

FORMULATION AND PREVENTION OF HERBAL MOUTHWASH

Sadiq Amin*¹, Tanya Sharma ², Kamalesh Mistry³, Dhananjay Mistry⁴, Noorul Huda⁵,
Shubham Singh⁶

¹Research Scholar, Faculty of Pharmaceutical Science, Mewar University, Gangrar,
Chittorgarh 312901, Rajasthan, India.

²Assistant Professor, Department of Pharmacy, Faculty of Pharmaceutical Science, Mewar
University, Gangrar, Chittorgarh 312901, Rajasthan, India.

³Associate Professor, Department of Pharmacy, Faculty of Pharmaceutical Science, Mewar
University, Gangrar, Chittorgarh 312901, Rajasthan, India.

⁴Lecturer, Department of Pharmacy, Faculty of Pharmaceutical Science, Mewar University,
Gangrar, Chittorgarh 312901, Rajasthan, India.

⁵Assistant Professor, Department of Pharmacy, Faculty of Pharmaceutical Science, Mewar
University, Gangrar, Chittorgarh 312901, Rajasthan, India.

⁶Student Dean Dayal Rustogi college of Pharmacy, Gurugram(HR), India.

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*Corresponding Author: Sadiq Amin

Research Scholar, Faculty of Pharmaceutical Science, Mewar University, Gangrar, Chittorgarh 312901, Rajasthan,
India.

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ABSTRACT

The current lifestyle has made children more susceptible to carious lesions all around the world. One of the major contributing microorganisms is *Streptococcus mutans*, which causes initiation of enamel caries and is a major etiological pathogen. For the prevention of caries and therapeutic purposes, mouthwashes or mouth rinses are promoted a lot. The first mentioned mouth rinse was seen around 2700 BC in Chinese medicine. Among antimicrobial agents, chlorhexidine is considered gold standard but it has side effects such as extrinsic stain formation and bitter taste. Glycyrrhiza (licorice), a herb, which has medicinal value and was used in ancient times as a remedy for a great diversity of ailments and sweetener. Roots of *Glycyrrhiza* contain a high concentration of saponin and glycyrrhizin, which are supposed to be sweetening agents. Green tea, which is very rich in fluoride, with its catechin content attribute to its benefits. Polyphenols and various flavonoids such as catechin, catechin gallate, and catechin proanthocyanidin are its main constituents. Hence, the aim of the study was to

evaluate and compare the antimicrobial activity of ethanolic licorice extract with and without green tea extract and sodium fluoride mouthwash against *S. mutans*.

KEYWORDS:

fusobacterium nucleatum; halitosis; phytotherapy; porphyromonas gingivalis; sulphur compounds.

Green tea mouthwash; Kidodent mouthwash; Licorice; Streptococcus mutans.

INTRODUCTION:

In India, the prevalence of dental caries ranges from 27% to 64% in children up to 12 years old and from 26% to 83% in adults. Dental caries is a multifactorial disease resulting from a complex interplay of factors, including oral microorganisms within dental plaque, dietary practices, and host-related determinants such as genetic predisposition, immune response, and various socioenvironmental influences.

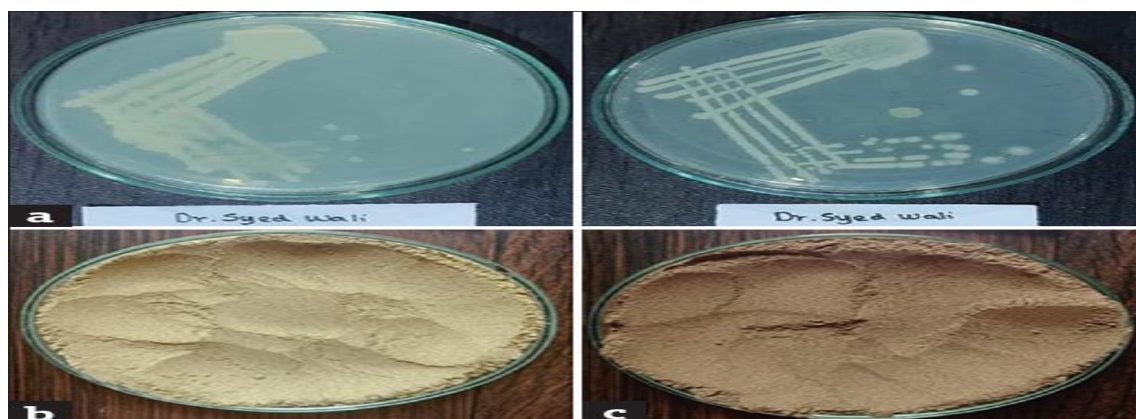
Although routine oral hygiene practices such as toothbrushing and flossing are effective in caries prevention, the complete eradication of *Streptococcus mutans* from anatomically challenging sites—such as pits, fissures, and interdental areas—remains difficult. The adjunctive use of chemotherapeutic agents, particularly antimicrobial mouth rinses, in combination with mechanical cleaning methods, has been shown to significantly reduce bacterial load.

Mouth rinses serve a critical role in the management of oral microbial populations and the inhibition of plaque accumulation. These formulations are generally classified into chemical and herbal categories. Among chemical agents, chlorhexidine is considered the gold standard due to its broad-spectrum antimicrobial activity and effectiveness against *S. mutans*. However, long-term use of chlorhexidine has been associated with several adverse effects, including mucosal irritation, increased calculus formation, altered taste sensation, and extrinsic staining of teeth and oral mucosa, limiting its applicability, especially in pediatric populations. These limitations underscore the necessity for developing alternative antibacterial mouth rinses that offer similar efficacy with a more favorable safety profile.

MATERIALS & METHODS:

Procurement of *Prevotella intermedia* and *Porphyromonas gingivalis*

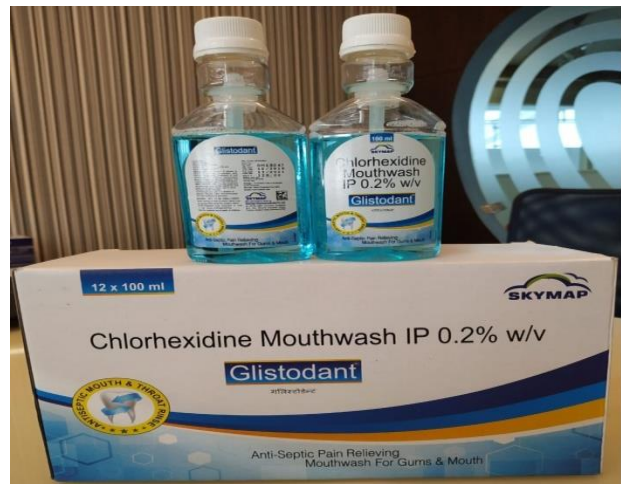
Test organisms highly significant with etiological characteristics associated with periodontitis were selected for the study. The selected bacteria (*Prevotella intermedia* and *Porphyromonas gingivalis*



The sample size was calculated to be 20 in each group (total: 60), considering $\alpha = 0.05$, effect size of 0.25, and power of 95%. Inclusion criteria consisted of patients with mild to moderate gingival inflammation (scores 1-2) with healthy periodontium aged between 9-12 years, compliant children (Frankl's behavior rating scale 3 or 4) capable of maintaining oral hygiene by themselves, and children without orthodontic appliances and prosthesis. Participants were excluded if they were uncooperative (Frankl's behavior rating scale 1 or 2), required special health care needs, took antibiotics for systemic diseases or had a history of using topical fluoride, mouthwash, or xylitol chewing gums in the past 4 weeks, had systemic diseases or conditions affecting saliva secretion, reported any known allergy to lemongrass oil, or had active carious- or intraoral-lesions.

Children were randomized into three groups by the lottery method. The test groups evaluated were:

- Group A: Lemongrass oil (organic *Cymbopogon citratus*; Hippocrates health institute, USA): 20 patients were randomly assigned to this group and received 0.25% lemongrass oil mouthwash to be used for the duration of 21 days
- Group B: CHX (Rexidin from Warren, Indoco Remedies Ltd, Mumbai, India): 20 patients were randomly assigned to this group and received 0.2% CHX mouthwash to be used for the duration of 21 days
- Group C:- Oral prophylaxis: 20 patients were randomly assigned to this group and only oral prophylaxis was performed for them.



THERAPEUTIC USES OF HERBS FOR PERIODONTITIS:

The bioactive polyphenol curcumin, isolated from turmeric (*C. longa*), has been established to possess anti-inflammatory, antibacterial, antioxidant, hepatoprotective, antiseptic, and analgesic activities. The active ingredients in turmeric include the flavonoid curcumin (diferuloylmethane) and various essential oils such as tumerone, atlanton, and zingiberone. Sugars, proteins, and resins are also present. Curcumin is the most extensively studied active antimicrobial and health-preserving ingredient in turmeric . It has been found to decrease histamine levels and potentially increase adrenal gland production of natural cortisol. Orally administered curcumin was found to be as effective as cortisone in acute inflammation, but less effective in chronic inflammation. It inhibits both neutrophil activity during inflammatory states and the synthesis of inflammatory prostaglandins from arachidonic acid. Due to its potent antioxidant properties, curcumin also defends against free radical damage. Turmeric and its curcumin component have powerful antioxidant properties that are comparable to those of vitamins C and E in water. According to Sharma and Kalsi (2016), the topical administration of *C. longa* as a supplement to subgingival scaling and root planning (SRP) may enhance currently used therapeutic methods for the management of plaque-induced periodontal disease. Additionally, curcumin enhanced cellular resilience to oxidative damage after 18 hours of incubation of bovine aortic endothelial cells. Curcumin's anti-inflammatory actions are explained by its inhibitory activity at several stages of the arachidonic acid inflammatory cascade. Research has demonstrated that curcumin can block a number of enzymes and cytokines connected to inflammation . Curcumin use in conjunction with SRP has been found to improve clinical indicators and have a beneficial impact on patients with chronic periodontitis. According to Nagasri et al. (2015) , the use of 1% *C. longa* L. extract had antibacterial efficacy against common periodontopathic bacteria. It

appears that a local drug administration system made of 2% whole turmeric gel can enhance SRP. The activity of the trypsin-like enzyme in the "red complex" species was greatly diminished.

DISCUSSION:

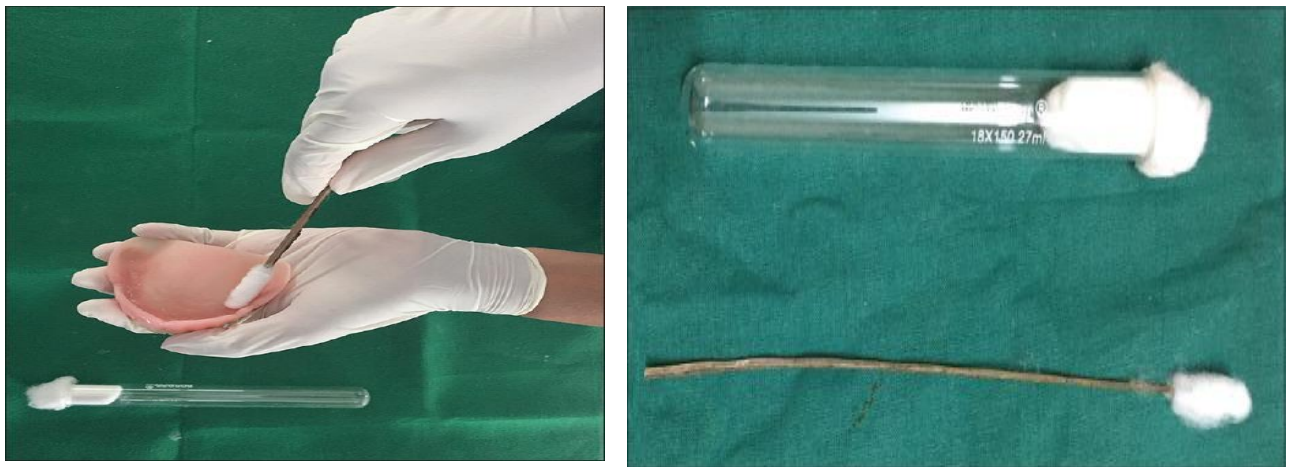
The importance of bioactive compounds like alkaloids, phenolics, flavonoids and some other secondary metabolites have been reported by Kala et al. The presence of these active ingredients would be an added advantage to the formulated toothpastes. Ihemeje et al. have reported the detection of observed phytochemical compounds in *D. tripetala* (pepper fruit) seed, which are the basis for its medicinal prowess. The presence of alkaloids, flavonoids, total phenols, saponins among other phytochemicals has been attributed to antifungal, antimicrobial, antiinflammatory and antidiabetes of its seed (Reihemann et al. Phytochemical compounds such as phenolics, alkaloids, and flavonoids were detected in *S. aromaticum* (clove) buds. In this study, the identified bioactive ingredients are valuable drug components that could be used as antimicrobial and analgesic. Hence, clove is a good pharmaceutical ingredient

The bioactive ingredient like; 9-octadecanoic acid (30.53%), trichloroacetic acid, undec-2-enyl ester (12.69%) and caryophyllene (0.83%) are present in the essential oil of *D. tripetala*, while, eugenol (49.71%) and caryophyllene (18.94%), were detected in the buds oil *S. aromaticum* and caryophyllene, eugenol. 9,12-octadecadienoic acid (Z, Z)- and octadecanoic acid among other compounds after found in the ethanolic extract of *S. aromaticum* The low quantity of essential oils in the two plant extracts could be due to the method of extraction but essential oils are mostly extracted when steam distillation method is adopted. Also, the process of concentrating the extracts through exposure to air could have reduced some of the extracted essential oils. All these factors could have lowered the number of essential oils detected by GC/MS analysis.

Microbial analysis of the dentures:

Swab samples were taken from experimental Groups I and II. Swab samples were taken by rolling sterile cotton swabs across the tissue surface of the upper and lower dentures . The swabs were placed in a test tube which was covered with a cotton plug . Then, using a 4-mm loop, the swab sample was immediately smeared onto the surface of culture plate with Sabouraud dextrose agar (SDA) (with 10% chloramphenicol). All plates were aerobically

incubated at 37°C for 72 h. Culture plates which were negative for candidal growth were left on the bench for further 72 h and then examined again before being discarded as negative.



RESULTS:

Formulation and stability check of mouthwash:

Erythritol was selected as a sweetener to mask the spicy flavour, while sodium benzoate was incorporated as a preservative at a concentration of 0.1% w/v. The antioxidant capacity of herbal mouthwash was assessed using DPPH radical scavenging assays, which revealed significant differences ($p < 0.05$). The CEOZnONPs mouthwash demonstrated moderate DPPH free radical scavenging activity, with values of 34.56%, 44.78%, 50.6%, and 55.90% for F1, F2, F3, and F4 formulations respectively.

Anti-oxidant activity of mouthwashes

Candidates	DPPH Scavenging activity (%)
F1	34.56%,
F2	44.78%,
F3	50.6%
F4	55.90%

Antibacterial activity of mouthwash:

The herbal mouthwashes demonstrated notable antibacterial activity against all three pathogens i.e. *S. mutans* MTCC890, *S. aureus* & *E. coli*. Herbal mouthwash with formulation F4 exhibited the highest zone of inhibition (ZOI) with a diameter of 64.2 ± 2.6 mm against *S. mutans*, followed by *S. aureus* (43.6 ± 1.0 mm), *E.*

coli (31.8 ± 1.5 mm) In comparison, the CMW exhibited comparatively lower ZOI i.e. 44.7 ± 2.3 mm, 43.6 ± 1.7 , 30.5 ± 1.4 against *S. mutans*, *S. aureus* and *E. coli* respectively. These findings indicate that CEOZnONPs, particularly at higher concentrations, enhance antibacterial efficacy.

ABBREVIATIONS:

CPNP

Commission cosmetic products notification portal

EWG

Environmental Working Group

GC/MS

Gas chromatography/mass spectrometry

SDS-PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

MTT

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

HaCaT

Human immortalized keratinocyte cell line

DMEM

Dulbecco's Modified Eagle Medium

DMSO

Dimethyl sulfoxide

DPPH

2,2-Diphenyl-1-Picrylhydrazyl

CHX

Chlorhexidine-digluconate

NADPH

Reduced form of nicotinamide adenine dinucleotide phosphate

NADH

Reduced form of nicotinamide adenine dinucleotide

ATP

Adenosine triphosphate

SARS-CoV-2

Severe Acute Respiratory Syndrome Coronavirus 2

BSA

Bovine serum albumin

FBS

Fetal bovine serum

RNA

Ribonucleic acid

FDA

United States Food and Drug Administration

CONCLUSION:

The purpose of the present study was to investigate the effects and intended usage of the ingredients included in commercially available mouthwashes as well as to identify any possible adverse impacts. According to the research on toothpaste, many compounds that might cause harmful effects and DNA and cell damage can also be ingested through mouthwash, hence boosting the body's concentration of these agents. It is crucial for human and environmental health that dental care products, which include various agents with hazardous effects, conduct research and development to purge themselves of these substances and make products based on this idea. Today, life expectancy is rising, people are paying more attention to their health as a result of social networks, and they are concerned with dental hygiene. Life expectancy has increased the amount of time that teeth remain in the mouth; thus, it is essential to optimise oral hygiene. People may thus prevent oral disorders such as dental caries and periodontitis by not only brushing their teeth but also employing other oral agents like mouthwash.

People can avoid the occurrence of oral diseases by utilising mouthwashes. However, mouthwashes alone are not as effective as using a toothbrush and toothpaste. In addition, individuals may lack enough awareness about the usage of mouthwashes. Although it is limited, there is research on the detrimental consequences of overuse. Therefore, the level of awareness about the use of mouthwash needs to be improved for both physicians and patients, and it is crucial to carry out some awareness-raising studies in this field. Additionally, clinicians should recognise the need of understanding and following the substance of mouthwashes.

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