World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Antimicrobial effect of marigold leaves extracts on periodontal pathogens: *Invitro* study

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World Journal of Advanced Research and Reviews, 2022, 15(01), 183–187

Publication history: Received on 02 June 2022; revised on 07 July 2022; accepted on 09 July 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.15.1.0678

Abstract

Aim and objective: The aim and objective of this study was to assess the efficacy of *Calendula officinalis (Linn.)* with commercially available chlorhexidine gel and tetracycline fibers against plaque bacteria so that it can eventually be incorporated into periodontal anti-infective therapy.

Materials and method: Plant extract was made by 10 grams of Preparation of plant extract dried and ground leaves were mixed with 100ml of methanol and ethanol used for the extraction. Periodontal ab plus and Hexi gel was used. Antibacterial activity for plaque sample was seen using agar well diffusion method.

Results: Minimum Inhibitory Concentration of plaque bacteria at 0.1g/mL by *Calendula officinalis (Linn.)* was 15mm, chlorhexidine gel was 18mm and Periodontal ab plus was 33mm. periodontal ab plus showed 51 percent more zone of inhibition than *C. officinalis* leaves extracts and 42 percent more that of chlorhexidine gel.

Conclusion: The results of the present study indicate that the *C. officinalis* extract possessed good antimicrobial potential. This study exhibited the significant antibacterial effect of *C. officinalis* but less than chlorhexidine gel and tetracycline fibers on plaque bacteria.

Keywords: *Calendula officinalis (Linn.)*; Herbal extract; Minimal inhibitory concentration; Periodontal pathogen

1. Introduction

Periodontal disease can be treated via surgical and non-surgical along with systemic antibiotics in certain selected cases. Various systemic antibiotics have been proven as a good adjunct but over usage led to development of resistance to these drugs in a various putative periodontal pathogens. In suitable vehicles these antibiotics are incorporated and delivered into the periodontal pocket locally. Organic/plant based antimicrobials are being developed in order to reduce the possibility of resistance development [1].

Marigold flower (*Calendula officinalis (Linn.)* of genus-species [Tagetes erecta] consist of a group of phenolic molecule (flavonoids) that are antimicrobial in nature. Other biologically active molecules of *Calendula officinalis (Linn.)* are saponins [2], carotenoids, triterpenic alcohols [3] carotenoid pigments, polyunsaturated fatty acids like calendic acid as well as tannins, anthraquinones, cardiac glycosides and steroids etc. *Calendula officinalis (Linn.)* has been proven already that it is effective against a various pathogens like Esherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus [4].

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The present study was an attempt to assess the efficacy of *Calendula officinalis (Linn.)* flower extract with chlorhexidine gel and tetracycline fibers against subgingival plaque bacteria in invitro so that it can eventually be incorporated into periodontal anti-infective therapy in substitute to tetracycline fiber.

2. Material and methods

2.1. Plant extract

The flowers of *Calendula officinalis (Linn.)* were obtained from botanical shop near Kelambakam and the leaves were separated and dried at room temperature. 10 grams of Preparation of plant extract dried and ground leaves were mixed with 100ml of methanol and ethanol used for the extraction. The sample was filtered and solvent was then evaporated. The concentrated/dried extract was transferred into a 2ml syringe and stored at 4 C for further analysis.

- **Periodontal ab plus –** 25mg collagen impregnated with 2gm tetracycline Hcl
- **Hexi gel –** 1.0 percent Chlorhexidine

2.2. Antibacterial activity

2.2.1. Plaque sample

Subgingival plaque sample was collected using gracey curette and stored in phosphate buffered saline solution of ph 7.4

2.2.2. Agar well diffusion method

Preparation of inoculum

Stock cultures were maintained at 4°c on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hrs at 37º C. The Assay was performed by agar well diffusion method.

Antibacterial activity

Antibacterial activity of sample was determined by well diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton agar medium was weighed as 3.8 gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured into sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Wells were made using corn borer. 100µl of sample (1, 2 and 3) was loaded in respective wells. These plates were incubated for 24 hrs at 37ºC. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

3. Results

Methanol and ethanol extracts of *Calendula officinalis (Linn.)* extract exhibited varying antimicrobial activity, as shown by the growth inhibition zones (IZ). The greater the inhibition zone, the greater the antimicrobial activity. The results from the well diffusion method indicated that the tested *Calendula officinalis (Linn.)* extracts had comparable antibacterial effects against both Grampositive and Gram-negative bacteria. Minimum Inhibitory Concentration of plaque bacteria at 0.1g/mL was *Calendula officinalis (Linn.)* was 15mm, Hexi gel was 18mm and Periodontal ab plus was 33mm. periodontal ab plus showed 51 percent more zone of inhibition than *Calendula officinalis (Linn.)* extracts and 42percent more that of hexi gel.

Table 1 Zone of Inhibition of *Calendula officinalis (Linn.)* leaves extracts, Hexi gel and Periodontal ab plus

Microorganisms	**Zone of Inhibition in mm**		
	Concentration 100µl		
	***Calendula officinalis* (Linn.) extracts**	**Hexi gel**	**Periodontal ab plus**
Plaque stain	15	18	31

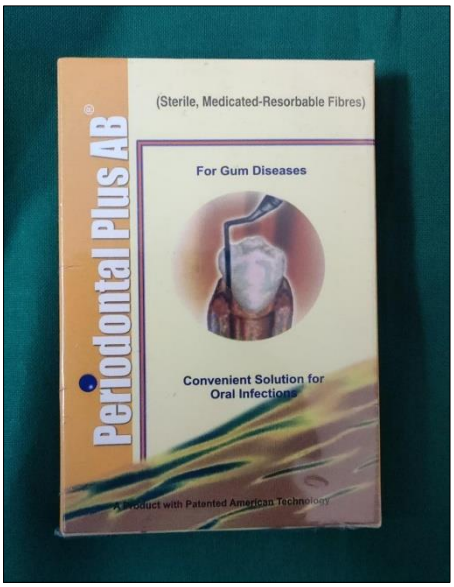

Figure 1 Periodontal ab plus

Figure 2 *Calendula officinalis (Linn.)* leaves extracts

Figure 3 Hexi gel

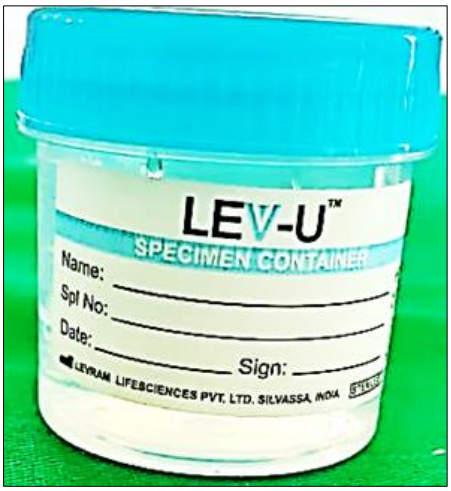

Figure 4 Plaque sample collected in sterile container

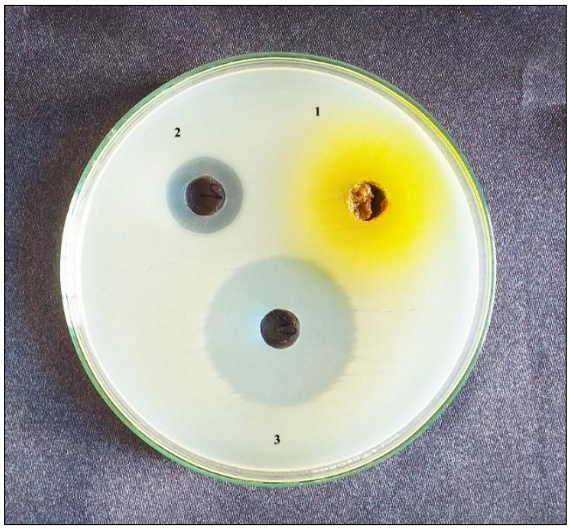

Figure 5 Minimum Inhibitory Concentration using well diffusion method

4. Discussion

Local administrations of antimicrobial agents are commonly preferred to systemic antimicrobials due to its higher drug availability in the periodontal pocket and decreasing systemic side-effects [5]. Chlorhexidine digluconate on the first day achieves a concentration greater than 100 ug/mL and maintained for an average of 6-9 days, and is much more than the MIC for chlorhexidine (0.10 ug/mL). Microorganisms that are high susceptibility to chlorhexidine include *P. gingivalis*, *P. intermedia*, *F. nucleatum*, *C. rectus*, *T. forsythia*, *H. aphrophilus*, and etc [6].

Tetracycline fibre are bacteriostatic and effective against rapidly multiplying bacteria. It also has activity of collagenase inhibition, property of anti-inflammatory, inhibits bone resorption and promote attachment of fibroblasts to root surfaces, also used as agents for conditioning root surfaces to enhance the regeneration of periodontal tissues [6]. In the present study, there was higher percentage of minimal inhibitory concentration compared chlorhexidine gel and *Calendula officinalis (Linn.)* gel.

Calendula officinalis (Linn.) a considerably low zone of inhibition of 15mm 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., [7] or its inability to test for susceptibility when it comes to certain fastidious micro-organisms [8]. *Calendula officinalis (Linn.)* having a lower MIC than Chlorhexidine gel and tetracycline fibers when tested in agar well diffusion. In another study involving chlorhexidine 0.12%, *Calendula officinalis (Linn.)* and other

herbs, *Calendula officinalis (Linn.)* exhibited a significantly lower MIC against *P. gingivalis* than against A.actinomycetemcomitans [9].

All studies have slightly different protocols for preparation *Calendula officinalis (Linn.)*, 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.

5. Conclusion

The results of the present study indicate that the *Calendula officinalis (Linn.)* extract possessed good antimicrobial potential. This study exhibited the significant antibacterial effect *Calendula officinalis (Linn.)* chlorhexidine gel and tetracycline fibers on plaque bacteria. Even though there is data on *Calendula officinalis (Linn.)* being incorporated in an antimicrobial local drug delivery agent along with other herbal agents, it still needs to be investigated as a standalone 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 *Calendula officinalis (Linn.)* 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 *Calendula officinalis (Linn.)* has to be included into mainstream periodontal anti-infective therapy.

Compliance with ethical standards

Acknowledgments

I acknowledge all my teachers for their constant support and encouragement

Disclosure of conflict of interest

All authors discloses no conflict of interest in publication of this article

References

[1] Silver LL. Challenges of antibacterial discovery. Clin Microbiol Rev, 2011, 24(1):71-109.

[2] Chakraborthy G. PHYTOCHEMICAL SCREENING OF CALENDULA OFFICINALIS LINN LEAF EXTRACT. International Journal of Research in Ayurveda & Pharmacy. 2010; 1(1): 131-134..

[3] Neukirch H, D'Ambrosio M, Dalla Via J, Guerriero A. Simultaneous quantitative determination of eight triterpenoid monoesters from flowers of 10 varieties of Calendula officinalis L. and characterisation of a new triterpenoid monoester. Phytochem Anal [Internet]. 2004; 15(1): 30–5.

[4] Shankar SM, Bardvalli SG, Jyotirmayee R, Chethana, Bhushan K, Kumar S. Efficacy of Calendula officinalis extract (marigold flower) as an antimicrobial agent against oral microbes: An invitro study in comparison with chlorhexidine digluconate. J Clin Diagn Res. 2017; 11(10): ZC05 - ZC10.

[5] Maurstad G, Stokke BT. Metastable and stable states of xanthan polyelectrolyte complexes studied by atomic force microscopy. Biopolymers [Internet]. 2004; 74(3): 199–213.

[6] Goodson JM, Offenbacher S, Farr DH, Hogan PE. Periodontal disease treatment by local drug delivery. J Periodontol [Internet]. 1985; 56(5): 265–72.

[7] Jenkins SG, Jerris RC. Critical assessment of issues applicable to development of antimicrobial susceptibility testing breakpoints. J Clin Microbiol [Internet]. 2011; 49(9): S5–10.

[8] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal [Internet]. 2016; 6(2): 71–9.

[9] Rodriguez-Garcia A, Galan-Wong LJ, Arevalo-Niño K. Development and in vitro evaluation of biopolymers as a delivery system against periodontopathogen microorganisms. Acta Odontol Latinoam [Internet]. 2010; 23(2): 158–63.