The Thai Journal of Pharmaceutical Sciences

Volume 38
Issue 0 *supplement issue*

Article 55

1-1-2013

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KEYWORDS: Hair tonic, *Phyllanthus emblica*, *Zanthoxylum limonella*, Physical stability

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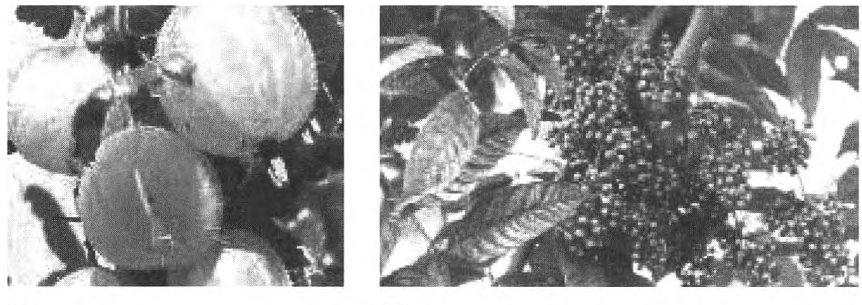
INTRODUCTION

Recently, the demand of herbal medicines is increasing rapidly due to their lack of side effects. Apart from traditionally documented applications, some modern trials have also established the utility of herbs in personal care products to help nourish and care for the skin. The herbal cosmeceutic products are formulated using various permissible cosmetic ingredients to form the base in which one or more herbal extracts are added as active ingredients. Hair tonic is a cosmeceutical product for using in the scalp to reduce hair loss and revitalize hair and scalp for healthy growth[1]. The active natural ingredients i.e., amino acids, vitamins, minerals, essential fatty acids, growth factors, and antioxidants (flavonoids, glycosides, etc.) could increase the hair cells and prevent the aging of the cells caused by the free radicals from modern life style including pollution, chemicals, stress and UV light[2-3].

Emblica (*Phyllanthus emblica* L., EUPHORBIACEAE), or "Ma-Kham Pom" in Thai, is a deciduous tree found throughout India, Nepal, South China, Thailand, Indochina, Laos and Malaysia to North Australia. The acidic fruits are eaten fresh or as condiments. The edible fruits are rich in tannins, vitamin C, polyphenols, alkaloids, flavoniods etc. The fruits possess anti-oxidant, anti-inflammatory, anti-microbial and tyrosinase activity. They are used for diverse internal ailments and in hair tonic in Asian medicine[4-9].

Ma-khwaen (*Zanthoxylum limonella* Alston, RUTACEAE) is an evergreen shrub of which non-toxic edible fruits are harvested in northern Thailand. The fruit has many active ingredients such as alkaloids, amides, coumarins, sterols and phenylpropanoid-lignans. The extracts of its roots, stem-barks, stems and fruits are widely used for antibacterial, anti-inflammatory, anesthetic properties and to inhibit tyrosinase activity [10-12].



(1a) (1b)

Figure 1 (1a) Emblica (*Phyllanthus emblica*) and (1b) Ma-khwaen (*Zanthoxylum limonella*).

The fruit extracts of Emblica and Ma-khwaen were mixed in a patented ratio to exhibit the best anti-microbial activity. TISTR has reported that the mixed extract was anti-oxidant (EC_{50} = 0.0079 µg/ml), potently anti-inflammatory (at 1.25 mg/ear) and could reduce croton oil-induced rat ear edema to 50% within 2 hr, which was better than diclofenac as the standard[13, 14]. As an alternative to commercial products, a patented hair tonic was formulated containing the mixed extract for its anti-microbial, anti-inflammatory and anti-oxidant benefits. In the present study, stability assessment was performed for the hair tonic using heating and cooling method which involves cycling the product through storage conditions of 45°C 24 hrs and 4°C 24 hrs for 6 cycles.

MATERIALS AND METHODS

Plant material The dry, powdered fruits of *P. emblica* and *Z. limonella* were provided by the Sakaerat Biosphere Research Center, Agricultural Technology Department, Thailand Institute of Scientific and Technological Research (TISTR).

Preparation of a patented mixed extract Specific extracts of *P. emblica* and *Z. limonella* were separately prepared by macerating the powdered fruits with ethanol-water and filtering through Whatman paper No. 41. The solvent was removed under reduced pressure using a rotary evaporator (Heidolph, Hei-VAP Precision) at 45 °C. An appropriate mixing ratio was used to prepare the mixed extract.

Formulation of hair tonic The hair tonic ingredients included 95% ethanol, menthol, propylene glycol, glycerin, panthenol, tocophery acetate, zinc pyrithione, crude extracts, disodium EDTA, fragrance and water. The active extract was dissolved in humectants and then mixed with water solution of ingredients. Chelating agent and alcohol were then added and mixed thoroughly. The hair tonic for adults should be acidic.

Stability testing of hair tonic The product stability in accelerated conditions was determined by heating and cooling method (45° C, 24 hrs and 4° C, 24 hrs for 6 cycles), as shown in **Figure 2**. The resulted physical and chemical properties of sample such as appearance, texture, color, odor, viscosity, pH, and phase separation were observed.

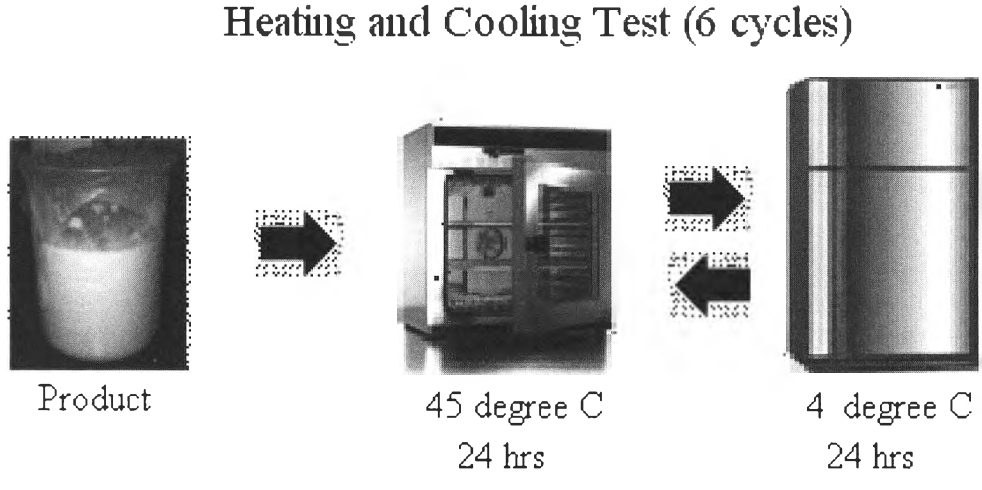


Figure 2 Diagram of stability testing under heating and cooling accelerated conditions.

Analysis of sample was performed in triplicate. The appearance, odor and separation of sample were evaluated by visual method. The viscosity of sample was evaluated by Brookfield viscometer (MLVT115, USA). The pH of sample was evaluated by pH meter (ph 700, German. The color of sample was investigated by color measurement (Miniscan EZ, USA).

RESULTS AND DISCUSSION

The stability and compatibility of a suitable hair tonic formulation containing the mixed extract from fruits of *P. emblica* and *Z. limonella* were evaluated under accelerated conditions (heating and cooling test, at 45 °C 24 hrs and 4 °C 24 hrs for 6 cycles). No phase separation occurred and the product showed no change in the appearance, odor and texture whereas pH, viscosity and color were slightly changed. The pH and viscosity were changed from 4.53 to 4.63 and from 41.23 to 36.83 CPs, respectively. The color of the products was analyzed according to three parameters including: L*, a*, b*. These values were slightly changed from 38.65, 22.08 and 46.09 to 36.29, 24.37 and 49.11, respectively, during the 6 cycle storage test.

Table 1 Physical and chemical changes of the hair tonic during heating and cooling test (45 °C 24 hrs and 4 °C 24 hrs, 6 cycles).

Properties	0 cycle	3 cycles	6 cycles
Appearance	Brown color, clear	Brown color, clear	Brown color, clear
Separation	None	None	None
Odor	Good	Good	Good
Texture	Good	Good	Good
pH	4.53 ± 0.01	4.47 ± 0.21	4.63 ± 0.06
Viscosity (CPs.)	41.23 ± 3.75	40.89 ± 2.96	36.83 ± 1.67
Color			
L*	38.65 ± 0.31	36.80 ± 0.66	36.29 ± 0.67
a*	22.08 ± 0.23	22.98 ± 0.73	24.37 ± 1.16
b*	46.09 ± 0.68	46.47 ± 0.83	49.11 ± 0.49

Note L = 100 (Reflecting diffuser, White), L* = 0 (Black)
a* = Positive a (red), Negative a (green)
b* = Positive b (yellow), Negative b (blue)

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CONCLUSION

The patented mixed extract from fruits of *P. emblica* and *Z. limonella* could be used as an active extract for hair tonic. The patented product was stable in appearances, odor and color with no phase separation whereas its pH, viscosity and color were slightly changed after storage under accelerated conditions (45 °C 24 hrs and 4 °C 24 hrs, 6 cycles). Stability of the sample product confirmed that it was suitable as a hair tonic formulation with beneficial effects of the active extract.

ACKNOWLEDGMENTS

The authors express their gratitude to the Thailand Institute of Scientific and Technological Research (TISTR) for providing research funds. We thank all TISTR colleagues who in one way or another help this project to succeed.

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