

## Anti-Microbial Efficacy Of *Ficus Benghalensis* And *Azadirachta Indica* Formulation - An In Vitro Study

Research Article

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### Abstract

**Aim:** The aim of the present study was to synthesize an antimicrobial solution against four common oral pathogens using two herbal formulations.

**Materials and methods:** *Ficus benghalensis* and *Azadirachta indica* leaves were dried and powdered, which were made into herbal formulation. The solution was synthesized using 1gm of banyan leaf and neem leaf extract and were mixed with 100 mL distilled water and boiled for 10 mins using a heating mandel at 70 to 80 degree celsius and the heated plant extract were filtered using whatman no 1.filter. Cytotoxicity effect of the herbal solution was tested. Later antimicrobial activity against four common pathogens was evaluated with three different concentration (25 µL, 50 µL, 100 µL and standard (AMOXRYRITE)) in agar diffusion methods.

**Results:** Antimicrobial efficacy was calculated using a zone of inhibition. Its showed better antimicrobial activity towards streptococcus mutans.

**Conclusion:** The herbal solution synthesized using neem and banyan formulations were effective against strains of *S. mutans* at all concentrations.

**Keywords:** Neem; Banyan; Antimicrobial Solution.

### Introduction

The most common oral health issues are dental caries and periodontal disease; however, other conditions such as oral cancer and oral mucosal lesions are also causes for concern.[1]. Dental caries is a widespread oral disease caused by Gram-positive bacteria such as *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus* spp., and some non-mutans streptococci forming plaque biofilms on tooth surfaces.[2, 3] Root caries and periodontal infections are caused by various bacterial species such as *Actinomyces* spp. and *Enterococcus faecalis* [4]. The rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, op-

portunistic infections in immunocompromised individuals, and financial considerations all contribute to the global need for alternative prevention and treatment options and products for oral diseases that are safe, effective, and cost-effective.

*Ficus benghalensis*, is commonly known as the banyan and Indian banyan tree. *Ficus benghalensis* is the national tree of India. *Ficus benghalensis* methanol and chloroform extracts have antibacterial activity against *Streptococcus mutans* and *Actinomyces viscosus* bacteria. The antibacterial activity on the extract is due to the presence of different phytochemicals. Sterols and flavonoids abound in *Ficus benghalensis* Linn. These phytochemicals are thought to be responsible for the plant's antibacterial properties.[5]

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Azadirachta indica, is commonly known as neem, nim tree or Indian lilac. It is typically grown in tropical and subtropical regions. As opposed to other dental caries-causing species including *S. salivarius*, *S. mitis*, and *S. sanguinis*, dried chewing sticks of Neem displayed the most antibacterial activity against *S. mutans* [6]. Muco adhesive dental gel containing Azadirachta indica has been found to be more effective than chlorhexidine gluconate mouthwash in reducing plaque index and salivary bacterial count [7].

In this study, the most common oral microbial species were considered: staphylococcus aureus is a gram-positive, round-shaped bacteria, streptococcus mutans is a facultatively anaerobic, gram-positive bacteria that occurs in the oral cavity, candida albicans is a yeast-like fungi that is common in human gut flora, and enterococcus faecalis is a gram-positive bacterium that can cause a variety of oral diseases [8]. Enterococcus faecalis is gram-positive bacterium that can cause a variety of nosocomial infection of urinary tract [10, 11]

The aim of the study was to investigate the antimicrobial efficacy of ficus benghalensis and azadirachta indica against four oral pathogens. Previously our team has a rich experience in working on various research projects across multiple disciplines [12-26] Now the growing trend in this area motivated us to pursue this project.

## Materials And Methods

### Preparation of herbal solution

Azadirachta indica and Ficus benghalensis leaves were collected from a university campus in Chennai, Tamil Nadu, in December. To remove dirt and dust from the surface of the leaves, they were thoroughly washed in running water. They were dried for 15 days and kept in the hot air oven at 60°C for 24-48 hours. These leaves were then ground to a fine powder. 1g Neem and banyan leaf extract powder were mixed with 100 mL distilled water and kept in an orbital shaker for 1 day. The solution was boiled for 10 mins at 70 to 80°C. The solution was heated and reduced upto 10 ML. It was filtered using whatman no 1 filter paper.

### Cytotoxic effect of newly introduced herbal solution

Cytotoxicity effect determines whether the bioactive compound is toxic to cells. Assay for the lethality of brine shrimp was assessed. The crustacean salina is a dependable and convenient method for assessing the cytotoxic effect of bioactive chemicals. Aquatic Remedies in Chennai supplied the brine shrimp eggs. In a hatching chamber, artificial sea water was created by combining 36 g of iodine-free salt with 1000 ml of water that had been distilled. The hatching chamber was divided into a dark area where shrimp eggs

were added, as well as lighting the area with the lamp above. The formalised paraphrase Brine shrimp hatch in two days and mature in two weeks. The hatched nauplii were used to evaluate the cytotoxic effect of herbal solution. In a 6 well ELISA (Enzyme Linked Immunosorbent Assay) plate, 10-12 mL of saline was added. *E. faecalis*, *S. aureus*, *S. mutans* were incubated at 37 degree celsius for 24 hours. *C. albicans*, a yeast like fungi, was incubated at 37 degree celsius for 48 hours. Four groups of microbial culture were included with the measurement of 25 µL, 50 µL, 100 µL and standard (AMOXYRITE). 10 nauplii were added at each well and the number of live nauplii observed after 24h incubation.

### Zone of inhibition

The antibacterial activity was carried out by disc diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in the solution and placed in the nutrient agar plate in (25 µL, 50 µL, 100 µL and standard (AMOXYRITE)) and kept for incubation at 37° for 24 hours and then zones of inhibition were measured.

## Result And Discussion

Based on the cytotoxic results, it has shown that 25 µL herbal solution had 10 shrimps nauplii still alive. The 50 µL herbal solution showed 9 nauplii still viable and the 100 µL had 7 nauplii viable.

As shown in table 1, a minimum of 10mm zone of inhibition was observed for three bacterial species *E. faecalis*, *C. albicans* and *S. aureus*, whereas *S. mutans* showed a minimum of 15mm in diameter. This results shows zone of inhibition is higher in *S. mutans*. These preliminary data indicated that neem and banyan extract have antibacterial activity.

Herbal medicines, according to WHO, serve the health needs of approximately 80% of the world's population, particularly millions of people in developing countries' rural areas. The beneficial medicinal effects of plant materials, including antibacterial activity, are typically attributed to secondary products present in the plant, rather than a single compound or a combination of metabolites [10, 27, 9].

Though the precise mechanism by which the active components of plant materials contribute to antibacterial activity is unknown, the antimicrobial effect could be mediated by one of several mechanisms, including inhibition of cell wall synthesis, cell membrane damage, inhibition of nucleic acid synthesis, inhibition of protein synthesis, and so on [28]. Antimicrobial phytochemicals are classified into several groups, including phenolics, polyphen-

**Figure 1. Preparation of plant extracts. (a) 1gm of neem and banyan extract were mixed with 100 ml distilled water (B) boiled for 10 mins using mandel until the solution is reduced to 10ml.**

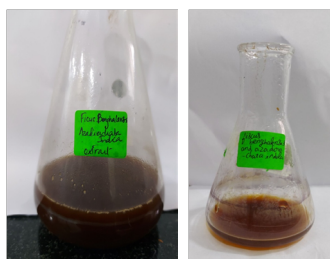


Figure 2. Cytotoxic effect observation.ELISA plate wells with different concentrations of neem and banyan extract observed for presence or absence of live nauplii after 24 hour incubation effect.

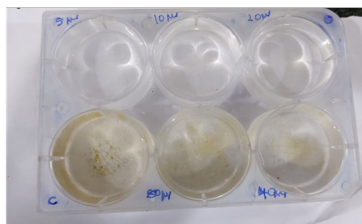


Figure 3. Antibacterial activity of Herbal formulations against pathogens by agar well diffusion method. (*E.Faecalis*,*S. mutans*).

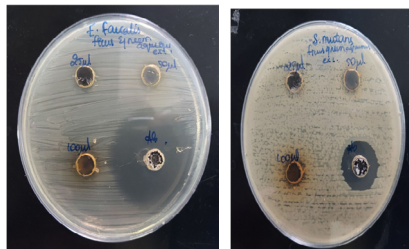


Figure 4. Antibacterial activity of Herbal formulations against pathogens by agar well diffusion method. (*S.aureus*,*C.albicans*).

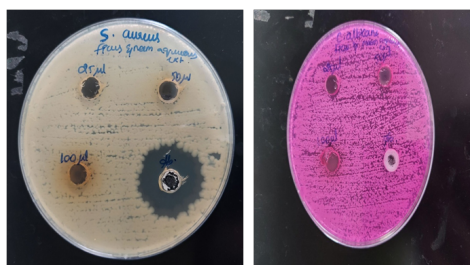
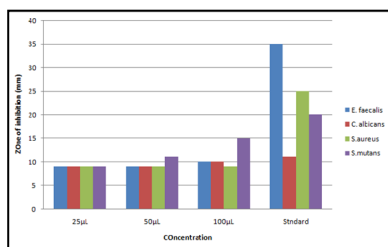


Figure 5. Anticariogenic activity of banyan and neem leaf extract.



nols, flavones, flavonoids, flavonols, quinones, tannins, coumarins, terpenoids, essential oils, alkaloids, lectins, and polypeptides. *Ficus benghalensis* Linn. is rich in sterols and flavanols. These phytochemicals are attributed to the plant’s antibacterial activity. Flavones, flavonoids, and flavonols complex with bacterial proteins and cell walls and exhibit antimicrobial activity [28, 29]. Ethanolic leaf extract of *Azadirachta indica* shows significant antibacterial activity against selected acidogenic oral bacteria. Presence of galotannins during the early stages of plaque formation could effectively reduce number of bacteria [11, 27, 30].

Zone inhibition of neem and banyan leaf extract is demonstrated against bacterial cultures. The antibacterial activity was done against the pathogenic bacteria such as streptococcus aureus, staphylococcus aureus, candida albicans and enterococcus faecalis [30]. There were three different concentrations (25 µL, 50 µL, 100 µL and standard (AMOXRYTE)) which were taken to kill the pathogenic bacteria. Overall, this study reported that herbal formulation mediated by *azadirachta indica* and *ficus benghalensis* plant extract demonstrated good antibacterial activity.

Zone of inhibition (ZOI) of neem and banyan extract demonstrated against microbial cultures. The zone of inhibition increased with concentration of herbal formulation, therefore, for streptococcus mutans 15MM shows that highest in 100 µL/ml, while the lowest ZOI for streptococcus aureus was 9MM shows that lowest in 100 µL/ml. Both herbal extraction was against all the major pathogens that cause dental caries [27, 30].

Our institution is passionate about high quality evidence based research and has excelled in various fields [16, 31-40].

### Conclusion

Our studies confirmed the antimicrobial effect of these natural products of *Ficus benghalensis* and *Azadirachta indica* extract. Antimicrobial activity against all the four oral microbial Pathogens with the highest effect at 100 µL of the extract. However, in the case of this plant, future research should focus on an alternative mechanism of synergistic effects of various natural substances when in combination with other antibiotics.

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## References

- Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol.* 2003 Dec;31(Suppl 1):3-24. Pubmed PMID: 15015736.
- Wang W, Tao R, Tong Z, Ding Y, Kuang R, Zhai S, et al. Effect of a novel antimicrobial peptide chrysothiosin-1 on oral pathogens and Streptococcus mutans biofilms. *Peptides.* 2012 Feb;33(2):212-9. Pubmed PMID: 22281025.
- Ruby JD, Li Y, Luo Y, Caulfield PW. Genetic characterization of the oral Actinomyces. *Arch Oral Biol.* 2002 Jun 1;47(6):457-63.
- Sarkonen N, Könönen E, Eerola E, Könönen M, Jousimies-Somer H, Laine P. Characterization of Actinomyces species isolated from failed dental implant fixtures. *Anaerobe.* 2005 Aug;11(4):231-7. Pubmed PMID: 16701573.
- Mathew M, Sghaireen MG. Study on antibacterial activity of dental cements with extracts of *Ziziphusspina-christi* on Streptococcus mutans: An in vitro study. *Int. J. Oral Health Dent.* 2020 Nov 1;12(6):568.
- Chava VR, Manjunath SM, Rajanikanth AV, Sri Devi N. The efficacy of neem extract on four microorganisms responsible for causing dental caries viz Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis and Streptococcus sanguis: an in vitro study. *J Contemp Dent Pract.* 2012 Nov 1;13(6):769-72. Pubmed PMID: 23404001.
- Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of Azadirachta indica leaf extract gel—a 6-week clinical study. *J Ethnopharmacol.* 2004 Jan;90(1):99-103. Pubmed PMID: 14698516.
- Kiran K, Rajeshkumar S, Roy A, Santhoshkumar J, Lakshmi T. In vitro cytotoxic Effects of Copper Nanoparticles Synthesized from Avocado Seed Extract. *Indian J Public Health Res Dev.* 2019 Nov 1;10(11):3497.
- Cowan MM. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 1999 Oct 1;12(4):564-82.
- Jose M, Cyriac MB, Pai V, Varghese I, Shantaram M. Antimicrobial properties of Cocosnucifera (coconut) husk: An extrapolation to oral health. *J Nat Sci Biol Med.* 2014 Jul;5(2):359-64. Pubmed PMID: 25097415.
- Siddeeqh S, Parida A, Jose M, Pai V. Estimation of Antimicrobial Properties of Aqueous and Alcoholic Extracts of *Salvadora Persica* (Miswak) on Oral Microbial Pathogens - An In vitro Study. *J Clin Diagn Res.* 2016 Sep;10(9):FC13-FC16. Pubmed PMID: 27790459.
- Govindaraju L, Gurunathan D. Effectiveness of Chewable Tooth Brush in Children—A Prospective Clinical Study. *J Clin Diagn Res.* 2017 Mar;11(3):ZC31-ZC34. Pubmed PMID: 28511505.
- Christabel A, Anantanarayanan P, Subash P, Soh CL, Ramanathan M, Muthusekhar MR, et al. Comparison of pterygomaxillary dysjunction with tuberosity separation in isolated Le Fort I osteotomies: a prospective, multi-centre, triple-blind, randomized controlled trial. *Int J Oral Maxillofac Surg.* 2016 Feb;45(2):180-5. Pubmed PMID: 26338075.
- Soh CL, Narayanan V. Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery—a systematic review. *Int J Oral Maxillofac Surg.* 2013 Aug;42(8):974-80. Pubmed PMID: 23702370.
- Mehta M, Deeksha, Tewari D, Gupta G, Awasthi R, Singh H, et al. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. *Chem Biol Interact.* 2019 Aug 1;308:206-215. Pubmed PMID: 31136735.
- Ezhilarasan D, Apoorna VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. *J Oral Pathol Med.* 2019 Feb;48(2):115-121. Pubmed PMID: 30451321.
- Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M, et al. The genetic basis of DOORS syndrome: an exome-sequencing study. *Lancet Neurol.* 2014 Jan;13(1):44-58. Pubmed PMID: 24291220.
- Sneha S. Knowledge and awareness regarding antibiotic prophylaxis for infective endocarditis among undergraduate dental students. *Asian J Pharm Clin Res.* 2016 Oct 1:154-9.
- Christabel SL, Linda Christabel S. Prevalence of type of frenal attachment and morphology of frenum in children, Chennai, Tamil Nadu. *World J Dent.* 2015 Oct;6(4):203-7.
- Kumar S, Rahman R. Knowledge, awareness, and practices regarding bio-medical waste management among undergraduate dental students. *Asian J Pharm Clin Res.* 2017;10(8):341.
- Sridharan G, Ramani P, Patankar S. Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma. *J Cancer Res Ther.* 2017 Jul 1;13(3):556-561.
- Ramesh A, Varghese SS, Doraiswamy JN, Malaiappan S. Herbs as an antioxidant arsenal for periodontal diseases. *J Intercult Ethnopharmacol.* 2016 Jan 27;5(1):92-6. Pubmed PMID: 27069730.
- Thamaraiselvan M, Elavarasu S, Thangakumaran S, Gadagi JS, Arthie T. Comparative clinical evaluation of coronally advanced flap with or without platelet rich fibrin membrane in the treatment of isolated gingival recession. *J Indian Soc Periodontol.* 2015 Jan;19(1):66-71.
- Thangaraj SV, Shyamsundar V, Krishnamurthy A, Ramani P, Ganesan K, Muthuswami M, et al. Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations. *PLoS One.* 2016 Jun 9;11(6):e0156582. Pubmed PMID: 27280700.
- Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J. In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. *Toxicol Mech Methods.* 2019 May;29(4):276-290. Pubmed PMID: 30461321.
- Ramakrishnan M, Shukri M. Fluoride, Fluoridated Toothpaste Efficacy And Its Safety In Children-Review. *Int J Pharm Res.* 2018 Oct 1;10(04):109-14.
- Deepak VN, Lakshminarayanan A, Anitha R, Rajeshkumar S, Lakshmi T, Ezhilarasan D, et al. Activity of Coconut Oil Mediated Effervescent Granules as a Denture Cleanser Against C. albicans, S. mutans and E. faecalis. *Indian J Public Health Res Dev.* 2019 Nov 1;10(11):3701.
- Sujatha J, Asokan S, Rajeshkumar S. Phytochemical analysis and antioxidant activity of chloroform extract of *Cassia alata*. *Res J Pharm Technol.* 2018;11(2):439-44.
- Ezhilarasan D, Subha M. Effervescent Denture Cleansing Granules Using Clove Oil and Analysis of Its In Vitro Antimicrobial Activity. *Indian J. Public Health.* 2019 Nov;10(11):3687.
- Deepak VN, Lakshminarayanan A, Anitha R, Rajeshkumar S, Lakshmi T, Ezhilarasan D, et al. Effect of Oregano Oil Mediated Effervescent Denture Cleansing Granules Against Oral Pathogens. *Indian J Public Health Res Dev.* 2019 Nov 1;10(11):3706.
- Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol.* 2019 Dec;90(12):1441-1448. Pubmed PMID: 31257588.
- Pc J, Marimuthu T, Devadoss P, Kumar SM. Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. *Clin. Implant Dent. Relat. Res.* 2018 Apr 6;20(4):531-4.
- Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. *J Periodontol.* 2018 Oct;89(10):1241-1248. Pubmed PMID: 30044495.
- Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJ. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. *Clin Oral Investig.* 2019 Sep;23(9):3543-50.
- Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. *J Oral Pathol Med.* 2019 Apr;48(4):299-306.
- Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial. *Clin Oral Investig.* 2020 Sep;24(9):1-6. Pubmed PMID: 31955271.
- Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent.* 2021 Mar;31(2):285-286. Pubmed PMID: 32416620.
- R H, Ramani P, Ramanathan A, R JM, S G, Ramasubramanian A, et al. CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2020 Sep;130(3):306-312. Pubmed PMID: 32773350.
- Chandrasekar R, Chandrasekar S, Sundari KKS, Ravi P. Development and validation of a formula for objective assessment of cervical vertebral bone age. *Prog Orthod.* 2020 Oct 12;21(1):38. Pubmed PMID: 33043408.
- Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. *Arch Oral Biol.* 2018 Oct;94:93-98. Pubmed PMID: 30015217.