

Antibacterial activity of *Syzygium aromaticum* Extracts (Clove Oil) on the Etiological Agent of Dental Caries

Jadhav Yashashree D., Garud Nandutai, Yojana Binawade and G.R. Pathade

*Krishna Vishwa Vidyapeeth, Deemed to be University Karad,
Dist. – Satara, Maharashtra, India*

(Received 10 November, 2022; Accepted 20 January, 2023)

ABSTRACT

Clove oil contains the active ingredient eugenol which is a natural anesthetic. Dental caries and dental plaque are among the most common diseases worldwide and are caused by a mixture of microorganisms and food debris. Specific types of acid-producing bacteria, especially *Streptococcus mutans*, colonize the dental surface and causes damage to the hard tooth. The decayed tooth of patients suffering from dental caries was taken as a starting material in our present investigation. The clinical specimen from dental caries patients were subjected to isolation of the etiological agent of dental caries by streak plate method on Blood agar. And two isolates were obtained. The antimicrobial activity of the extract of *Syzygium aromaticum* against both isolated organisms was studied. The aqueous extract of *Syzygium aromaticum* did not show antimicrobial activity against both isolated organisms. But methanol extract of *Syzygium aromaticum* showed significant antimicrobial activity against both organisms.

Key words: Antimicrobial, *Syzygium aromaticum*, Dental caries, Clove oil.

Introduction

Dental caries is a widely spread and predominant disease. Dental caries cause serious problems in the oral cavity. It is associated with many pathogenic microorganisms, including *Streptococcus mutans*, and *Candida albicans*. The *Streptococcus mutans* is the main etiological agent of dental caries because it can produce high levels of dental caries-causing lactic acid and extracellular polysaccharides.

Dental caries is caused due to the destruction of dental hard cellular tissue by acidic by-products from the bacterial fermentation of dietary carbohydrates and sugars (Gupta *et al.*, 2011). Dental plaque is known to be the primary cause of dental caries and other oral infections. It exists not only on the

tooth surface but also under the gums. Eugenol is most popular in dentistry because of its sedative properties.

For thousands of years, clove oil (eugenol) has been used in dentistry. Eugenol has been used topically in dental practice to relieve pain arising from a variety of sources, including pulpitis and dentinal hypersensitivity. Interestingly, eugenol exhibits irritant action in addition to its analgesic effect as found in certain studies (Thosar *et al.*, 2013).

Eugenol is the main antimicrobial component present in *Syzygium aromaticum*, and it shows antibacterial activity against many pathogens. Eugenol in clove can disrupt bacterial membranes (Devi *et al.*, 2010). The main aim of this study is to evaluate the antimicrobial efficacy of extracts of *Syzygium*

aromaticum against dental caries-causing microorganisms. In addition, the comparison of aqueous and solvent extracts of clove oil against dental caries.

Materials and Methods

Sample collection

Clinical specimens (decayed teeth) of patients suffering from dental caries were collected from the local hospitals.

Isolation of microorganisms from clinical samples

Clinical specimens for isolation of etiological agent of dental caries were obtained from the decayed tooth of a patient with the help of a sterile cotton swab in sterile saline. From the suspension of the clinical specimen, a smear was prepared on a glass slide and gram staining was done. Isolation of the etiological agent was done by the streak plate method using blood agar plates and nutrient agar plates. The plates were incubated at 37°C for 48 h. The representative isolates were streaked on blood agar slants and preserved at 4 °C.

Preparation of clove bud extract:

- a. Aqueous extraction.
- b. Organic solvent extraction

a) Aqueous extraction method: (Nikousaleh *et al.*, 2016)

Clove buds were ground into a coarse powder with the help of a mixer. A fixed quantity, i.e. 10 g of clove bud powder was weighed out and taken in a glass beaker, 10 ml of boiled distilled water was added to it. Mixed Properly, then filtered through the filter paper. The filtrate was used as an aqueous extract of Clove buds.

b) Solvent extraction method: (Nikousaleh *et al.*, 2016).

The 10 g of clove bud powder was weighed out and taken in a glass beaker in which an equal amount of methanol was added and kept on the shaker for 3

hours. The solution was filtered through the muslin cloth. The filtrate was taken in the petri dish and kept overnight for drying. A dried filtrate was dissolved in the dimethyl sulfoxide (DMSO). The solution was taken as methanol extract for further study.

Detection of antimicrobial activity of clove bud extract by Agar diffusion method

From the stock culture, a thick suspension of each isolate was prepared and 1 ml of each of suspension was spread on blood and nutrient agar plates. The plates were bored with the help of a borer. On each plate, in one well 0.1 ml Clove extract was added as the test with help of a micropipette under sterile conditions. Then plates were kept in the refrigerator for 20 - 25 minutes for diffusion. The blood agar plate was incubated an aerobically (in a candle jar) at 37 °C for 24 h. while nutrient agar plates were incubated at 30 °C for 24 h. After incubation zones of clearance were observed and zones of inhibition were measured.

Results and Discussion

When these two isolates were gram-stained to study gram nature and morphology, It is clear from Table 1 that isolate S1 was observed as Gram-positive cocci and isolate A1 was observed as Gram-positive rods.

It was observed from Table 2 that isolate S1 fermented both sugars, i.e Mannitol and Sorbitol with the production of acid. Isolate A1 fermented both sugars. (Sucrose, fructose). Isolate S1 did not ferment lactose and growth was not shown in 4%,6% NaCl concentration and gave a negative result for catalase. From the above results, the isolated organisms were found to be *streptococcus mutans* and *Actinomycetes* which S or A.

S1= *Streptococcus mutans*

A1=*Actinomycetes*.

It was seen from Table 3 and photoplate-1 (a,b) that the aqueous extract of Clove buds did not show any antimicrobial activity against the *Streptococcus mutans* and *Actinomycetes* species.

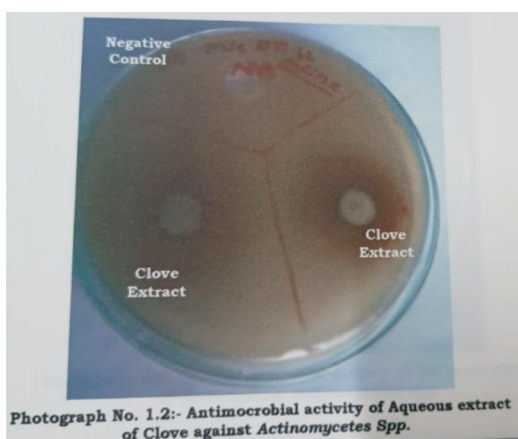
Table 1. The cultural and morphological characteristics of the isolates from samples

Isolate No.	Size	Shape	color	Elevation	Margin	Opacity	Consistency	Gram nature and motility
S1	1mm	circular	White	convex	entire	opaque	moist	gram +vecocci, on-motile
A1	4mm	irregular	white	flat	Ceret	opaque	moist	gram +ve rod



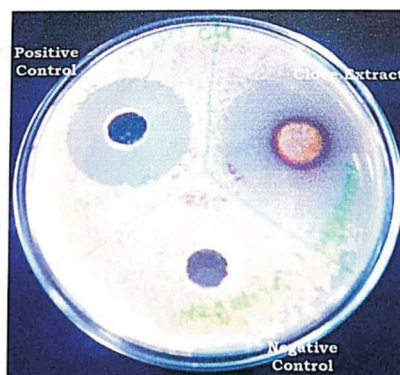
Photograph No. 1.1:- Antimicrobial activity of Aqueous extract of Clove against *Streptococcus mutans*

Plate -1 a



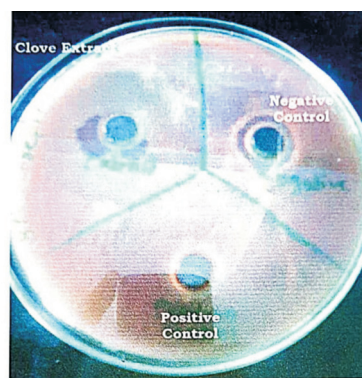
Photograph No. 1.2:- Antimicrobial activity of Aqueous extract of Clove against *Actinomyces Spp.*

Plate 1 b



Photograph No. 1.4:- Antimicrobial activity of Methanol extract of Clove against *Actinomyces spp.*

Plate 2a



Photograph No. 1.3:- Antimicrobial activity of Methanol extract of Clove against *Streptococcus mutans*

Plate 2b

Table 2. The biochemical characteristics of the isolates from the sample.

Isolated microorganism	Biochemical test	Results
S1	Mannitol fermentation	Acid production
	Sorbitol fermentation	Acid production.
	Lactose fermentation	No acidification
	Sucrose fermentation	Acid production
	Catalase production	Negative
	4% NaCl	No growth
	6% NaCl	No growth
	A1	Sucrose fermentation
	Fructose fermentation.	Acid production

Table 3. The antimicrobial activity of aqueous and methanol extracts against *streptococcus mutans* and *actinomyces* spp isolates.

Sr. Isolate No.	Negative Control (saline)	Zone of inhibition (in cm)		
		Positive control (Antibiotic) streptomycin	Clove extract	
			Aqueous extract	Test Methanolic extract
1. <i>Streptococcus mutans</i>	negative	2.9 cm	Negative	2.7 cm
2. <i>Actinomyces</i>	negative	2.9 cm	negative	3.3cm

+ = zone of inhibition.- = no zone of inhibition

It was observed further from Table 3, zones of inhibition of 2.7 cm and 3.3 cm respectively were seen with that methanol extract clove buds showed against both the organisms viz, *Streptococcus mutans*, and *Actinomyces* species involved in the pathogenesis of Dental caries. The inhibition zones with methanol extract and standard antibiotics 2.9,2.7,3.3 cm. are comparable. Clove essential oil exhibited antibacterial activity. The MIC for clove essential oil was at 0.078% (v/v) for all strains tested (Ginting *et al.*, 2021).

Conclusion

The methanol extract of *Syzygium aromaticum* showed antimicrobial activity against both organisms viz; *Streptococcus mutans*, *Actinomyces* species. The plant *Syzygium aromaticum* buds extract may prove to be a source for obtaining chemotherapeutic agents for Dental caries. From the above results, it can be concluded that clove can be used as a potential antimicrobial agent against organisms that are the main cause of dental caries.

Acknowledgement

We are very much thankful to the management of the Krishna Institute for inspiration and for providing all necessary laboratory facilities for the present research work.

Conflict of Interest - no conflict of interest is there amongst the author.

References

- Aida, P., Rosa, V., Blame, F., Tomas, A. and Salvador, C. 2001. Antifungal activity of Paraguayan plants used in traditional medicine. *J Ethnopharm.* 16: 93-98. doi: 10.1016/s0378-8741(01)00214-8
- Charu, G., Amar, P.G. and Ramesh, C.U. 2008. Antimicrobial activity of some herbal oils against common food-borne pathogens. *African Journal of Microbiology Research.* 2(10) : 258-261.
- Devi, K.P., Nisha, S.A., Sakthivel, R. and Pandian, S.K. 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of Ethnopharmacology.* 130(1) : 107-115.
- Fiori, A.C.G., Schwan-Estrada, K.R.F., Stan Garlin, J.R., Vida, J.B., Scapim, C.A. and Guz, M.E.S. 2000. Antifungal activity of leaf extracts and essential oils of some medicinal plants against didymellabryonia. *J Phytopathology.* 148: 483-487. doi: 10.1046/j.1439-0434.2000.00524.x
- Gupta, C., Garg, A.P., Uniyal, R.C. and Kumari, A. 2008. Antimicrobial activity of some herbal oils against common food-borne microbes. *African J Microbiol. Res.* 2 : 258-261.
- Gupta, C., Kumari, A., Garg, A.P., Catanzaro, R. and Marotta, F. 2011 . Comparative study of cinnamon oil & clove oil on some oral microbiota. *Acta Biomed.* 82(3): 197-199.
- Kamel, C., Hafedh, H., Tarek, Z., Amel, B.K.N., Mahmoud, R. and Kacem, M. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): A short review. *Phytother Res.* 21(6): 501- 506. doi: 10.1002/ptr.2124.
- Nikousaleh, A. and Prakash, J. 2016. Antioxidant components and properties of dry heat treated clove in different extraction solvents. *Journal of Food Science and Technology.* 53(4) : 1993-2000.
- Thosar, N., Basak, S., Bahadure, R.N. and Rajurkar, M. 2013. Antimicrobial efficacy of five essential oils against oral pathogens: An *in vitro* study. *European Journal of Dentistry.* 7(S 01): S071-S-077.
- Yadav, K. and Prakash, S. 2016. Dental caries: A review. *Asian J Biomedical Pharmaceut Sci.* 6(53) : 1-7.
- Ginting, E.V., Retnaningrum, E. and Widiasih, D.A. 2021. Antibacterial activity of clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum burmannii*) essential oil against extended-spectrum γ -lactamase-producing bacteria. *Veterinary World.* 14(8): 2206.