Antibacterial and anti-inflammatory properties of *Plantago ovata* Forssk. leaves and seeds against periodontal pathogens: An *in vitro* study

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Abstract

Background: *Plantago* commonly called as *Isabgol* (*Plantago ovata* Forssk.) is a perennial herb that belongs to the family *Plantaginaceae*. A range of biological activities has been found from plant extracts, including wound healing activity, anti-inflammatory, analgesic, antioxidant, weak antibiotic, immunomodulating and anti-ulcerogenic activity. Periodontal disease is a complex condition as a result of interaction between microorganisms and host inflammatory mediators. Hence, the extract of *Isabgol* is tested for its antibacterial and anti-inflammatory properties against periodontal disease. **Aim:** The aim of this *in vitro* study is to evaluate the antibacterial property of *Isabgol* leaves and seeds against periodontal pathogens, namely *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis, Prevotella intermedia,* and *Fusobacterium nucleatum* and anti-inflammatory property against matrix metalloproteinase-2 (MMP-2) and MMP-9. **Materials and Methods:** In this *in vitro* study, aqueous extract of *Isabgol* is tested for its antibacterial property against the stock cultures of specified periodontal pathogens using the tube dilution method and anti-inflammatory property against MMP-2 and MMP-9 using zymogen gel electrography. **Results:** Minimum concentration at which the sensitivity of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia,* and *F. nucleatum* for the extract observed was 50 μl/ml, 0.8 μl/ml, 0.4 μl/ml and 12.5 μl/ml, respectively, concentrations below these showed no effect on the microorganisms. Zymogen electrographic test for anti-inflammatory activity showed percentage inhibition of 30% and 40% against MMP-2 and MMP-9, respectively. **Conclusion:** *Isabgol* is effective against the periodontal pathogens and inflammatory mediators which are responsible for periodontal disease.

Keywords: Antibacterial, anti-inflammatory, *Isabgol*, matrix metalloproteinases, periodontitis, *Plantago*

Introduction

Plantago commonly called as *Isabgol* is a perennial herb that belongs to the family *Plantaginaceae*. The leaves of *Plantago ovata*[1] have long been used in wound healing and are still being used in traditional medicine.[1] Greek physicians described its usage in wound healing in the first century A.D.[2] In traditional medicine, the juice from the leaves of this plant is used to treat superficial wounds.[3] Norwegian and Swedish people called this plant "groblad" which means "healing leaves."[4] A range of biological activities has been found from plant extracts, including wound healing activity, anti-inflammatory, analgesic, antioxidant, weak antibiotic, immune-modulating and anti-ulcerogenic activity.[4]

P. ovata is used in many parts of the world in treating skin diseases, infectious diseases and problems concerning the digestive organs, respiratory organs, reproduction, against tumors, for pain relief and for reducing fever.[4]

It is commonly used as a diuretic agent in India and China.[3] In some parts of India, the plant is used as Ayurvedic medicine to treat cut wounds, fever, weakness, respiratory infections and digestive system associated problems. In homeopathic medication, it is used to treat disorders of the epidermis, headache, earache and toothache.[1] Although the plant is known for its antibacterial and anti-inflammatory properties, it has been never evaluated for its efficacy on pathogens and inflammatory mediators resulting in periodontal disease.

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Hence, the aim of this study is to evaluate the antibacterial property of *Isabgol* against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* and anti-inflammatory property against matrix metalloproteinase-2 (MMP-2) and MMP-9.

Materials and Methods

In this *in vitro* study, extract of *Isabgol* leaves and seeds were tested for its antibacterial property against the stock cultures of specified periodontal pathogens using the tube dilution method and anti-inflammatory property against MMP-2 and MMP-9 using the zymogen gel electrography.[4] Extract of *Isabgol* was purchased from the VHCA herbals through online portal www.ayurvedacart.com. The extract was the aqueous type and was made by the cold maceration process, manufactured according to GMP (goods manufacturing practices) guidelines by the seller VHCA Ayurveda, Gaharaunda, Haryana, India.

Evaluation of antibacterial property

Antibacterial property was evaluated using the minimum inhibitory concentration (MIC) against three periodontal pathogens, i.e., *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *F. nucleatum* using the tube dilution method.

Minimum inhibitory concentration

Stock cultures of the mentioned organisms were obtained from the Department of Microbiology, Bapuji Pharmacy College, Davangere, Karnataka, India. Tube dilution method was carried out to evaluate the antibacterial property.

Tube dilution method

Nine dilutions of each drug was done with thioglycollate broth for MIC.

Initially, 20 µl of the extract was added to 380 µl of thioglycollate broth. For dilutions, 200 µl of broth was added in nine tubes separately. A volume of 200 µl from the initial tube containing extract was added to the first tube, this was 10^{-1} dilution.

From 10^{-1} diluted tube, 200 µl was transferred to the second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution. Thus, the concentrations of the extract obtained after serial dilutions were 0.2, 0.4, 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50, and 100 µl/ml, respectively.

From the maintained stock cultures of required organisms, 5 µl was taken and added into 2 ml of thioglycollate broth. In each serially diluted tube, 200 µl of above culture suspension was added. Tubes were incubated in anaerobic jar at 37°C for 48–72 h and observed for turbidity[5] [Figure 1].

Evaluation of anti-inflammatory property

Anti-inflammatory property against two MMPs, i.e., MMP-2 and MMP-9 were evaluated *in vitro* through the zymogen electrographic method.

Sample preparation

Excised inflamed tonsil specimen was used as a source of the inflammatory sample. The sample was chopped, and 5 ml of Tris (tris[hydroxymethyl] aminomethane) buffer was added to it and centrifuged at 3000 RPM for 15 min and stored at −20° for further use.

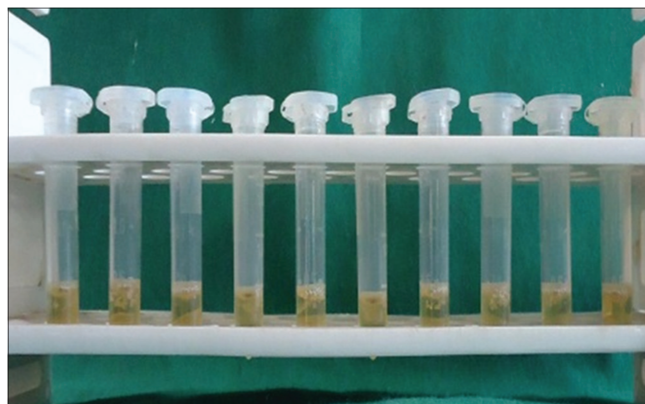

Figure 1: Tube dilution method

Zymography

Proteolytic activity was examined on 10% polyacrylamide gels-containing 0.05% gelatin. The tissue sample was added to equal volume of nonreducing buffer (2.8 mL distilled water +1 ml 0.5M Tris HCL (pH 6.8) +0.8 ml glycerol +3.2 ml 10% SDS +0.2 ml 0.2% bromophenol blue). 20 µL of the mixture sample was loaded into each well and subjected to electrophoresis.

After electrophoresis, the gel was removed and put into a plastic dish and washed with zymogram renaturing buffer, i.e., 2.5% Triton x-100 for 1 h to remove SDS from the gel and allow proteins to denature. Decanting of the zymogram renaturing buffer and the gel was incubated in zymogram incubation buffer at 37°C overnight. The gel was then stained with Coomassie blue R-250 for 1 h, and then the gels were destained using appropriate destaining solution. After staining, the gels were observed for the white bands which indicate the presence of gelatinases. The anti-inflammatory activity of tested compounds lightens or clears the white bands against the dark background [Figure 2].

Inhibition of metalloproteinase activity by the extract

To examine the effect of *Isabgol* extract on enzyme activity, conditioned medium containing MMPs was loaded on preparative gelatin-containing polyacrylamide gels. After electrophoresis, the gels were incubated at 37° for 16 h in Tris-$CaCl_2$ buffer containing the extract. The concentration used was 100 µl/ml. After adding the extract to the solution, the pH was adjusted to 7.4, the gels were extensively washed in 2% Triton X-100, and reincubated in Tris-CaCl2 solution at 37°C for 16 h. To quantify the relative inhibition of MMPs by $ZnSO_4$ and $CuSO_4$, electrophoretic bands were scanned, and the transmittance (the transmittance values of the zymogen and active form were added) was analyzed with the SigmaGel software (Sigma – Aldrich Merck, Germany). The percentage inhibition of the extract was determined by comparing the activity of MMPs with control reactions.

MMPs sample without any compound was used for the negative control. MMPs sample with tetracycline stored for 1 h was used as the positive control.

Results

Of all the concentrations tested, the concentration at which the organisms have started showing sensitivity is recorded as MIC [Table 1]. The results varied among the organisms. *A. actinomycetemcomitans* showed more resistance when compared to other three organisms as the MIC required for *A. actinomycetemcomitans* was 50 µl/mL, whereas *P. intermedia* showed sensitivity at the lowest concentration when compared to others that was at 0.4 µl/mL. *P. gingivalis* and *F. nucleatum* showed sensitivity at a concentration of 0.8 µl/mL and 12.5 µl/mL, respectively.

Anti-inflammatory results were interpreted in percentage (%) inhibition of MMP-2 and MMP-9 when compared to the positive control (tetracycline) and negative control (no compound).

Isabgol showed 30% inhibitory activity against MMP-2, which was three times lesser than that of positive control but three times more than that of the negative control. Against MMP-9 *Isabgol* showed 40% inhibitory concentration which was 2.5 times lesser than that of positive control and two times more than that of the negative control [Table 2].

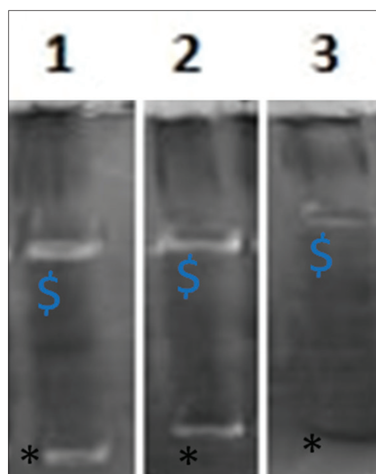

Figure 2: Zymogen gel electrography. $Matrix metalloproteinase-2, *matrix metalloproteinase-9. 1: *Isabgol*, 2: Negative control, 3: Positive control

Discussion

Recent ethnopharmacological studies reported that *Plantago Ovata* is used in many parts of the world, in the treatment of a number of diseases.[6] *Plantago* leaves and seeds contain carbhohydrates,[7-10] lipids,[11] alkaloids,[12] caffeic acid derivatives,[13,14] flavonoids,[15] iridoid glycosides,[16] vitamins and other organic substances owing to its diverse medicinal properties. Each of the constituents has unique medicinal property. The polysaccharide called plantaglucid extracted from *Isabgol* has shown to reduce the ulcer index in the rat stomach.[9] Clinical and histological studies showed that saturated C26–C30 primary alcohols with even numbers of carbon atoms from the *n*-hexane extract and the nonhydrolysable fractions of the *n*-hexane extract made from isabgol leaves had powerful curative effects on superficial injuries in rabbits.[17] Aucubin which is one of the iridoid glycosides present in leaves is known to have anti-inflammatory property through the inhibitory effect of TPA (12-o-tetradecanoylphorbol-13-acetate).[18] *Isabgol* has also been tested for wound healing properties by assessing the proliferation and migration of oral epithelial cells *in vitro* and the results showed that the extracts of *Isabgol* have beneficial effects on proliferation of epithelial cells suggesting its wound healing properties.

Studies showed that the extracts made from *Isabgol* have shown antibacterial activity against many bacteria such as *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis* and methicillin-resistant *S. aureus*. The exact mechanism is not understood, but this antibacterial activity can be majorly attributed to a caffeic acid derivative called plantamajoside.[19] Plantamajoside is also known to have anti-inflammatory activity through its inhibitory effect on arachidonic acid metabolism,[20] and is also known to have anti-oxidant effect[21] and radical scavenging property.[22] The present study was undertaken to assess the efficacy of *Isabgol* on periodontal pathogens, and the results show that *Isabgol* is effective against *P. gingivalis*, *A. Actinomycetemcomitans* and *F. nucleatum*. Reason for selecting specific microorganisms is that the flora present in the dental plaque is predominated by anaerobic bacteria such as *P. gingivalis, F. nucleatum*, *A. actinomycetemcomitans* and has shown to be associated with onset and progression of periodontal disease.[23] However, it is now recognized that during active periodontitis, degradation of gingival tissue (mainly collagen) is due in part to MMPs expressed *in situ* by inflammatory cells and resident cells.[24,25]

Table 1: Minimum inhibitory concentration

Isabgol (*Plantago ovata* Forssk.)	100 (µl/ml)	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Aa	S	S*	R	R	R	R	R	R	R	R
Pg	S	S	S	S	S	S	S	S*	R	R
Pi	S	S	S	S	S	S	S	S	S*	R
Fn	S	S	S	S*	R	R	R	R	R	R

*MIC of the extract found using the tube dilution method. MIC for *Aa* is 50 µl/ml, *Pg* is 0.8 µl/ml, *Pi* is 0.4 µl/ml, *Fn* is 12.5 µl/ml, respectively.
S: Sensitive, R: Resistant, *Aa: Aggregatibacter actinomycetemcomitans, Pg: Porphyromonas gingivalis, Pi: Prevotella intermedia, Fn: Fusobacterium nucleatum*, MIC: Minimum inhibitory concentration

Table 2: Anti-inflammatory activity

Sample name	Anti-inflammatory activity against MMP-2 (%)	Anti-inflammatory activity against MMP-9 (%)
Isabgol (*Plantago ovata* Forssk.)-1st	30%*	40%*
PC	90%#	100%#
NC	10%$	20%$

*Percentage inhibition of MMP-2 and MMP-9 by isabgol is 30% and 40%, respectively, #Percentage inhibition of MMP-2 and MMP-9 by positive control (tetracycle) is 90% and 100% respectively, $Percentage inhibition of MMP-2 and MMP-9 by negative control (no compound) is 10% and 20% respectively. PC: Positive control, NC: Negative control, MMP: Matrix metalloproteinase

The proteolytic activity of MMP's is under the control of endogenous tissue-specific inhibitors, the tissue inhibitors of metalloproteinases, as well as α2-macroglobulin.[26] Imbalance between these results in pathological processes. Hence, it has been tested for its anti-inflammatory properties against MMP-2 and MMP-9. The results showed its effect on these MMPs is weak when compared with the positive control. MMP-2 and MMP-9, also known as gelatinases A and B, respectively, are active in the degradation of denatured fibrillar collagens, elastase, and several other components of the extracellular matrix[27-29] MMP-2 and MMP-9 were selected because there are several evidence indicating that MMP-2 and MMP-9 play an important role in tissue destruction during periodontal disease.[28] Periodontitis patients have significantly higher levels of MMP-2 and MMP-9 than healthy participants, and the amount of gelatinases decreases after periodontal treatment.

As it is tested only against MMP-2 and MMP-9, the results cannot be extrapolated to other MMP's.

Conclusion

Within the limitations of the study, *Isabgol* extract has shown to be an effective antibacterial and a weak anti-inflammatory agent. This is the first time, it is tested for its efficacy against periodontal pathogens and MMPs. Further confirmatory studies need to be conducted to prove it as an effective alternative for regularly used antibiotics.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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