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Determination of Minimal Inhibitory Concentration of Gel containing combination of Picrorhiza Kurroa and Ficus Benghalensis for Treatment of Periodontitis

Research Article

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Abstract

Background: The therapeutic effects of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. for treatment of chronic inflammatory diseases is well known. Objective: The purpose of the present study is assessment of the efficacy of gel containing combination of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. against periodontopathogens and hydroalcoholic extracts of *Picrorhiza kurroa* Royle ex benth (PK) and *Ficus benghalensis* L. (FB) against periodontopathogens. Material & Methods: The preparation of the herbal Water extracts, Alcohol extracts and Hydro Alcoholic extracts was done by Soxhlet extraction method. MIC of different extracts of PK and FB and its combination was determined. Results: The MIC of the PK water extract against Aggregatibacter actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg) was 0.8 µg/ml. For Aa and Pg, the MIC of water extract of FB was 1.6 µg/ml and 6.25 µg/ml, respectively. The combination of PK and FB alcoholic extract was shown to have a MIC of 1.6 µg/ml. Conclusion: The presence of antibacterial activity could be confirmed in periodontopathogens species which were assayed in this study. However, the MIC for the species employed showed a very large range, and were mostly very high. Nevertheless, traditional knowledge might provide some leads to elucidate potential candidates for future development of new antibiotic agents.

Keywords: Minimum Inhibitory Concentration (MIC), Antibacterial, *Picrorhiza kurroa, Ficus benghalensis*, Ayurvedic medicine, Antioxidants.

Introduction

Periodontal diseases affect the supporting tissues of the teeth manifesting as gingival inflammation and perhaps advancing to loss of alveolar bone and tooth loosening. It is associated with a dysbiosis of microorganisms in subgingival biofilm. Porphyromonas gingivalis (Pg) has been designated as a keystone pathogen by modifying the biofilm towards a pathogenic one via modulation of the host response. (1) The other important microbe that is generously associated with aggressive periodontitis includes Aggregatibacter actinomycetemcomitans (Aa).

A range of treatment medications have been tried and tested slowing the advancement of periodontal inflammatory process and that would aid in the regeneration of the lost periodontal tissues. Clinical trials for a number of additional herbal-based treatments focused on their ability to combat periodontopathogens are under underway. Local delivery of chemo-therapeutic agents into the pockets via a syringe or irrigating device has been shown to be effective against subgingival flora.

Picrorhiza kurroa (P. kurroa) is a well-known as *Kutki* in Ayurvedic medicine and has antioxidant, anti-inflammatory, anti-allergic and immunomodulatory properties. (2-6) Its anti-inflammatory activity is mediated through the suppression of macrophage-derived cytokine and mediators via suppression of NF-κB signalling. (7)

The *Ficus benghalensis* L. root extract has been used in medicine since ages to boost the immune system. The methanolic and water extracts of ficus have immunostimulatory properties and enhances the phagocytic potential of PBMCs. It also induces the proliferation of lymphocytes and hence the generation of cytokines that activate other immune cells. The hydroalcoholic leaf extracts of *Ficus benghalensis* L. significantly increased the phagocytic activity of human neutrophils and hence engulfment and clearance of microorganisms by leukocytes, along with free radical scavenging properties and reduction of oxidative stress, thereby showed immunomodulatory, anti- inflammatory and antioxidant activity. (8,9) Traditionally, *Ficus benghalensis* L. is used as astringent, haemostat, as anti-inflammatory and anti-septic agent and in other ailments.

A drug's minimum inhibitory concentration (MIC) is the lowest concentration at which it may

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prevent a test organism from growing visibly. (10) A drug's in vivo use is guided by the in vitro determination of its MIC against infections. Clinicians use MIC scores to decide which antimicrobial agents are best for individuals with certain illnesses and to determine a suitable dosage of the medication. A. actinomycetemcomitans and Pg are the potential periodontopathogens hence determination of the MIC of the antimicrobial agent against these pathogens is mandatory for the particular agent to be effective for periodontal therapy.

As there is a paucity of literature assessing the effectiveness of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. for treatment of chronic periodontitis, this study was designed with the aim to evaluate the minimal inhibitory concentration(MIC) of water and alcoholic extracts of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. against Periodontopathogens Pg and Aa.

Materials and Methods

An in vitro experimental design was adopted to conduct the study. It was conducted at Department of Periodontics, Ranjeet Deshmukh Dental College and Research Centre, Nagpur from September 2022 to February 2023.

Selection and collection of plant materials

The selection and the collection of plant material are important in making efficient phyto-constituent isolation. The disease free and healthy plants were selected for the plant extraction. The drying process is important for the extraction of plant materials having the active enzymes which produces the active constituent's intermediates and metabolic reactions. The selected plant materials were dried.

Grinding and size reduction

Grinding and size reduction is the essential for the Soxhlet extraction process because smaller the particle size greater the surface area of the powdered particles. Large surface area improves the contact of the powdered particles with the solvent used for extraction and hence efficient extraction takes place. The dried plant material was grounded for size reduction using mortar pestle.

Table 1: Composition of gel

Ingredients	Gms/litre
Calf brain, infusion	200.00
Beef heart, infusion	250.00
Proteose peptone	10.00
Dextrose	2.00
Sodium chloride	5.00
Disodium phosphate	2.50
Final pH (at 25ºC)	7.4+/-0.2

Procedure of preparation of the herbal extracts

Soxhlet extractor: The Soxhlet extractor setup consisted of a round bottom flask, siphon tube, distillation path, expansion adapter, condenser, cooling water inlet, cooling water outlet, heat source and thimble. In this method, powdered sample was enclosed in a porous bag or "thimble" made from a strong filter paper or cellulose, which was placed in thimble chamber of the Soxhlet apparatus. Extraction solvent in the round bottom flask was heated by using heating mantle. The heating temperature was built on the solvent employed to extraction. Due to heat the solvent in the bottom flask vaporised into the condenser and then dripped back to the sample thimble. When liquid content reached the siphon arm, the liquid contents emptied into the bottom flask again and the clear solution in the siphon tube was obtained.

Post extraction process

After the Soxhlet extraction process, the extracted materials were exposed to further processes like concentrate the extract, evaporate the solvent and storage of the extract. The distillation process was used for the isolation of extract from solvent. After collecting the extract, it was stored in a well closed container covered with an aluminum foil and refrigerated.

Water extraction, Alcohol extraction and Hydro Alcoholic extraction for *Ficus benghalensis* L.

Procedure: 10 g of the air dried powder with 100 ml water was refluxed with Soxhlet apparatus for 6 hours. The water extraction yield of *Ficus benghalensis* L. found to be 9.23 %. The alcohol extraction yield of *Ficus benghalensis* L. was found to be 1.34 % and the hydro alcoholic extraction yield of *Ficus benghalensis* L. was found to be 14.87 %.

Water extraction, Alcohol extraction and Hydro Alcoholic extraction for *Picrorhiza kurroa* Royle ex benth

Procedure: 10 g of the air dried powder with 100 ml water reflux with Soxhlet apparatus for 6 hours. It was filtered rapidly taking precautions against the loss of solvent. Evaporate of the filtrate was in a tarred flat bottomed shallow dish at 1000 with the help of thermostatic water bath and weigh the percentage of water soluble extractive calculate. The water extraction yield of *kutki* was 9.46%. The alcohol extraction yield of *kutki* was found to be 1.87 % and the hydro alcoholic extraction yield of *kutki* was 15.78 %.

Antimicrobial effects of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L.

The antimicrobial effects of both the herbal extracts have been investigated and is reported to be effective against the anaerobic periodontopathogens. We have determined the minimal inhibitory concentration (MIC) of different extracts of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. and its combination. The control used was Moxifloxacin <0.125 µg/ml. HIMEDIA M210-500G Brain Heart Infusion Broth (BHI) was used for culture. Calf brain infusion 200 gms/litre, Beef heart infusion 250 gms/litre, Proteose peptone 10 gms/litre, Dextrose 2.00gms/litre, Sodium chloride 5.00 gms/litre, Disodium phosphate 2.50gms/ litre. Final pH obtained (at 25ºC) was 7.4+/- 0.2.

Procedure

MIC Test (Aerobic): Nine dilutions of each drug were 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 into the next nine tubes separately. Then from the initial tube 200 microliter was transferred to the first tube containing 200 microliters 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, 5 microliters were taken and added into 2ml of BHI broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity.

Table 2: MIC of gels and combination of both the gels

Extract	MIC of *Picrorhiza kurroa* Royle ex benth.		MIC of *Ficus benghalensis* L.		MIC of combination of *Ficus benghalensis* L. and *Picrorhiza kurroa* Royle ex benth.	
	Aa	Pg	Aa	Pg	Aa	Pg
Water Extract	0.8 µg/ml	0.8 µg/ml	1.6 µg/ml	6.25 µg/ml	-	-
Alcoholic Extract	1.6 µg/ml	25 µg/ml	-	-	1.6 µg/ml	1.6 µg/ml
Hydro-alcoholic Extract	0.8 µg/ml	3.12 µg/ml	1.6 µg/ml	1.6 µg/ml	-	-

Results

The MIC of different extracts of both the herbs is shown in Table 1. The water extract of *Picrorhiza kurroa* Royle ex benth had MIC of 0.8 µg/ml against Aa and Pg. The MIC of *Ficus benghalensis* L. for Aa was 1.6 µg/ml and Pg was 6.25 µg/ml. The alcoholic extract of *Picrorhiza kurroa* Royle ex benth showed MIC of 1.6 µg/ml against Aa and 25 µg/ml against Pg. MIC of combination of alcoholic extract of *Ficus benghalensis* L. and *Picrorhiza kurroa* Royle ex benth was 1.6 µg/ml against both the microbes. The hydroalcoholic extract of *Picrorhiza kurroa* Royle ex benth showed MIC of 0.8 µg/ml against Aa and 3.12 µg/ml against Pg while *Ficus benghalensis* L. showed MIC of 1.6 µg/ml against both the periodontopathogens. The MIC of the control Moxifloxacin was found to be around 0.12 µg/ml for Aa and 0.06 µg/ml.

Discussion

P. kurroa is known to possess anti-oxidant, anti-diabetic, anticancer, antifungal, immunomodulatory properties. Phytoconstituents such as glycosides, alkaloids, cucurbitacins, iridoids, phenolics, and terpenes in *Picrorhiza kurroa* Royle ex benth have shown promising pharmacological potential. The diverse pharmacological activities of *Picrorhiza kurroa* Royle ex benth, a medicinally significant endangered plant, have led researchers to develop practical techniques for its in vitro mass multiplication. (11-13) *Ficus benghalensis* L. also helps in dealing with bleeding gums, dental abscesses, throat infections, canker sores or mouth ulcers. The higher analgesic and anti-inflammatory effects of various extracts of *Ficus benghalensis* L. tested might back to the presence of flavonoids and phenolic compounds. These data suggest that the different extracts of the bark of *Ficus benghalensis* L. produce analgesic, anti-inflammatory and antipyretic activities that could be due to the effects of bioactive components in the extract (14).

Along with this the hydroalcoholic leaf extracts of *Ficus benghalensis* L. significantly increased the phagocytic activity of human neutrophils and hence engulfment and clearance of microorganisms by leukocytes, along with free radical scavenging properties and reduction of oxidative stress, thereby showed immunomodulatory and antioxidant activity.

Periodontal disease chronic multifactorial infectious disease of the supporting tissue of the teeth and is characterised by destruction of periodontal connective tissue and alveolar bone.

Many local drug delivery agents have been effectively used in periodontal therapy. For a drug to be delivered locally needs to possess antimicrobial properties against periodontopathogens, anti-inflammatory and immunomodulatory properties. The present study evaluated the Efficacy of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. against Pg and A actinomycetemcomitans. The results of the study demonstrated that the water, alcoholic and hydroalcoholic extracts of both the herbs were effective against both the pathogens. Thus, indicating its effectiveness in periodontal therapy. However, the MIC values of *Ficus benghalensis* L. obtained for Pg and Aa was higher than *Picrorhiza kurroa* Royle ex benth indicating higher concentrations of the herbal drug in any formulation for being effective in periodontal therapy.

The current in vitro MIC investigation enabled us to concentrate on an intervention strategy for designing and conducting a clinical trial to ascertain *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. positive effects on people at risk for periodontitis. Owing to their inherent limitations, in vitro values of MIC might not be reliable for in vivo research. Microorganisms proliferate exponentially in a lab setting but may not develop at all in their natural environment. (15) Although the in vitro MIC is used as an alternative measure to attempt to quantify the

pharmacological activity, it does not represent the actual activity of the medicine at the site of infection. (16)

Understanding the etiopathogenesis of periodontal disease scientifically has placed a new obligation on dentists to care for current and prospective periodontitis patients not just for their oral health, but also for their overall health. Within the constraints of the current investigation, the lowest concentration of *Picrorhiza kurroa* Royle ex benth and *Ficus benghalensis* L. was shown to be efficacious for both A. actinomycetemcomitans and Pg. However, because periodontitis is a polymicrobial disease, the drug's susceptibility to other periodontal infections must be determined.

Despite the well-established long-term safety profiles of herbal drugs, more research is necessary to: (a) Examine the safety of administering to individuals with other systemic ailments in order to cure periodontitis. (b) Compare in vivo effectiveness with those of other commonly prescribed antimicrobials used for periodontal care.

Conclusion

The results of the MIC study indicating that the combination of the *Ficus benghalensis* L. and *Picrorhiza kurroa* Royle ex benth alcoholic extract is effective against both the periodontopathogens. Hence this combination can be effectively used in chronic periodontitis patients as a local delivery drug.

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