

# **Evaluation and comparison of anti-bacterial efficacy of commercially available various ayurvedic mouth ulcer gels: An in-vitro study**

## **ABSTRACT**

**Aim:** To evaluate and compare the anti-bacterial efficacy of commercially available various ayurvedic mouth ulcer gel against *Streptococcus mutans*.

**Study design:** In-vitro study

**Background:** Mouth ulcer, also known as aphthous ulcer, is a type of oral ulcer that can have a variety of symptoms like pain, redness, swelling and discomfort which occur due to numerous etiological factors. Mouth ulcer gels alleviate the symptoms associated with mouth ulcers. Oral ulcer gels also have antibacterial properties that aid to prevent ulcer infection. A number of mouth ulcer gels are available on the market to treat mouth ulcers. The goal of this study was to assess and compare the antibacterial activity of ayurvedic mouth ulcer gels against oral bacteria.

**Materials and methods:** The commercially available mouth ulcer gel formulations used are SMYLE GEL, MUCOPAIN GEL, HIMALAYA HIORA-SG GEL, COLO MOUTH ULCER GEL and CURE NEXT GEL. *Streptococcus mutans* were used for the study. Direct agar well diffusion was performed by transferring a loop full of cells from the stock cultures to test tube of nutrient broth for bacteria incubated at 37C for 24 hours and adding 0.1 ml of the gels to each well and dilute with water in ratio 1:1, 1:5, 1:10.

**Results:** Commercially available various ayurvedic mouth ulcer gels were tested against *Streptococcus mutans* in which HIMALAYA HIORA-SG GEL showed substantial inhibitory activity in various concentration which was determined by direct agar diffusion method.

**Conclusion:** In the present study, it was observed that HIMALAYA HIORA-SG GEL was more effective when compared to other mouth ulcer gels.

*Keywords – [Ayurvedic, Antibacterial, Mouth ulcer, Streptococcus mutans]*

## **1. INTRODUCTION**

“Recurrent aphthous stomatitis (RAS) is a common oral mucosal illness characterised by recurrent and painful ulcerations on the non-keratinized”<sup>[1]</sup> or moveable oral mucosa.<sup>[2]</sup> “In the general population, the prevalence of RAS ranges from 5% to 66 percent, with a mean of 20%”.<sup>[3]</sup> Kleinman et al.,<sup>[4]</sup> reported “a point prevalence of 1.23% and a lifetime prevalence of 36.5% for RAS. There are three different forms of RAS: minor, major, and herpetiform. The most frequent kind of RAS is minor

RAS, which affects 80 percent of RAS patients. Aphthous stomatitis is idiopathic and multifactorial in nature; however it is thought to be caused by activation of the cell-mediated immune system. Aphthous ulcers are not communicable since they are not caused by acute infections. Local trauma, emotional or physiologic stress, allergy or sensitivity (to sodium lauryl sulphate found in toothpaste and oral hygiene products, foods like cinnamon, cheese, citrus, figs, or pineapple), toxin exposure (nitrates in drinking water), menstruation, or changes in the oral microbiome can all trigger aphthous stomatitis. Hematinic deficiencies (iron, folate, vitamin B6 and B12) account for up to 20% of cases, while additional deficiencies such as vitamin D, zinc, or thiamine may also be present".<sup>[6]</sup> "Aphthous ulcers are more common in non-smokers and smokers who have quit, and less common in people who maintain good oral hygiene" <sup>[6]</sup>

"Around 20% of the general population suffers from aphthous stomatitis. It is more prevalent among girls and women, as well as in rich socioeconomic classes and countries. The disease does not appear to be influenced by race. The onset age can be as early as childhood, although it is more frequent in the second and third decades of life, and it becomes less common as people get older".<sup>[7]</sup> "Behcet syndrome, systemic lupus erythematosus, reactive arthritis, or inflammatory bowel illness can all cause aphthous stomatitis" <sup>[8]</sup> (especially Crohn disease). Systemic signs and symptoms may be used to rule out certain illnesses. <sup>[9]</sup>.

The use of antibacterial mouth ulcer gel has been recommended in order to alleviate the symptoms produce by mouth ulcer. There is no definite aetiology and pathology known for aphthous; although some factors are considered important such as topical trauma, bacterial and viral infections, genetics, nutrition, immunological, hormonal and psychological factors, allergies, medications and etc. Some generally suggested aphthous treatments are: antibiotics and antiseptics, herbal treatments, local analgesics, immunological mediators, both steroidal and non-steroidal anti-inflammatory drugs.

An aphthous ulcer has been linked to a number of different bacterial species.<sup>[10]</sup> A few studies have been conducted to learn more about the link between bacteria and ulcers. <sup>[11]</sup> These investigations used a culture-independent molecular technique to quantify bacterial diversity in oral lesions.<sup>[12]</sup>

*Streptococcus mutans*, a gram positive facultative anaerobic bacterium, considered being one of the major pathogens involving in causing dental caries; however certain literatures also state that the organism is also involved in causing mouth ulcer. However, *Streptococcus mutans* link among the ulcer relation is not proven and it is still under discussion.

These mouth ulcer gels are primarily used to treat canker sores and are sold over-the-counter as a general oral anaesthetic. As a result, the goal of this study was to see how well commercially available mouth gel formulations worked on *S. mutans* using appropriate in vitro test models.

## **2. MATERIALS AND METHODS**

### **2.1 AGAR WELL DIFFUSION METHOD**

“The antibacterial activity of plants or microbial extracts is commonly assessed using the agar well diffusion method. The agar plate surface is inoculated by spreading a volume of microbial inoculum over the entire agar surface, similar to the approach employed in the disk-diffusion method”.<sup>[13]</sup>

## 2.2 PREPARATION OF INOCULUM

Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hrs at 37° C.

## 2.3 ANTIBACTERIAL ACTIVITY

“Antibacterial activity of sample was determined by well diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton agar medium was weighed as 3.8 grams and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured into sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Wells were made using cork borer. Samples” (Abbott, Smyle, Novita, Himalaya and Benzocaine gel) of three different concentrations (1:1, 1:5 and 1:10) was placed on MHA plates and Streptomycin (1mg/ml - 20 µl) loaded in respective wells. These plates were incubated for 24 hrs at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

## 3. RESULTS

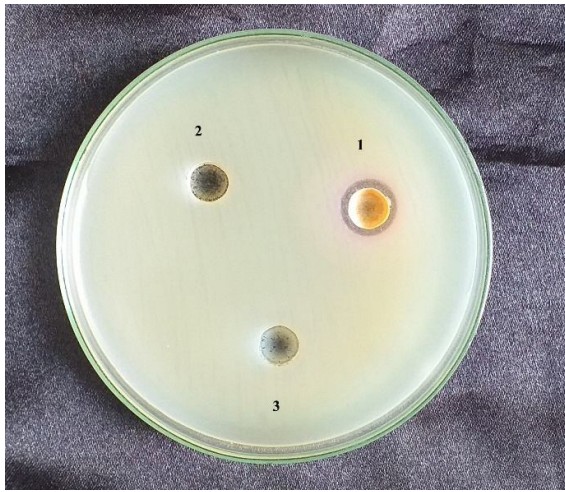
The antibacterial activity of the different gels tested against *S. mutans* is shown in table 1.

Table 1 : illustrating the zone of inhibition

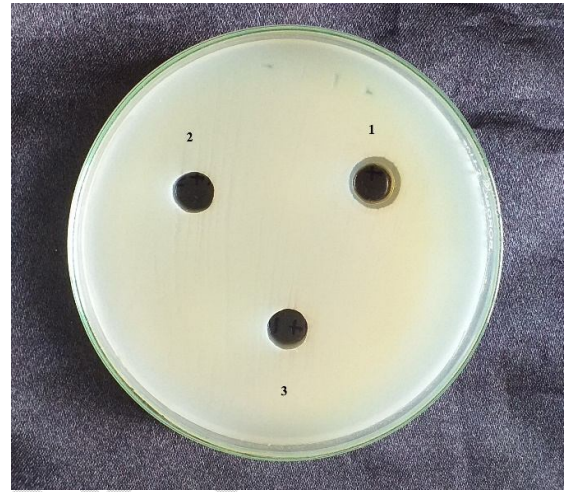
Microorganisms/Sample Concentration (µl)	Zone of Inhibition in mm		
	1:1	1:5	1:10
<b><i>Streptococcus mutans</i></b>			
Cure next gel (AG)	12	-	-
Smyle (SG)	12	-	-
Colo gel (NG)	-	-	-
Himalaya gel (HG)	17	13	12
Mucopain gel (BG)	-	-	-

When comparing the zone of inhibition of various oral ulcer gel formulations (Figure1) that were used in the study, HIMALAYA HIORA-SG GEL(HG) showed a substantial amount of zone of inhibition of bacterial activity when provided in ratios of 1:1, 1:5, 1:10 of values 17,13,12 mm, respectively, whereas the other oral ulcer gel formulations (CURE NEXT GEL,SMYLE, COLO MOUTH ULCER GEL, MUCOPAIN GEL) that were used in the study and performed by direct agar diffusion method

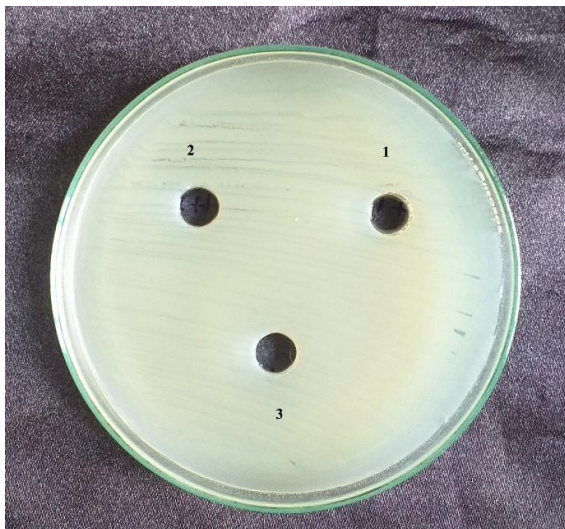
have not shown any significant zone of inhibition. This shows that HIMALAYA GEL that were used showed significant reduction of *Streptococcus mutans* activity for mouth ulcer as oral gel formulation.



(a)



(b)



(c)



(d)



(e)

Figure 1 shows zone of inhibition of mouth ulcer gels, a. Curenext gel, b. Smyle mouth ulcer gel, c. Colo mouth ulcer gel, d. Himalaya Hiora – sg gel, e. Mucopain gel

#### 4. DISCUSSION

The present study is to evaluate and compare the anti-bacterial efficacy of SMYLE GEL, HIMALAYA HIORA-SG GEL, COLO MOUTH ULCER GEL, CURENEXT ORAL GEL, MUCOPAIN GEL against *Streptococcus mutans* which is performed by direct agar diffusion method.

The study showed that HIMALAYA GEL as an oral gel formulation has showed inhibition of *Streptococcus mutans* activity when samples were performed in three different concentrations of 1:1,1:5,1:10. This might be due to contents of HIMALAYA GEL against the *Streptococcus mutans*. Various studies from Valgas C.,<sup>[13]</sup>Baker M<sup>[14]</sup> have shown that Licorice, a component of HIMALAYA GEL have shown a strong inhibitory effect against *Streptococcus mutans*.<sup>[15]</sup>Triphala, another component is also alleviated inflammation caused by mouth ulcer. A study shown that Triphala, is used to reduce to pain against ulcer<sup>[16]</sup> and it shows significant effect against anaerobic and aerobic bacteria present in the oral cavity.<sup>[17]</sup> The unique component (Triphala) has antiplaque efficacy and acts inflammatory inducers similar result to CHX.<sup>[18]</sup>*Sushruta Samhita* has emphasized that Triphala has haemostatic, anti-inflammatory, analgesic, and wound-healing properties.

Other gel formulation that was used for the study were, CURE NEXT GEL which were prepared in the concentration of 1:1,1:5,1:10 have shown that zone of inhibition is 12 mm in 1:1 concentration while no change is shown in other concentration. The zone of inhibition that was seen in the initial concentration might be due to presence of Turmeric, which is natural anti-oxidant reducing the pain and inflammation. Similarly, SMYLE an oral gel also shows same amount of inhibitory activity of *Streptococcus mutans*.

While the other oral ulcer gel formulation COLO GEL and MUCOPAIN GEL have shown no inhibitory activity against *streptococcus mutans*. This might be due to presence of only flavouring agent and sweetening in the presence of bad breath, anaesthetic agent that might numb the ulcerated area in the oral cavity, but little or absence of medicated substances that might alter the *Streptococcus mutans* activity in the ulcerated area.

#### 5. CONCLUSION

HIMALAYA GEL that was tested against *Streptococcus mutans* has showed substantial inhibitory activity in various concentrations which was determined by direct agar diffusion method. While other gel formulation showed no inhibition of *Streptococcus mutans* activity. Other gels, on the other hand, could incorporate antibacterial agents in their formulation to limit bacterial activity, potentially reducing aphthous ulcer symptoms.

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