

Calendula :- A Blooming Alternative Against Periopathogens

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Abstract : Periodontitis is a chronic inflammatory disease of the tooth and supporting structures with signs of alveolar bone and connective tissue loss and is mediated by combination of periodontal pathogens and host defense system. The most common treatment for periodontal disease is scaling and root planing (SRP) which has been confirmed as a gold standard treatment of periodontitis. However, the limitations of SRP are the inability to access deeper areas of gingival sulcus which leads to early microbial recolonization and recurrence of periodontitis. In the recent years, most of the researcher's interest has shifted in evaluating the antimicrobial properties of different parts of the plant researchers to investigate regarding local delivery of drugs prepared from them which can reach the inaccessible areas of the gingival sulcus, to decrease the current problems associated with the wide scale misuse of antibiotics that induce microbial drug resistance.

Keywords: Antibacterial agents, calendula, drug resistance, periodontal diseases, periodontitis

I Introduction

Periodontitis is a chronic inflammatory disease of the tooth and supporting structures with signs of alveolar bone and connective tissue loss and is mediated by combination of periodontal pathogens and host defense system.¹ More than 700 different bacterial species colonize the oral cavity, but only a few of these are thought to be potential periodontal pathogens.² *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia* are known as the main pathogens of periodontal disease and treatment of periodontal disease is associated with success in removing and reducing these microorganisms.^{3,4,5} The most common treatment for periodontal disease is scaling and root planing (SRP) which has been confirmed as a gold standard treatment of periodontitis.⁶ However, the limitations of SRP are the inability to access deeper areas of gingival sulcus which leads to early microbial re-colonization and recurrence of periodontitis.^{7,8} This has led researchers to investigate regarding local delivery of drugs which can reach the inaccessible areas of the gingival sulcus.

In the recent years, most of the researcher's interest has shifted in evaluating the antimicrobial properties of different parts of the plant. A lot of research has been carried out on the utilization of medicinal plants in the treatment of a variety of ailments, especially in the last two to three decades. The interest in plants with antibacterial and anti-inflammatory activity has increased as a consequence of the current problems associated with the wide scale misuse of antibiotics that induce microbial drug resistance^{9,10} and cytotoxic effects on the host cells.

Calendula officinalis is commonly known as marigold. It is an annual flower in Asteraceae family,¹¹ and is native of Asia and southern Europe. According to the secondary sources, calendula has been used medicinally since 12th century, mainly in the Mediterranean. It has been used topically to treat minor wounds, burns, naturopathy, homeopathic and powder form of the plant petals is occasionally used as an inexpensive alternative to saffron for coloring and flavoring foods.¹²

In laboratory research, calendula has hypoglycemic effects,¹³ and also have antihypersensitivity¹⁴, anti-inflammatory¹⁵, Antiprotozoal¹⁶, Antispasmodic¹⁷ and antimicrobial activity¹⁸. Dietary Supplementation of *Calendula officinalis* counteracts the oxidative stress and liver damage resulted from aflatoxin.¹⁹ It has been shown to have activity against HIV²⁰, vesicular stomatitis virus and rhinovirus as well as the Epstein - Barr virus. It has Immunosuppressant²¹, sedative²², have antioxidant²³, Antineoplastic²⁴, cytotoxicity and genotoxicity²⁵ properties. *Calendula officinalis* produces a dual *in vitro* effect i.e., cytotoxic anti-tumor activity and lymphocyte activation²⁶. It has been reported high industrial value in food and Drug Company. The US

Food and Drug administration listed calendula and recognized as safe when used as a spice, natural seasoning, and flavouring²⁷.

Mouth rinsing with calendula will allow its anti-inflammatory properties to work against the swollen, irritated gums and its antibacterial properties deal with the periodontopathic microorganisms²⁸. **The present study was carried out to evaluate the antimicrobial activity of Calendula Officinalis against various periopathogens (Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Tanerella forsythia).**

II Materials And Method

2.1 Plant material

The part of *calendula officinalis plant* used in this study was flower. **Calendula Oil** is extracted from the flowers of Calendula of marigold family through the steam distillation process.

2.2 Steam distillation process

In this process, the steam was used to pass through the organic plants to extract the essential oils from it. The organic compounds such as plants and herbs can decompose easily by passing the steam and then the essential oil which is not soluble in hot water can be easily separated from water. With the effect of hot steam, the tiny glands of plant which contain essential oils release the oil molecules easily and then this oil was separated by using the Florentine separator from the mixture of hot water and oil. By using this process, the natural essence and aroma was kept intact with the extracted oil.

2.3 Test Organism

The bacterial culture were collected from *Maratha Mandal's* Nathajirao G. Halgekar institute of Dental Sciences and Research Centre, Belgaum. The strains of bacteria selected to assess susceptibility against extracts prepared in the study included were *Porphyromonas gingivalis*, *Tanerella forsythia*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*.

2.4 Determination of MIC by tube dilution method.

9 dilutions of each drug have to be done with BHI for MIC. In the initial tube 20 microliter of drug was added into the 380 microliter of BHI broth. For dilutions 200 microliter of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200 microliter was transferred to the first tube containing 200 microliter of BHI broth. This was considered as 10⁻¹ dilution. From 10⁻¹ diluted tube 200 microliter was transferred to second tube to make 10⁻² dilution. The serial dilution was repeated up to 10⁻⁹ dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth. In each serially diluted tube 200 microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity(fig 1)

Note: For facultative anaerobes, tubes were incubated at 37°C for 48-72 hrs in Co₂ Jar. For strict anaerobes, tubes were incubated in anaerobic jars at 37°C for 48-72 hrs.

III Results

Calendul a	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
Pg	S	S	S	S	S	S	S	S	R	R
Tf	R	R	R	R	R	R	R	R	R	R
Fn	S	R	R	R	R	R	R	R	R	R
Aa	S	S	S	R	R	R	R	R	R	R

Note:S-Sensitive ; R-Resistant

Porphyromonas gingivalis showed sensitivity at the concentration of 0.8 µg/ml, **Aggregatibacter actinomycetemcomitans** showed sensitivity at the concentration of 25µg/ml, **Fusobacterium nucleatum** showed sensitivity at the concentration of 100µg/ml and **Tanerella forsythia** showed resistance through out.

IV Discussion

Calendula officinalis is a well known and versatile herb in herbal medicine. It is a remedy for skin problems and is applied externally to bites, stings, sprains, wounds, sore eyes and varicose veins etc. It is also a cleansing and detoxifying herb and is taken internally in treating fever and chronic infections.^{29,30} Its flowers were examined for source of extracts with antioxidant activity and also showed anti-inflammatory activity with possible mechanism of action. The results of their investigation showed that anti-inflammatory response of flower extract may be mediated by the inhibition of proinflammatory cytokines and cox-2 and subsequent prostaglandin synthesis. Effect of *Calendula officinalis* flower extract on acute phase proteins, antioxidant

defense mechanism and granuloma formation during thermal burns was analyzed. The histopathological analysis of skin tissue gave the evidence of the increased healing potential of the extract after burn injury.³¹

A study which reported that a calendula containing mouthwash (plandula) was effective in reducing the PI score by 23.9% and the GI score by 62.3% during a 14day study period.³² . It is known that periodontal infections can cause more or less serious damage, from gum inflammation to the loss of alveolar bone and eventually the loss of teeth, it is necessary to begin a series of preventative measures in patients at risk. Information on the antibacterial activity of medicinal plants against anaerobic and facultative aerobic bacteria present in the oral cavity that often cause periodontal infections is very scarce.

A study have given useful information about the positive effects of calendula toothpaste on decreasing gingivitis indices. It is suggested that the use of the toothpaste containing calendula flower alcoholic extract for gingivitis could be a useful aid to obtaining a significant reduction of parameters like Plaque Index, Gingival Index and Bleeding on probing compared to the placebo paste. It can be recommended as an adjunct treatment to daily oral hygiene procedures.³³

A study demonstrated that the calendula flower extract possesses a high degree of antimicrobial activity against 18 different strains of anaerobic and facultative aerobic periodontal bacteria *in vitro*, suggesting that it may have an inhibitory effect on the bacteria causing pathogenesis of the supporting structures of the tooth.³⁴ In a *in vitro* study, it showed antifungal activity of calendula when compared to nystatin against different species of *Candida* including those causing oral candidiasis.³⁵ Like chlorhexidine, calendula also has the property of substantivity as demonstrated by Schmidgall *et al* in *ex-vivo* laboratory model. Calendula has strong bioadhesion to porcine buccal membrane. This property has been attributed to polysaccharides and mucilage content in the herb. Such effects suggest that it can be used for the therapeutic application in treatment of canker sore, aphthous ulcer, sore throat, gingivitis, etc., However, a study comparing the substantivity between chlorhexidine and calendula needs to be analyzed in future.³⁶ It has also been suggested that calendula accelerates wound healing through reepithelialization and collagen maturation..³⁷

The present study carried out to evaluate the minimum inhibitory concentration of *Calendula officinalis* against various periopathogens like *Porphyromonas gingivalis* showed sensitivity at the concentration of 0.8 µg/ml, *Aggregatibacter actinomycetemcomitans* showed sensitivity at the concentration of 25µg/ml, *Fusobacterium nucleatum* showed sensitivity at the concentration of 100µg/ml and *Tanerella forsythia* showed resistance throughout.

V Figures



Fig 1

VI Conclusion

Periodontal disease is a multifactorial disease, caused by the interaction of host, microbial agents and environmental factors. It is important to remember that *in vitro* tests do not reflect the real condition found in periodontal pockets. They do not consider the above factors into consideration. From the present study, it can be concluded that calendula possess antibacterial activity against periodontal pathogens. As the antibacterial activity is assessed against single microorganism, the exact interaction cannot be assessed by *in vitro* studies. Further *in vivo* studies has to be conducted to prove its antibacterial activity against periodontal pathogens without causing major local or systemic adverse effects. Indeed, further work has to be done to elucidate main chemical compounds and their exact mechanism of action responsible for the antibacterial activity.

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