



Proceeding

Oral Liquid Preparation of *Clinacanthus Nutans*: Formulation

Development

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Background and objective: Leaves of *Clinacanthus nutans* (CN) is one of the most well-known herbal plants listed in Thai Herbal Pharmacopoeia, exerting anti-inflammatory and antiviral activities. One of its use which is currently stated in the Thai national list of essential medicine (NLEM) is topical oral applications for mouth sore from aphthous ulcer, chemotherapy and radiotherapy-induced oral ulcer or herpetic gingivostomatitis. Unacceptable appearance, odor and taste of the current hospital formula of the CN topical oral solution, listed in NLEM, indicate a need to revise the formulation. Mucoadhesive polymers are shown to improve the quality and functions of topical oral preparations. This study intended to improve the CN topical oral solution using a mucoadhesive polymer and compare with the current hospital formula. The modified formula would be registered as the hospital formula. This study aimed to modify oral bases intended to improve CN topical oral solution for use as a modified hospital formula.

Methods: The selection of CN for use as the herbal resources was first attempted. Concentrate crude extracts of CN (CNC) were obtained after maceration of dried and grinded CN in ethanol followed by evaporation. The Folin–Ciocalteu colorimetric method was used to quantify the total phenolic content of CNC using gallic acid as the standard. Apparent solubility of CNC was determined for solvent selection. Sodium carboxy methylcellulose and volatile oils are attempted to modify the formula. The modified formula was physically evaluated for its appearance, flavor and pH. In-house satisfaction was blinded evaluated at the Department of Pharmacy of a community hospital.

Results: The modified formula of CN topical oral solution was green and viscous liquid with a pH of 6-7. It contained an average total phenolic content of 0.11 mg GAE/g. There was no precipitate found in the modified formula but various small brown precipitates from the current hospital formula. All of the in-house testers (n = 10) accepted the odor and flavor of the modified formula but not those of the current hospital formula.

Conclusions: Addition of a mucoadhesive polymer and volatile oils to the current hospital formula enhance the performance of the CN topical oral solution.

Keywords: *Clinacanthus nutans*; topical oral solution, modified hospital formula

Introduction

Topical oral preparations containing herbal extracts have become very popular and well accepted by clinicians and patients as mouth sore or oral ulcers occurred from various sources can interfere or deteriorate the clinical symptoms. Oral ulcers may self-heal, but these ulcers cause painful discomfort which interfere oral functions and quality of life. Oral ulcers from recurrent aphthous stomatitis which affects up to 25% of the global population¹ or those occurred during long-term hospitalization or treatments with radio- or chemotherapy² or infections such as HSV-2³ may lead to serious consequences and/or hospitalization due to inappropriate care. These include infection, malnutrition, poor oral hygiene, etc. The first line therapy of oral ulcers from viral infection is acyclovir 200 mg five times daily for 7-10 days⁴. The drugs of choices for oral ulcer are usually topical corticosteroids (short course) and a chlorhexidine mouthwash. Corticosteroids are not safe for long-term use as fungal infection can become a serious side effect⁵. Chlorhexidine mouthwash is not palatable and must be formulated as alcohol free preparations. Topical oral antiviral preparations may not be applicable for long-term use in all cases. Thai health care system has encouraged the use of herbal medicine, thus, community hospitals are required to seek opportunities to use herbal medicine. Topical oral preparations by herbal medicine should be one of the products of interest by community hospitals as these are immediate products that can reduce pain with antiviral activities. Also, there were a lot of Thai traditional medicines which were recorded and should be further evaluated. This study was tempted to improve a topical oral preparation from an herbal origin for the hospital use.

Clinacanthus nutans (Burm.f.) Lindau or *Clinacanthus burmanni* Nees (family Acanthaceae)⁶, with several local Thai names including Phaya Yo or Saled Pangpon Tua Mea, is currently listed as one of the herbal hospital formulary⁷. Leaves of *C. nutans* (abbreviated as CN) possess *in vitro* anti-inflammatory⁸, *in vivo* anti-inflammatory and antiviral activities⁹, and approved clinical efficacy¹⁰. Acute toxicity investigation of ethanol extract of CN leaves in mice at the highest dose, 1.3 g/kg of body weight (g/kg BW) did not appear any signs of the animals toxicity and no any abnormalities of internal organs from histopathological examination that could be due to the effect of the extract in sub chronic toxicity study¹¹. A clinical study showed that CN healed the ulcer faster than placebo and had no adverse effect¹². The hospital formula of CN for topical oral applications contains crude extracts of CN and glycerin. Crude extracts of CN was obtained by maceration of dried CN in 70% alcohol, as shown in the herbal drug list for topical drugs local treatments in the 3rd Thai National List of Essential Medicine (NLEM). There are various dosage forms of the preparations containing CN listed as hospital formularies in the NLEM, i.e. lotions, topical oral solutions, ointments and tinctures¹³. Among these, the formula of CN topical oral solution is indicated for aphthous ulcer, chemotherapy and radiotherapy-induced oral ulcer, herpetic gingivostomatitis¹³. This can support the philosophy of self-sufficiency and encourage agriculturists for harvesting herbs as raw materials¹⁴.

Mucoadhesive polymers can form attractive bonds between the polymers and the biological tissues¹⁵. Sodium carboxy methylcellulose (SCMC) is one of the mucoadhesive polymers used in formation of oral gels¹⁵. If the formula of CN can prolong contact to the oral mucosa by addition of SCMC, it should improve the penetration of the active ingredients of CN.



To promote the use of herbs in prevention and treatment of diseases or symptoms, acceptable formulas are essential for patients who require herbal self-care, particularly topical oral applications. CN is approved for use with an advantageous over corticosteroids, but its use is limited due to the unpleasant taste of the current hospital formula of CN and its high cost. Therefore, it is tempted to improve CN topical oral solution for use as a modified hospital formula. This is conducted by modifying oral bases from the HHHF formula by additions of mucoadhesive polymer and volatile oils. The mucoadhesive polymer is expected to promote dissolution of the herbal ingredients and the volatile oils are intended to improve the flavor, and thus the taste of the finished products.

Methods

Chemicals and materials

A commercial product of CN in glycerin (in accordance to and NLEM, Thai Herbal Product Co., LTD., Ayutthaya Province, Thailand.), Folin–Ciocalteu reagent (Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), Na_2CO_3 (Loba Chemie, Mumbai, India), sodium carboxy methylcellulose (B.L. Hua & Co., Ltd., Bangkok, Thailand), glycerin (S. Tong Chemicals Co., Ltd., Bangkok, Thailand), filter paper (GE Healthcare, Buckinghamshire, UK), peppermint oil (B.L. Hua & Co., Ltd., Bangkok, Thailand), PEG 400 (B.L. Hua & Co., Ltd., Bangkok, Thailand), propylene glycol (S. Tong Chemicals Co., Ltd., Bangkok, Thailand), and Eucalyptus oil (B.L. Hua & Co., Ltd., Bangkok, Thailand) were used as received.

Plant sources and extraction

Two sources of CN grown in Roi-et and Chaiyapum were used. Fresh CN were collected and dried by herbal medicine manufacturer (Moryathai Co., Ltd., Roi-et, Thailand). The dried CN were pulverized to powder which was macerated, with frequent agitation for 7 days, in 70% ethanol using a weight ratio of 1:4, respectively. The liquid extract of CN was obtained by filtration through a filter paper and then 50% of its volume was evaporated to obtain a CN concentrate (CNC).

Total phenolic content by Folin–Ciocalteu colorimetric method

The CNC was quantified by the Folin–Ciocalteu colorimetric method for total phenolic content expressed as gallic acid equivalents (GAE). In brief, 0.04 ml of the sample was diluted with 3 ml of distilled water, reacted with 0.2 ml of Folin–Ciocalteu reagent for 5 min and then 0.6 ml of saturated Na_2CO_3 solution was added and incubated (40 °C) for 30 min. The absorbance of the resultant was read at 765 nm using a UV–vis spectrophotometer (UV-1201, Shimadzu, Japan) and calculated for the concentration by comparing to gallic acid (0.05–0.9 $\mu\text{g/ml}$) used as the standard.

The method was validated by analyzing gallic acid standards for intra- and interday samples.

Formulation

The apparent solubility of the CN concentrates were determined for choosing a suitable solvent. Afterward gelling agent, SCMC was used to prepare formula. The flavoring agents were peppermint oil and eucalyptus oil. Glycerin was the sweetening agent for this study. Sodium benzoate was used to preserve the formula at typical usage level. All formula were evaluated by physical properties that are appearance, flavor, pH.

In house satisfaction study was performed at department of pharmacy, Phukhieo Chalermprakit Hospital by 10 of pharmacy staff tasted the modified formula and current formula.

Microscopic study

Microscopic study was conducted using microscope (Olympus cx31, Japan) for precipitates observation.

Results

The HHHF defines the content of CN oral topical solution to contain 2.5 – 4 % ethanol extract of dried leaves of *C. nutans* in glycerin (Handbook of Herbal Hospital Formulary (HHHF)). The modified formula intentionally set the concentration of CNC at 4%. The source of CN was the main concern. It was found that fresh CN gave about 42% of dried CN which were extracted. The dried CN gave a 50% yield of CNC.

The quantification of total phenolic content of the samples was calculated in equivalent to standard curves of gallic acid by Folin–Ciocalteu colorimetric method. The method was shown to be validated as it was repeatable based on the intra-day data and precise based on the inter-day data (Table 1). Also, the relative standard deviation (RSD) of the data obtained from intra-day and inter-day analysis were found to be 5.88% and 3.15%, respectively. The standard equation used for the quantification of total phenolic content was, as follows:

$$\text{UV absorbance at 765 nm} = (0.5571 \times \text{conc}) + 0.0615$$

It was found to be linearly correlated as a coefficient of 0.9995 was obtained.

The total phenolic content of three batches of CNC were 2.71 ± 0.09 , 2.72 ± 0.10 , 2.63 ± 0.23 , respectively. The modified formula contained an average total phenolic content of 0.11 mg GAE/g (Table 2).

Table 1 Intra-day (repeatability) and inter-day precision of the quantification of total phenolic content by Folin–Ciocalteu colorimetric method (theoretical concentration of 0.126 mg/ml)

Precision	Experimental concentration (mg/ml)	Mean Recovery (%)	RSD (%)
Intra-day (n = 6)	0.127 ± 0.007	100.79	5.88
Inter-day			
Day 1 (n = 3)	0.127 ± 0.007	100.79	4.96
Day 2 (n = 3)	0.126 ± 0.007	100.00	2.45
Mean \pm SD (n = 6)	0.127 ± 0.004	100.79	3.15

Table 2 Total phenolic contents (mean \pm SD) of three batches of CN extraction taken samples from CNC of each batch (n = 3).

batch	Total phenolic content (mg GAE/ g)		
	Dried CN	CN concentrate (CNC)	Modified formula
1	1.71 \pm 0.06	2.71 \pm 0.09	0.12
2	1.53 \pm 0.06	2.72 \pm 0.10	0.11
3	1.60 \pm 0.14	2.63 \pm 0.23	0.11

The microscopic study showed that commercial product of CN in glycerin (in accordance to and NLEM) was observed to contain small crystals (Figure 1 (left)). There was not observable precipitate in the modified formula prepared from the CNC, as shown in Figure 1

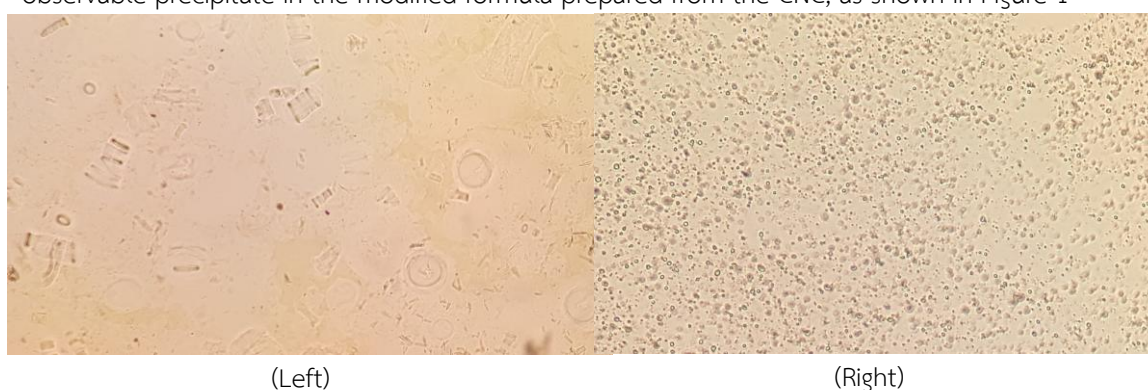


Figure 1 (left) Small crystals observed in the CN oral topical solution (HHHF formula, containing 4% of CN ethanolic extract in glycerin) from a commercial product; (right) clear solution of CNC from this improved formula. (10 \times)

In-house satisfaction of the modified formula conducted at the Department of Pharmacy showed that 50% complained about the unpleasant herbal smell of commercial CN topical oral solution. The modified formula was well accepted for pleasant taste and the herbal smell was able to be masked by volatile oils.

The modified product was subjected to the hospital regulations set by the Ministry of Health.

Discussion

The particles of the topical oral solution of CN in glycerin formulation (HHHF formula) are potentially precipitates from the crude extract which may reduce its absorption into the body. The release and penetration of active ingredients could play important roles in exerting activities and should be considered. As a herbal formulary, this has never been shown. Total phenolic contents, as well as other physicochemical properties, i.e. color, pH, appearance, apparent viscosity, should be used in quality control for pharmacy preparation of CN products. Stability, safety and permeation will be further studied to provide substantial information for the hospital service.

Herbal tastes of CN is one of the major complaints that prevented patients' compliance shown in the previous study¹². Herbal products with fewer manufacturing companies tend to be expensive, thus the commercial CN oral product in glycerin is rather expensive. Data from searching the website of Drug and Medical Supply Information Center, Ministry of Public Health shows the price of 41.31 bath/ 10 ml. This is more expensive than triamcinolone (12.98 bath/ 5 g) and acyclovir cream (7.5 bath/5g)¹⁶.

Mucoadhesion affects saliva secretion and drug absorption in the oral cavity¹⁷. Orabase is a common formulation used in oral preparation such as topical steroids. Treatment of recurrent aphthous stomatitis using Orabase with mucoadhesion of CN gave good results but some volunteers complained about Orabase adhesion¹². Several mucoadhesive agents are used in a commercial product, including gelatin, pectin, SCMC, and plasticized hydrocarbon gel (polyethylene and mineral oil)¹⁸. As the crude extracts of CN is alcoholic extracts, it may not be homogeneously mixed with the hydrocarbon gel, water insoluble material¹⁹, therefore SCMC, a water soluble material, was used. SCMC was well mixed with CNC and should increase oral deposition. It may also mask the unpleasant taste of CNC which help in improving patient compliance and allow revision of the hospital formula without changing the main content.

In addition, the new formula should avoid using paraben as in commercial product due to controversies in safety of parabens. In animal study, parabens have been showed the estrogenic effects by uterotrophic (uterine growth) assays in mice and rats. It has been assumed that the estrogenic activity of parabens may promote breast cancer development. Today the European Union (EU) has set up limits on paraben use at concentrations of 0.4% for any individual paraben and 0.8% for total paraben concentrations²⁰. In consequence, paraben should be skip and use glycerin as self preservative²¹.

The modified hospital formula of CN topical oral liquid would be further compared with the HHHF formula, containing CN ethanolic extract in glycerin in the improving of patient acceptability and permeation which should promote herbal usage in hospitals.

Conclusion

The modified formula of CN intended for topical oral applications could reduce precipitate of insoluble ingredients from the extract. It can also mask the unpleasant herbal taste of CNC and improve acceptability. Total phenolic content of the CN was sufficiently high and can be used as one of the quality control indicators. No adverse event was observed throughout the in-house satisfaction study.

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