 mg/ml** for *Pseudomonas aeruginosa*.

Table 4: Metal ions activity with methanolic extract of clove against *Staphylococcus aureus*.

Metal ions	Zone of inhibition (mm)		
	10%	20%	30%
Calcium	20	20	18
Zinc	**26**	25	26
Copper	21	20	20
Magnesium	21	20	20
Lead	19	18	17
Iron	20	19	19

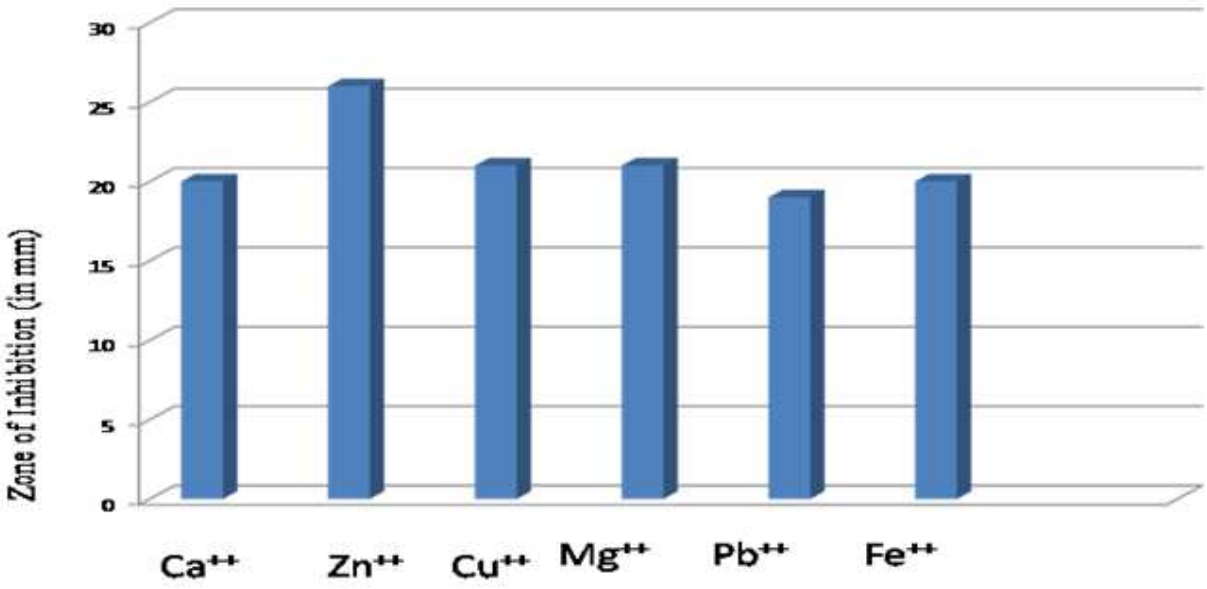


Chart showed that methanolic extract of clove were subjected to get the maximum zone of inhibition against *Staphylococcus aureus* in the presence of **zinc** whereas minimum in the presence of lead.

Table 5: Metal ions activity with methanolic extract of clove against *E. coli.*

Metal ions	Zone of inhibition (mm.)		
	10%	**20%**	**30%**
Calcium	17	17	16
Zinc	18	18.5	19
Copper	21	19	18
Magnesium	**24**	22	18
Lead	18	19	20
Iron	18	20	15

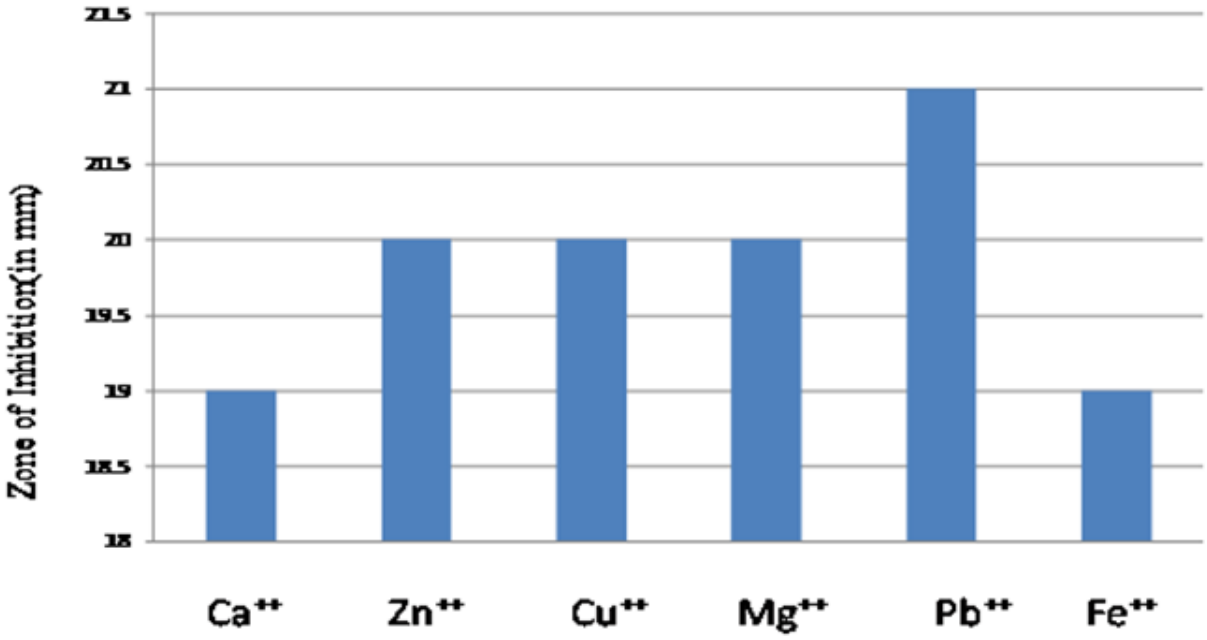


Chart showed that methanolic extract of clove were subjected to get the maximum zone of inhibition against *E. coli* in the presence of **magnesium** whereas minimum in the presence of calcium.

Table 6: Metal ions activity with methanolic extract of clove against *Pseudomonas aeruginosa*.

Metal ions	Zone of inhibition (mm.)		
	10%	**20%**	**30%**
Calcium	19	18	20
Zinc	20	20	23
Copper	20	20	19
Magnesium	20	20	20
Lead	**21**	21	19
Iron	19	19	23

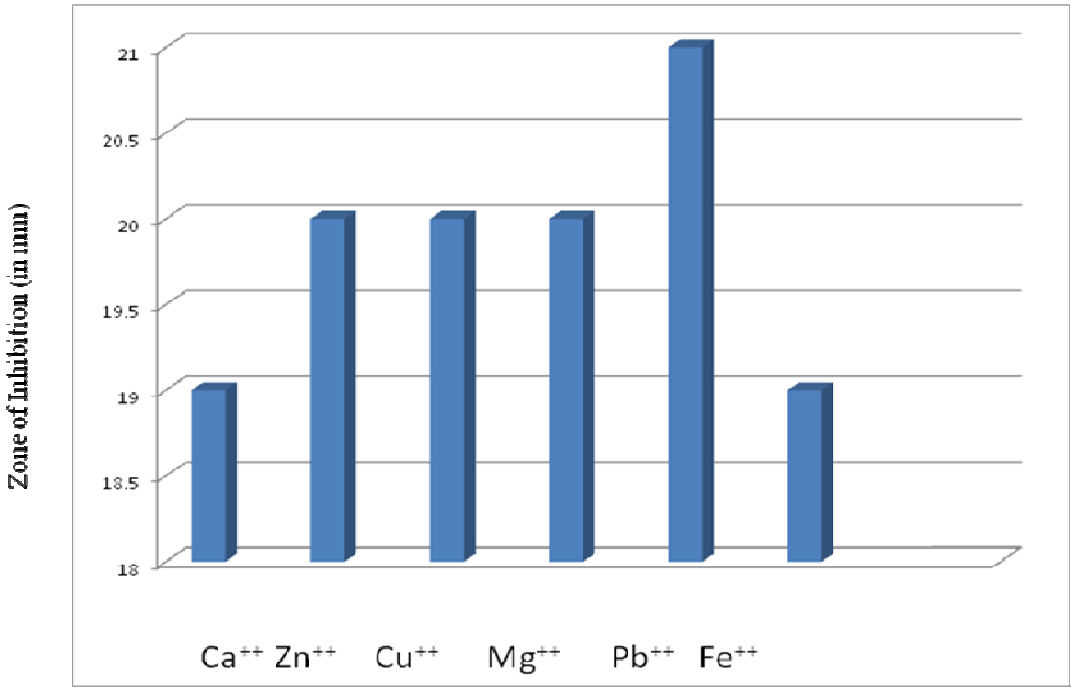
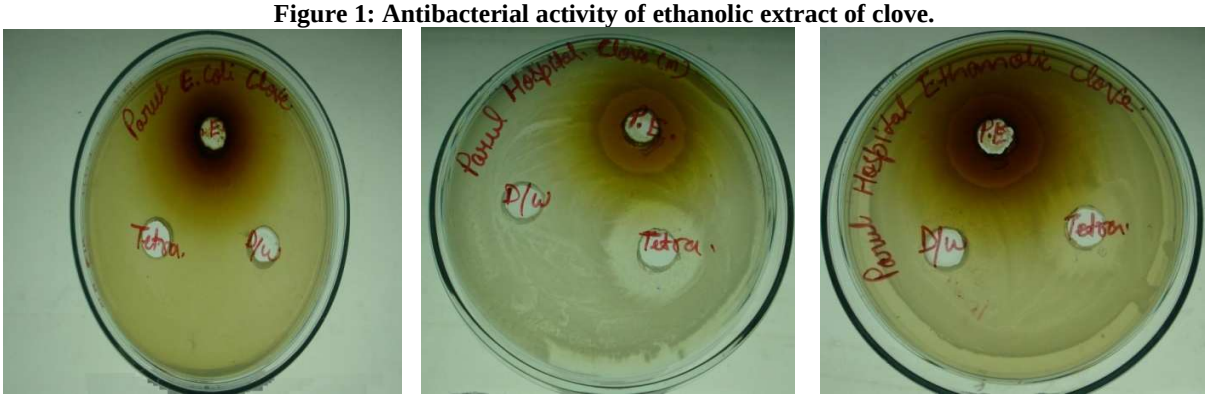


Chart showed that methanolic extract of clove were subjected to get the maximum zone of inhibition against *Pseudomonas aeruginosa* in the presence of **lead** whereas minimum in the presence of calcium.

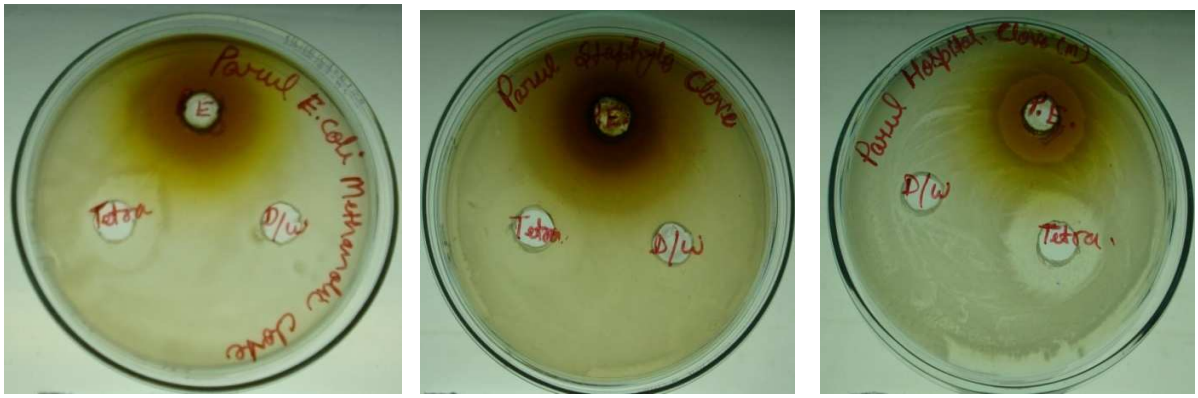
Figure 1: Antibacterial activity of ethanolic extract of clove.



Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Result indicates that ethanolic extract of clove sample was having maximum antibacterial activity against *Staphylococcus aureus* but it was less as compared to methanolic extract.

Figure 2: Antibacterial activity of methanolic extract of clove.

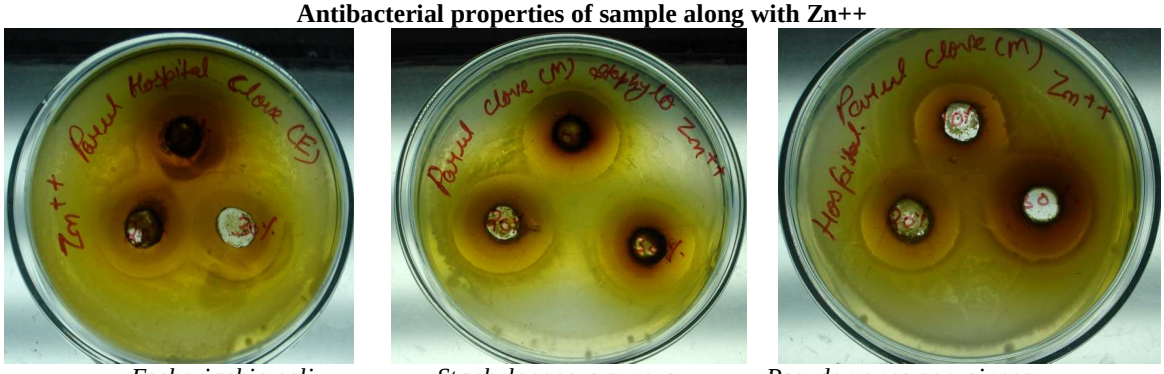


Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Result indicates that in ethanolic extract only diffusion occur while in methanolic extract clear zone of inhibition occur and *Staphylococcus aureus* is having maximum zone of inhibition as compared to *Pseudomonas aeruginosa* and *E. coli.*

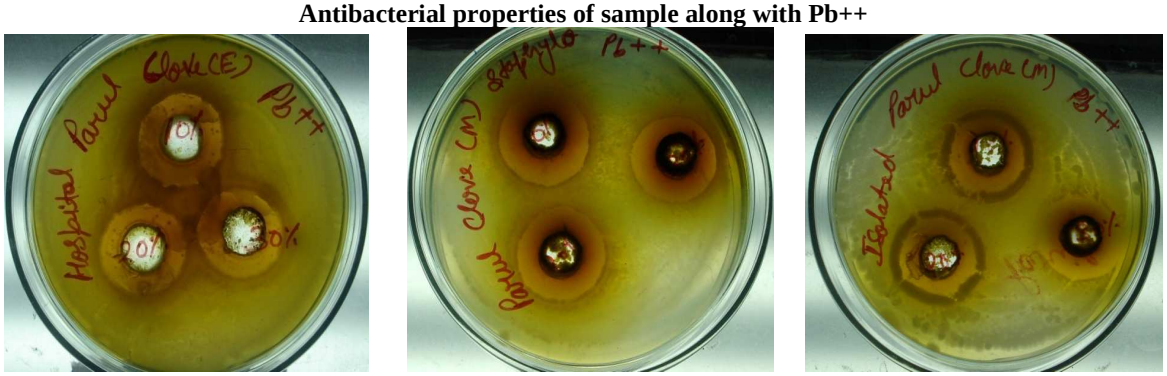
Figure 3: Effect of metal ion on antibacterial properties of clove (methanolic extract).

Antibacterial properties of sample along with Zn++



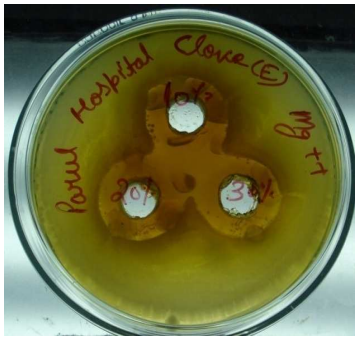
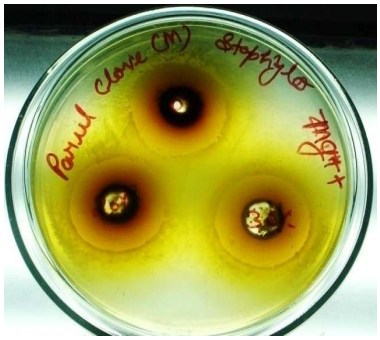
Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Antibacterial properties of sample along with Pb++



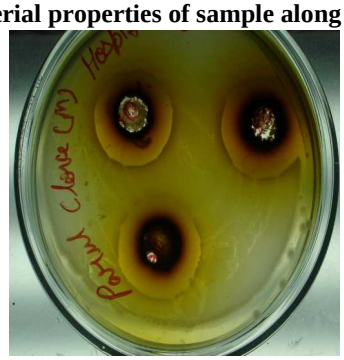
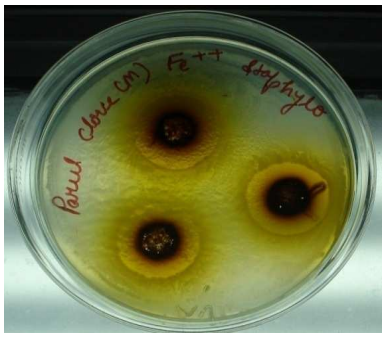
Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Antibacterial properties of sample along with Mg ++

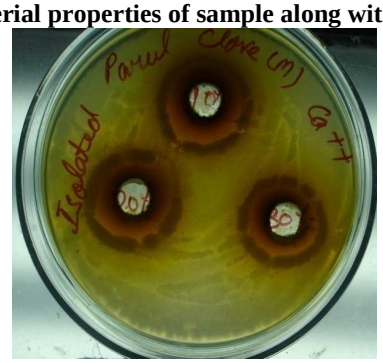
Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Antibacterial properties of sample along with Fe++

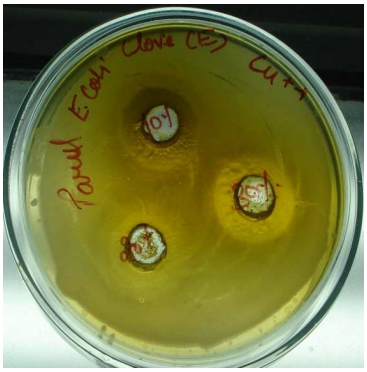
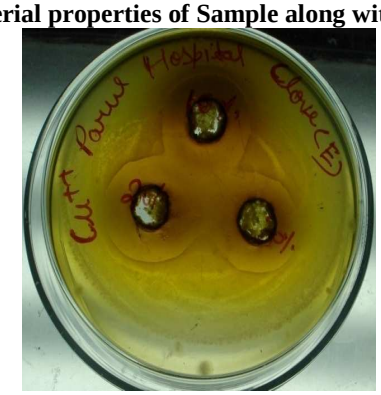
Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Antibacterial properties of sample along with Ca++


Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

Antibacterial properties of Sample along with Cu ++


Escheriachia coli *Staphylococcus aureus* *Pseudomonas aeruginosa*

CONCLUSION

Antimicrobial component of clove *i.e.* Eugenol, carvacrol and thymol are phenolic compounds found in cinnamon, cloves, sage and oregano. The presence of these compounds in cinnamon and cloves, when added to bakery items, function as mold inhibitors in addition to adding flavor and aroma to baked products**.** The currently used antibiotics (such as ampicillin, chloramphenicol, co-trimoxazole) are becoming insensitive to these infections which has resulted in the treatment of the infectious diseases quite difficult. Hence it is required to look for newer antimicrobial agent. It is clear from this study and earlier reports that clove appears to satisfy all of the criteria for antibacterial agents. This study attempts to increase the antibacterial activity of clove by introducing metal ions with crude sample in varying ratios. Most of the antimicrobial active compounds that have been identified were soluble in polar solvents such as methanol and ethanol instead of water [14].The result of antibacterial susceptibility assay shows promising evidence for the antibacterial effect of clove methanolic and ethanolic extract against three food associated (*Staphylococcus aureus*, *E.coli* and *Pseudomonas aeruginosa*) gram positive and gram negative bacteria respectively. Antimicrobial activity of methanolic extract of clove was better than the ethanolic extract of clove against all the test organisms which were showing better result compare to [15].Methanolic extract of clove showed maximum zone of inhibition 24mm against *S. aureus* while minimum was 19 mm against *P. aeuginos.* Ethanolic extract of clove showed maximum zone of inhibition 20 mm against *P. aeuginosa* while minimum was 18mm against *E. coli.* Thus, MIC assay are capable of verifying that the compound has antibacterial activity and that it gives reliable indication of the concentration of drug required to inhibit the growth of microorganisms. Methanolic extract of clove was subjected to get the MIC against test organisms and it was found to be 2.31 mg/ml for *E. coli,* 0.385 mg/ml for *S. aureus* and 0.010 mg/ml for *P.aeruginosa* which was very less as compare to [15]. The addition of metal ions and its positive results against test organisms showed that at various concentrations it can not increase the antibacterial activity of samples compare to 10%, 20%, 30% almost zone of inhibition were equal slightly changes were present**.** Methanolic extract of clove were subjected to get the maximum zone of inhibition against *S. aureus* in the presence of zinc, copper and magnesium whereas minimum in the presence of lead, iron and calcium.

Methanolic extract of clove demonstrated zone of inhibition of 20mm against *E.coli* but in the presence of metal ions *i.e.* copper (10%) and magnesium (10% & 20%), plant extracts demonstrated zone of inhibition of 21mm, 24mm and 22mm respectively.

Acknowledgement
I wish to express my immense gratitude to Mr. Manoj Verma, Director, MRD LifeSciences (P) Limited, Lucknow. I am very grateful and my heartiest thanks to Mr. R.P. Mishra (Research Scientist), Mr. Jahir Alam Khan (Research Scientist) & Ms. Chanda Sinha (Research Scientist), MRDLS, Lucknow, for there kind support throughout the research work. I am also thankful to the almighty without whose blessings nothing is possible.

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