

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NEEM AND CLOVE EXTRACT AGAINST S.MUTANS AND C.ALBICANS-AN INVITRO STUDY

Dr. Sandesh Nagarajappa, Dr. Pratiksha Bathija*, Dr. Prashant Mishra, Dr. Vaibhav Bansal, Dr. Sonali Gupta and Dr. Shantanu Sontakke

¹Professor and Head of the Department, Public Health Dentistry, Sri Aurobindo College of Dentistry and PG Institute Indore.

²Postgraduate Student, Department of Public Health Dentistry.

³Reader, Department of Public Health Dentistry.

⁴Senior Lecturer, Department of Public Health Dentistry.

⁵Postgraduate Student, Department of Public Health Dentistry.

⁶Postgraduate Student, Department of Public Health Dentistry.

Article Received on
14 Jan. 2018,

Revised on 05 Feb. 2018,
Accepted on 26 Feb. 2018,

DOI: 10.20959/wjpr20185-10806

*Corresponding Author

Dr. Pratiksha Bathija

Postgraduate Student. Public
Health Dentistry.

ABSTRACT

Introduction: Dental caries is one of the public health concerns as oral health is integral to general well-being and relates to the quality of life. It is a chronic disease which often requires an invasive treatment along with antibiotic regimen. Since the microbial resistance to traditionally used antibiotics is increasing day by day, there is urgent need for development of new drug molecules which are effective affordable and non toxic. **Objective:** Present study was aimed to detect antibacterial and antifungal activity of Neem and Cloves against *Streptococcus*.

Mutans and *Candida.Albicans*. **Material And Method:** Strains of S.mutans and C.Albicans and selective media for growing micro-organisms were procured. Antimicrobial activity was assessed using two methods, by determining Minimum Inhibitory Concentration (MIC) using Broth Dilution Method and determining Zone of Inhibition using well diffusion method on Mitis Salivarius Bacitracin (MSB) selective for S.mutans and Saboraud's dextrose agar plates for C.albicans. One way ANOVA with post hoc analysis was done to compare the Antimicrobial activity of extracts and 0.2% Chlorhexidine. **Results:** MIC of neem was found to be 4.2mg/ml and 5mg/ml against S.mutans and C.albicans and 5.5mg/ml for cloves. Neem had highest antibacterial activity with a mean zone of inhibition of 11.4 mm followed by

Chlorhexidine and Cloves whereas antifungal activity was highest for Chlorhexidine (14.4 mm)followed by Neem and Clove. **Conclusion:** The results of present work established that both plant extracts possess antimicrobial activity against common microbes present in oral cavity responsible.

KEYWORDS: Neem, Clove, Minimum Inhibitory Concentration, Zone of Inhibition.

INTRODUCTION

Scientific heritage of Indian medicinal plants continue to be a rich source of natural antibiotic supplements and a safe treatment modality for various microbial diseases. Being the largest global producer of medicinal herbs, India is appropriately called the ‘botanical garden’ of world. About 8000 medicinal herbs have been documented in AYUSH, 1110 species in Charaka Samhita and 1270 in Sushruta Samhita.^[1]

Rising disease incidence and financial considerations in developing countries call for an alternative treatment and prevention options for oral diseases that are safe, effective and economical.^[2]

Additionally, prolonged use of antibiotics creates a microbial resistance as and exposes the immunocompromised persons to a variety of oppourtunistic infection.

Thus there is a urgent need for the development of new drug molecules and a new treatment modality for treatment of dental infections.

Presently there is a renewed interest in traditional or the ‘green medicine’ that is safe and more dependable than the costly synthetic drugs, many of which have adverse side-effects.^[3]

Neem also known as Azadirachta Indica (A.Indica) belongs to botanic family Meliaceae and is commonly referred to as “Village Dispensary”.^[4] Different parts of neem has been used for their various pharmacological action such as antioxidant, antimutagenic, anti inflammatory, anticarcinogenic, antidiabetic properties.^[5]

Cloves (*Syzygium aromaticum*) are dried aromatic unopened floral buds, belonging to the family Myrtaceae. Clove is a natural antibiotic with broad antibacterial, antifungal, antiviral and antimycosal activity.^[6] It has been used by dentists as a dressing in dentistry for minor wounds, and an analgesic in painful and infective diseases of the oral cavity. It has also been

used as an analgesic, anti spasmodic, and as a general antiseptic in medical and dental practices.^[7]

Neem is known to inhibit of bacterial adhesion to saliva-conditioned hydroxyapatite, a composite of bone and enamel. Neem extract also inhibited insoluble glucan synthesis thereby reducing the adherence of streptococci to tooth surfaces.

Azadirachtin, Terpenoid chief constituent of neem is mainly responsible for antibacterial properties of neem.^[8]

Clove extract was more effective against *S.mutans* with a zone of inhibition of 22mm in a study done by Sweta VR, Geetha RV(2015) whereas neem was found to be having highest antibacterial activity at 6.25mg/ml in a study done by Hala A Mohammad and Al Fadhil A. Omer(2015).

Antibacterial activity of neem extract of varying concentration has been assessed using disc diffusion method and measuring zone of inhibition which displayed variable results in previously reported literature.

So the present research was conducted with an intention to investigate antibacterial and antifungal properties of neem and clove against two common oral pathogens *S. mutans* and *C.albicans*. They were chosen for the study as they are responsible for caries initiation and progression.^[9]

Screening of these medicinal plant for bioactive compounds may lead to development of less expensive new antimicrobial agents with improved safety and efficacy. These two herbs are widely available in rural and urban areas of India and are accepted traditionally by majority of people.

So the aim of present study was to determine the Antibacterial and Antifungal activity of Neem and Cloves against the *Streptococcus.mutans* and *Candida.albicans* and to compare it with 0.2% Chlorhexidine.

MATERIALS AND METHOD

Study was conducted from a period of March 2016 to May 2016, in Department Of Microbiology.

Preparation Of Extract

The leaves of neem and buds of clove, were identified and collected from a botanical garden, washed and dried in sunlight and then powdered to prepare a fine powder of 500 grams. An aqueous extract was prepared by heating dried powder in 1000 ml of distilled water. Then extract was filtered through Whatmann filter paper and centrifuged at 15000 rpm for 15 minutes. Successive concentration filtration and extraction of this filtrate powders was done with the help of Soxhlet apparatus. The concentrated extract was stored at 4°C in airtight bottles for further use.

Procuring microbes and Revival of Microorganisms

Strains of *S. mutans* (ATCC 25175) and *C. Albicans* were obtained from HiMedia Lab, Pvt Ltd, Bombay and cultured fresh on Selective media for the purpose of study.

Vial containing *S. mutans* was broken and powder containing lyophilized bacteria was added to flask containing autoclaved enriched nutrient broth in Laminar Air Flow chamber. The flask was then kept in incubator for 48 hours at 37°C. After 48 hours it was checked for turbidity on the surface indicative of revival and growth of bacteria.

Similar procedure was carried for revival of *C.albicans* with simple nutrient broth.

Identification and confirmation of bacteria

The bacterial and fungal isolates were suspended in peptone broth and incubated at 37° C for 3-4 hours were used as inocula. One loopfull colony of *S. mutans* was picked up using inoculating loupes and streaked over surface of cooled MSB plates and then plates were placed in anaerobic gas jar using anaerobic gas packs, for 24 hours. Similarly colonies of *C. Albicans* was picked up using sterile gauze sticks and spread over the slants of Saboraud's Dextrose agar and incubated aerobically for 48 hours, in a incubator. They were then confirmed on basis of morphological and colonial characteristic.

Determination of Antimicrobial Activity

Antimicrobial and antifungal activity of extracts against *S.mutans* and *C.albicans* was determined using Minimum inhibitory concentration and zone of inhibition created. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.^[10]

Minimum Inhibitory Concentration was determined using Broth dilution method. Serial dilution of extract was done in a concentration ranging from 70% to 10%.

One loopfull colony of *S.mutans* was picked up using inoculating loop from the prepared culture and inoculated in all the test tubes prepared for serial dilution. Suspensions of pure colonies in broth at 10^6 CFU/ml from growth on the plates were made using Mcfarland's turbidity standards. All the tubes were then vortexed for 10 seconds in a sterilized chamber. They were then placed in a test tube rack and allowed to stand in incubator at 37°C for 48 hours and checked for turbidity at surface. The test tube showing minimum turbidity was recorded as Minimum Inhibitory Concentration of neem and cloves for *S.mutans* and *C.Albicans*. This particular concentration of extract was preserved and used to compare the zone of inhibition with positive control Chlorhexidine.

For checking zone of inhibition

Well diffusion method was employed to determine zone of inhibition against bacteria and fungus.

A total of 5 plate each of MSB and SDA were prepared to assess mean zone of inhibition of extracts carrying MIC of neem and clove comparing it with 0.2% chlorhexidine.

A sterile cotton swab was inserted into the bacterial and fungal suspension, rotated, and then compressed against the wall of the test tube to express any excess fluid. The swab was then passed on surface of agar plate twice or thrice to ensure a uniform, confluent growth.

The agar plates were allowed to dry and three wells (6mm diameter) were punched out on each plate with a sterile borer in the inoculated agar. 100 µl neem and clove extract was poured in the wells using a micropipette. 0.2% Chlorhexidine was used as positive control and poured in third well. Colonies were then allowed to grow an aerobically for *S. mutans* and aerobically for *C. Albican*. The diameter of zone of inhibition is measured and the antimicrobial activity of the extract was reported accordingly. One way ANOVA with post hoc Tukey analysis was used to compare the antimicrobial activity of extracts and control Chlorhexidine, (SPSS version IBM 22, Chicago)

RESULTS

Minimum Inhibitory Concentration of neem was more for *Candida* (5 mg/ml) as compared to *S.mutans* (4.2 mg/ml). MIC of clove was equal against both the test organisms (5mg/ml).

Neem had highest antibacterial activity with a mean zone of inhibition of 11.4 mm±4.03mm followed by Chlorhexidine (9.2mm±1.095mm) and Cloves (3.8mm±3.633).

Post hoc test revealed that there was significant difference between the antibacterial activity of Clove and Neem as well as Clove and Chlorhexidine(Table 1).

Antifungal activity was highest for Chlorhexidine with a mean zone of inhibition of 14.4 mm followed by Neem and then Clove 5.8 mm, 3.88 mm respectively. Comparison of Antibacterial activity of Neem, Cloves, Chlorhexidine against *S. mutans*.

Post hoc test revealed that there was significant difference between the antifungal activity of Clove and Chlorhexidine as well as Neem and Chlorhexidine(Table 2).

Legends for Tables

Table 1: Comparative results of mean zone of inhibition of Neem, Clove and Chlorhexidine against *S. mutans*.

EXTRACTS	No.of plates	Mean Zone of Inhibition	Standard Deviation	f-Value	Significance	Tukey P value
CHX	5	9.2	1.095	7.47	0.0078*	0.899
NEEM	5	11.4	4.037			0.021*
CLOVE	5	3.8	3.633			0.021*

Table 2: Comparative results of mean zone of inhibition of Neem,Clove and Chlorhexidine against *C. Albicans*.

EXTRACTS	No.of plates	Mean Zone of Inhibition	Standard Deviation	f-Value	Significance	Tukey P value
CHX	5	14.4	5.550	8.7786	0.0045*	0.019*
NEEM	5	5.8	4.266			0.005*
CLOVE	5	3.8	2.280			0.732

DISCUSSION

The era of herbal treatment is coming back and herbal ‘renaissance’ is happening all over the world. They are not only effective in the treatment for infectious diseases but also mitigate many of the side effects that are often associated with synthetic antimicrobials.^[11]

Hence, the purpose of the present study was to evaluate the role of antimicrobial agents of plant origin in inhibition of the growth of *S.mutans* and *C.Albicans* which plays a vital role in tooth decay.

Neem extract has been shown to have a wide spectrum of antibacterial and anti mycotic activity. It is extremely effective against *Candida*.^[12]

Nimbidin, a principle component of neem is responsible for its antibacterial and anti-inflammatory action. Polyphenolic tannins present in the extract, effectively bind to the surface associated bacterial proteins, resulting in bacterial aggregation and loss of glucosyltransferase activity. This bacterial aggregate effectively reduces the count of *S. mutans*.^[13]

Previous studies reported that the ethanolic extract of Neem is very useful orally to treat many diseases caused by bacteria. Neem extract produced the maximum zone of inhibition on *Streptococcus mutans* at 50% concentration.^[14]

Subapriya R, Bhuvanewari V and Nagini S (2005) reported that presence of high concentration of azadirachtin, quercetin and beta sosterol in *A. Indica* leaves might be responsible for strong antibacterial and antifungal properties.^[15]

In the present study neem exhibited a significant antibacterial and antifungal activity against *S. mutans* and *C. Albicans*. Minimum Inhibitory concentration of Neem was more for *Candida* (5mg/ml) as compared to *S. mutans*(4.2 mg/ml).

Aqueous extract of Neem exhibited a mean zone of inhibition of 11.4 mm against *S. mutans* and 6mm against *C. albicans* whereas Clove had a zone of inhibition of 3.8 mm both against neem and clove. Chlorhexidine displayed a better antifungal activity than neem with a mean zone of inhibition of 14.4 mm.

Cloves was equally effective against bacteria and the fungus (MIC was 5 mg/ml) in a study done by Kamal Rai and Radhika Joshi(2010). The antimicrobial activity of clove and clove bud oil were investigated by agar well diffusion method against five dental caries causing microorganisms which reported MIC of clove and clove oil to be 12.5 and 3.5 mg/ml respectively.

Eugenol comprises 72-90% of the essential oil extracted from cloves. Kaempferol and myricetin present in clove are supposed to have significant growth inhibitory effect against periodontal pathogens.^[16]

Clove oil is often used to relieve pain caused by dry socket, a possible complication of tooth extraction. To relieve toothache a plug of cottonwool soaked in the oil, is applied to the cavity of the decayed tooth.^[17] It also helps to decrease infection in the teeth due to its antiseptic properties. Cai *et al.* reported preferential activity of crude methanolic extract of clove against Gram-negative anaerobic oral pathogens which cause periodontal diseases.

The antibacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. The present results, therefore, offer a scientific basis for commercialization of these plant products against oral pathogens.

Chlorhexidine is a cationic agent that exhibits broad spectrum antimicrobial activity. It kills bacteria by disrupting the cell membrane.^[18] In the present investigation, chlorhexidine proved to be a better antifungal than antibacterial. Although it has been used to prevent dental caries for several decades, it is associated with some side effects such as staining of teeth and addiction. Thus, there is no perfect antimicrobial agent to prevent dental caries until now.

When used in appropriate concentrations, herbal drugs do not interrupt or alter the natural flora. Therefore, care must be taken in selecting herbal antimicrobials, with consideration about the effect of herbs in oral tissues, the mechanism of action, and side effects.

Herbal medicine form a comprehensive system, that is both promotive and preventive in its approach. Apart from healing and reducing the microbial count in the oral cavity, they help in strengthening the immunity. As dental practitioners we have to understand these products and imply them in our clinical practice.

More importantly study of these species is a commendable way of preserving potential of these medicinal plants as well as move toward developing indigenously made herbal medicine instead of importing foreign manufactured drugs.

CONCLUSION

Neem and Clove demonstrated a potent antibacterial and antifungal activity. The antibacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. The present results, therefore, offer a scientific basis for traditional use and marketing of clove and neem as phytomedicine against oral microorganisms.

REFERENCES

1. Seal M, Rishi R, Satish G, Divya K.T, Talukdar P, Maniyar R. Herbal Panacea: the need for today in dentistry. *Journal of International Society of Preventive and Community Dentistry*, 2016; 6(2): 105-109.
2. Salam R, Sarker B.K, Md. Razwanur Haq, Khokon J.U. Antimicrobial activity of medicinal plant for oral health and hygiene. *International Journal of Natural and Social Sciences*, 2015; 2(1): 01-12.
3. Nagarajappa R, Batra M, Sharda A.J, Asawa K, Sanadhya S, Daryani H, Ramesh G. Antimicrobial effect of *Jasminum Grandiflorum L.* and *Hibiscus Rosa-sinensis L.* Extracts against pathogenic oral microorganisms – an *in vitro* comparative study. *Oral Health Preventive Dentistry*, 2015; 13(4): 341-348.
4. Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of *Azadirachta indica*, *Mimusops elengi*, *Tinospora cardifolia*, *Ocimum sanctum* and 2% chlorhexidine gluconate on common endodontic pathogens: An *in vitro* study. *European Journal of Dentistry*, 2014; 8(2): 172–177.
5. Biswas K, Ishita C, Ranajit K, Bandhyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science*, 2002; 82(11): 1336-1345.
6. Sweta V.R, Geetha R. In vitro Antibacterial activity of clove and pepper on *Streptococcus mutans*. *Asian Journal of Pharmaceutical and Clinical Research*, 2015; 8(5): 269-270.
7. Cai L, Wu CD. Compounds from *Syzygium Aromaticum* possessing growth inhibitory activity against oral pathogens. *Journal of Natural Products*, 1996; 59(10): 987-990.
8. George SS, Shetty A, Hegde A. Assessment of Antifungal properties of crude neem extract versus neem mouthwash – An *In Vitro* study. *IOSR Journal of Dental and Medical Sciences*, 2016; 15(5): 101-105.
9. Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S *et al.* Symbiotic relationship between *S.mutans* and *C.albicans* synergizes virulence of In Vivo plaque biofilms. *Infection and Immunity*, 2014; 82(5): 1968-1981.
10. Andrews JM. Determination of Minimum Inhibitory Concentration. *Journal of Antimicrobial Chemotherapy*, 2001; 48(1): 5-16.
11. Rios JL, Recio MC. Medicinal plants and Antimicrobial activity. *Journal of Ethnopharmacology*, 2005; 100(1-2): 80-84.
12. Raghvendra SS, Balsaraf KD. Antifungal efficacy of *Azadirachta indica* (neem)-An *in vitro* study. *Brazilian Journal of Oral Sciences*, 2014; 13(3): 242-245.

13. Wolinsky LE, Mania S, Nachnani S, Ling S. The inhibiting effect of aqueous Azadirachta Indica (neem) extract upon bacterial properties influencing *in vitro* plaque formation. *Journal of Dental Research*, 1996; 75(2): 816–822.
14. Prashanth GM, Chandu GN, Murulikrishna KS, Shafiulla MD. The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: An *in vitro* study. *Indian Journal of Dental Research*, 2007; 18(4): 148-151.
15. Subapriya R, Bhuvaneshwari V, Nagini S. Ethanolic neem(*Azadirachta Indica*)leaf extract induces apoptosis in the hamster buccal pouch carcinogenesis model by modulation of Bcl-2, Bim, caspase 8 and caspase 3. *Asian Pacific Journal of Cancer Prevention*, 2005; 6(4): 515-20.
16. Dhinahar. S, Lakshmi. T. Role of Botanicals as Antimicrobial agents in management of dental infections – A review. *International Journal of Pharma and Bio Sciences*, 2011; 2(4): 690-704.
17. Barnes J, Anderson LA, Phillipson JD. *Herbal medicines*. 3rd Ed.London: Pharmaceutical Press, 2007; 84-86.
18. Linares MR, Ferrer-Luque CM, Moliz TA, Castro P, Aguado B, Baca P. Antimicrobial activity of alexidine, chlorhexidine and cetrимide against *Streptococcus mutans* biofilm. *Annals of Clinical Microbiology and Antimicrobials*, 2014; 13(41).