DOI: 10.7860/JCDR/2024/67836.19311

Original Article

Dentistry Section

Assessment of Minimum Inhibitory Concentration and Anti-biofilm Activity of *Plectranthus amboinicus* Solvent Extract against Pure Strains of Putative Periodontal Pathogens: An In-vitro Study

S LAVANYA[1], T JESSICA CHRISTELLA[2], NIZAR AHMED[3], SK BALAJI[4], VAMSI LAVU[5]

ABSTRACT

Introduction: Antiseptic agents used in periodontics as anti-plaque and anti-gingivitis agents are primarily chemical substances such as Bis-biguanide derivatives (chlorhexidine) or essential oils. Herbal derivatives have gained prominence in the recent past due to their activity against putative periodontal pathogens; however, only a few have achieved commercialisation. This study focusses on determining the efficacy of an extract from a widely available herb, Indian mint-*Plectranthus Amboinicus* Methanolic extract (PAM), which has known anti-microbial and anti-inflammatory properties against periodontal pathogens in-vitro.

Aim: To assess the Minimum Inhibitory Concentration (MIC) and anti-biofilm property of PAM solvent extract against pure strains of putative periodontal pathogens, namely *Porphyromonas gingivalis* (American Type Culture Collection-ATCC 33277), *Fusobacterium nucleatum* (ATCC 25586), and *Aggregatibacter actinomycetemcomitans* (ATCC 43718).

Materials and Methods: The extract of PA was prepared using methanol and a Soxhlet extractor. An in-vitro analysis of the MIC and anti-biofilm efficacy of the extract was performed against standard strains of *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans,* and *Fusobacterium nucleatum* using the broth dilution method and microtitre-crystal violet assay, respectively. The MIC activity of the PAM extracts was compared with Chlorhexidine as a standard.

Results: The MIC value of *P. amboinicus* extract was nearly similar to Chlorhexidine as assessed by the broth dilution method. The MIC of *P. amboinicus* extract for A.a and P.g was 0.4 µg/mL, F.n was 0.8 µg/mL, and the Chlorhexidine values against all three periodontal pathogens were 0.2 µg/mL. The anti-biofilm activity of The extract of PAMwas evaluated using the microtitre-crystal violet assay, and the Optical Density (OD) values were reduced after exposure to the extract, with a significant reduction ($p<0.001$) of the biofilm-forming bacteria observed.

Conclusion: The methanol extract of PAM demonstrated a noteworthy MIC, exhibiting effectiveness at a low concentration of 0.4 µg/mL against *Aggregatibacter actinomycetemcomitans* in three repeated trials. Moreover, this extract displayed significant inhibitory effects on the biofilm formation of periodontal pathogens *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* (Pg, Aa, Fn), suggesting its potential as an alternative to conventional chemical anti-microbials.

Keywords: *Aggregatibacter actinomycetemcomitans*, Anti-biofilm, Concentration, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*

INTRODUCTION

Severe periodontal disease ranks as the eleventh most globally prevalent condition, and it is associated with dental plaque biofilm [1]. A biofilm is a consortium of microorganisms attached to a biotic or abiotic surface. Sessile cells within a biofilm exhibit phenotypic and physiological differences compared to planktonic cells [2]. One distinctive property illustrating the contrast between sessile and planktonic cells is the significantly higher concentrations of antimicrobial agents needed to eradicate sessile cells, in comparison to planktonic cells [2]. Dental plaque was the first biofilm studied for its microbial composition and susceptibility to antimicrobial agents [3]. The development of a biofilm such as dental plaque proceeds through a series of events including attachment, growth, removal, and re-attachment of bacteria, resulting in continuous re-organisation [4]. Extracellular polymeric substances impede the mass transport of antibiotics through the biofilm, contributing to antimicrobial resistance [5]. Biofilm bacteria are phenotypically different from planktonic cells, with increased tolerance to antimicrobial agents being a key factor. Microorganisms can develop antibiotic resistance through mutations, the presence of drug efflux pumps, and the production of neutralising enzymes. Even inherently sensitive organisms can exhibit apparent resistance when growing on a biofilm surface [6].

In the recent past, multiple drug resistance has emerged against many microbial infections due to the in-discriminate use of commercial antimicrobial drugs [7]. Furthermore, antibiotics are occasionally linked to adverse effects on the host, including hypersensitivity, immune suppression, and allergic reactions [8]. Therefore, a necessity exists to explore natural alternatives to existing antimicrobial agents by evaluating herbal extracts from alternative systems of medicine such as Ayurveda. Plectranthus, a large genus with over 300 species from the *Lamiaceae* family, exhibits a rich diversity of medicinal uses [9]. *P. amboinicus*, locally known as PA or by names like bangun-bangun, bebangun, hati-hati hijau, or sedingin, is an indigenous vegetable that can be consumed fresh. The leaves of PA are said to possess anti-oxidant, anti-bacterial, anti-microbial, anti-inflammatory, and anti-fungal properties [9]. Sabrina EMN et al., reported that the essential oil of PAM exhibited significant antimicrobial activity and suggested that this activity may be due to the presence of major monoterpenoid compounds,

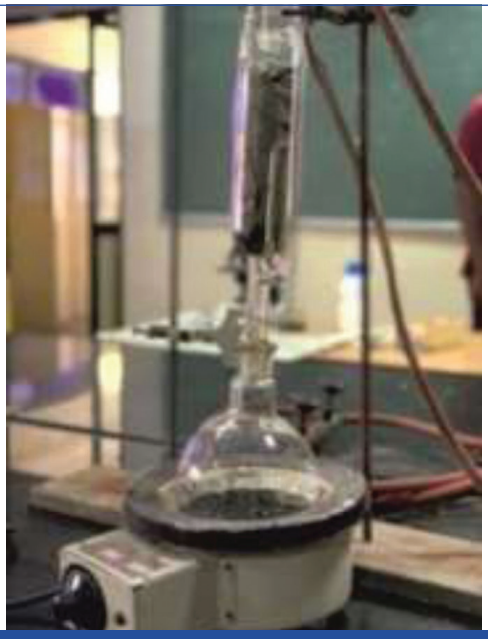
carvacrol, and camphor [9]. Arumugam G et al., evaluated the PA plant and demonstrated a wide range of biological properties, proving its effectiveness in treating respiratory, cardiovascular, oral, skin, digestive, and urinary diseases [10]. Manimekalai K et al., assessed the antibiofilm efficacy of PAM against *Streptococcus pyogenes* isolated from pharyngitis patients and concluded that the methanol extract of *P. amboinicus* leaves contained pharmacologically active components that could be used as an anti-biofilm agent at minimal concentrations, successfully preventing biofilm formation [11]. Sivaranjani D et al., reported that PAM solvent extracts exhibited good antimicrobial activity against most human pathogenic bacteria and only one fungal yeast, *Candida albicans* [12]. The aim of the present study was to assess the MIC of PAM solvent extract against pure strains of putative periodontal pathogens *Porphyromonas gingivalis* (ATCC 33277), *Fusobacterium nucleatum* (ATCC 25586), and *Aggregatibacter actinomycetemcomitans* (ATCC 43718). Additionally, the antibiofilm activity of PAM solvent extract against the selected periodontal pathogens was also evaluated.

MATERIALS AND METHODS

An in-vitro study was conducted between December 2022 and March 2023. The study involved investigators from three departments: the Department of Periodontology at Sri Ramachandra Dental College and Hospital, the Department of Pharmacognosy at Sri Ramachandra Institute of Higher Education in Chennai, and the Research Department of Molecular Biology and Immunology at Maratha Mandal's NGH Institute of Dental Sciences and Research Centre in Belgaum, Karnataka, India. The ethics approval for the study was obtained from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (CSP/22/MAR/106/69).

Commercially available PA dry powder was used (powder collected from Herbal Medicines and Botanicals in Tirunelveli). The PA leaf powdered samples were hermetically sealed in separate polythene bags until the time of extraction. The extract was prepared as mentioned below.

One hundred grams of powdered PA leaves were extracted successively with 400 mL of methanol in a Soxhlet extractor until the extract was clear [Table/Fig-1]. The extracts were subjected to evaporation until dry, and the resultant paste was preserved by storing it in a refrigerator at 4°C for future use [12].



[Table/Fig-1]: Soxhlet extractor used for the preparation of methanolic extract of *Plectranthus Amboinicus* (PAM).

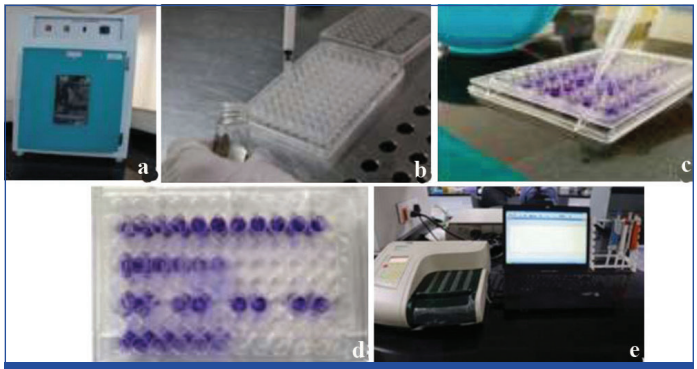
Three bacterial strains (*P. gingivalis* ATCC-33277, *F. nucleatum* ATCC-25586, *A. actinomycetemcomitans* ATCC-43718) were obtained from Maratha Mandal's Central Research Laboratory in Belgaum.

Procedure

Stock preparation was conducted with 10 mg of the extract, which was then dissolved in 10% Dimethyl Sulfoxide (DMSO). The MIC of the plant extract was evaluated following the protocol available in the published literature [13]. In summary, the bacterial strains *P. gingivalis* (ATCC-33277), *F. nucleatum* (ATCC-25586), *A. actinomycetemcomitans* (ATCC-43718) were added to thioglycollate medium with Hemin and Vitamin K supplemented with the PAM or methanolic extract of PA at 10 different dilutions ranging from 0.2 to 100 µg/mL. Three test groups were established: Group-1 (Broth+extract+*Pg*), Group-2 (Broth+extract+*F.n*), Group-3 (Broth+extract+*A.a*). The positive control used was Chlorhexidine+Broth+Organism. The tubes were then incubated for 48-72 hours in an anaerobic jar at 37°C and observed for turbidity [Table/Fig-2]. The experiment was repeated in triplicate.

[Table/Fig-2]: Determination of MIC of methanolic extract of *Plectranthus amboinicus* (PAM) using thioglycolate medium: (a) Thioglycolate broth; (b) Addition of Chlorhexidine to the Eppendorf tube containing the broth with the bacteria; (c) Addition of PAM extract to the Eppendorf tube containing the broth with the bacteria.

A multispecies biofilm was established in a 96-well microtitre plate at baseline using Pg, Fn, Aa, and incubated for three days. The suspensions were removed from all the wells and washed with Phosphate Buffered Saline (PBS) (slowly to remove loosely attached biofilms). To determine anti-biofilm activity, a comparison was made between baseline and after exposure to the extract. Four groups were included in this assay: (i) only broth; (ii) Broth+Extract; (iii) Broth+Mix of organisms; (iv) Broth+Organisms+Extract (test group). The extract was added only in the test group and the broth+extract group, incubated for 30 minutes, and then washed with PBS. A 0.1% crystal violet solution was added to all the wells and incubated for 10 minutes, followed by a PBS rinse. Subsequently, 70% ethanol was added to dissolve all the crystal violet that was attached. This ethanol was collected in another microtitre plate, and OD values were assessed. Readings were taken in a spectrophotometer to determine the OD values [Table/Fig-3].



[Table/Fig-3]: Determination of antibiofilm activity by Microtitre-crystal violet assay: (a) Incubator for the growth of multispecies biofilm; (b) Addition of PBS to incubated microtitre plate; (c) Addition of ethanol to dissolve crystal violet; (d) Varying colour saturation between the groups in the well, and (e) Spectrophotometer to determine OD values.

STATISTICAL ANALYSIS

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 20.0 software. Pearson's Chi-square test and Analysis of Variance (ANOVA) were employed to calculate the differences in the MIC of the PAM as compared to Chlorhexidine and the antibiofilm activity of the PAM against pure

strains of periodontal pathogens, respectively. Values were deemed significantly different if the p-value was ≤0.05.

RESULTS

The MIC was determined for *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans,* and *Fusobacterium nucleatum (P.g, A.a, F.n)* species treated with different concentrations of the PAM using the broth dilution method. The MIC was conducted in triplicates for each group. The MIC of PAM for *A.a* and *P.g* was 0.4 µg/mL, and for *F.n* was 0.8 µg/mL, as indicated by the absence of bacterial growth at these concentrations. The MIC of Chlorhexidine against all three periodontal pathogens was 0.2 µg/mL. The MIC values of the PAM against the three putative periodontal pathogens were lower than Chlorhexidine [Table/Fig-4]. The Chi-square test was used to assess the difference in MIC values between the PAM extract and Chlorhexidine against *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans,* and *Fusobacterium nucleatum (P.g, A.a, F.n)* species. When comparing both interventions, statistical significance could not be demonstrated, with p-values for *Porphyromonas gingivalis being* 0.173, *Aggregatibacter actinomycetemcomitans being* p=0.285, and *Fusobacterium nucleatum* was p=0.032 [Table/Fig-5].

The MIC of 1.6 µg/mL was selected for the antibiofilm assay. The difference in the OD values between the control groups (Broth+compound 0.85±0.03), (Broth+Mix of organisms 1.99±0.01), and the test group (Broth+Organisms+Extract 0.90±0.08) was assessed by ANOVA and was found to be statistically significant (p-value=0.0001) [Table/Fig-6].

DISCUSSION

The null hypothesis of the present study was that the PAM extract would not exhibit antimicrobial effects against the chosen periodontal pathogens. However, based on the aforementioned results, the null hypothesis stands rejected. Bacterial biofilm, considered the fundamental cause of periodontitis, is associated with poor oral hygiene as it promotes bacteria accumulation [5]. To limit bacterial resistance to antibiotics, antimicrobial agents from plant extracts are emerging as a potential mode to control the progression of periodontitis, as they demonstrate significant abilities to restrict bacterial biofilm formation and reduce the virulence of colonising microorganisms [14]. Despite Chlorhexidine being universally recognised as the standard antimicrobial agent in periodontal treatment regimens, it can cause side effects such as teeth staining and altered taste sensation when used over an extended period [15]. Plant extract-derived antimicrobial agents have been increasingly utilised on a global scale. Studies by Choi HA et al., and Mehdipour A et al., on the use of plant extracts as antibacterial and antibiofilm agents have illustrated the resurgence of medicinal plant extracts in dentistry, showcasing their efficacy and safety as alternatives to synthetic compounds, which may exhibit certain side effects with long-term use [16,17].

Literature exists highlighting the anti-bacterial, anti-inflammatory, and anti-oxidant properties of PAM [18-22]. Leu WJ et al., reported in an in-vitro study that a *P. amboinicus* extract (PA-F4) exhibited inhibitory activity on the activation of the NLRP3 inflammasome and also inhibited ATP-induced signaling pathways in LPS-primed

S. No.	Broth+organism+varied concentrations of PAM extract/CHX	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
P.g (1)											
1	PAM 01	S	S	S	S	S	S	S	S	S	R
2	PAM 02	S	S	S	S	S	S	S	S	R	R
3	PAM 03	S	S	S	S	S	S	S	S	S	R
4	CHX	S	S	S	S	S	S	S	S	S	S
F.n (2)											
1	PAM 01	S	S	S	S	S	S	S	R	R	R
2	PAM 02	S	S	S	S	S	S	S	S	R	R
3	PAM 03	S	S	S	S	S	S	S	S	R	R
4	CHX	S	S	S	S	S	S	S	S	S	S
A.a(3)											
1	PAM 01	S	S	S	S	S	S	S	S	S	R
2	PAM 02	S	S	S	S	S	S	S	S	S	R
3	PAM 03	S	S	S	S	S	S	S	S	S	R
4	CHX	S	S	S	S	S	S	S	S	S	S

[Table/Fig-4]: Antibacterial efficacy of varied concentrations of *P. amboinicus* methanolic extract (PAM) (in triplicate) against chosen periodontal pathogens with chlorhexidine as positive control.
*Aa: Aggregatibacter actinomycetemcomitans; Fn: Fusobacterium nucleatum; PAM: Methanolic extract of Plectranthus amboinicus; Pg: Porphyromonas gingivalis; S: Sensitive; R: Resistant; CHX: Chlorhexidine

	Minimum Inhibitory Concentration (MIC) for test group (µg/mL)	Minimum Inhibitory Concentration (MIC) for control group chlorhexidine (µg/mL)	p-value
Aggregatibacter actinomycetemcomitans	0.4	0.2	0.285
Fusobacterium nucleatum	0.8	0.2	**0.032**
Porphyromonas gingivalis	0.4	0.2	0.173

[Table/Fig-5]: Tabular representation of antimicrobial activity of the P.amboinicus solvent extract and Chlorhexidine in broth dilution method.
*Aa: Aggregatibacter actinomycetemcomitans; CHX: Chlorhexidine; Fn: Fusobacterium nucleatum; MIC: Minimum inhibitory concentration; Pg: Porphyromonas gingivalis

Number of repeats	Experiment 1	Experiment 2	Experiment 3	Mean+Standard deviation	p-value*
Group-1-Only Broth	0.195	0.191	0.190	0.19±0.00	**0.0001**
Group-2-Broth+extract	0.826	0.894	0.854	0.85±0.03	
Group-3-Broth+organisms	1.978	2.010	1.984	1.99±0.01	
Group-4-Broth+organisms+extract	0.907	1.103	1.008	0.90±0.08	

[Table/Fig-6]: Optical density values in varying groups (Group-1-Only Broth), (Group-2-Broth+compound), (Group-3-Broth+Mix of organisms), (Group-4-Broth+Mix of Organisms+extract).

cells [18]. Swamy MK et al., demonstrated the presence of various soluble bioactive compounds in *P. amboinicus* leaf extracts contributing significantly to antioxidant and antimicrobial activities [19]. The authors also noted that the methanolic extract contains more soluble phytocompounds, supporting the traditional medicinal use of *P. amboinicus* in treating various diseases [19]. Therefore, in the present study, the authors prepared a methanol extract of *P. amboinicus*.

An in-vitro study by Nazliniwaty N and Laila L, reported that the antibacterial activity test of the 2% PA Spreng mouthwash successfully inhibited the growth of both *Staphylococcus aureus* and *Streptococcus mutans* [20]. Some studies have identified active compounds such as p-cymene, thymol, β-caryophyllene, γ-terpinene, and secondary metabolites such as steroids, tannins, flavonoids, and alkaloids in extracts of PA (Lour) that have demonstrated anti-bacterial efficacy against *Staphylococcus aureus* [21,22].

In the present study, key periodonto-pathogens such as *Porphyromonas gingivalis* (ATCC 33277), *Aggregatibacter actinomycetemcomitans* (ATCC 43718), and the bridging organism *Fusobacterium nucleatum* were chosen among the various putative periodontal pathogens. The antimicrobial activities of the extract were analysed by assessing MIC, after exposing the selected bacteria in the broth to *P. amboinicus* solvent extract at various dilutions. The present study results have shown that the MIC of PAM against *P. gingivalis* and *A. actinomycetemcomitans* was similar to chlorhexidine (CHX) and less effective against *F. nucleatum*. These results are consistent with the observations of Thaniarasu R et al., where the authors elucidated that the MIC of ethanolic extracts of *Plectranthus bourneae* possess antibacterial activities against certain Gram-positive and Gram negative organisms [23]. A recent study by Chandra K et al., demonstrated that the ethanolic extract of *P. amboinicus* was effective at a low concentration of 50 µg/mL against four periodontopathogenic bacteria, namely *Aggregatibacter actinomycetemcomitans (Aa)*, *Prevotella intermedia (Pi)*, *Fusobacterium nucleatum (Fn)*, and *Porphyromonas gingivalis (Pg)* [24]. However, *Pg, Fn,* and *Pi* organisms were found to be resistant at concentrations lower than 0.2 µg/mL, and *Aa* was found to be resistant at concentrations lower than 25 µg/mL. The present study results contradict the findings of Chandra K et al., where the MIC of the methanolic extract of *P. amboinicus* against *A. actinomycetemcomitans* was reported to be 0.2 µg/mL [24]. The discrepancies in the results may be attributed to differences in the methods of preparation of the *P. amboinicus* extract used in the two studies.

Biofilm formation is crucial for the development of periodontal disease. The crystal violet microtiter assay was used to quantify the formed biofilm based on the optical density obtained. The antibiofilm activity of PAM was assessed at the chosen MIC of 1.6 µg/mL (this concentration was where all three periodontal pathogens assessed in this study showed sensitivity to the PAM). The extract-treated cells (Broth+organisms+extract) exhibited disorganisation of the biofilm, as identified by the reduced OD values compared to the control group (Broth+mix of organisms). This finding is in agreement with the observations of the previous study by Manimekalai K et al., where the authors demonstrated that the PAM extract inhibited the biofilm biomass significantly ($p<0.05$) in a dose-dependent manner at sub-MIC levels against *S. pyogenes* [11]. Similar findings were reported by Harini K et al., against *Streptococcus mutans* using the aqueous extract of *Vaccinium oxycoccos* and PAM [25]. To the best of our knowledge, the present study is the first to provide evidence of the antibiofilm activity of PA against selected periodontal pathogens.

The clinical implications of the study observations indicate that PAM extracts can be incorporated into mouthwashes and dentifrices as part of patient maintenance. *P. amboinicus* may serve as an effective alternative to synthetic antimicrobials like Chlorhexidine, which have long-term side effects.

Limitation(s)

As this is an in-vitro study, additional research efforts are required to apply the findings in in-vivo conditions. Since periodontitis is a polymicrobial disease, further detailed investigations should be aimed at understanding the effectiveness of the PAM extract in a multispecies community.

CONCLUSION(S)

In conclusion, the PAM demonstrated a noteworthy MIC, showing effectiveness at a low concentration of 0.4 µg/mL against *Aggregatibacter actinomycetemcomitans* in three repeated trials. Furthermore, this extract exhibited significant inhibitory effects on the biofilm formation of periodontal pathogens *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum*, suggesting its potential as an alternative to conventional chemical antimicrobials. Additionally, these results are the first of their kind in elucidating the role of the well known medicinal plant *P. amboinicus* in controlling biofilm formation by periodontal pathogens. However, further research is necessary to develop formulations containing *P. amboinicus* solvent extracts and to conduct human clinical trials to assess the efficacy of reducing biofilm formation by periodontal pathogens.

REFERENCES

[1] Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global prevalence of periodontal disease and lack of its surveillance. Scientific World Journal. 2020;2020:2146160. Doi: 10.1155/2020/2146160. PMID: 32549797; PMCID: PMC7275199.

[2] Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358(9276):135-38. Doi: 10.1016/s0140-6736(01)05321-1. PMID: 11463434.

[3] Marsh PD. Dental plaque as a microbial biofilm. Caries Res. 2004;38(3):204-11. Doi: 10.1159/000077756. PMID: 15153690.

[4] Marsh PD, Bradshaw DJ. Microbiological effects of new agents in dentifrices for plaque control. Int Dent J. 1993;43(4 Suppl 1):399-406. PMID: 8282422.

[5] Marsh PD. Dental plaque as a biofilm and a microbial community-implications for health and disease. BMC Oral Health. 2006;6(Suppl 1):S14. Doi: 10.1186/1472-6831-6-S1-S14. PMID: 16934115; PMCID: PMC2147593.

[6] Gilbert P, Maira-Litran T, McBain AJ, Rickard AH, Whyte FW. The physiology and collective recalcitrance of microbial biofilm communities. Adv Microb Physiol. 2002;46:202-56. PMID: 12073654.

[7] Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, et al. Antibiotic resistance: A rundown of a global crisis. Infect Drug Resist. 2018;11:1645-58. Doi: 10.2147/IDR.S173867. PMID: 30349322; PMCID: PMC6188119.

[8] Upadhyay RK. Tulsi: A holy plant with high medicinal and therapeutic value. International Journal of Green Pharmacy. 2017;11(1):S1-S12.

[9] Sabrina EMN, Razali M, Mirfat AHS, Mohd Shukri MA. Antimicrobial activity and bioactive evaluation of Plectranthus amboinicus essential oil. Am J Res Commun. 2014;2(12):121-27.

[10] Arumugam G, Swamy MK, Sinniah UR. Plectranthus amboinicus (Lour.) Spreng: Botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016;21(4):369. Doi: 10.3390/molecules21040369. PMID: 27043511; PMCID: PMC6274163.

[11] Manimekalai K, Srinivasan P, Dineshbabu J, Guna G, Teepica Priya Darsini D. Anti-biofilm efficacy of Plectranthus amboinicus against streptococcus pyogenes isolated from pharyngitis patients. Asian J Pharm Clin Res. 2016;9(4):348-54.

[12] Sivaranjani D, Saranraj P, Manigandan M, Amala K. Antimicrobial activity of Plectranthus amboinicus solvent extracts against Human Pathogenic Bacteria and Fungi. J Drug Deliv Ther. 2019;9(3):36-39.

[13] Schwalbe R, Steele-Moore L, Goodwin AC. (Eds.). (2007). Antimicrobial Susceptibility Testing Protocols (1st ed.). CRC Press. Available from: https://doi.org/10.1201/9781420014495.

[14] Joseph RA, Sabarish R, Muthukumar S, Bhat K, Balaji SK. Comparative evaluation on the effect of herbal mouthwash on putative periodontal pathogens-In-vitro study. RJPT. 2023;16(1):97-102.

[15] McCoy LC, Wehler CJ, Rich SE, Garcia RI, Miller DR, Jones JA. Adverse events associated with chlorhexidine use: results from the department of veterans affairs dental diabetes study. J Am Dent Assoc. 2008;139(2):178-83. Doi: 10.14219/jada.archive.2008.0134. PMID: 18245686.

[16] Choi HA, Cheong DE, Lim HD, Kim WH, Ham MH, Oh MH, et al. Antimicrobial and Anti-Biofilm Activities of the Methanol Extracts of Medicinal Plants against Dental Pathogens Streptococcus mutans and Candida albicans. J Microbiol Biotechnol. 2017;27(7):1242-48. Doi: 10.4014/jmb.1701.01026. PMID: 28478657.

[17] Mehdipour A, Ehsani A, Samadi N, Ehsani M, Sharifinejad N. The antimicrobial and antibiofilm effects of three herbal extracts on Streptococcus mutans compared with Chlorhexidine 0.2% (in-vitro study). J Med Life. 2022;15(4):526-32. Doi: 10.25122/jml-2021-0189. PMID: 35646170; PMCID: PMC9126453.

[18] Leu WJ, Chen JC, Guh JH. Extract from plectranthus amboinicus inhibit maturation and release of interleukin 1β through inhibition of NF-κB nuclear translocation and NLRP3 inflammasome activation. Front Pharmacol. 2019;10:573. Doi: 10.3389/fphar.2019.00573. PMID: 31191313; PMCID: PMC6546882.
[19] Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM, Sinniah UR. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of malaysian plectranthus amboinicus leaves. Evid Based Complement Alternat Med. 2017;2017:1517683. Doi: 10.1155/2017/1517683. Epub 2017 Mar 23. PMID: 28424737; PMCID: PMC5382359.
[20] Nazliniwaty N, Laila L. Formulation and antibacterial activity of plectranthus amboinicus (lour.) spreng leaves ethanolic extract as herbal mouthwash against halitosis caused bacteria. Open Access Maced J Med Sci. 2019;7(22):3900-03. Doi: 10.3889/oamjms.2019.529. PMID: 32128001; PMCID: PMC7048360.
[21] Costa J, Pereira C, Rodrigues F, De Lima S. Chemical composition, antibacterial and fungicidal activities of leaf oil of plectranthus amboinicus (Lour.) Spreng. J Essent Oil Res. 2010;22(2):183-85.
[22] Ismayil S, Nimila PJ. Antimicrobial activity of plectranthus amboinicus (lour.) Against gram negative bacteria klebsiella pneumoniae and shigella flexneri and their phytochemical tests. Int J Health Sci Res. 2019;9(5):304-11.
[23] Thaniarasu R, Senthil Kumar T, Abubacker MN, Rao MV. Preliminary phytochemical screening and evaluation of antibacterial activity of different solvent extracts of Plectranthus bourneae Gamble (Lamiaceae). Asian J Pharm Clin Res. 2015;8(1):79-82.
[24] Chandra K, Thomas R, Shah R, Mehta DS. Efficacy of Plectranthus Amboinicus (Indian Borage) gel as an adjunct to scaling in management of patients with chronic gingivitis. WJPMR. 2021;7(1):163-68.
[25] Harini K, Janani K, Teja KV, Mohan C, Sukumar M. Formulation and evaluation of oral disintegrating films using a natural ingredient against Streptococcus mutans. J Conserv Dent. 2022;25(2):128-34. Doi: 10.4103/jcd.jcd_143_21. Epub 2022 May 4. PMID: 35720822; PMCID: PMC9205350.

PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Student, Department of Periodontics, Sri Ramachandra Dental College and Hospital, Chennai, Tamil Nadu, India.
2. Postgraduate Student, Department of Periodontics, Sri Ramachandra Dental College and Hospital, Chennai, Tamil Nadu, India.
3. Associate Professor, Department of Periodontics, Sri Ramachandra Dental College and Hospital, Chennai, Tamil Nadu, India.
4. Professor, Department of Periodontics, Sri Ramachandra Dental College and Hospital, Chennai, Tamil Nadu, India.
5. Professor, Department of Periodontics, Sri Ramachandra Dental College and Hospital, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Vamsi Lavu,
Professor, Department of Periodontology, Sri Ramachandra Dental College and Hospital, Porur, Chennai-600116, Tamil Nadu, India.
E-mail: vamsilavu@sriramachandra.edu.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 09, 2023
- Manual Googling: Dec 12, 2023
- iThenticate Software: Feb 14, 2024 (15%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 09, 2023**
Date of Peer Review: **Dec 14, 2023**
Date of Acceptance: **Feb 16, 2024**
Date of Publishing: **Apr 01, 2024**