THE EFFECTIVENESS OF THAI HERBAL LOZENGE ON REDUCTION OF ORAL PATHOGENS IN HEALTHY VOLUNTEERS

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ABSTRACT

Decrement of oral microbials would provide the prevention of dental caries and oral illness. Thai herbal lozenge from multi-herbal formula was antitussive and expectorant properties. This research was aimed to assess the antimicrobial effectiveness against oral pathogens of Thai herbal lozenge that was contained multi-herbal, which was included, liquorice root, Indian gooseberry, coriander seed, gall of chebulic myrobalans, cumin seed, belleric myrobalan fruits and karanda fruits as in certain ratio. Evaluation of lozenge qualities was included physical appearance and tablet testing. The elimination of oral pathogens in participants (n = 30) before and after product usage (per instruction) were assessed a modified dip slide test. Physical appearance of lozenge was almost preferable. Therefore, lozenge may ease to broken. The quantity of oral microbial colonies on the slides was significantly decreased ($p < 0.05$) after the lozenge usage.

Keywords: anti-microbial; dental caries, lozenge, oral microbials, herbal mixture

INTRODUCTION

Oral healthcare constitutes a financial burden, and in nations with high economic status, a notable proportion of total healthcare expenditures, approximately 5%, is designated for this specific realm. The World Health Organization is approximate that more than 50% of population is suffer from oral illness [1]. Tooth decay is a pathological condition that is associated with a different condition. The condition, such as a diet that is conducive to caries, suboptimal oral hygiene practices, elevated levels of cariogenic microorganisms, the presence of dental plaque, insufficient salivary flow, and inadequate exposure to fluoride are among the environmental risk factors that contribute to the development of dental caries [2, 3, 4]. The etiology of dental caries is also intricately linked to the involvement of microorganisms [5, 6]. Reducing the levels of microorganisms within the buccal cavity would provide an additional justification for the prevention of dental caries. Traditional herbal medicine has gained significance in healthcare facilities in Southeast Asia, specifically in Thailand [20]. Thai herbal lozenge from multi-herbal formula was antitussive and expectorant properties. Currently, we are engaged in the development of a novel UM formula that incorporates karanda (*Carissa carandas* L.) fruits as a replacement for *Aristolochia* sp. [7, 8, 9] and developed in lozenge form [9, 10]. This research was conducted to assess the antimicrobial effectiveness against oral pathogens of lozenge that contained multi-herbal extract.

MATERIALS AND METHODS

Plant identification

Medicinal plants were collected from Samut Songkhram Campus, Suan Sunandha Rajabhat University, Thailand (100.0370572°E, 13.4200603°N). Botanical characteristics of herbs were authentic details as following: liquorice, (*Glycyrrhiza glabra*) root, Indian gooseberry (*Phyllanthus emblica* Linn.) fruits, coriander (*Coriandrum sativum*) seeds, the gall of chebulic myrobalans (*Terminalia chebula* Retz.), cumin (*Cuminum cyminum*) seeds and the Karanda (*Carissa carandas* L.) fruits. The process of herbal identification followed the methods outlined in the Saralamp publication (1996) [11] and the Thai Pharmacopoeia (2019) [12]. Consistent with the formulation procedure, all herbs were subjected to washing and drying until achieving a stable weight.

Formulation and extraction

Dried Thai herbs were formulated in certain ratio, and included, liquorice root (35 g), Indian gooseberry, coriander seed, gall of chebulic myrobalans, cumin seed, belleric myrobalan fruits and karanda fruits (7 g for each). The multi-herbal mixture was grinded in powder form and extracted by 95% ethanol maceration for three days. Herbal mixture extract was filtered, concentrated to dryness under vacuum and dried by rotary evaporator.

Ingredients, development and qualities of lozenge

The lozenges were composed of herbal mixture extract (10%), spearmint, avicel, aerosol, xylitol, menthol, microcrystal cellulose, and magnesium stearate in a specific ratio. The extract and other ingredients were thoroughly blended in a step-by-step manner and then compressed into lozenges (300-350 mg/each tablet) using a tablet pressing machine. A total of 30 lozenges were randomly selected for assessment of tablet qualities, such as physical appearance, weight variation, tablet thickness, tablet hardness, tablet friability, disintegration time, and loss on drying [13].

In vivo anti-microbial test

The elimination of oral pathogens in a group of healthy individuals was assessed by employing a modified dip slide test (MU test kit, Mahidol University, Thailand) [14]. A case-control investigation was undertaken on a sample of healthy participants (N = 30). This evaluation was conducted both prior to and subsequent to the utilization of lozenges per instruction. Oral pathogens were decreased following the application of oral care products. Enumeration of microorganisms was performed using Mutans s*treptococci*, *Candida* sp., and *Lactobacilli* sp., with each count being conducted three times. The dependent *t*-test was utilized to analyze the difference in microbial colonies before and after the lozenge usage, and statistical significance was determined at a *p*-value of less than 0.05. This measurement was permission by the Ethic Committees at Suan Sunandha Rajabhat University, Thailand (COA.1-060/2020 and COA.1-118/2022).

RESULTS AND DISCUSSION

Physical characteristics of lozenges

Physical appearance of herbal lozenges was roughly pinkish-white tablet without fragile or broken. There were produced few fined foaming with spearmint-like odor. On tablet evaluation, weight variation and disintegration time were within reference range, which were implied that tablet was dissolved properly. Therefore, this lozenge was prone to broken or unstable due to lower thickness and hardness; and higher friability and loss on drying (Table 1).

Anti-microbial activity against oral microbials in healthy participants

The oral microorganisms in healthy participants were assessed prior to and following the applying of lozenge by using a modified dip-slide test. The quantity of oral microbial colonies on the slides, including Mutans *streptococci*, *Candida* sp., and *Lactobacilli* sp., exhibited a decrease subsequent to the application of lozenge. These reductions were quantified as colonies forming units per milliliter (CFU/ml) and are presented in Table 2. In addition, all of oral microorganisms were statistically significant decrease at a level of $p < 0.05$

Table 1 Physical appearance and tablet characteristics of herbal lozenges

Physical appearance	**Results**	**Tablet picture**
Color	Pinkish-white	
Odor	Spearmint like	
Foaming	Few/ fine foaming	
Visual inspection	Tablet forming/roughly surface without broken or fragile	
Tablet evaluation	**Results**	**Reference range**
Weight variation	< 10%	< 10%
Tablet thickness	4.1-4.8 mm	3.0-3.5 mm
Tablet hardness	1.9-2.2 kilopond	> 4.0-8.0 kilopond
Tablet friability	0.5-2.0%	<1.0%
Disintegration time	25.24-26.04 min	< 30 min
Loss on drying	10.6-11.5%	< 10%

Table 2 Number of oral microbials in participants before and after lozenge usage

Oral Microbials	**Lozenge**	
	Before	**After**
***Mutans* streptococci**		
Colony counts	25.6±0.56	6.6±0.32
CFU/ml	2.13 x 10^3	0.55 x 10^3
***Candida* sp.**		
Colony counts	32.8±0.74	16.6±0.88
CFU/ml	2.73 x 10^3	1.38 x 10^3
***Lactobacilli* sp.**		
Colony counts	15.4±0.35	12.0±0.14
CFU/ml	1.25 x 10^3	1.0 x 10^3

DISCUSSION

Our findings revealed that lozenge was exhibited favorable physical appearances and demonstrated effectiveness in their antimicrobial properties. However, the herbal lozenge was more prone to breakage due to deviations from the reference ranges of certain tablet standard parameters. In our investigation, the presence of Mutans *streptococci*, *Candida* sp., and *Lactobacilli* sp. was demonstrated a decrease subsequent to the utilization of lozenge. This discovery aligns with a previous study that focused on children afflicted with severe early childhood caries, wherein green tea and chlorhexidine mouthwashes were prescribed. In this study, it was observed that all three oral microorganisms underwent a reduction [15]. The potential anti-cariogenic property of lozenge may also be associated with the synergistic effect of herbal mixture extract [16, 17]. However, the reagent base of products was not included as a control in our study on antimicrobial testing in each participant. Consequently, the potential interference of other chemical ingredients on the antimicrobial activity of the products were remained unverified.

CONCLUSION

We were concluded that lozenge contained multi-herbal extract was met to quality on physical appearance. However, herbal lozenge was prone to broken. Use of lozenge in oral health practices have been found to have anti-microbial effectiveness against the cariogenic *S. mutans*, *Candida* sp. and *Lactobacilli* sp.

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