International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Impact Factor (2018): 5.426

Antibacterial Activity of Hexane and Methanol Extract of Clove (*Syzygium aromaticum* L.)

Swathi Patrata*, Prof. A. J. Solomon Raju

Department of Environmental Sciences, Andhra University, Visakhapatnam-530003, India

Abstract: ***In the present study, to investigate antibacterial activity of hexane and methanol extract of clove (Syzygium aromaticum L.). The in vitro antibacterial activity was performed by agar well diffusion method. The clove extracts inhibit the growth of bacterial species such as two gram negative E.coli, Pseudomonas aeruginosa and gram positive Staphylococcus aureus, Bacillus cereus. Methanol extract of clove revealed highest zone of inhibition in E.coli, whereas hexane extract of clove showed highest activity against Bacillus cereus. The results obtained from this study suggest that the methanol and hexane extracts of Syzygium aromaticum L. revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulation.***

Keywords: clove, agar well diffusion, antibacterial activity

1. Introduction

Traditional medicines have been used for many centuries by a substantial proportion of the population of India (9). Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods (11) (12). The active ingredients of plants are secondary metabolites (*i.e.*alkaloids, glycosides, Etc.) that are present in abundance in herbs and spices commonly used in Indian food preparations. Commonly used spices are cloves, cinnamon, cardamom, nutmeg, Etc., in Indian cuisine.

Cloves (*Syzygium aromaticum* L.) are belonging to the family Myrtaceae, which are dried unopened floral buds of an evergreen tree (2). Clove is used as a flavouring agent and as a spice. It has known as aromatic, stimulant, and carminative, used for dyspepsia and gastric irritations. Antimicrobial and antioxidant properties of clove have known to clove buds and their essential oils (3). The antimicrobial activity of clove is attributable to eugenol, oleic acids, and lipids found in its essential oils (5). Clove oil is widely used as a perfume and food flavouring (4), as an analgesic, antispasmodic, and as a general antiseptic in medical dental practices (6). Its antimicrobial potential was established when its essential oil extracts killed many Gram positive and Gram negative organisms including some fungi. (7) (8)

Food borne pathogens (e.g. viruses, bacteria, parasites) are biological agents that can cause a food borne illness event. A food borne disease outbreak is defined as the occurrence of two or more cases of a similar illness resulting from the ingestion of a common food (10). Food borne pathogens are causing a great number of diseases with significant effects on human health and the economy. The most common food borne illness is caused by *Salmonella, Staphylococcus* spp., *Escherichia spp., Vibrio parahemolyticus, Clostridium perfringens, Clostridium botulinum, Bacillus sp., and Enteroviruses.*

The aim of present study antimicrobial activity of hexane and methanolic extract of clove against selected food borne pathogens such as *E.coli, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus,* were isolated from the edible fish tissue sample.

2. Materials and Methods

Collection of Sample:

The spice clove (*Syzygium aromaticum* L.) was collected from the local market of Visakhapatnam in 2019.

Preparation of Extracts by Soxhlet method:

The dried and powdered materials (150 gm) were extracted with 200ml of each solvent separately by using a Soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the solvent. The solvents were Hexane and Methanol used for the extraction. The extracts were distilled by the distillation unit and were transferred to amber glass bottles and kept at 4^0C. Extracts were dissolved in Dimethyl sulphoxide (DMSO) to prepare different concentrations (50 µl, 100 µl, 150 µl, 200 µl).

Screening of antimicrobial activity:

Tested organisms:

Test organisms bacteria were isolated from the fish tissue sample. Gram positive ***Staphylococcus aureus, Bacillus cereus*** and gram negative ***E.coli, Pseudomonas aeruginosa.***

Agar well –diffusion method.

24 hrs of fresh inoculums were spread on freshly made nutrient agar plates with sterile glass spreader. After that five wells of 8mm diameter was bored on culture plates with borer, four wells for aliquots of clove extract with different concentrations (50 µl, 100 µl, 150 µl, and 200 µl) and one for the standard antibiotic solution. Each well filled with 20 µl clove extract with different concentrations and the same portion of antibiotic solution was filled. The antibiotic erythromycin used as standard. The plates were incubated at 37^0C for 24hrs.

3. Results and Discussion

The results of the antimicrobial activity assays indicated that clove had inhibitory activity on *Staphylococcus aureus, Bacillus cereus, E.coli* and *Pseudomonas aeruginosa.* The methanolic and hexane extract of clove was showed good antimicrobial activity compare with selected antibiotic (erythromycin).

The antibacterial activity of clove sample with hexane and methanol extract ranged from 12mm to 19 mm zone of inhibition against all four bacterial pathogens. Minimum zone of inhibition 12 mm was observed in 50 µl concentrations against *staphylococcus aureus, E.coli* in hexane extract and *Pseudomonas aeruginosa* in methanol extract. Shown in table 1& 2

Table 1: Zone of inhibition methanolic extract of clove

Zone of inhibition					
Organisms	**Erythromycin**	**Methanol extract**			
		50µl	**100 µl**	**150 µl**	**200 µl**
E.coli	14	18	18	19	18
Pseudomonas aeruginosa	18	12	12	14	13
Bacillus cereus	29	14	13	14	16
Staphylococcus aureus	16	15	15	14	14

Table 2: Zone of inhibition Hexane extract of clove

Zone of inhibition					
Organisms	**Erythromycin**	**Hexane extract**			
		50µl	**100 µl**	**150 µl**	**200 µl**
E.coli	14	12	12	13	14
Pseudomonas aeruginosa	18	13	14	14	14
Bacillus cereus	29	15	17	17	19
Staphylococcus aureus	16	12	12	12	12

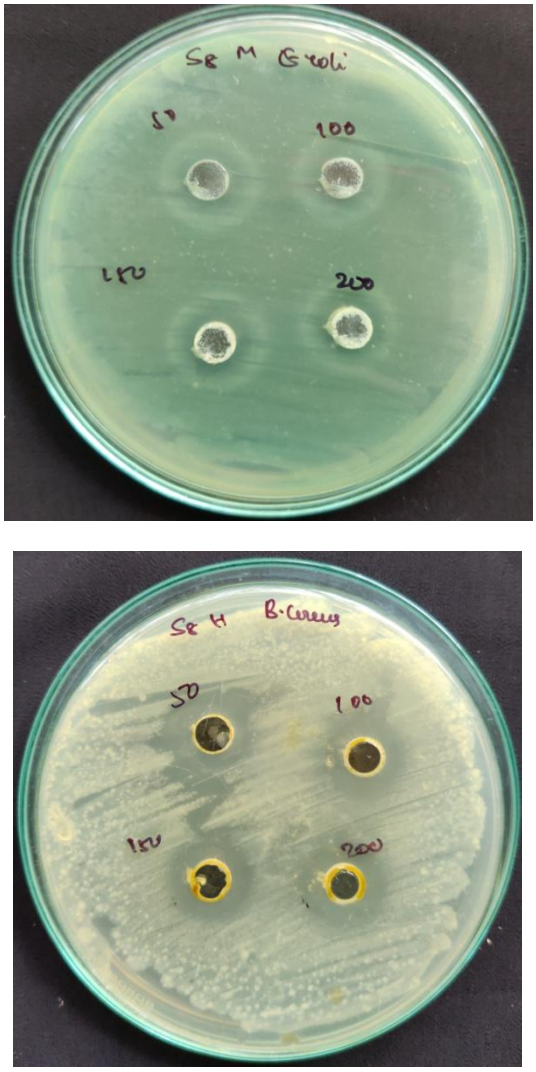

Figure 1: Antibacterial activity of methanol extract of *Syzygium aromaticum* L recorded zone of inhibition of 19 mm against *E.coli* and hexane extract of clove showed zone of inhibition of 19mm against *B.cereus*.

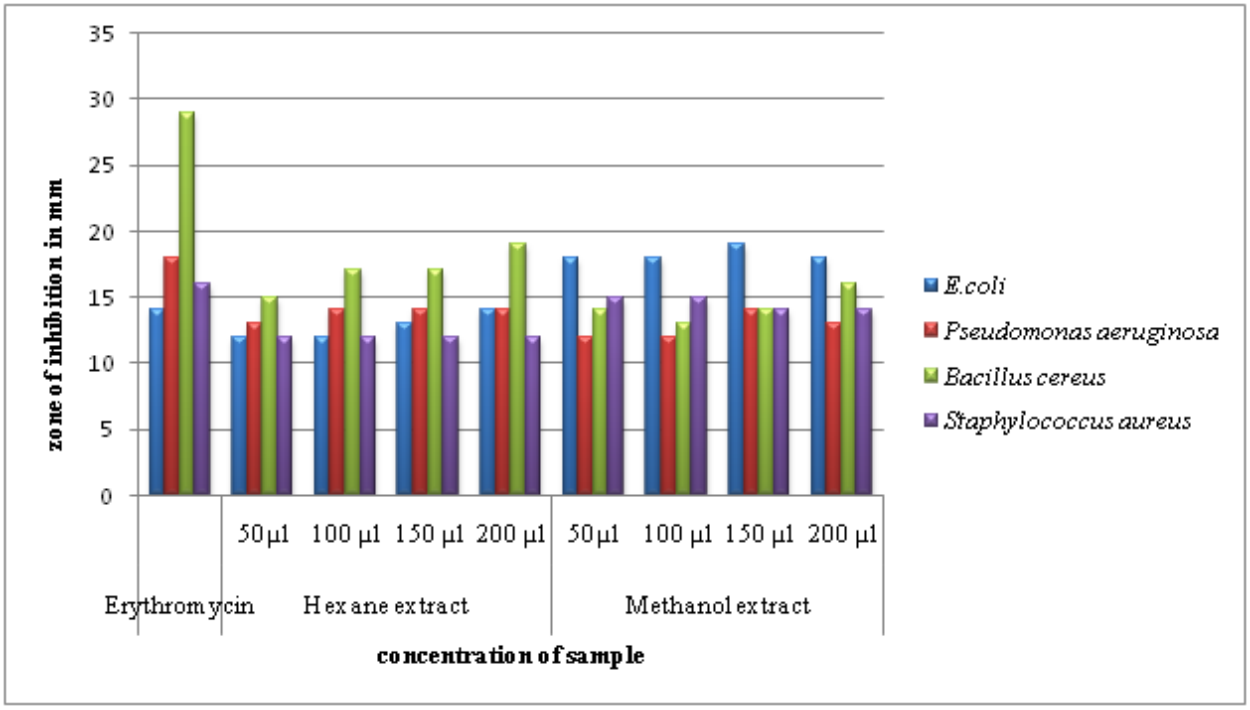

Figure 2: Antibacterial activity of *Syzygium aromaticum* L

The maximum zone of inhibition 19 mm was observed in 200 µl concentration against *Bacillus cereus* in hexane extract, and *E.coli* in methanol extract at 150 µl concentrations. Shown in figure no 1. The antimicrobial activity of both hexane and methanol extract was ranged from 12mm to 19mm against all four bacterial pathogens.

The microorganism susceptibility to different extracts did not correlated with the susceptibility or resistance to a particular antibiotic within the same species. This fact was evident for *S. aureus, Bacillus.cereus and E.coli.* These results were comparable with standard antibiotic erythromycin which was showed inhibition zone ranged from 14 – 29 mm. According to the study of Claudiu et al 2009 various chemical compounds including volatile oils, alkaloids, tannins and lipids presented in herbs tissues. The effect of clove inhibits the growth of microorganisms.

4. Conclusion

Antibacterial activity of clove extract was studied by selecting four types of bacteria (two gram negative and two gram positive) and found that the hexane and methanol extract of clove has an evident effect on bacteria. Higher effect of clove extract was appeared on *Bacillus cereus, E.coli* and lower impact on *Staphylococcus aereus* and *Pseudomonas aeruginosa*. The overall evaluation of this study concludes that the clove extract have high antibacterial activity potential.

References

[1] Claudiu N M and Maria M M, Antimicrobial effect of seed extract of coriander. J. Agroalimentary processes and technologies. 2009; 15 (2): 298-300.
[2] Shyamala, M. P., Venukumar, M.R. and Latha, M.S. Antioxidant potential of this *Syzygium aromaticum* (Gaertn.) Linn. (Cloves) in rats fed with high fat diet. Ind. J. Pharmacol.2003; 35: 99-103.
[3] Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S. and Efferth T. Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother. Res.2007; 21: 989-994.
[4] Kalemba D. and Kunicka A. Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry.2003; 10: 813-829.
[5] Hammer KA, Carson CF, Riley TV. Antimicrobial Activity of Essential Oils and Other Plant Extracts. J Appl Microbiol 1999; 86: 985-990
[6] Cai, L. and Wu, C.D. Compounds from *Syzygiumaromaticum* possessing growth inhibitory activity against oral pathogens, *J.* Natural Products. 1996; 59: 987-990.
[7] Gijzen M, Efraim L, Savage T, Croteau R. Bioactive Volatile Compounds from Plants. In: Teranishi R, Buttery RG, Sugisawa H, ed. Conifer Monoterpenes: Biochemistry and Bark Beetle Chemistry Ecology. Ch. 2 ed. Washington DC: American Chemistry Society, 1991.
[8] Gislene GF, Paulo C, Giuliana L. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic Resistant Bacteria. Braz J Microbiol 2000; 31: 314-325.
[9] Consumer information from USDA, *Food Safety and Inspection Service*, Food Safety and Consumer Education Office. 1997
[10] CDC, What is a foodborne disease outbreak and why do they occur, 2012.
[11] Arora D S, Kaur J, *International Journal of Antimicrobial Agents*. 1999:12,257.
[12] Shelef L A, *Journal of Food Safety*. 1983: 6, 29