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Harnessing the potential of botanical extracts in inhibiting *Porphyromonas gingivalis* and alleviating Periodontitis and Rheumatoid arthritis symptoms

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ABSTRACT
Periodontitis(PD),a bacterial induced inflammatory disease of the periodontium, is commonly observed in Rheumatoid arthritis(RA) patients. The potential trigger of the disease is Porphyromonas gingivalis(Pg),a Gram negative anaerobe. This study aimed to evaluate the efficacy of herbal extracts against the pathogen to overcome the emergence and spread of antibiotic resistance in pathogens. In this study,200 patients(50 each) were selected and grouped into four categories(PD+RA+,PD+RA-,PD-RA+, Healthy individuals). P.gingivalis was isolated anaerobically from subgingival samples of the respective patients. Further antimicrobial potential of twenty-five herbal extracts were screened against fifteen isolates of P.gingivalis and one reference strain of P.gingivalis ATCC33277 being used throughout the study. The agar well diffusion method was used to study the antimicrobial properties of ethanolic herbal extracts..The results showed that the samples from 42.0% of the total subjects of each group had presence of P. gingivalis, the PD and RA group showed presence of P. gingivalis in 32.0% samples. However, the healthy subjects did not have presence of P. gingivalis. The mean diameter of inhibition zone between different categories was compared using one-way analysis of variance(ANOVA).The screening of herbal extracts resulted that out of twenty five extracts, five extracts (Zinziber officinale, B. propolis, Psidium guajava, Phyllanthus emblica and Eucalyptus globules) exhibited excellent antimicrobial properties against P.gingivalis. The results of ANOVA revealed that there is statistically no difference in the average zone of inhibition value of different herbal extracts.Thus, this study concluded that these extracts could act as a possible sources for preparation of herbal products to control infection caused by P. gingivalis.
Keywords:*Antimicrobial agent, Zinziber officinale, Phyllanthus emblica, Porphyromonas gingivalis, Periodontitis, Rheumatoid arthritis.*

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INTRODUCTION

Periodontitis is a persistent inflammatory condition that results in the loss of teeth and deterioration of the gums and bone surrounding them. Clinically, dental plaque is described as a resilient, structured material with a yellow-grayish appearance that firmly attaches to the hard surfaces inside the mouth. Dental plaque is a potential risk factor for the development of periodontal diseases. The primary method of preventing periodontal disease and gingivitis is the thorough removal of plaque from the gum area[1]. Periodontitis is caused by various microorganisms, including a key pathogen called *Porphyromonas gingivalis* (Pg)[2]. This bacterium is a gram-negative, rod-shaped, obligate anaerobe that resides in the oral cavity[3]. Pg possesses the ability to evade the host's immune response and spread from cell to cell, leading to chronic inflammation in different organs outside the mouth[4]. Infection with Pg has been linked to conditions such as cardiovascular disease, diabetes mellitus, respiratory infections, rheumatoid arthritis, osteoporosis, obesity, and preterm birth[5].Periodontitis and Rheumatoid arthritis (RA) are both common chronic inflammatory diseases that share similar mechanisms of pathogenesis mediated by the host's immune response[6].

Periodontitis is characterized by the destruction of both soft and hard tissues surrounding the teeth, eventually resulting in tooth loss[7]. On the other hand, RA involves the destruction of cartilage and bone in the joints, facilitated by similar bone-resorptive cytokines and proteinases[6,8]. Ultimately, both conditions cause significant morbidity. The infection caused by *P. gingivalis* is a significant concern in

clinical settings due to its numerous associations with potentially life-threatening diseases. Previous reviews and clinical studies have established a link between *P. gingivalis* infection and Rheumatoid arthritis(RA)[9]. Furthermore, periodontal disease (PD) has been identified as a statistically significant risk factor for autoimmune diseases such as RA[10,11]. Recent studies have focused on investigating the pathogenic association between PD and RA, particularly emphasizing the role of *P. gingivalis*. Currently, PD is typically treated with a combination of systemic and local antibiotics, with occasional surgical intervention to address deep-pocket inflammation[12]. There are several antimicrobial drugs available that have demonstrated positive clinical outcomes in improving conditions caused by anaerobic bacteria, including *P. gingivalis*. Recommended antibiotics for PD treatment include metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin, tetracycline, and fluoroquinolones[13,14].

One of the primary challenges in managing oral infections is drug resistance, which arises due to the formation of biofilms by dental plaque. To overcome drug resistance observed in oral pathogens, herbal-based therapies have been documented. Natural products, particularly herbal extracts, have attracted attention as potential sources for the treatment of oral infections. Numerous medicinal plants have been recognized as valuable resources for natural antimicrobial compounds, offering alternative options for effectively treating bacterial infections[15]. Identifying promising herbal extracts as antimicrobial agents represents the initial step in utilizing these extracts optimally against oral pathogens. Many plants have been utilized due to their inherent antimicrobial properties, attributed to the phytochemicals synthesized in their secondary metabolism[16,17]. Plants contain a wide variety of secondary metabolites, such as tannins, alkaloids, phenolic compounds, and flavonoids, which have demonstrated in vitro antimicrobial properties[18,19]. Assessing the antimicrobial efficacy of herbal extracts against oral cavity colonizers could provide an effective strategy for preventing periodontal diseases and managing RA. Thus, the aim of the present study was to evaluate the in vitro antimicrobial effectiveness of various herbal extracts against clinical isolates of *P. gingivalis* and a reference strain of *P. gingivalis* ATCC33277.

MATERIAL AND METHODS

Selection of individuals

The purpose of this research was to investigate the antimicrobial properties of different plant extracts against *P. gingivalis*. To isolate the pathogen, a total of 200 patients from four distinct categories were selected. The patient's ages ranged from 30 to 59 years, with a mean age of 41.8 years. Females constituted the majority, accounting for 58.6% of the participants. Each group consisted of 50 individuals. The groups were categorized as follows:

1. RA Group - Rheumatoid Arthritis without Periodontitis
2. PD Group - Periodontitis without Rheumatoid Arthritis
3. PD and RA Group - Periodontitis and Rheumatoid Arthritis patients
4. Healthy Group (Control group)

The exclusion criteria for Periodontitis excluded individuals who had been diagnosed with diabetes, HIV infection, or were pregnant. Additionally, those who were using immunosuppressant medications, any form of tobacco, or had received antibiotic therapy within the last 3 months were also excluded from the study. Patients who underwent antibiotic treatment or dental procedures for any reason during the study period were also excluded. The inclusion criteria consisted of the presence of gingival inflammation, bleeding upon probing, and probing depth of ≥5 mm.

Regarding Rheumatoid arthritis patients, the exclusion criteria included a history of total joint replacement within the last 24 months. The inclusion criteria consisted of morning stiffness around the joint, swelling in the wrist joint, and the evaluation of patients through X-ray films.

Collection of Subgingival plaque sample and bacterial microbiology

The participants for this study were selected from private Dental clinics and the Department of Periodontology in Government Dental colleges in and around the Nagpur region. Subgingival plaque samples were collected from the active sites of periodontal patients, Rheumatoid arthritis patients, and healthy individuals using a universal curette inserted into the periodontal pocket. The collected subgingival plaque samples were directly placed into 2 ml of Brucella broth (supplemented with 0.4 µl/ml vitamin K1 and 5 µl/ml hemin). They were then diluted and plated onto Trypticase soy agar (supplemented with 10% defibrinated horse blood, 5 µl/ml hemin, and 0.4 µl/ml vitamin K1). The plates were incubated in a Mclntosh and Filde's anaerobic jar for a period of 7 to 10 days.

Following the incubation period, the plates were examined for colony characteristics, such as pigmentation. Species identification was carried out through biochemical tests, including IMViC test, catalase, indole, and nitrate reductase. Culturing the subgingival samples on blood agar plates in the anaerobic jar resulted in colonies with black pigmentation, which were observed to have haemolytic

zones around them. Once the species identification of *P. gingivalis* was confirmed, the antimicrobial activity of twenty-five herbal extracts on the isolated pathogen was evaluated using the agar well diffusion method.

Molecular characterization of *P.gingivalis*

To conduct molecular identification of the clinical isolate, 16S rDNA sequence analysis was employed. Genomic DNA was extracted from an overnight bacterial culture using the Genei Pure Bacterial DNA purification kit from GeNei Laboratories Pvt. Ltd, India. The extraction protocol provided by the manufacturer was followed accordingly. Amplification of the 16S rDNA region was carried out using the isolated DNA and universal primers specific to the 16S rRNA gene. GeNei PCR Mastermix, containing recombinant Polymerase from GeNei Laboratories Pvt. Ltd, India, was utilized for the amplification process. The thermal cycler used for PCR was the T100 system from Biorad, USA.

Following amplification, sequencing reactions were performed using the ABI 3500XL system and the Big Dye Terminator version 3.1 cycle sequencing Kit from Thermo Fisher Scientific, USA. The obtained sequencing data were subsequently processed and analyzed using Mega X software and BLASTn. These tools were used to identify the bacterium and determine its closest genetic match.

Preparation of plant extracts

A total of twenty-five different herbs were selected to evaluate their anti-*P. gingivalis* activity. The selected plant species included Ginger (*Zinziber officinale),* Fennel seed (*Foeniculum vulgare*), Betel leaf (*Piper Betel*), Cinnamon (*Cinnamomum verum*), Bee propolis (*B.propolis*), Mushroom (*Agaricus bisporus*), Green tea (*Camellia sinensis*), Mulethi (*Glycyrrhiza glabra*), Amla (*Phyllanthus emblica*), Eucalyptus (*Eucalyptus globulus*), Guava (*Psidium guajava*), Clove (*Syzygium aromaticum*), Black pepper (*Piper nigrum*), Harad (*Terminalia chebula*), Bay leaves (*Laurus nobilis*), Pomegranate (*Punica granatum*), Turmeric (*Curcuma*), Aloevera (*Aloe barbadensis miller*), Papaya seed (*Carica papaya*), Jamun seed (*Syzygium cumini* L.), Garlic (*Allium sativum*), Triphala (*Emblica officinalis*), Giloy stem (*Tinospora cordifolia*), Safaed museli (*Chlorophytum borivilianum*), and Ashwagandha roots (*Withania somnifera*).

The herbal powders of the selected plants were purchased from a local market (Bhatia Herbals, Jabalpur). Each product (50 gms) was packed in muslin cloth and subjected to continuous hot extraction using a Soxhlet extractor with absolute ethanol for 72 hours. The ethanolic extracts were filtered first through muslin cloth and then through Whatman-1 filter paper. The filtrate was further processed by vaporization under reduced pressure and temperature using a rotary evaporator (Buchi Rotavapor R-200). The resulting dried plant extracts were re-dissolved in ethanol at a concentration of 0.2 g/ml and used for the antibacterial susceptibility assay.

Antimicrobial susceptibility assay of Ethanolic extract on *P.gingivalis*

The agar well diffusion method is a commonly used technique to assess the antimicrobial activity of herbal extracts. To perform this method, a lawn culture of P. gingivalis was prepared on blood agar plates using a sterilized cotton swab. Subsequently, 6mm diameter wells were aseptically punched into the agar using a sterile cork borer. Freshly prepared extracts, measuring 50 µL, were added to each well. The plates were then placed in a McIntosh and Fildes anaerobic jar and incubated for 5 to 7 days.

After the incubation period, bacterial colonies were observed on the surface of the agar plates, except in areas surrounding the wells where growth inhibition occurred. Extracts that resulted in clear zones around the wells were considered to have an inhibitory effect on the bacterium, indicating antimicrobial activity. Conversely, extracts that did not produce clear zones were considered to have no inhibitory effect on the bacterium.

Statistical Analysis of Data

The collected data were subjected to various statistical analyses using appropriate tests. Descriptive statistics, including frequency, mode, percentage, mean, standard deviation, standard error, and more, were calculated to summarize the data. To compare different groups, a one-way analysis of variance (ANOVA) was employed. The chosen level of significance was set at 0.05 (or 5%).

For conducting the statistical analysis, the Statistical Package for Social Sciences (SPSS) software version 18.0 was utilized. This software provided the necessary tools and functions to perform the statistical calculations and generate the required results based on the collected data.

RESULTS

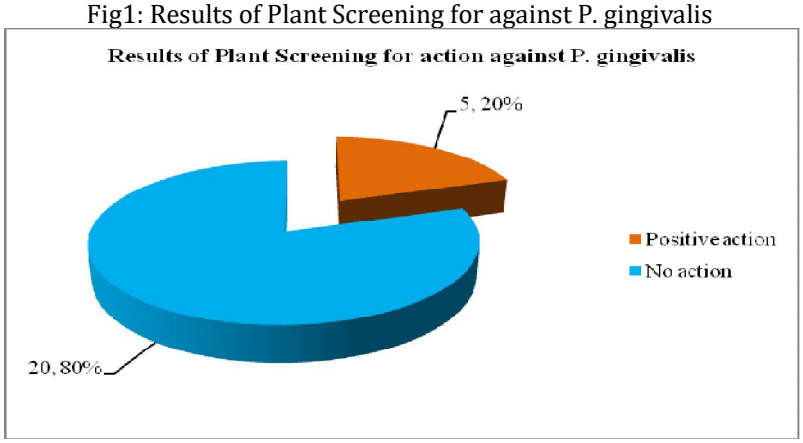
Out of the total subjects, 42.0% were found to have the presence of *P. gingivalis* in their subgingival samples. Among the PD and RA groups, 32.0% of the samples showed the presence of *P. gingivalis*. However, the healthy subjects did not exhibit the presence of the pathogen. A total of fifteen isolates of *P. gingivalis* were included in the study. The cultivation of the samples on blood agar plates within an anaerobic jar resulted in the growth of colonies with black pigmentation, accompanied by haemolytic zones. The identification of the desired bacteria was confirmed through biochemical tests, where the

results showed negative catalase, positive indole, negative urease, negative motility, and positive glucose fermentation. Molecular characterization through 16S rDNA sequence analysis further confirmed the identity of the isolated bacteria.

Among the various plant extracts tested, *Zinziber officinale, B. propolis, Psidium guajava, Phyllanthus emblica*, and *Eucalyptus globulus* extracts exhibited the highest zone of inhibition against the tested pathogen. On the other hand, extracts from *Withania somnifera, Cinnamomum verum, Glycyrrhiza glabra, Syzygium aromaticum, Piper nigrum, Foeniculum vulgare, Piper Betel, Agaricus bisporus, Camellia sinensis, Terminalia chebula, Laurus nobilis, Curcuma, Aloe barbadensis miller, Punica granatum, Carica papaya, Syzygium cumini L, Allium sativum, Emblica officinalis, Tinospora cordifolia,* and *Chlorophytum borivilianum* were found to be inactive against the bacteria.

The screening of plant extracts for their antibacterial activity against P. gingivalis revealed that among the total fifteen isolates, 66.6% of them were sensitive to the extracts of *Zinziber officinale, B. propolis, Psidium guajava,* and *Phyllanthus emblica*. Furthermore, 53.3% of the isolates showed sensitivity to the extract of Eucalyptus globulus.

Fig1: Results of Plant Screening for against P. gingivalis



Antibacterial action of herbal extracts against *P. gingivalis*

Table 1: Screening of herbal extracts as antibacterial agents against Reference strain (***P. gingivalis* ATCC33277)** and *P. gingivalis* isolates.

Plant extract	**Zone of inhibiton (in mm) of Reference strain *P. gingivalis* ATCC33277**	**Average zone of inhibition (in mm) of *P. gingivalis* isolates from patients**
Zinziber officinale	24	**20.5±1.4**
Foeniculum vulgare	Resistant	Resistant
Piper Betel	Resistant	Resistant
Cinnamomum verum	16	Resistant
B. propolis	25	**19.3±2.8**
Agaricus bisporus	Resistant	Resistant
Camellia sinensis	Resistant	Resistant
Glycyrrhiza glabra	20	Resistant
Phyllanthus emblica	20	**18.2±1.6**
Eucalyptus globulus	22	**19.2±1.3**
Psidium guajava	16	**18.0±2.4**
Syzygium aromaticum	19	Resistant
Piper nigrum	16	Resistant
Terminalia chebula	Resistant	Resistant
Laurusnobilis	Resistant	Resistant
Curcuma	Resistant	Resistant
Aloe barbadensis miller	Resistant	Resistant
Punica granatum	Resistant	Resistant
Carica papaya	Resistant	Resistant
Syzygiumcumini L.	Resistant	Resistant
Allium sativum	Resistant	Resistant
Emblica officinalis	Resistant	Resistant
Tinospora cordifolia	Resistant	Resistant
Chlorophytum borivilianum	Resistant	Resistant
Withania somnifera	20	Resistant

Table 2: Descriptive Statistics (one- way ANOVA) for comparison of extent of antibacterial action of various plant extracts against *P. gingivalis.*

Plant (extracts)	Mean	±SD	SE	95% CI for Mean		Min	Max	F ratio and p
				LB	UB			
Zinziber officinale	20.5	±1.4	0.5	19	22	18	22	2.521 p>0.05 (NS)
B. propolis	19.3	±2.8	0.9	17	21	16	24	
Psidium guajava	18.0	±2.4	0.8	16	20	14	22	
Phyllanthus emblica	18.2	±1.6	0.5	17	19	16	20	
Eucalyptus globulus	19.2	±1.3	0.4	18	20	18	22	

SD: Standard deviation; **SE**: Standard error; **CI**: Confidence interval; **LB**: Lower bound; **UB**: Upper bound; **Min**: Minimum; **Max**: Maximum; **p**: Probability; **NS**: Not significant

Table 2 represents the result of statistical analysis of various outcomes. The statistical analysis of the zone of inhibition data for different plant extracts indicated the following average values: *Zinziber officinale* extract had an average zone of inhibition of 20.5 ± 1.4 mm, *B. propolis* extract had an average zone of inhibition of 19.3 ± 2.8 mm. Additionally, the average zone of inhibition for *Psidium guajava, Phyllanthus emblica*, and *Eucalyptus globulus* extracts were 18.0 ± 2.4 mm, 18.2 ± 1.6 mm, and 19.2 ± 1.3 mm, respectively. Moreover, the results of the comparative assessment using ANOVA showed no statistically significant difference in the average zone of inhibition values among the various plant extracts. This suggests that the extent of antibacterial action was relatively similar for all the herbal extracts derived from the mentioned plants.

DISCUSSION

The association between oral microbial flora and infectious oral diseases, such as dental caries and periodontal disease, is widely recognized. Among these conditions, periodontal disease is primarily linked to anaerobic Gram-negative rods, including *A. actinomycetemcomitans, P. gingivalis, Tannerella forsythus, Bacteroides, Prevotella*, and *Fusobacterium* species. These pathogenic bacteria are commonly found in the periodontal pockets of individuals with periodontitis. In this particular study, *P. gingivalis* was chosen as an opportunistic periodontopathogen, as it has been consistently isolated from dental plaque or periodontal pockets in patients with periodontitis, as documented in numerous reports[20, 21].To combat this pathogen, alternative therapies have been explored, with a significant focus on investigating the antibacterial effects of natural herbs against *P. gingivalis*. Various studies have delved into the potential of natural herbs to inhibit the growth or activity of *P. gingivalis*, aiming to develop alternative treatment options for periodontal diseases.

Numerous clinical studies have provided evidence supporting a higher prevalence of periodontitis (PD) in patients with rheumatoid arthritis (RA) compared to those without RA [22]. Schmickler *et al*. conducted a study demonstrating that individuals with RA exhibited poorer oral health, including a higher incidence of missing teeth and periodontal conditions, when compared to healthy controls [23]. The relationship between RA and *P. gingivalis* has been extensively investigated in various studies, with most of them reporting a positive association between the two [24]. Furthermore, PD has been identified as a statistically significant risk factor for autoimmune diseases such as RA [10,11]. A meta-analysis examining the link between PD and RA revealed a significantly elevated risk of PD in individuals with RA compared to non-RA controls [25].

The relationship between *P. gingivalis* and Rheumatoid arthritis (RA) is a complex and intriguing area of research. *P. gingivalis*, a common microbial pathogen found in periodontal disease, has been implicated in the development and progression of RA. One of the key mechanisms by which *P. gingivalis* is thought to contribute to RA is through its ability to citrullinate host and bacterial peptides [26,27]. Citrullination is a process that converts arginine residues to citrulline, leading to the generation of autoantigens and triggering an immune response that can contribute to the development of RA[28].

Furthermore, there is evidence suggesting that oral bacteria, including *P. gingivalis*, or their genetic material may have the ability to migrate from the oral cavity to the joints [29]. This migration could potentially trigger an inflammatory response in the joints and contribute to the pathogenesis of RA. A specific enzyme produced by *P. gingivalis*, called peptidyl arginine deaminase (PAD), has been identified as a factor associated with increased susceptibility to rheumatoid arthritis. PAD is responsible for the citrullination process mentioned earlier and has been found to be present in higher levels in individuals with RA compared to healthy individuals.

Targeting the arginine-dependent gingipain (Rgp) enzyme produced by *P. gingivalis* could hold promise as a therapeutic and preventive strategy for diseases caused by this pathogen[30]. Inhibiting Rgp activity could potentially disrupt the pathogenicity of *P. gingivalis* and reduce its ability to contribute to the onset and progression of periodontitis and RA.Top of FormBottom of Form Periodontal disease is typically treated using chemotherapeutic drugs and mechanical methods. Chlorhexidine mouthwash is a commonly used and effective agent for treating periodontitis. However, it has some drawbacks, including discoloration of the tongue and teeth, a burning sensation in the oral cavity, dryness of the mouth, and potential taste loss. Additionally, bacterial species often develop resistance to antibacterial drugs [31]. These limitations have prompted the search for newer and safer herbal-based agents as alternatives. Plant-derived medicine has been used for centuries in many developing countries as a traditional healthcare therapy. Herbal medicines offer a wide range of properties, including antibacterial, antioxidant, and anti-inflammatory effects, making them attractive as alternative, stable, safe, and bioactive treatments compared to chemical medications. In this study, a screening process was conducted to evaluate the antibacterial activity of twenty-five herbal extracts against *P. gingivalis*. Among them, five extracts (*Zinziber officinale, B. propolis, Psidium guajava, Phyllanthus emblica*, and *Eucalyptus globules*) exhibited the maximum zone of inhibition against fifteen clinical isolates and one reference strain of *P. gingivalis* ATCC33277.

The use of these herbal extracts with potent antibacterial properties may provide a promising avenue for the development of alternative treatments for periodontal disease. By harnessing the benefits of herbal medicine, it is possible to explore more sustainable, safe, and bioactive options that could potentially overcome the limitations associated with conventional chemotherapeutic drugs. Further research and development in this field may lead to the formulation of effective herbal-based therapies for the management of periodontal disease caused by pathogens such as *P. gingivalis*.

Ginger extract, widely used in traditional medicine and human clinical trials, has shown no significant side effects [32]. While there is limited literature on the use of ginger extract as a drug delivery system for the treatment of periodontal diseases, its antimicrobial properties against various pathogens such as *Candida albicans, Candida krusei, Candida tropicalis, Candida glabrata,* and *Escherichia coli* have been observed [32,33,34,35]. Our study also demonstrated that *Zingiber officinale* (ginger) extract exhibited significant antimicrobial effects against the major periodontopathogen *P. gingivalis*.

Another plant species that demonstrated antibacterial activity against *P. gingivalis* was *B. propolis*. Propolis is known to possess anti-inflammatory, antimicrobial, and immunostimulating activities [36]. Similar to the findings on ginger extract, *B. propolis* has been reported to exhibit antibacterial and antifungal effects against strains of *Pseudomonas* spp., *Enterobacteriaceae, Lactobacillus plantarum*, as well as yeasts like *S. cerevisiae* and *Debaryomyces hansenii*, and *Fusarium oxysporum* [37]. The present study aligns with the work by Gebara *et al*. (1996), which highlighted the promising antibacterial activity of propolis extracts against periodontopathic bacteria [38].

Regarding guava plant extracts, previous studies have shown no antibacterial effect on gram-negative bacteria such as Escherichia coli and *Salmonella enteritidis* [39]. However, another study demonstrated the antibacterial effect of guava extract against both gram-positive bacteria (*Micrococcus luteus, Bacillus subtilis, S. aureus*, and *Streptococcus* sp.) and gram-negative bacteria (*E. coli, Pseudomonas aeruginosa*, and *Salmonella typhimurium)*[40]. In our study, the ethanolic extract of guava exhibited good bactericidal activity against the gram-negative microorganism *P. gingivalis*.

In addition to the mentioned plant extracts, Amla extract (*Phyllanthus emblica*) also exhibited promising antibacterial activity against gram-negative bacteria in our in vitro study. A previous analysis has reported that the investigated oils derived from *Phyllanthus emblica* L. were more effective against Gram-positive bacteria compared to Gram-negative bacteria[41]. Furthermore, *Phyllanthus emblica* seeds have shown anti-infective potential against various human-pathogenic bacteria, including *C. violaceum*, *S. marcescens*, *P. aeruginosa*, and *S. aureus* [42].

The extract prepared from *Eucalyptus globulus* plant parts also demonstrated notable antibacterial activity, particularly against the *P. gingivalis* isolates obtained from the study participants. Previous studies have reported the antibacterial activity of essential oils derived from the leaves of *Eucalyptus globulus* against bacteria such as *Escherichia coli* and *Staphylococcus aureus* [43]. Moreover, the antibacterial action of *Eucalyptus globulus* oil has been investigated against microorganisms such as *Pseudomonas aeruginosa*, *Salmonella* sp., *Staphylococcus aureus*, *Proteus vulgaris, Escherichia coli*, and *Candida albicans* [44].These findings indicate that Amla extract from *Phyllanthus emblica* and *Eucalyptus globulus* extracts possess significant antibacterial properties against various bacterial strains, including gram-negative bacteria. Further research is needed to explore the potential mechanisms of action and evaluate their effectiveness in clinical settings for the treatment of bacterial infections, including those caused by *P. gingivalis*.

CONCLUSION

The reviewed literature indicates that the tested herbal extracts primarily exhibit inhibitory effects against gram-positive bacteria, some gram-negative bacteria, yeast, and fungal strains. However, there is limited research on the potential of these herbs against the major gram-negative oral pathogen, P. gingivalis. In contrast, our study confirmed the excellent inhibitory activity of the herbal extracts against P. gingivalis. It is worth noting that there are only a few reports available from India regarding the isolation and characterization of P. gingivalis due to the complex growth conditions required for anaerobic cultures.

Further research is necessary to explore the potential antibacterial properties of these herbal extracts against other oral pathogens, such as their ability to inhibit or eradicate biofilms. The present study focused on the antimicrobial activity of ethanolic extracts of *Zinziber officinale, B. propolis, Psidium guajava, Phyllanthus emblica,* and Eucalyptus globules against P. gingivalis. These herbs have shown clear utility as adjunctive therapies to mechanical treatments in the prevention and treatment of infections caused by P. gingivalis. The findings suggest the possibility of developing a mouthwash that combines these extracts, considering their high growth inhibitory properties against P. gingivalis. as P. gingivalis has been linked to various diseases, including cardiovascular disease, diabetes mellitus, respiratory infections, rheumatoid arthritis, osteoporosis, and obesity, the potential applications of these results are significant.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONFLICTS OF INTEREST

The authors reveal no conflicts of interest concerning the work reported in this article.

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