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Antimicrobial Activity Of Polyherbal Extract: An In Vitro Study

Research Article

M. Kavyashree[1], Arvina Rajasekar[2*]

[1] Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai- 77, India.
[2] Senior Lecturer, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai- 77, India.

Abstract

Background: Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. Plant extracts or phytochemicals that inhibit the growth of oral pathogens, reduce the development of biofilms and dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases.

Aim: To assess the antimicrobial activity of polyherbal extract containing of *Salvia officinalis* (Sage), R*osemaryinus officinalis* (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon), *Mentha arvensis* (Mint).

Materials and Methods: The leaves of *Salvia officinalis* (Sage), *Rosemaryinus officinalis* (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon), *Mentha arvensis* (Mint) were collected and dried. 100gm of dried leaves of each herb was powdered finely and mixed together. 100 ml of distilled water was added with the polyherbal extract powder and then it was filtered with filter paper and boiled at the temperature 50 degree celsius. The agar diffusion method was used to determine the antimicrobial activity of different concentrations of the polyherbal extract (25µL 50µL,100µL) against *S. mutans*, *C. albicans*, *E. faecalis* and *S. aureus*. Antibody (Amoxicillin) was used as positive control and the zones of inhibition were recorded in each plate. Zones of inhibition obtained for different microorganisms at various concentrations of polyherbal extract were compared using ANOVA test.

Result: At 25µl, 50µl and 100µl, the antimicrobial activity against *S.mutans* was found to be statistically significant when compared to the standard ($p<0.05$). At 50µl and 100µl, the antimicrobial activity against *S.aureus* was found to be statistically significant when compared to the standard ($p<0.05$). At 25µl and 100µl, the antimicrobial activity against *E.faecalis* was found to be statistically significant when compared to the standard ($p<0.05$).

Conclusion: The present study suggests that the polyherbal extract containing *Salvia officinalis* (Sage), R*osemaryinus officinalis* (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon), *Mentha arvensis* (Mint) showed antibacterial activity against *S. mutans*, *E. faecalis* and *S. aureus*.

Keywords: Antimicrobial Activity; Bacteria; Green Synthesis; Innovative; Oral Pathogens; Polyherbal.

Introduction

Man is turning towards nature as natural herbal products are being increasingly used in prophylaxis and treatment of different diseases [1]. Because of its low incidence of serious adverse effects, low cost and their perceived efficacy, herbal medicine is gaining more importance [2] caries and periodontal problems are the most common chronic diseases worldwide. Dental caries is defined as an infectious bacterial disease that results in destruction of the calcified tissues of the teeth [3-6]. It seems that *S. mutans* is one of the primary organisms associated with dental caries in humans. A caries prevention method is a complex process comprising multiple aspects. To reach this goal, limiting substrate, disrupting of plaque formation with brushing and flossing, modifying tooth surface with different forms of fluoride, stimulating the saliva flow, restoring cavitated tooth surface and modifying cariogenic microflora to non-cariogenic ones with topical fluoride

***Corresponding Author**:
Dr. Arvina Rajasekar,
Senior Lecturer, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai- 77, India.
Tel: +91 9486442309
E-mail: arvinar.sdc@saveetha.com

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treatment, antibiotic treatment or bactericidal mouth rinses such as chlorhexidine can be applied [7]. The golden standard for the mouth rinses is a diguanidohexane with pronounced antiseptic properties, named chlorhexidine [8, 9].

Recently there has been a renewed interest in the use of herbal mouth rinses oral care products [10]. In The recent past, there has been an increased interest in the therapeutic properties of some medicinal plants and natural compounds which have demonstrated anti-cariogenic activities in both *in vitro* and *in vivo* conditions. Among these phytoconstituents, several polyphenolic compounds like tannins (catechins) and flavonoids seem to be the most promising biomolecules [11]. Research in the field of caries prevention has been focusing on ways for reducing or totally eradicating cariogenic flora from the oral cavity. Studies have shown that caries can be prevented by regular tooth brushing and flossing. However, most of the studies have shown it difficult to eliminate *S. mutans* from the pits, fissures, and approximal surfaces by mechanical means alone. For effective caries control, these methods should be combined with the chemoprophylactic agents. These agents, e.g., chlorhexidine and antibiotics, act by lowering the number of microorganisms or inhibiting dental plaque formation. However, they have several undesirable side effects, including tooth staining and emergence of bacterial resistance. These side effects stimulate the search for alternative agents [12]. Another study evaluated the antibacterial activity of *S. rebaudiana* leaves extracted using various solvents against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi*, and *Vibrio cholera* and it was found in the study that the acetone extract showed greater activity against Gram-positive bacteria than Gram-negative bacteria [13, 14].

Herbal medicines are an important source of nutrients that promote health. Various herbal products such as Propolis and Azadirachta indica have shown significant advantages in reducing signs of gingival and periodontal inflammation. The use of plants and their derivatives which possess preventive and therapeutic effects could contribute to the oral health [15-25]. Herbal medicine is useful in preventing cavity, toothache, gingivitis, mouth ulcers, swollen tonsil, oral thrush and hairy tongue (Al-Somaiday, Al-Samaray and Al-Samydai, 2020). The formulation of *Salvia officinalis (Sage), Rosemaryinus officinalis (*Rosemary*), Thymus serpyllum (*Thyme*), Cinnamomum zeylanicum (*Cinnamon*), Mentha arvensis (*Mint*)* has good antibacterial activity against dental pathogens [26]. *Malus Domestica* (Apple) are often utilized in titanium implant coating in dental implantology, and *Cissus Quadrangularis* (Veldt grape) and *Carthamus tinctorius* (Safflower) are recommended for periodontal filler in periodontal regeneration [27].

Our team has extensive knowledge and research experience that has translated into high quality publications [28-47]. Extensive literature search, it was revealed that there is a lack of adequate studies testing the antimicrobial activity of *Salvia officinalis* (Sage), *Rosemaryinus officinalis* (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon), *Mentha arvensis* (Mint).Henceforth the aim of this research was to assess the antimicrobial activity of polyherbal extract containing *Salvia officinalis* (Sage), *Rosemaryinus officinalis* (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon), *Mentha arvensis* (Mint).

Materials and Methods

The leaves of *Salvia officinalis* (Sage), *Rosemaryinus officinalis* (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon), *Mentha arvensis* (Mint) were collected and dried. 100gm of dried leaves of each herb was powdered finely and mixed together. 100 ml of distilled water was added with the polyherbal extract powder and then it was filtered with filter paper and boiled at the temperature 50 degree celsius.

Evaluation of antimicrobial activity

The agar diffusion method was used to determine the antimicrobial activity of prepared polyherbal extract. Oral pathogens like *S. mutans*, *C. albicans*, *E. faecalis* and *S. aureus*. The fresh bacterial suspension was dispersed on the surface of Muller Hinton agar plates. Different concentrations of the polyherbal extract (25µL 50µL, 100µL) were incorporated into the wells and the plates were incubated at 37°C for 24 hrs. Antibody (Amoxicillin) was used as positive control and the zones of inhibition were recorded in each plate.

Results

Zone of inhibition using different concentrations of polyherbal extract shows the antimicrobial activity against *S. mutans* (Figure 1), *S. aureus* (Figure 2) *E. faecalis* (Figure 3), *C. albicans* (Figure 4). Against *S. mutans*, 25µl showed 20mm of zone of inhibition, 50µl showed 25mm of zone of inhibition and 100µl showed 27mm of zone of inhibition. 31mm of zone of inhibition was noted against the antibody. Against S. aureus, 25µl showed 20mm of zone of inhibition, 50µl showed 27mm of zone of inhibition and 100µl showed 29mm of zone of inhibition. 22mm of zone of inhibition was noted against the antibody. Against E. faecalis, 25µl showed 10 mm of zone of inhibition, 50µl showed 20mm of zone of inhibition and 100µl showed 20mm of zone of inhibition. 49mm of zone of inhibition was noted against the antibody.

Against *C. Albicans*, 25µl showed 11mm of zone of inhibition, 50µl showed 11mm of zone of inhibition and 100µl showed 11mm of zone of inhibition. 11mm of zone of inhibition were

Figure 1. Zone of inhibition of polyherbal extract by disk diffusion method showing antimicrobial activity against *S. mutans*.

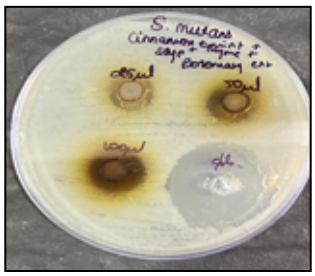

Figure 2. Zone of inhibition of polyherbal extract by disk diffusion method showing antimicrobial activity against *S. aureus*.

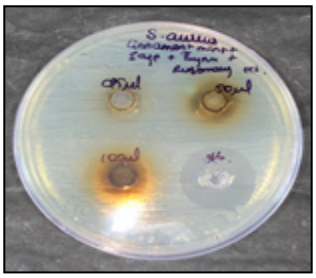

Figure 3. Zone of inhibition of polyherbal extract by disk diffusion method showing antimicrobial activity against *E. faecalis*.

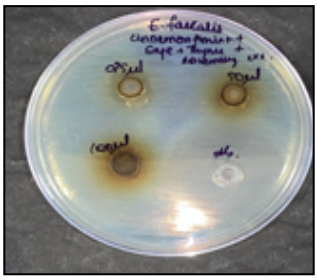

Figure 4. Zone of inhibition of polyherbal extract by disk diffusion method showing antimicrobial activity against *C. albicans*.

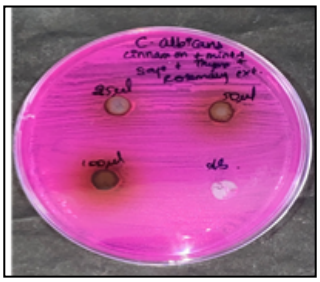

Figure 5. Bar graph shows the antimicrobial activity of polyherbal extract at various concentrations along with positive control (amoxicillin). The concentration was plotted on the X axis and the zone of inhibition was plotted on Y axis. The blue colour in the bar depicts the *S.mutans* and the green colour denotes *S.aureus* and the brown colour denotes the *E.faecalis* and the purple colour represents the *C.albicans*. At 25µl, 50µl and 100µl, the antimicrobial activity against *S.mutans* was found to be statistically significant when compared to the standard ($p<0.05$). At 50µl and 100µl, the antimicrobial activity against *S.aureus* was found to be statistically significant when compared to the standard ($p<0.05$). At 25µl and 100µl, the antimicrobial activity against *E.faecalis* was found to be statistically significant when compared to the standard ($p<0.05$) (One way ANOVA followed by post hoc analysis).

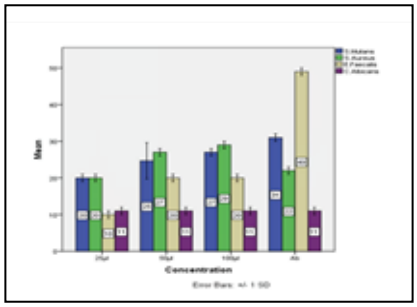

Table 1. Zone of inhibition using different concentrations of polyherbal extract against *S. mutans*, *S. aureus*, *E. faecalis* and *C. albicans*.

Concentration (micro litres)	*S. mutans*	*S. aureus*	*E. faecalis*	*C. albicans*
25 µl	20	20	10	11
50µl	25	27	20	11
100µl	27	29	20	11
Standard	31	22	49	11

Table 2. ANOVA test for antimicrobial activity

		Sum of Squares	df	Mean Square	F	Sig.
S. mutans	Between Groups	190	3	63.333	6.268	.006*
	Within Groups	54.667	8	6.833		
	Total	244.667	11			
S.aureus	Between Groups	159	3	53	53	.000*
	Within Groups	8	8	1		
	Total	167	11			
E.Faecalis	Between Groups	2552.5	3	850.75	850.75	.000*
	Within Groups	8	8	1		
	Total	2560.25	11			
C.albicans	Between Groups	0	3	0	0	1
	Within Groups	8	8	1		
	Total	8	11			

***(p<0.05)**

Table 3. Post hoc for antimicrobial activity.

Dependent variable	Concentration (I)	Concentration(J)	Mean differences	Std.Error	Sig.
Zone of inhibition of *S. mutans*	25	50µl	-4.667	2.134	0.207
		Antibiotic	-11.000	2.134	0.004*
	50	100µl	-2.333	2.134	0.000*
		Antibiotic	-6.333	2.134	0.000*
	100	Antibiotic	-4.000	2.134	0.000*
		25 µl	-7.000	0.816	0.000*
Zone of inhibition of *S. aureus*	25	100µl	-9.000	0.816	0.000*
		Antibiotic	-2.000	0.816	0.144
	50	100	-2.000	0.816	0.144
		Antibiotic	5.000	0.816	0.001*
	100	Antibiotic	7.000	0.816	0.000*
		25 µl	-10.000	0.816	0.000*
Zone of inhibition of *E. faecalis*	25	100µl	-10.000	0.816	0.000*
		Antibiotic	-39.000	0.816	0.000*
	50	100µl	0.000	0.816	1
		Antibiotic	-29.00	0.816	1
	100	Antibiotic	-29.000	0.816	0.000*
		25 µl	0.000	0.816	1
Zone of inhibition of *C. albicans*	25	100µl	0	0.816	1
		Antibiotic	0	0.816	1
	50	100µl	0	0.816	1
		Antibiotic	0	0.816	1
	100	Antibiotic	0	0.816	1
		25µl	0	0.816	1

***(p<0.05)**

noted against the antibody. Zone of inhibition by disk-diffusion method shows antimicrobial activity in different concentrations of polyherbal extract. Zones of inhibition obtained for different microorganisms at various concentrations of polyherbal extract were compared using ANOVA test. The result obtained for antimicrobial activity against S. mutans, S. aureus and E. faecalis were found to be statistically significant with the p value of <0.05. (Table 1, Table 2 and Table 3).

Discussion

The present study was done to assess the antimicrobial activity of polyherbal extract containing *Salvia officinalis* (sage), *Rosemaryinus*

officinalis (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon) and *Mentha arvensis* (Mint).

Jonatas Rafael de Oliveiria *et al.,* assessed the antimicrobial activity of salvia officinalis extract against bacterial and fungal species from the oral cavity. This study evaluated the antimicrobial activity of *Salvia officinalis* (sage) extract on clinical samples isolated from the oral cavity and reference strains of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Candida albicans, Candida tropicalis and Candida glabrata.* Minimum inhibitory, minimum bactericidal and minimum fungicidal concentrations and the cytotoxic effect of *S. officinalis* extract were determined. *S officinalis* extract presented antimicrobial activity on all isolates of *Staphylococcus spp, S mutans* and *Candida spp* and no cytotoxic effect was observed [48].

Ghezelbash GR *et al.,* evaluated the antimicrobial activity of the *S. officinalis* on *Bacillus anthracis*, *Bacillus cereus, Escherichia coli*, and *Staphylococcus aureus bacteria.* Three solvent extracts (deionized distilled water, Acetone and Ethanol) of the plant were investigated by using disc diffusion method. The results indicated that the inhibitory effects of acetone extract of *S. officinalis* with MIC= 10 mg/ml for *B. anthracis* and MIC=30 mg/ml for *S. aureus.* Gram-negative microorganisms presented larger sensitivity for the extracts. As a result, organic solvent extracts (especially acetone leaves extracts) of this plant can be used as natural antimicrobial product [49].

Biljana Bozin *et al.,* evaluated the antimicrobial and antioxidant properties of Rosemary and Sage essential oils. Antimicrobial activity was tested against 13 bacterial strains and 6 fungi, including *Candida albicans* and 5 dermatomycoses. It was found out that both the tested essential oils had strong antimicrobial property and antioxidant property [50]. Also, Aziza Kamal Geneana *et al.,* confirmed the antioxidant, antibacterial and antifungal activities of the Rosemary leaf extracts against *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* [51].

Chenchen Cai *et al.,* prepared and assessed the antimicrobial activity of thyme essential oil and the findings indicated that the thyme essential oil acts as a natural bacteriostatic agent and has the potential to be widely used in the food processing industry.[52]. Monika Sienkiewicz *et al.,* investigated the antimicrobial activity of thyme essential oil against *Staphylococcus, Enterococcus, Escherichia* and *Pseudomonas genus.* Agar diffusion was used to determine the microbial growth inhibition of bacterial growth at various concentrations of oil from *Thymus vulgaris.* Susceptibility testing to antibiotics was carried out using disk diffusion. Thyme essential oil strongly inhibited the growth of the clinical strains of bacteria tested [53].

Linda SM Ooi *et al.,* studied the antimicrobial activity of cinnamon oil against *Staphylococcus aureus, E. coli, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae, Vibrio parahaemolyticus and Salmonella typhimurium, C. albicans, C. tropicalis, C. glabrata*, and *C. krusei* and was found out that cinnamon exhibited antimicrobial activity against all the organisms that are tested [54]. Yasser Shabhazi *et al.,* investigated the chemical composition and antibacterial activity of essential oil from the leaf of *Mentha spicata* plant against *Staphylococcus aureus* and it was found out that mint exhibited antimicrobial activity against the organisms that were tested [55]. Basheer Al-Sum *et al,* investigated antimicrobial activity of aqueous extract of mint plant against seven selected pathogenic bacteria: *Bacillus fastidiosus, Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris, Salmonella choleraesuis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Serratia odorifera.* Menth extract at different concentrations (1:1, 1:5, 1:10, and 1:20) was active against all tested bacteria except for *S.aureus* and the highest inhibitory effect was observed against *S. mutans* using the well diffusion method [56].

The findings of the present study are in accordance with the previous studies as the polyherbal extract tested showed antibacterial activity against *S. mutans, E. faecalis* and *S. aureus* at various concentrations. However, clinical trials needed to be conducted to confirm these findings.

Conclusion

Within the limitations, the present study suggests that the polyherbal extract containing *Salvia officinalis* (Sage), *Rosemaryinus officinalis* (Rosemary), *Thymus serpyllum* (Thyme), *Cinnamomum zeylanicum* (Cinnamon), *Mentha arvensis* (Mint) showed antibacterial activity against *S. mutans, E. faecalis* and *S. aureus.*

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