



ANTIMICROBIAL ACTIVITY OF BOSWELLIA SERRATA EXTRACT AGAINST PERIODONTAL PATHOGENS- AN IN-VITRO STUDY

Periodontology

Dr Jyoti I Pattanashetti	M.D.S, Professor, Department of Periodontics, P.M.N.M. Dental College and Hospital, Bagalkot-587103, Karnataka - INDIA
Dr. Shweta Sonkusle*	Post-graduate, Department of Periodontics, P.M.N.M Dental College and Hospital, Bagalkot-587103, Karnataka - INDIA. *Corresponding Author
Dr Nagaraj B Kalburgi	M.D.S, Professor and Head of Department of Periodontics, P.M.N.M. Dental College and Hospital, Bagalkot-587103, Karnataka - INDIA.
Dr Varsha Singh	Post-graduate, Department of Periodontics, P.M.N.M. Dental College and Hospital, Bagalkot-587103, Karnataka - INDIA.

ABSTRACT

Introduction – Periodontitis being the main cause of tooth loss is caused by periodontopathic bacteria in periodontal tissues. Among them, *Aggregatibacter actinomycetemcomitans*(A.a), *Porphyromonas gingivalis*(P.g), *Prevotella intermedia*(P.i), *Fusobacterium nucleatum*(F.n) and *Tanerella forsythia*(T.f) are considered to be important consortium in the progression of periodontitis. Active component of *Boswellia serrata* inhibits 5-lipo-oxygenase pathway which provides anti-inflammatory & anti-microbial effect.

Aim - To assess antimicrobial activity of *Boswellia serrata* extract against A.a, P.g, P.i, F.n and T.f

Methodology - Bacterial strains, namely *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *Tanerella forsythia* were cultured on Thioglycollate broth. Minimum inhibitory concentration was determined, by serial broth dilution.

Results – *Boswellia serrata* impeded the growth of all selected periopathogens in a dose-dependent manner and suppressed completely at low concentrations of *Boswellia serrata*.

Conclusion – *Boswellia serrata* has a property of antibacterial activity against periodontopathic bacteria and may be effective agent for preventing periodontitis.

KEYWORDS

Boswellia serrata, Frankincense, periodontitis, antimicrobial activity

INTRODUCTION

With the breakthrough of antibiotics, the healthcare community believed that the battle with contagious diseases was victorious. However, it was soon understood that the microorganisms were capable of developing resistance against any kind of antimicrobial drugs in use.¹ Herbal plants have been used since time immemorial for the management of systemic diseases. They are specifically favored on account of their minimal side effects.² In the last few decades, researchers have turned their attention to investigate the potential of traditional medicinal plants for their remedial properties.³

Boswellia serrata has distinct names in numerous languages. It is called as Indian oli-banum, Salai guggul, Frankincense and Sallaki in Sanskrit. Commonly found in dry mountainous regions of India, Northern Africa and Middle East, it is indigenous to Iran.⁴ Since time immemorial, the gum resin of *Boswellia serrata* has been used as incense in religious ceremonies.⁵

Traditionally, the resin extract of *Boswellia serrata* has been used for therapy of various inflammatory diseases like arthritis, diabetes, asthma, inflammatory bowel disease and cancer.⁶ The promising results can be attributed to the anti-inflammatory, antioxidant, immunomodulatory, anti-bacterial, anti-fungal and broad antiviral activity.⁷

Around 12 distinct boswellic acids have been recognized. Out of these, only AKBA (3-O-acetyl-11-keto- β -boswellic acid) and KBA (11-keto- β -boswellic acid) have received pronounced pharmacological heed.⁸

Periodontal disease is a polymicrobial infection resulting from concomitant interaction of different bacterial species. Scaling and root planing is the first recommended step in the management of periodontal diseases, but there are factors such as accessibility or presence of plaque in retentive areas that can limit instrumentation.⁹ Local drug delivery has been advocated to complement the etiopathogenic phase of treatment. Numerous antimicrobials such as metronidazole, chlorhexidine, tetracycline, minocycline and doxycycline are being used to eliminate periodontopathogens. On the contrary, the major disadvantage is the emergence of antimicrobial resistance resulting from extensive utilization of antibiotics.¹⁰

Taking into consideration the anti-inflammatory and antibacterial effects, low-cost, availability of *Boswellia serrata*, this study was designed to assess the antimicrobial effect of *Boswellia serrata* extract against periodontal pathogens.

In the present study, we evaluated the anti-bacterial activity of *Boswellia serrata* extract against periodontopathic bacteria such as *Aggregatibacter actinomycetemcomitans*(ATCC 29523), *Porphyromonas gingivalis*(ATCC 33277), *Prevotella intermedia*(ATCC 25611), *Fusobacterium nucleatum* and *Tanerella forsythia*(ATCC 43037).

MATERIAL AND METHODS

The present study was outlined with the aim to determine Minimum Inhibitory Concentration (MIC) which was accomplished as per the guidelines of Clinical and Laboratory Standards Institute. The bacterial cultures of periodontopathogens employed in this study were obtained from Maratha Mandal's Nathajirao G. Halgekar's Institute of Dental Science & Research Center, Belagavi and were germinated at 37°C to a stationary phase for 24 h.

Stock solution of *Boswellia serrata* extract was concocted in dimethyl sulfoxide to ensure complete solubilization. For obtaining MIC, nine dilutions of each drug were done with Thioglycollate broth.

In the first tube, 20 microlitres (μ l) of drug was added into 380 μ l of Thioglycollate broth. In order to obtain the dilutions, 200 μ l of Thioglycollate broth was added into the next 9 tubes separately. From the initial tube containing the drug, 200 μ l was transferred to the first of 9 tubes accommodating 200 μ l of Thioglycollate broth. The resulting dilution was considered as 10^{-1} dilution. This procedure was replicated until 10^{-9} dilution was obtained for each drug.

A culture suspension was prepared by adding 5 μ l from the maintained stock cultures of required organisms (A.a, P.g, P.i, F.n and T.f) into 2ml of Thioglycollate broth. 200 μ l of prepared culture suspension was added into each serially diluted tube. The tubes were incubated for 48-72 hours in anaerobic jar at 37°C and observed for turbidity. The minimum concentration of the extract showing no turbidity was recorded as MIC.

RESULTS

The Minimum Inhibitory Concentration of *Boswellia serrata* extract against the tested micro-organisms is shown in Table 1

Sl. No.	Samples	100 µg/ ml	50 µg/ ml	25 µg/ ml	12.5 µg/ ml	6.25 µg/ ml	3.12 µg/ ml	1.6 µg/ ml	0.8 µg/ ml	0.4 µg/ ml	0.2 µg/ ml
	Boswellia serrata extract										
01	Pg	S	S	S	S	S	S	S	R	R	R
02	Pi	S	S	S	S	S	S	S	R	R	R
03	Aa	S	S	S	S	S	S	S	R	R	R
04	Tf	S	S	S	S	S	S	S	S	R	R
05	Fn	S	S	S	S	S	S	S	S	R	R

S: Sensitive, R: Resistant

The tests were triplicated to obtain and ensure maximum accuracy. *Boswellia serrata* extract suppressed the growth of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* at a low concentration of 1.6 µg/ml whereas, *Tanarella forsythia* and *Fusobacterium nucleatum* were suppressed at 0.8 µg/ml concentration. The test agent was sensitive for all 5 strains at 1.6 µg/ml.

DISCUSSION

Periodontal diseases are chronic inflammatory diseases of the investing and supporting tissues of the teeth. Plaque biofilm is accountable for the gingival inflammation.¹¹ Around 700 distinct species of bacteria exist in harmony in the oral cavity. *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Tanarella forsythia* and *Fusobacterium nucleatum* are the principal gram negative bacteria which exist in anaerobic environment of the deep periodontal pockets and are responsible for progressive attachment loss.^{12,13} In the present study, we evaluated the minimum inhibitory concentration of *Boswellia serrata* extract against these bacteria with a goal to utilize it as a local drug delivery agent in the forthcoming future. *P. gingivalis* is a Gram-negative, non-motile obligatory anaerobic rod. *P. intermedia* is Gram-negative, non-motile, obligatory anaerobes. *T. forsythia* is an anaerobic, Gram-negative bacterial species in red complex. *A. actinomycetemcomitans* is an immobile microaerophilic, facultative anaerobic, Gram-negative coccoid rod.¹⁴ *F. nucleatum* is a gram-negative anaerobe, an intermediate colonizer bridging the interactions between gram positive & gram-negative organisms.¹⁵

Boswellia serrata extract showed anti-microbial effect against all five periopathogens which is in accordance with a study conducted by Raja et al (2011) where they proved anti-bacterial effect of boswellic acids against all oral cavity pathogens in a dose dependent manner.¹⁶

Maraghehpour et al (2016) concluded that *Boswellia serrata* extract is effective against *A.a* which should be taken into account for the formation of oral hygiene products. The MIC was found to be 512 µg/ml against *A.a*.¹⁷ Recent studies reported by Alluri et al¹⁸ and Goma et al¹⁹ confirmed that *Boswellia serrata* did not exhibit mortality or toxicity in rats up to 2000mg/kg.

Samani et al proved the effects of *Boswellia serrata* in the form of chewing gum in gingivitis among students which may be attributed to anti-inflammatory effect caused by boswellic acids leading to inhibition of pro-inflammatory enzymes.²⁰

Another study conducted by Khoshbakht et al showed significant decrease in GI, PI and Gingival Bleeding Index after 21 days following intervention with mouthwash containing *Boswellia serrata* which may be attributed to its anti-bacterial, anti-biofilm and anti-inflammatory properties.²¹ A review by Cochrane has given contrasting opinion about the anti-inflammatory properties of Boswellic Acids.²²

CONCLUSION

Our study upholds the usage of *Boswellia serrata* as a potential adjuvant with periodontal therapy as a mouthwash, topical gel and local drug delivery agent owing to its low-cost availability, low toxicity, anti-bacterial, anti-plaque, anti-biofilm and anti-inflammatory properties. Although in-vitro and in-vivo studies have already suggested *Boswellia serrata* to be a potential therapeutic anti-inflammatory agent. Further clinical studies are needed to realize its

true potential against various periodontal diseases. Therefore, we conclude that *Boswellia serrata* can be used an adjuvant to standard periodontal therapy in order to overcome the side effects of anti-inflammatory agents.

REFERENCES

- Reygaert WC. An overview of the antimicrobial resistance of bacteria. *AIMS Microbiol.* 2018;4(3):482-501.
- Nadkarni KM, Nadkarni AK. Indian Materia Medica – with Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies. *Popular Prakashan Private Ltd., Bombay, India.* 1999;1:142-9.
- Cowan MM. Plant Products as Antimicrobial Agents. *Clinical Microbiol. Reviews* 1999; 12(4):564-82.
- Kathpalia H, K.K.Shreya. Topical nanoemulgel formulation of *Boswellia serrata*. *Indian J Pharm Sci* 2018; 80(2):261-67.
- Lemenih M, Teketay D. Frankincense and Myrrh resources of Ethiopia: II. Medicinal and Industrial Uses. *Ethiop J Sci* 2003;26(2):161-72.
- Roy NK, Deka A, Bordoloi D, Mishra S, Kumar AP, Sethi GK. The potential role of boswellic acids in cancer prevention and treatment. *Cancer Lett.* 2016;377:74-86
- Goma AA, Mohamed HS, Abd-ellatif RB. Boswellic acids/*Boswellia serrata* extract as a potential COVID-19 therapeutic agent in the elderly. *Inflammapharmacol* 2021;29:1033-48.
- Katragunta K, Siva B, Kondepudi N, Vadaparthi PR, Rama Rao N et al. Estimation of Boswellic acids in herbal formulations containing *Boswellia serrata* extract and comprehensive characterization of secondary metabolites using UPCL-Q-ToF-MS/MS. *J Pharm Anal* 2019;9(6):414-22.
- Theilade E, Wright WH, Jensen SB, Loe H. Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. *J Periodontol Res.* 1966; 1:1-13.
- Da Rocha HA, Silva CF, Santiago FL, Martins LG, Dias PC, Magalhaes. Local drug delivery systems in the treatment of periodontitis: A literature review. *J Int Acad Periodontol* 2015;17(3):82-90.
- Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. *Periodontol* 2000 1997;14:21648.
- Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: Current concepts. *J Periodontol* 1992;63(4):32231
- Slots J, Rams TE. New views on periodontal microbiota in special patient categories. *J Clin Periodontol* 1991;18:41120.
- Bomdya RS, Shah MU, Doshi YS, Shah VA, Khirade SP. Antibacterial activity of curcumin (turmeric) against periopathogens-An in vitro evaluation. *J Adv Clin Res Insights* 2017;4:175-80.
- Signat B, Roques C, Poulet P, Duffuat D. Role of *Fusobacterium nucleatum* in periodontal health and disease. *Curr Issues Mol Biol* 2011;13:25-36.
- Raja AF, Ali F, Khan IA, Shawl AS, Arora DS. Acetyl-11-keto-β-boswellic acid (AKBA); targeting oral cavity pathogens. *BMC Research Notes* 2011;4:40.
- Maraghehpour B, Khayamzadeh M, Najaf S, Kharazifard M. Traditionally used herbal medicines with antibacterial effect on *Aggregatibacter actinomycetemcomitans*: *Boswellia serrata* and *Nigella sativa*. *J Indian Soc Periodontol* 2016;20(6):603-7.
- Alluri VK, Dodda S, Kilari EK. Toxicological assessment of a standardized *Boswellia serrata* gum resin extract. *Int J Toxicol* 2019;38:423-35.
- Gomaa AA, Makboul R, Al-Mokhtar M. Polyphenol-rich *Boswellia serrata* gum prevents cognitive impairment and insulin resistance of diabetic rats through inhibition of GSK3β activity, oxidative stress and pro-inflammatory cytokines. *Biomed Pharmacother* 2019;109:281-92.
- Samani MK, Mahmoodian H, Moghadamnia AA, Bejeh Mir PA, Chitsazan M. The effect of Frankincense in the treatment of moderate plaque-induced gingivitis: A double blinded randomized clinical trial. *Daru* 2011;19(4):288-94.
- Khoshbakht Z, Khashabi E, Khodaie L, Torbati M, Lotfipour F, Hamishehkar H. Evaluation of herbal mouthwashes containing *Zataria Multiflora* boiss, Frankincense and combination therapy on patients with gingivitis: A double blind, randomized, controlled, clinical trial. *Galen Med J* 2019;15:e1366.
- Chande N, McDonald JW, Macdonald JK. Interventions for treating collagenous colitis. *Cochrane Database Syst Rev*;2008:CD003575.