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Anticariogenicity of *Picrorhiza kurroa* Root Extract when used as a Mouthwash in High Caries Risk Patients: Randomized Controlled Clinical Trial

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Abstract

Background: Imbalances in the oral microbiota or the presence of certain pathogenic microorganisms can lead to discomfort and various oral health issues such as dental caries, gingivitis, mucositis, oral ulcers and thrush, halitosis, and abscess. Plants and their derivatives are gaining more interest in the pharmaceutical industry. Mouth wash preparations provide a significant antimicrobial activity, promote tissue regeneration, and reduce inflammation. The mouthwash could be considered as a potential natural antimicrobial agent for treating infections caused by cariogens.

Aim: To investigate the efficiency of *Picrorhiza kurroa* root extract as an anticariogenic mouthwash in high caries risk patients.

Materials & methods: A fresh concentrated herbal mouth rinse of 10% was prepared by diluting it in sterile distilled water. Forty patients with high risk for caries were selected to assess the efficacy of the mouthwash. They were randomly assigned to two groups. Salivary pH, buffering capacity, and microbial count were evaluated before mouthwash use, after 10 min, and 20 mins.

Results: Alter in the pH, buffering capacity, and a decrease in microbial count.

Conclusion: *Picrorhiza kurroa* root extracts used as a mouthwash seem to be an effective anticaries agent.

Keywords: *Picrorhiza kurroa*; Mouthwash; anticariogenic; Dental Caries

Introduction

Plants play a crucial role in human healthcare systems, providing a vast array of medicinal compounds that have been utilized for thousands of years in traditional medicine practices. Many rural communities throughout the world regularly use medicinal plants for traditional purposes of curing ailments [1]. The traditional medical system is still growing and makes a significant contribution to the pharmaceutical industry despite global industrialization and urbanization. Every component of medicinal plants has therapeutic value and aids in the development of novel medications [2]. India has a rich history of traditional medicine, comprising various components such as Ayurveda, siddha, unani, and homoeopathy, and is the world's leading producer of medicinal herbs [3].

Sustaining proper oral and dental hygiene is the primary prerequisite for living a healthy lifestyle. Bad nutrition, diet, and hygiene practices lead to dental cavities, plaque, gum disease, and other disorders [4]. Dental caries is primarily caused by a pathogenic microbial community in the oral cavity, which is dominated by a large number of facultative and obligate anaerobic bacteria [5]. However, the primary oral bacterium responsible for the onset and progression of dental caries is *Streptococcus mutans* [6]. Whereas, for the development of periodontal disease, the keystone pathogen is the gram-negative anaerobe bacterium Porphyromonas *gingivalis* [7]. *Candida* species causes oral candidiasis in immunosuppressive individuals, denture wearers, and HIV-infected individuals [8, 9].

Tooth adherent cariogenic microbes and dietary sources of sucrose or refined sugar colonize a tooth surface that is susceptible to decay, resulting in tooth decay. Carbohydrate fermentation, lactic acid production by bacteria, melts the tooth's hydroxyapatite crystal structure and causes caries [10, 11]. Dental caries and periodontal diseases are managed using mechanical and chemical plaque control. Use of fluoridated toothpastes and varnishes, antimicrobial therapy such as chlorhexidine mouth rinse, are the methods involved in the management of dental caries. Even though conventional therapies are effective in treating the disease, continuous use of the currently available drugs only shows a decrease in clinical illness rather than a total eradication of the infection [12]. Thus, Oral pathogenic biofilms need alternative therapeutic approaches. Due to the complexity of biofilm formation, a new class of agents with anti-biofilm activity needs to be found. Novel oral products that have anti-biofilm formation, anti-caries, and anti-periodontal qualities are urgently needed in light of these challenges.

Medicinal plants are a valuable and rich source of active natural metabolites that vary greatly in their biological and structural characteristics and are crucial in the etiology of a wide range of human illnesses, including oral diseases [13]. Several plants with ethnopharmacological backgrounds were tested for their potential against pathogenic microbes, and the positive results demonstrated their potent anti-cariogenic activity [14].

The well-known medicinal plant *Picrorhiza kurroa* is a member of the Scrophulariaceae family. This perennial alpine herb is used in Indian Ayurveda medicine to treat respiratory and liver conditions. It possesses an elongated root with a bitter taste that is rich in iridoid glycosides. It has been used for thousands of years in traditional medicine and is thought to have many therapeutic uses, including cardiovascular, brain tonic, antifungal, antioxidant, and anti-inflammatory. In addition, it is used to treat chronic hepatitis, dyspepsia, fever, asthma, flatulence, and cardiac complaints [15]. Numerous secondary metabolites, including kutkin, kutkoside, picroside V, pikuroside, mussaenosidic acid, bartsioside, and boschnaloside, are found in the active ingredient, iridoid glycosides. According to reports, Kutkin is responsible for the pharmacological value [16].

Review of Literature

Review of literature reveals that an abundant number of studies conducted to manage dental caries by utilizing the therapeutic potential of phytocompounds and its metabolites. In a study conducted to screen the antibacterial efficacy of the 15 selected medicinal plants against four cariogenic bacteria. Among them, *Eucalyptus globulus* had the potential to treat dental caries [17]. In another study, the antibacterial potential of 100 native Korean plant species' extracts was evaluated against *Streptococcus mutans* during cariogenesis of those, *Streptococcus mutans* growth was inhibited by extracts from five different plants: *ArctiiFructus*, *Caryopterisincana*, *Aralia continentalis*, *Symplocarpus nerifolius*, and *Lamium amplexicaule*. The minimal inhibitory concentration and minimal bactericidal concentration of each of the five extracts were assessed separately. Remarkably, a synergistic antibacterial activity between the plant extracts and sodium fluoride was noted. *Streptococcus mutans* was exposed to escalating concentrations of the plant extracts in order to assess their anti-biofilm activity [18]. The anticariogenicity of a 0.5% extract of *Stevia rebaudiana* leaves were evaluated in 46 high risks of dental caries. Two groups were assessed: group I: chlorhexidine (CHX) mouthwash, group II for S. rebaudiana (0.5% extract of Stevia bio) mouth wash. Salivary pH, buffer, and microbial count were assessed before the patients were asked to use the mouthwashes. Patients were prescribed the mouthwash/extract twice a day for 7 days. On the 8th day, post rinse salivary pH, buffer analysis, and *Streptococcus mutans* and *Lactobacilli* count were done [19].

These kinds of anticariogenicity studies influenced us to do this study, to analyze the anticariogenicity of *Picrorhiza kurroa* root extract as a mouthwash in high carries risks patients as a randomized controlled trial.

Though we have various chemically formulated derivatives to treat oral hygiene, but some potential risks, and there is a need to look for alternate control agents, like those that are naturally present in plant materials, in order to increase the safety and quality of dental care.

Aim

To investigate the efficacy of the root extracts of *Picrorhiza kurroa* as an anticariogenic mouthwash.

Objectives

- To prepare the *Picrorhiza kurroa* root extract and to investigate the anticariogenic potential of *Picrorhiza kurroa* root extract in caries risk patients
- To assess the change in salivary pH, buffering capacity of saliva, and microbial count before and after the use of mouthwash.

Materials & Methods

- ***Root material collection:*** The root material was procured from the herbal drug store in Chennai. It was then identified and authenticated from the plant taxonomist. A voucher specimen deposited in the central research lab of the college for future reference.
- ***IEC approval:*** Approval from the Institutional Ethics committee (Human studies) was obtained before starting this study.
- ***Preparation of herbal syrup:*** 5 gms of powdered root were added to 500ml of double distilled water and boiled at 80°C gets reduced to one-fold reduction. The concentrated solution was filtered using clean muslincloth and cooled to normal temperature. This herbal mouthwash was utilized as mouthwash against risks patients. Solutions were prepared freshly whenever required.
- ***Type of study:*** Randomized controlled trial
- ***Study area & Population:*** Out-patient (OP) Department of oral medicine and radiology of a Tertiary Dental College and Hospital
- ***Sample size:*** A total of 40 volunteers satisfied the selection criteria of high caries risk was included in the study. They were informed and explained about study protocols in regional language and obtained informed written consent.
- ***Selection criteria:*** 40 patients with high caries risk were chosen. Consent was obtained after the protocol had been explained clearly.

Inclusion criteria

- Male and female Patients aged between 18-35 years were assessed for caries risk.
- Patients those who gave informed consent and compiled with the study were selected.

Exclusion criteria

- Subjects were taking antibiotics or any other drugs within last 3 months.
- Pregnant women and lactating mothers.
- Medically compromised patients.
- Smokers.
- Patient with a known history of allergy to chemicals or any herbal products.

Sample Collection

Selection criteria for caries risk assessment

Based on the History and Clinical Examination, the criteria for the caries risk assessment as follows [19]:

History	
Family tendency	Yes/No
Susceptible age factor	Yes/No
Past history of restoration for carious teeth	Yes/No
Past history of extractions of carious teeth	Yes/No
Medical history predisposing to caries	Yes/No
Socio economic status and dental awareness	Good/Bad / Moderate
Dietary habits	Good/Bad / Moderate
Oral habit status	Good/Bad / Moderate
Oral hygiene status	Good/Bad / Moderate
Oral hygiene habits status	Good/Bad / Moderate
Clinical Examination	
Presence of active carious lesions	Yes/No
Presence of more than two proximal Caries/anterior caries	Yes/No
Presence of root stumps in need of extraction	Yes/No
Presence of restorations done for carious reason	Yes/No

Table 1: Criteria for caries risk assessment.

The high risk categories were based on the following assessment such as:

High risk: At least two categories must have at least 70% of the total score, with salivary analysis or clinical examination serving as one of the categories.

Moderate risk: Score of 50-70% in two categories at least, with salivary analysis or clinical examination serving as one of the categories.

Low risk: Less than 50% score in all categories.

Analysis for salivary pH, buffering capacity and microbial analysis

Salivary analysis for pH: Unstimulated saliva samples were collected and transferred to the collecting jar, and analyzed for the pH. The pH test paper was dipped in the sample for at least 10s and the color changes were compared with the chart provided by the manufacturer (GC Saliva check) and the values were recorded.

Salivary analysis for buffer: Stimulated saliva samples were collected for the buffer. The patient was instructed to chew a piece of paraffin wax for 30s. Saliva that was secreted initially was discarded and the later was collected for the analysis. Using the pipette, a drop of saliva was placed on the test pad. Following two minutes, the manufacturers chart was consulted to compare the color of the strip and the values were noted.

Salivary microbial analysis: In the same way as for the buffer test, saliva samples were collected from patients for microbial analysis. The method employed to evaluate the microbial content was the spread plate and dilution method [20]. For each of the 40 patients, saliva samples were collected twice: once prior to rinsing with *Picrorhiza kurroa* root extract and once 10 minutes following the rinse. After diluting the samples with saline, they were streaked onto petri plates that were filled with the proper medium—LB agar for *Lactobacilli* and MSB agar for *Streptococcus mutans*. At 35°C, the plates were incubated for 72 hours. Counting the colonies was done after incubation. The patients were instructed to rinse their mouths with the 10% concentrated extract and hold it there for 40 seconds before expectorating it. After this, the patients were not permitted to drink anything or rinse with water for 20 minutes. At twenty minutes, the microbiological analysis was repeated. The outcomes were noted.

After tabulating the results of the pH, buffering, and microbial analyses, the means and standard deviations were computed.

Group I: The prerinse sample with the 10 min post rinse sample.

Group II: The prerinse sample with the 20 min post-rinse sample.

Observations & Results

		Mean	*N*	*Std Deviation*	*Std. Error Mean*
Pair 1	Pre Rinse	6.3490	40	0.16800	0.02656
	Post Rinse -10mins	7.2110	40	0.12197	0.01928
Pair 2	Pre Rinse	6.3490	40	0.16800	0.02656
	Post Rinse 20 Mins	7.4423	40	0.24410	0.03860
Pair 3	Post Rinse -10mins	7.2110	40	0.12197	0.01928
	Post Rinse 20 Mins	7.4423	40	0.24410	0.03860

Table 2: Mean and SD of salivary pH values.

	Salivary Buffer			
No of samples	***Post-rinse***		***Pre-rinse***	
			10minutes	20minutes
40	Mean	6.6300	7.2160	7.4356
40	Std deviation	0.1658	0.1803	0.1858

Table 3: Salivary buffering capacity.

For both groups mean and standard deviation (SD) of the pre- and post-rinse pH and buffer were calculated accordingly. (Tables 2 and 3). There is a significant difference ($p<0.0001$) in the pre and post-rinse salivary parameters (Table 2). Using the paired T test, the groups differences in the mean and standard deviation of the pH and buffer changes following the rinse were examined (Table 3) In table 4, based on the Minimum inhibitory concentration (MIC) and the microbial colony count pre and post rinse sample 68% and 70

% of reduction were seen against *Streptococcus mutans* and *Lactobacilli* respectively. 37 samples taken into account since three plates are fully opaque and not able to count the colonies.

No of Samples	*Percentage Reduction in Streptococcus Mutans Count*	*Std Deviation in %*	*Percentage Reduction in Lactobacilli Count*	*Std Deviation in %*
37	68	60	70	68

Table 4: Percentage of Reduction in *Streptococcus mutans* and *Lactobacilli*.

Discussion

Dental caries, or tooth decay, is primarily caused by the breakdown of tooth enamel due to acids produced by bacteria in the mouth. These bacteria thrive on sugars from food and drinks, leading to the formation of plaque and eventually, cavities [21]. Use of natural products is gradually increasing nowadays, which is also seen in the dental caries management. Investigating natural treatments that may help prevent or lessen the incidence of cavities is part of using plant products as alternatives to allopathic medicine for dental caries [22]. The shift towards plant products in dental health aims to minimize the adverse effects associated with some allopathic treatments, such as allergies, side effects, or antibiotic resistance. Medicinal plants are a rich source of antimicrobial agents, found to be helpful in the treatment of a variety of diseases, including bacterial diseases [23]. By incorporating medicinal plants into clinical practice and utilizing their inhibiting action against microbes, we can minimize, if not completely eradicate, the disease entity.

Picrorhiza kurroa is a small perennial herb from the plantain family (Plantaginaceae). The plant is well-known in traditional medicine systems like ayurveda. The rhizome, or underground stem, is thick and tuberous with a bitter taste, which is the main medicinal part of the plant. *Picrorhiza kurroa* is primarily known for its therapeutic benefits. In Ayurvedic medicine, the rhizome is used for a variety of purposes, such as hepatic dysfunction, as an immune modulator, aid in digestive issues, and for respiratory illness [24].

In a study conducted, the methanol extract of *Picrorhiza kurroa* rhizome was effective against bacterial strains and fungal strains. The cup plate method showed that the methanol extracts outperformed Ciprofloxacin against bacterial strains, while the aqueous extract outperformed Fluoconazole against fungal strains [25]. In another study, antimicrobial potentials of *Picrorhiza kurroa* hydroalcoholic extract against *Salmonella Bongori*, *Enterococcus faecalis*, and *Klebsiella pneumonia* using the Agar well diffusion method was determined. The extract showed a potential antimicrobial activity against the tested organisms [26].

High caries risk is biologically indicated by salivary pH and buffer [27]. The saliva's acidic pH is caused by the lactic acid produced by *Streptococcus mutans* metabolism of the readily fermentable sugars. This ultimately contributes to the growth of acidogenic and aciduric organisms and causes the tooth structure to become less mineralized [28]. Various polyol-containing sugar substitutes, like xylitol, are non-fermentable sugars, and their anticariogenic properties have been demonstrated. On the other hand, there isn't much data regarding the *Picrorhiza kurroa root extract in* vivo effects on salivary pH and buffer.

Thus, the purpose of this study was to identify the anticariogenic potential of the *Picrorhiza kurroa* root extract and its effectiveness and biocompatibility without causing harm to human health. The result presented here shows the strong antimicrobial activity against the two important cariogenic pathogens in the post rinse session, which means that the extract was effective in favorably altering the pH and buffer. The prerinse salivary pH was acidic as it was collected from high caries risk patients, which came close to near normal after the rinse with the *Picrorhiza kurroa* root extract. The presence of various photoactive agents like tannins, steroids, glycosides, terpenoids, and flavones [2] may be responsible for the anti-inflammatory, antioxidant, antimicrobial, and anticariogenic potential. Presence of tannins indicates the astringent property, to arrest the local bleeding in the oral route along with the infection.

Thus, it can be inferred from the current study that *Picrorhiza kurroa* extract is not only non-acidogenic but also tends to lower the acidic pH of saliva. It is conferred that pH was reduced in pair 1 and 2 test, whereas, comparing the time duration of 10 and 20 minutes in pair 3 test, there is no much difference in the activity. If the time duration may increase like 60 and 90 minutes, it might still clearly

study the effect of the root extract, which is one of the limitations of the present study.

Conclusion

Picrorhiza kurroa aqueous root extract, when used as a mouth wash in high caries risk individuals had exhibited the following:

- Improved the pH and buffering capacity of the saliva in high caries risk patient.
- Good inhibition of cariogens exhibiting the antimicrobial efficacy.

But, in order to demonstrate its substantivity, more extensive clinical trials are needed.

Summary

Plants are indeed rich sources of nutrients essential for human health. They provide a wide array of vitamins, minerals, fibers, and phytochemicals, each playing unique roles in maintaining and promoting health. Naturally, Plants contain biologically active compounds like Flavonoids, carotenoids, and polyphenols which help to neutralize the harmful effects of free radical damage and various other diseased conditions. Our findings showed the pH maintenance and good antimicrobial actions of *Picrorhiza kurroa* aqueous root extract against the two selected cariogens, such as *Streptococcus mutans* and *Lactobacillus*. Since this is a preliminary study, further quantification investigation is necessary to make the best of this root extract in various pharmacological formulations including anticariogenic agent.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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ICMR-STS.

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