

Antibacterial activity of syzygium aromaticum (Clove) against oral microorganisms- an *in – vitro* study

Swati Balsaraf¹, Revant H Chole², Swati Balsaraf^{1*}

¹ Professor, Department of Public Health Dentistry, College of Dental Sciences & Hospital, Rau, Indore, Madhya Pradesh, India

² Department of Oral and Maxillofacial surgery and Diagnostic Sciences, Faculty of Dentistry, Najran University, Saudi Arabia

Abstract

Introduction: The present century in medical sector is flooded with various allopathic options for treatment of diseases. It is indeed a boon but have introduced synthetic chemicals in human body. It has many side effects and microbes too are developing resistance to such drugs. Antimicrobial activity of clove oil is attributed by disturbing the cell wall and membrane cell lysis, leakage of cellular contents and inhibition of proton motive force. The aim of this study was to check efficacy of clove extract against streptococcus mutans.

Methodology: The efficacy of clove will be assessed by agar well diffusion method. Results: clove extract was effective at 100% concentration.

Conclusion: Clove oil is a popular remedy for toothache due to its potent antiseptic and analgesic activity.

Keywords: Clove, streptococcus mutans, oral microorganisms, phytochemicals, tannins

Introduction

Ayurveda and Herbs: The present century in medical sector is flooded with various allopathic options for treatment of diseases. It is indeed a boon but have introduced synthetic chemicals in human body. It has many side effects and microbes too are developing resistance to such drugs ^[1].

Although Allopathic medicines is the mainstream, the knowledge of Ayurveda originated in India and the Ayurvedic treatments were compiled in the form of Charaka Samhita and Sushruta Samhita.

Uses of herbs and traditional practices will continue to play a significant role in the socio-cultural life of village communities. The term “Herb” includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and bark ^[2].

The herbal medicines presents with enormous unexplored therapeutic potential as antimicrobials. The antimicrobial property of herbs is attributed to the various phytochemicals such as alkaloids, flavanoids, tannins, phenolic compounds, glycosides, terpenoids, quinines, saponins, and coumarins ^[3].

Alkaloids are phytochemicals commonly found in Angiosperm and rarely found in Gymnosperm. Caffeine, quinine, cineline, strychnine, brucine, emetine and narcotine are few examples of alkaloids that have known medicinal values ^[4].

Phytochemical analysis of these plants revealed presence of tannins, alkaloids, flavanoids, terpenoids and glycosides. Antimicrobial activity of clove oil is attributed by disturbing the cell wall and membrane cell lysis, leakage of cellular contents and inhibition of proton motive force. The aim of this study was to check efficacy of clove extract against streptococcus mutans ^[5].

Materials and Methods

Agar

Test tubes

Petri dishes

Conical flask

Clove's powder

Microorganisms

Streptococcus Mutans

Methodology

Cloves were purchased from local shop. Although dry it was again dried under sun shade and ground to powder and sieved. The fine powder thus obtained was perfectly measured at 25 mg, 50 mg, and 100 mg and mixed well with water and stirred regularly. It was kept for one day. It was boiled using conical flask and burner so that the phytochemicals are extracted from it. The water was thus filtered and aqueous solution in the concentration of 25%, 50% and 100% were obtained by adding 100 ml of water to each concentration.



Picture 1: Image showing preparation of extract of clove powder in distilled water

Culture revival

Ampoules of freeze dried form of *Streptococcus mutans* (890) were obtained from MTCC (Microbial Type Culture Collection), Chandigarh. The culture was revived by adding the ampoule contents into nutrient broth and incubated for 24 hours at 37°C. Confirmation of growth was done by McFarland's turbidity standards.

The efficacy testing by agar well method. The culture media used was BHI agar enriched with blood which was prepared according to manufacturer's instructions. The growth obtained was transferred to BHI agar plates to test the efficacy by streaking method. Four wells were made on the 10 agar plates and 10 µl of each concentration of extract was transferred to each 3 wells with the help of micropipette and chlorhexidine mouth wash in one well which was used as positive control.

The plates were incubated and later on the zone of inhibition was measured and studied further.

Results



Picture 2: Antimicrobial efficacy of Clove extract in different concentrations

Table 1: Zone of Inhibition recorded at different concentration against *S. Mutans* P>0.01

<i>S.mutans</i>	25%	50%	100%	Chlorhexidine
1 Clove extract	02mm	2mm	3mm	3mm
2 Clove extract	1.5mm	2.5mm	4mm	02mm
3 Clove extract	2mm	2mm	2.5mm	02mm

Discussion

In a study done by Gupta C and Prakash D *Syzygium aromaticum* (Family-Myrtaceae) contains many compounds such as eugenol which is considered as one of essential component of clove oil and is known to possess antimicrobial activity against many pathogens. The other chemical constituents are eugenol acetate 4-allyl-2-methoxyphenol acetate β-caryophyllene; trans – (1R, 9S)-8-methylene-4, 11, 11-, bicycloundec-4-ene trimethylbicycloundec-4-ene, and 60-90% of another secondary compounds [6].

In a study done by Thosar N and Basak S it was quoted that the clove plant grows in warm climates and is cultivated commercially in Tanzania, Sumatra, the Maluku (Molucca) Islands and South America. The principal constituent of distilled clove bud oil (60-90%) is eugenol (4-allyl-2-methoxyphenol). The oil also contains about 10% acetyleneugenol and small quantities of gallic acid, sesquiterpenes, furfural and vanillin and methyl-n-amyl ketone. Other constituents include flavonoids, carbohydrates, lipids, oleanolic acid, rhamnetin and vitamins [7].

In a study done by Bansal V *et al* the mean zone of inhibition for Clove extract was found out to be 3.8 ± 3.633 mm. Whereas in our study mean zone of inhibition was found to be 3.1mm at 100 Microgram/ml [8].

A herbal mouth wash containing clove oil by Agar well diffusion method showed a maximum zone of inhibition against *S. mutans* and *S. salivarius* (19 mm and 22 mm), respectively [9].

Conclusion

Clove oil is a popular remedy for toothache due to its potent antiseptic and analgesic activity. It is a potent antimicrobial agent for external use because it is not inactivated by dilution or affected by organic matter. It has strong antioxidant and antiviral activities. Natural products have been recently investigated more thoroughly as promising agents for prevention of oral diseases. This study shows that clove extract is effective at high concentration (100%)

References

- Rose M, bhagya B, Shantaram M. Ethnomedicinal herbs used in oral health and hygiene in costal dakshina Kannada. JOHCD, 2011, 5(3).
- Gandhiraja N, Sriram S, Meenaa V, Srilakshmi JK, Sasikumar C, Rajeswari R, *et al*. Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. *Ethnobot Leaflets*, 2009;13:618-24.
- Arokiyaraj S, Sripriya N, Bhagya R, Radhika B, Prameela L, Udayprakash NK. Phytochemical screening, antibacterial and free radical scavenging effects of *Artemisia nilagirica*, *Mimosa pudica* and *Clerodendrum siphonanthus*- An *in-vitro* study. *Asian Pacific Journal of Tropical Biomedicine*, 2012;2(2):S601-S604.
- Muthukumaran P, Padmapriya P, Salomi S, Umamaheshwari R, Kalaiarasan P, Malarvizhi C. *In vitro* antimicrobial activity of leaf powder. *Asian J Pharm Res*, 2011;1:108-10.
- Srivastava V, Sharma A, Alam I. A review on ethnomedical and traditional uses of *mimosa pudica* (Chui-Mui). *Int Res J Pharm*, 2012, 3.
- Gupta C, Prakash D. Comparative study of the antimicrobial activity of clove oil and clove extract on oral pathogens. *Dent Open J*, 2021;7(1):12-15.
- Thosar N, Basak S, Bahadure R, Rajurkar M. Antimicrobial efficacy of five essential oils against oral pathogens: An *in vitro* study. *Eur J Dent*, 2013;7(Suppl 1):S71-S77.
- Bansal V, Gupta M, Bhaduri T, Shaikh SA, Sayed FR, Bansal V, *et al*. Assessment of Antimicrobial Effectiveness of Neem and Clove Extract

Against *Streptococcus mutans* and *Candida albicans*:
An *In vitro* Study. Niger Med J,2019;60(6):285–289.

9. Rakshanaa TVR, Lakshmi T. Antibacterial efficacy of herbal mouthwash against oral microbes - *in vitro* assay. J Adv Pharm Edu Res,2017;7(1):31-33.