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Preliminary study of artificial saliva containing herbs: physical and staining the teeth properties

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Introduction

Xerostomia is escorted by oral discomfort and impaired oral functions and artificial saliva or saliva substitutes are widely used to alleviate oral symptoms that caused by many factors, including the side effects of the medicine, some diseases, such as Sjogren's syndrome, diabetes and radiation therapy for head and neck cancer patients.[1,2] Nowadays, artificial saliva products are imported and expensive in Thailand. It was interesting to develop the local products. Many herbs in Thailand could be used for increasing oral comfort. There have been very few studies on artificial saliva containing herb extract and its effect on teeth staining. In this study, artificial saliva containing herbs using hydroxyethylcellulose as viscosity increasing agent were prepared. Three herbs: *Glycyrrhiza glabra*, *Phyllanthus emblica* and *Clinacanthus nutans* extracts were added in the formulations. Physical appearance, pH, viscosity, contamination test and staining the teeth of the products were evaluated.

Methods

Preparation of artificial saliva

The composition of artificial saliva was shown in Table 1. All electrolytes were dissolved in buffer. The extracts, 1%HEC, humectant, sweetener and preservative were added. Finally, the formulation was adjusted with buffer.

Table 1. Composition of artificial saliva (adapted from Subari *et al*., 2012)[3]

Ingredient	Blank formulation	Formulation containing *C. nutans*	Formulation containing *P. emblica*	Formulation containing *G. glabra*
C. nutans extract		2 g		
P. emblica extract			2 g	
G. glabra extract				2 g
1%HEC	50 g	50 g	50 g	50 g
Electrolytes				
NaCl	0.04 g	0.04 g	0.04 g	0.04 g
$CaCl_2$	0.09 g	0.09 g	0.09 g	0.09 g
KCl	0.04 g	0.04 g	0.04 g	0.04 g
Sweetener				
Saccharin sodium	0.30 g	0.30 g	0.30 g	0.30 g
Preservative				
Paraben conc.	1 mL	1 mL	1 mL	1 mL
Humectant				
Sorbitol	20 mL	20 mL	20 mL	20 mL

Ingredient	Blank formulation	Formulation containing *C. nutans*	Formulation containing *P. emblica*	Formulation containing *G. glabra*
C. nutans extract		2 g		
P. emblica extract			2 g	
G. glabra extract				2 g
Vehicle Phosphate buffer pH 6.2 to	100 mL	100 mL	100 mL	100 mL

Viscosity Measurement

Thai herbal artificial saliva formulation was evaluated the viscosity by a Brookfield viscometer (Brookfield engineering laboratories Inc, USA) at 25 ± 2ºC. It was measured in triplicate.

Determination of pH

SevenEasy S-20 pH meter (Mettler Toledo, Switzerland) was used to determine the pH value of artificial saliva containing herbs. It was measured in triplicate.

Contamination Test

The formulation was diluted with 0.9% sodium chloride solution into serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) and then spread onto Petri-dish that contained each plate count agar (bacteria contamination test) or potato dextrose agar (fungi contamination test) for contamination test. They were incubated at 37±2ºC for 24 hours.

Stability Test

Artificial saliva containing herbs formulation was performed by storage at 2-8°C for 10 weeks. The appearance, pH value and viscosity of all formulations were determined every 2 weeks.

Teeth staining Test

Dentures were immersed into each formulations, chlorhexidine solution (as positive control) or 0.9% sodium chloride solution (as negative control) at and storage at 2-8°C for 16 weeks. Photos of the dentures were taken every week comparing with the standard dentures**.**

Statistical Analysis

Data, when applicable, are presented as a mean ± standard deviation (SD) from at least three samples unless indicated. The data were compared using analysis of variance (ANOVA) followed by a One-Way ANOVA to determine the difference between data sets. All statistical comparisons were calculated using SPSS software version 17 (SPSS Inc., Chicago, IL, USA). A *p*-value < 0.05 was considered statistically significant.

Results

Four formulations were successfully prepared; blank formulation, formulation containing *C. nutans*, formulation containing *P. emblica* and formulation containing *G. glabra*. The results showed physical appearance was acceptable. It was different colour. Blank formulation was colourless solution, while the formulation containing *C. nutans*, formulation containing *P. emblica* and formulation containing *G. glabra* were green, brown and yellow, respectively. Viscosity of all formulations was in range of 230–280 cP (Figure 1). The pH value of all formulations was in range of 5.60 – 6.10 (Figure 2).

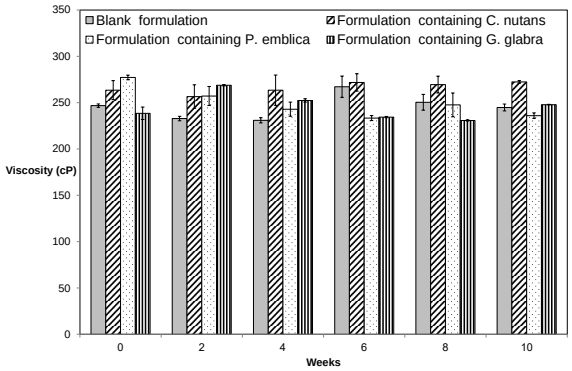


Figure 1. Viscosity of artificial saliva containing herbs.

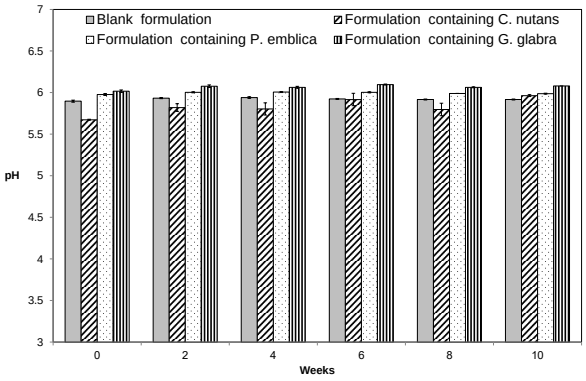
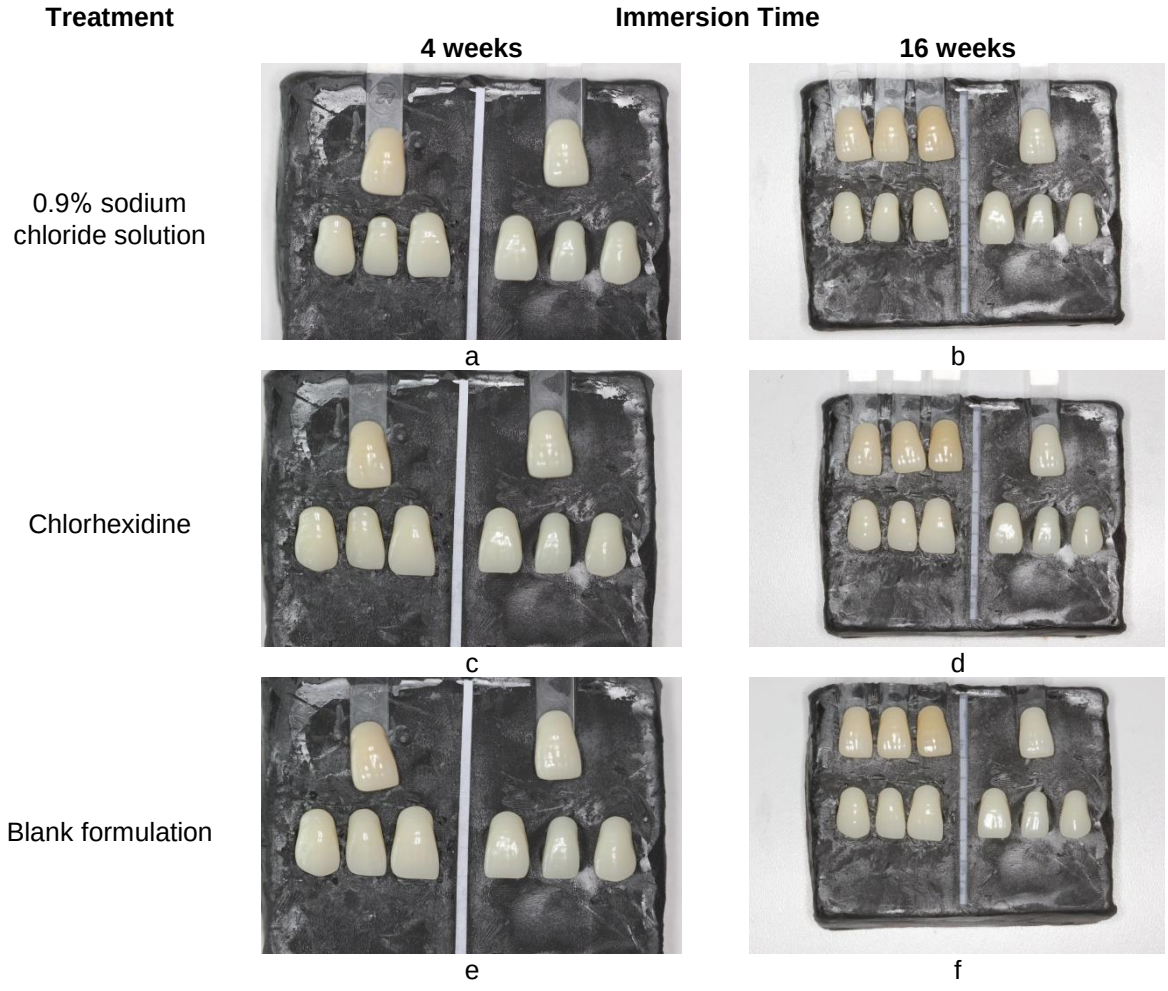


Figure 2. The pH value of artificial saliva containing herbs.

Bacteria and fungi were not found in all formulations. All formulations had good stability after storage at 2-8°C for 10 weeks. For the teeth staining test, the white dentures (A1) were chosen to use in this test. No colour changed in all treatments after immersed for 1 week. Figure 3 showed the dentures after immersed in 0.9% sodium chloride solution (a&b), chlorhexidine solution (c&d), blank formulation (e&f), formulation containing *C. nutans* (g&h), formulation containing *P. emblica* (i&j) and formulation containing *G. glabra* (k&l) at 4 and 16 weeks. The dentures in each picture divided into four groups; standard brownish yellow denture A2, A35 and A3, respectively (upper left), standard white denture A1 (upper right), dentures after immersed in the treatment (lower left) and standard white dentures A1 (lower right). At the 4st week, the colour of dentures immersed in chlorhexidine solution and formulation containing *C. nutans* were changed to brownish yellow similar A2. They got brownish yellow (similar the brownish yellow denture, A3) at 16st week.



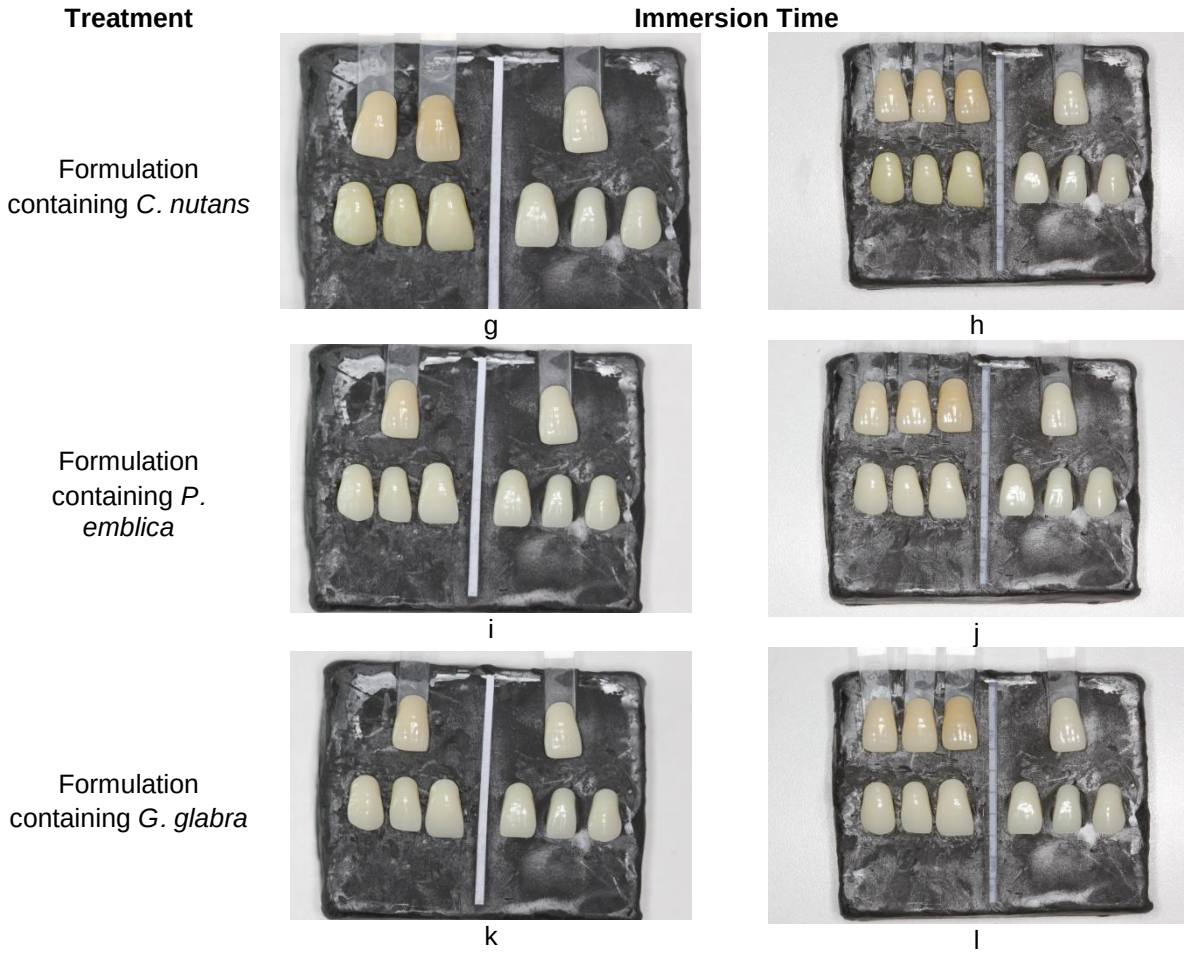


Figure 3. Teeth staining test of artificial saliva containing herbs.

Discussion

Artificial saliva containing herb extracts could be successfully prepared. The colour of herb extract had the effect on the appearance of formulation, so the formulation had the same colour as the added extract. Their physical appearances were acceptable and the data showed no contamination of bacteria and fungi before and after storage at 2-8°C for 10 weeks. The pH of all formulations was in range of 5.60 – 6.10. The alkaline or acidity of saliva depends on the flow rate. The pH of saliva is usually within the range 5.5-7.9.[4] Viscosity of all formulations was in range of 230–280 cP. They had the same appearance, pH value and viscosity when compared with the fresh preparation. From the viscosity data, all formulations could be used as spay product for oral cavity. It was found that formulation containing *C. nutans* staining the teeth like chlorhexidine (positive control). In this study, *C. nutans* extract could stain the teeth, while *P. emblica* and *G. glabra* extracts could not. It indicated that all colour herb extract could not stain the teeth.

Conclusion

We could conclude that artificial saliva containing herbs could be successfully prepared. Their physical appearances were acceptable and no contamination of bacteria and fungi. *G. glabra* and *P. emblica* extracts could not stain the teeth. Formulation containing both extracts might be good candidate for future study.

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