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Original Article

Dentistry Section

Efficacy of *Calendula officinalis* Extract (Marigold Flower) as an Antimicrobial Agent against Oral Microbes: An Invitro Study in Comparison with Chlorhexidine Digluconate

SHRINIDHI MAJI SHANKAR[1], SOUMYA GURURAJ BARDVALLI[2], RUKMINI JYOTIRMAYEE[3], CHETHANA[4], KALA BHUSHAN[5], SHIV KUMAR[6]

ABSTRACT

Introduction: *Calendula officinalis* is a member of the family Asteraceae containing flavonoids, and essential oils etc. It is known to be effective against certain gram negative and gram positive clinical pathogens. Incorporating natural plant extracts into periodontal antiinfective therapy is a wise alternative in light of rampant antibiotic resistance amongst periodontal pathogens.

Aim: To assess the antimicrobial efficacy of *C.officinalis* against five oral microbes, as compared to gold standard chlorhexidine digluconate.

Materials and Methods: The inhibitory action of the two test agents, chlorhexidine digluconate 0.2% and *C.officinalis* raw extract was assessed using tests to determine minimum inhibitory concentration, minimum bactericidal concentration, and inhibition zones using agar diffusion method. Time kill curve method was used to assess the time in which drug was inhibitive against five oral microbes namely, *A.actinomycetemcomitans, P.gingivalis, P.intermedia, F.nucelatum* and *S.mutans.*

Results: *P.gingivalis* and *P.intermedia* were sensitive to *C.officinalis*, while *F.nucleatum* and *A.actinomycetemcomitans* exhibited reduced sensitivity. *C.officinalis* was highly effective against *S.mutans* (3.12 µg/mL), its inhibitory concentration close to that of chlorhexidine digluconate 0.2% (6.25 µg/mL). *C.officinalis* took longer than chlorhexidine digluconate 0.2% to exhibit lethality against all the organisms that it inhibited. Chlorhexidine exhibited immediate lethality (zero minute) against *S.mutans* while it inhibited the other microbes at 5 minutes. *A.actinomycetemcomitans* and *P.intermedia* were inhibited by *C.officinalis* at 30 minutes, *S.mutans* at 10 minutes. *F.nucleatum* was inhibited by *C.officinalis* at 2 hours and no inhibition of *P.gingivalis* by *C.officinalis* was observed.

Conclusion: *C.officinalis* showed antimicrobial efficacy against most organisms tested, yet its efficacy was not on par with chlorhexidine digluconate 0.2%. However, its performance could still be used as evidence to spur on further human clinical trials, in patients undergoing phase I or IV therapies.

Keywords: Antibiotic resistance, Anti-infective therapy, Complementary/alternative medicine, Non surgical periodontal therapy

INTRODUCTION

Periodontal disease affects more than 50% of Indian population [1]. Treatment includes surgical and non surgical options along with systemic antibiotics in selected cases. When scaling and root planing alone do not result in lasting changes in the sub gingival microbiota, systemic antibiotics have proven a good adjunct. However, over enthusiastic use of systemic antibiotics has led to a rapid development of resistance to these drugs in a number of putative periodontal pathogens. These antibiotics have also been incorporated into suitable vehicles and delivered locally into the periodontal pocket. Faced with the threat/reality of antibiotic resistance [2], it has not only become necessary to locally deliver the drug to the site but perhaps switch to more organic/plant based antimicrobials in order to reduce the possibility of resistance development. Plants are known to synthesize aromatic substances, usually phenols or their derivatives [3] which are intensely antimicrobial and antioxidant in nature [4]. They also produce flavones, flavonoids and flavonols in response to infection. These are phenolic structures which contain one carbonyl group and complex with extracellular soluble proteins and bacterial cell walls. They are also capable of disrupting microbial membranes based on their lipophilic nature [5]. Marigold flower (*C.officinalis*) also produces a molecule group of phenols (flavonoids), which is antimicrobial in nature. Biologically active molecules of *C.officinalis* are phenolic compounds (flavonoids), saponins [6], carotenoids, triterpenic alcohols [7,8] carotenoid pigments [9], polyunsaturated fatty acids like calendic acid [10] as well as tannins, anthraquinones, cardiac glycosides and steroids [11] etc.

C.officinalis has already shown to be effective against a range of drug resistant pathogens like *Esherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* [12]. The present study was an attempt to assess the efficacy of *C.officinalis* against some common oral microbes so that it can eventually be incorporated into periodontal anti-infective therapy.

MATERIALS AND METHODS

The present invitro study was carried out and completed within the month of January of 2017 after obtaining clearance from the Ethical Clearance Committee of Sharavathi Dental College and Hospital, Shimoga, India. (Clearance number: SDC/SMG/2016/722/A) For the purpose of this study, stock cultures of *A.actinomycetemcomitans* (ATCC No. - 43718), *P.gingivalis* (ATCC No. - 33277, 53978), *P.intermedia* (ATCC No. - 25611), *S.mutans* (ATCC No. - 25175) and *F. nucleatum* (ATCC No. - 25586) (from Himedia laboratories) were obtained from the Department of Microbiology, Nathaji Rao G Halgekar's Institute of Dental Sciences and Research Center, Belgaum, India. Chlorhexidine 0.2% mouthrinse (Warren Pharma) was used as a positive control for the study.

Fine marigold flakes were procured from Aum Agri Freeze Foods, Baroda, India. Flakes were dissolved in dimethyl sulfoxane to obtain a crude form for ease of use in the experiments [13,14].

Minimum Inhibitory Concentration (MIC) Procedure

MICs are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation or 24 hour incubation. Microbroth dilution method was used to determine the MIC.

A 200 µL of Brain Heart Infusion (BHI) broth was added into the 9 tubes separately. For drug dilution procedure (using the microbroth dilution method) 9 dilutions of chlorhexidine and *Calendula officinalis* were prepared with BHI. An initial tube contained 20 µL of the test agent to which 380 µL of BHI broth was added. To start the dilution process, 200 µL from the initial tube (tested agent and BHI) was transferred to the first tube containing 200 µL of BHI broth. This was considered as 10^1 dilution. From 10^1 diluted tube, 200 µL was transferred to the next (second) tube to make it 10^2 dilution. The serial dilution was repeated up to 10^9 dilution for each tested agent. About 5 µL from the maintained stock culture of each organism, was taken and added into 2 mL of BHI broth and made into a suspension and the turbidity was adjusted with reference to the standard 0.5 McFarland unit such that the inoculum would contain 1×10^8 CFU/mL. Turbidity was evaluated after incubating for 24 hours [15].

The test was interpreted as follows: Clear broths were interpreted as the target organism being visibly sensitive to the drug (no growth); turbid broths were interpreted as the target organism being visibly resistant (growth is apparent) to the tested agent.

Minimum Bactericidal Concentration (MBC) Procedure

To determine bactericidal concentration, the MIC dilution tubes with no visible growth (no turbidity) and the control tube were subcultured onto the respective media (Brain Heart Infusion agar for *A.actinomycetemcomitans* and blood agar for the remaining microbes) and incubated for 24 hours anaerobically at 37°C and the colonies were counted the next day. The organisms' growths from the control tubes were then compared with the organism growth from the MIC test tubes.

Inoculum Preparations

The colonies were transferred from the plates to the BHI broth with a sterilized straight nichrome wire. The turbidity was visually adjusted with BHI broth to equal that of a 0.5 McFarland unit turbidity standard that has been freshly prepared [16].

The test was interpreted as follows: If there were similar number of colonies it indicated bacteriostatic activity only, while reduced number of colonies denoted a partial or slow bactericidal activity. No growth was observed if the whole inoculum has been killed.

Agar Well Diffusion Procedure

Well plate method was used for agar diffusion procedure. After adjusting the inoculum to a 0.5 McFarland unit turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of the blood agar plate was swabbed three times, rotating plates approximately 60° between streaking to ensure even distribution. The inoculated plate was allowed to stand for at least 3-15 minutes before punching the wells in agar plate.

A heated hollow tube of 5 mm diameter was pressed on the inoculated agar plate and removed immediately to punch wells. Five such wells were made on each plate. Two such plates for each organism were prepared. A 50 µL/mL of *C.officinalis* was added into the respective wells on each plate. The plates were incubated within 15 minutes of drug application for 18-24 hours at 37°C anaerobically. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to the nearest whole millimeter by using calipers. Two plates for each organism were prepared. A zone of 12 mm was considered the reference zone of inhibition above which test microbes were considered sensitive and below which they were considered resistant to the test drugs [17].

Time Kill Curve

The dilutions were done in a manner similar to that of MIC. The broth (BHI, 100 µL volume) and the test compounds were mixed in equal proportions and inoculated with the test microbes. The tubes were then placed in a CO_2 jar and the baseline was noted at 0 hours. At every 5 minutes, 10 minutes, 30 minutes and 2 hours the mixture was cultured or plated. Incubation was done according to growth requirement, i.e., in CO_2 anaerobic jar. After 48-78 hours of incubation, the plates were removed and the colonies were counted [17]. Simple statistics was used for the calculations.

RESULTS

Minimum Inhibitory Concentration

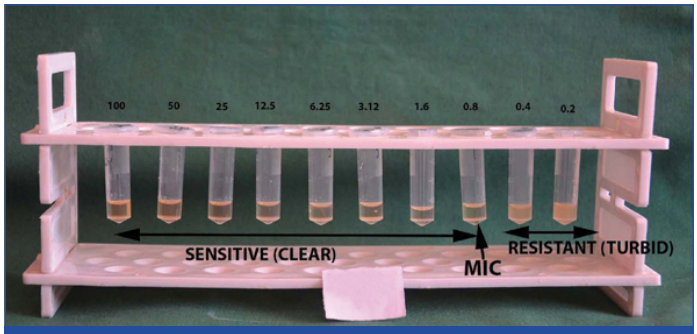
A.actinomycetemcomitans as well as *F.nucleatum* was sensitive to *C.officinalis* at 100 µg/mL and was resistant at all other concentrations [Table/Fig-1]. On the other hand MIC of *C.officinalis* at 0.8 µg/mL for *P.gingivalis* [Table/Fig-2] as well as *P.intermedia* (50 µg/mL) was lower than chlorhexidine which was at 1.6 µg/mL [Table/Fig-3]. *C.officinalis* MIC for *S.mutans* was at 3.12 µg/mL as compared to chlorhexidine which inhibited growth at 6.25 µg/mL.

Minimum Bactericidal Concentration

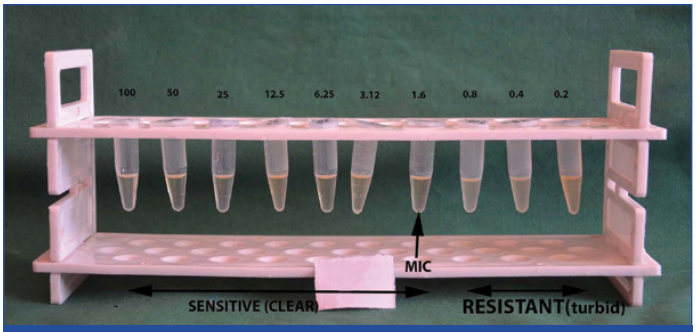
C.officinalis failed to inhibit the growth of *P.gingivalis* as well as *P.intermedia* at any concentration, while 6.25 µg/mL of

Quantity (µg/mL)	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
A.actinomycetemcomitans										
Chlorhexidine digluconate 0.2%	S*	S	S	S	R†	R	R	R	R	R
C.officinalis	S	R	R	R	R	R	R	R	R	R
P.gingivalis										
Chlorhexidin edigluconate 0.2%	S	S	S	S	S	S	S	R	R	R
C.officinalis	S	S	S	S	S	S	S	S	R	R
P.intermedia										
Chlorhexidine digluconate 0.2%	S	S	R	R	R	R	R	R	R	R
C.officinalis	S	S	S	S	S	S	S	S	R	R
F.nucleatum										
Chlorhexidine digluconate 0.2%	S	S	R	R	R	R	R	R	R	R
C.officinalis	S	R	R	R	R	R	R	R	R	R
S.mutans										
Chlorhexidine digluconate 0.2%	S	S	S	S	S	R	R	R	R	R
C.officinalis	S	S	S	S	S	S	R	R	R	R

[Table/Fig-1]: Minimum Inhibitory Concentration.
*Sensitive, †Resistant



[Table/Fig-2]: Minimum Inhibitory Concentration (MIC) of *Calendula officinalis* against *P.gingivalis*.
*Turbidity observed in tubes containing 0.4 and 0.2 µg/mL of broth indicating resistance. Clear broth observed in all the concentrations above 0.4 µg/mL indicating sensitivity to *C.officinalis*. Arrow indicates minimum inhibitory concentration of 0.8 µg/mL of chlorhexidine against *P.gingivalis*. Note: Concentration expressed in µg/mL.



[Table/Fig-3]: Minimum Inhibitory Concentration (MIC) of chlorhexidine 0.2% against *P.gingivalis*.
*Turbidity observed in tubes containing 0.8, 0.4 and 0.2 µg/mL of the broth, indicating resistance. Clear broth observed in all the concentrations above 0.8 µg/mL, indicating sensitivity to chlorhexidine 0.2%. Arrow indicates MIC of 1.6 µg/mL of chlorhexidine against *P.gingivalis*.
Note: Concentration expressed in µg/mL

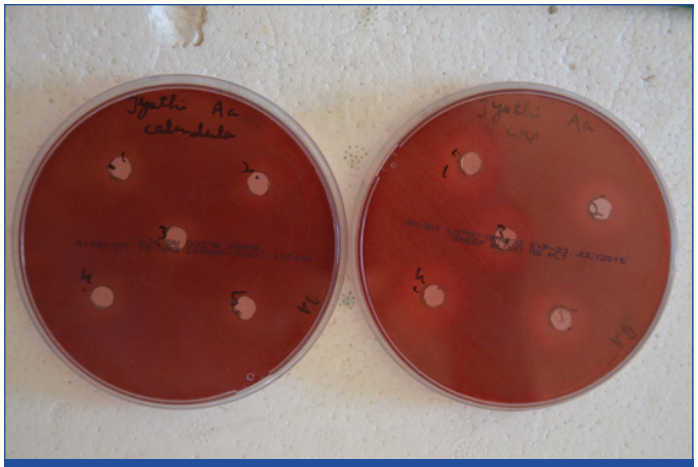
Quantity of drug(ug/mL)	0.2	0.42	0.8	1.6	3.12	6.25	12.5	25	50	100
P.gingivalis										
Chlorh exidine digluconate 0.2%	120	100	80	50	30	NG	NG	NG	NG	NG
C.officinalis	250	230	200	180	150	130	130	120	110	100
P.intermedia										
Chlorhex idine digluconate 0.2%	200	180	170	150	80	NG	NG	NG	NG	NG
C.officinalis	300	280	250	230	200	150	130	110	80	50
A.actinomycetemcomitans										
Chlorh exidine digluconate 0.2%	300	270	250	230	180	NG	NG*	NG	NG	NG
C.officinalis	300	290	280	260	230	200	180	150	120	NG
F.nucleatum										
Chlorhexi dine dig luconate 0.2%	300	260	230	180	150	NG	NG	NG	NG	NG
C.officinalis	300	280	225	200	150	130	120	110	100	80
S.mutans										
Chlorhexi dine digl uconate 0.2%	180	120	80	50	50	NG	NG	NG	NG	NG
C.officinalis	200	180	150	100	10	NG	NG	NG	NG	NG

[Table/Fig-4]: Minimum bactericidal concentration.
*NG-No Growth

chlorhexidine 0.2% turned out to be bactericidal for both *P.gingivalis* and *P.intermedia*. *C.officinalis* was bactericidal to *A.actinomycetemcomitans* at 100 µg/mL, to *S.mutans* at 6.25 and was otherwise not bactericidal to other microbes tested. Chlorhexidine was bactericidal to both *A.actinomycetemcomitans* and *F.nucleatum* at 6.25 µg/mL. For *S.mutans* MBC was identical for both test drugs at 6.25 µg/mL [Table/Fig-4].

Agar Well Diffusion Method

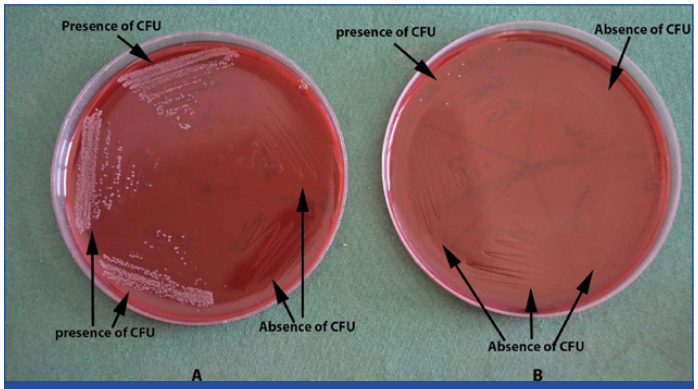
Both the agents were poured in the wells [Table/Fig-5] and resultant zones of inhibition were noted. *A.actinomycetemcomitans* and *P.gingivalis* were considerably more sensitive to *C.officinalis* than to chlorhexidine. The other organisms exhibited more sensitivity towards chlorhexidine than to calendula. *F.nucleatum* was the least sensitive to chlorhexidine amongst all the organisms tested [Table/Fig-6].



[Table/Fig-5]: Agar Well Diffusion- Wells punched deposited with 50 µL of the test drugs: a) *C.officinalis;* and b) Chlorhexidine.

Quantity Added (µL)	50	50	50	50	50
P.intermedia					
Chlorhexidine digluconate 0.2%	20 mm*	20 mm	20 mm	20 mm	20 mm
C.officinalis	15 mm	10 mm	10 mm	10 mm	10 mm
P.gingivalis					
Chlorhexidine digluconate 0.2%	20 mm	20 mm	20 mm	20 mm	20 mm
C.officinalis	22 mm	22 mm	22 mm	22 mm	22 mm
A.actinomycetemcomitans					
Chlorhexidine digluconate 0.2%	13 mm	13 mm	13 mm	13 mm	13 mm
C.officinalis	20 mm	20 mm	20 mm	20 mm	20 mm
S.mutans					
Chlorhexidine digluconate 0.2%	20 mm	20 mm	20 mm	20 mm	20 mm
C.officinalis	15 mm	15 mm	15 mm	15 mm	15 mm
F.nucleatum					
Chlorhexidine digluconate 0.2%	18 mm	18 mm	18 mm	18 mm	18 mm
C.officinalis	12 mm	12 mm	12 mm	12 mm	12 mm

[Table/Fig-6]: Agar Well diffusion.
*Zone of inhibition of growth of microorganisms measured in millimeters



[Table/Fig-7]: Time kill curve- a) Colony Forming Units (CFU log10) counted 72 hours later. Growth observed at 0, 5 and 10 minutes and no growth at 30 minutes and 2 hours for *C.officinalis;* b) Colony Forming Units of 110 (CFU log10) counted 72 hours later. Growth observed at 0 minutes and no growth at other time intervals for chlorhexidine.

Time Kill Curve Method

Chlorhexidine exhibited lethality at 5 minutes for all periodontal pathogens. *C.officinalis* exhibited lethality against *A.actinomycetemcomitans* at 30 minutes [Table/Fig-7] and *P.intermedia* at 30 minutes, but failed to be bactericidal to *P.gingivalis* at any time interval, however, decreasing colony forming units were observed with increasing time intervals. *C.officinalis* showed lethality

Time	0 minute	5 minutes	10 minutes	30 minutes	2 hours
A.actinomycetemcomitans					
Chlorhexidine digluconate 0.2%	110	NG*	NG	NG	NG
C.officinalis	180	165	130	NG	NG
P.gingivalis					
Chlorhexidine digluconate 0.2%	120	NG	NG	NG	NG
C.officinalis	120	110	90	70	55
P.intermedia					
Chlorhexidine digluconate 0.2%	40	NG	NG	NG	NG
C.officinalis	90	70	40	NG	NG
S.mutans					
Chlorhexidine digluconate 0.2%	NG	NG	NG	NG	NG
C.officinalis	20	10	NG	NG	NG
F.nucleatum					
Chlorhexidine digluconate 0.2%	50	NG	NG	NG	NG
C.officinalis	150	140	130	90	NG

[Table/Fig-8]: Time kill curve.
*NG-No Growth

to *F.nucleatum* at 2 hours and to *S.mutans* at 10 minutes as against chlorhexidine at 5 minutes and at 0 minutes respectively [Table/Fig-8].

DISCUSSION

A.actinomycetemcomitans was found to be fairly sensitive to *C.officinalis*, almost on par with chlorhexidine in its ability to inhibit or kill *A.actinomycetemcomitans*. While MIC of *C.officinalis* for *A.actinomycetemcomitans* in this study was a high 100 µg/mL it contrasted with an MIC of as low as 25 µg/mL in another study [18]. *C.officinalis* however exhibited a 20 mm zone of inhibition as against a 13 mm of chlorhexidine against *A.actinomycetemcomitans*. In another study that compared the antimicrobial activity (against *A.actinomycetemcomitans* and *P.gingivalis*) of a hydroalcoholic extract of *C.officinalis* along with a host of other plant products and chlorhexidine 0.12%, the MIC and MBC of *C.officinalis* against *A.actinomycetemcomitans* was far higher than chlorhexidine 0.12% [19].

The solvent used to make *C.officinalis* preparation in this study was dimethyl sulfoxide, certain other studies have used methanolic extracts of *C.officinalis* and it was found to be effective against even Multi Drug Resistant Pathogens (MDR pathogens) [12]. Methanolic extraction of *C.officinalis* enables it to have a higher content of polyphenols and antioxidants. However, we have used *C.officinalis* in an aqueous solution dissolved in dimethyl sulfoxide. *C.officinalis* in combination with a few other herbs was tested against *A.actinomycetemcomitans* in a pilot study. There was no significant reduction in plaque or gingival index neither was there a significant reduction in *A.actinomycetemcomitans* [20], yet this warrants further studies with larger sample size of volunteers.

P.gingivalis has shown a few conflicting results against *C.officinalis*. *C.officinalis* exhibited an MIC of 0.8 µg/mL which was slightly better than chlorhexidine at 1.6 µg/mL and yet did not show lethality against *P.gingivalis* at any concentration in the sub culturing, but exhibited a considerably high zone of inhibition of 22 mm in agar well diffusion. It needs to be noted here that agar well diffusion method does have a few limitations, such as the issue pertaining to the type of antibiotic used in the test and its break points etc., [21] or its inability to test for susceptibility when it comes to certain fastidious micro-organisms [22]. *C.officinalis* is rich in polyphenols, while certain other herbs, also rich in polyphenols, are known to be specifically inhibitory to virulence factors of *P.gingivalis* [23], no such data exists for *C.officinalis*. In this study, *C.officinalis* failed to be bactericidal to *P.gingivalis* even at two hours in the time kill curve despite having a lower MIC than Chlorhexidine and relatively high zone of inhibition of all the microbes tested in agar well diffusion. In another study involving chlorhexidine 0.12%, *C.officinalis* and other herbs, *C.officinalis* exhibited a significantly lower MIC against *P.gingivalis* than against *A.actinomycetemcomitans* [19].

P.intermedia, a Gram-negative, obligate anaerobic pathogenic bacterium, was considerably sensitive to *C.officinalis* at an MIC of 0.8 µg/mL as against 50 µg/mL of chlorhexidine. However, *C.officinalis* failed to exhibit total lethality to *P.intermedia* at any concentration despite reduction in colony forming units. *C.officinalis* has exhibited considerable anti-microbial activity against *P.intermedia* in an earlier study [24].

Socransky and Haffajee's study compared chlorhexidine, listerine and an herbal mouthrinse containing *C.officinalis* against about 40 oral micro organisms. *C.officinalis* exhibited an MIC of 16 µg/mL for *P.intermedia* which was significantly high compared to the MIC in this study. However, mouthrinse used in their study, contained other herbal extracts and MIC dilution tube preparation protocol also differed from our study [25].

S.mutans, a primary aetiologic agent of human dental caries, is particularly effective at forming biofilms on the hard tissues of the human oral cavity. It adheres to primary colonizers by cell-to-cell interaction. *S.mutans*' cariogenicity is well known and some of it is attributed to its property of adhesion to tooth surface or pellicle [26]. Several herbal products have been shown to be effective against *S.mutans*. Green tea mouthrinse for example, has shown significant antimicrobial activity against cariogenic micro organisms like *S.mutans* and *Lactobacillus* [27]. Garlic with lime has also shown promise with in vitro antimicrobial activity against *S.mutans* [28]. In the present study, *C.officinalis* showed significant antimicrobial activity against *S.mutans*, with a lower MIC than chlorhexidine and fewer colonies of *S.mutans* were observed for *C.officinalis* at a dilution identical to chlorhexidine and yet zone of inhibition was much smaller as compared to chlorhexidine. *C.officinalis* in the form of tincture (in varying dilutions) has shown considerable antimicrobial activity against *S.mutans* in a study that compared it with another herbal extract and chlorhexidine 0.12%. The zones of inhibition ranged from 0-12.5 mm as against chlorhexidine which exhibited upto 14 mm [29] which is again similar to our study where chlorhexidine had higher zones of inhibition than *C.officinalis* against *S.mutans*. Similarly, when *C.officinalis* was compared with chlorhexidine 0.12% and Listerine it was observed that *C.officinalis* had a far lower MIC than listerine against *S.mutans* but higher MIC compared to chlorhexidine [25].

All studies have slightly different protocols for preparation *C.officinalis*, therefore large differences in MIC should be viewed keeping in mind the different constituents, protocols, vehicles and concentrations that make each study design different despite using the same plant extract.

C.officinalis was completely ineffective against *F.nucleatum*, a nonmotile, and gram negative, anaerobic micro-organism, at all concentrations when tested for MBC. However, with *C.officinalis*, it does seem like longer the drug is in contact, the more effective its antimicrobial activity is, as exhibited by the antimicrobial activity

at two hours. Time kill curve data for a potential antimicrobial agent becomes important in terms of the target micro-organisms it is intended to have antimicrobial activity against. If the target microbes (periodontal pathogens in this instance) have an average regrowth period of few minutes while the potential drug possesses a time kill curve of two hours, it is not desirable irrespective of what the minimum inhibitory or bactericidal concentration is. *C.officinalis* also did not fare well in another study where it was used as a methanolic extract. It exhibited an extremely high MIC against *F.nucleatum* as compared to the other organisms in this particular study [30].

In the present study, *C.officinalis* was considerably effective against *S.mutans*, an organism that relies on adhesion to tooth surface as one of its virulence factors [31]. Cranberry juice, because of its high molecular polyphenol content is able to inhibit the adhesion of S.mutans [32] similar to it's the antiadhesive action against *E.coli* in preventing urinary tract infection in women [33]. Also, polyphenols of *Myrothamnus flabellifolia* were shown to reduce *P. gingivalis* adhesion and invasion upto about 50% by interacting with certain proteins that were specific to *P.gingivalis* [34]. Since, polyphenols are one of the chief constituents of *C.officinalis*, its antimicrobial potential and anti adhesive properties could as well translate to anti-plaque applications in vivo, further studies are required to confirm the same. If *C.officinalis* has to prove its worth against other key periodontal pathogens, animal model studies have to precede safety and efficacy studies in human clinical trials.

LIMITATION

All procedures were carried out only once, hence relevant analysis for accurate determination of efficacy could not be done.

CONCLUSION

This study exhibited the significant antibacterial effect *C.officinalis* had, on 4 major periodontal pathogens and *S.mutans*. However, its efficacy was most evident against *A.actinomycetemcomitans*, and least against *F.nucleatum*. Even though there is data on *C.officinalis* being incorporated in an antimicrobial mouthwash along with other herbal agents, it still needs to be investigated as a stand alone antimicrobial agent. More in vivo studies in different carrier vehicles need to be carried out to assess its longevity and feasibility in oral hygiene maintenance applications as well as locally delivered agent in a periodontal pocket. The key components/molecules in *C.officinalis* that are responsible for its antimicrobial efficacy still remain unexplored. Studies involving multiple repetitions of the tests as well against more number of micro-organisms would be the next step if *C.officinalis* has to be included into mainstream periodontal anti-infective therapy.

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Department of Microbiology, Maratha Mandal Dental College and Research Center, Belgaum, Karnataka. India.

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PARTICULARS OF CONTRIBUTORS:
1. Professor and Head, Department of Periodontics, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India.
2. Professor, Department of Periodontics, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India.
3. Postgraduate Student, Department of Periodontics, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India.
4. Reader, Department of Periodontics, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India.
5. Professor, Department of Periodontics, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India.
6. Reader, Department of Periodontics, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Soumya Gururaj Bardvalli,
710, 3rd a Cross, I Block, 7th Main, HRBR Layout, Kalyanagar, Bengaluru-560043, Karnataka, India.
E-mail: drbgsoumya@gmail.com

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