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Itsara Keeta

Nahathai Sawang

Buppachart Potduang

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Itsara Keeta[1], Nahathai Sawang[2] and Buppachart Potduang[1,*]

[1]*Pharmaceutical and Natural Products Department, Thailand Institute of Scientific and Technological Research (TISTR), Technopolis, Klong 5, Klong Luang, Pathumthani 12120, Thailand; E-mail: buppachart@tistr.or.th*
[2]*School of Cosmetic Science, Mae Fah Luang University, Muang, Chaing Rai 57100, Thailand*

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INTRODUCTION

Hair tonic is a cosmeceutical product for use on the scalp to reduce hair loss and revitalize hair and scalp. Marketed hair tonics claim to improve blood flow and restore small hair follicles to increase the delivery of oxygen and nutrients for hair follicles.[1] The product benefits from active natural ingredients as amino acids, vitamins, minerals, essential fatty acids, growth factors, and antioxidants (flavonoids, glycosides, etc.) which increase the hair cells and prevent the aging of the cells causing by the free radicals from modern life style including pollution, chemicals, stress and UV light[2].

Fruits of Ma-khampom (*Phyllanthus emblica* Linn., EUPHORBIACEAE) are consumed as fruit or in the form of food products. The fruit extracts possess several pharmacological activities, such as anti-oxidant, anti-tumor and anti-inflammation, attributable to its high vitamin C and polyphenolic content. The fruits have long been used as hair growth nourishment in traditional Tibetan and Ayurvedic medicine. Recent *in vivo* studies suggested *P. emblica* as possessing hair growth promoting activity as it is a component in the herbal formulations that effectively enlarge the size and prolong the anagen phase of hair follicles. However, its biological effects on follicular cells are still largely unknown[3-6].

Ripe fruits of Ma-khwaen (*Zanthoxylum limonella Alston*, RUTACEAE) are harvested and commercialized in local markets as a popular spice in the northern part of Thailand. Vitamin E has been found in the seed oil. The essential oil from these fruits affects the gastrointestinal system and initiates smooth muscle contraction by a non-specific mechanism. The oil contains sabinene, which is potently bactericidal against the multi-drug resistant bacteria.[7, 8]

This research aimed to develop a hair tonic comprising a patented mixed extract from the fruits of *P. emblica* and *Z. limonella* which are anti-oxidant, anti-inflammatory and anti-microbial[9]. The product also benefits from the fruit extract of *P. emblica* as it is reported to promote proliferation in dermal papilla cells of human hair follicle[4]. The development of hair tonic was prepared from water and alcohol as base ingredient (water as diluents and alcohol as vehicle), together with other ingredients as humectants and nutrients. Excipient compatibility, organoleptic characteristics and stability in accelerated conditions were tested to select the best hair tonic formulation.

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 of 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 with anti-microbial activity, as tested by agar diffusion assay.

Formulation of hair tonic Four formulas of hair tonic consisting of the *P. emblica* and *Z limonella* mixed extract were formulated. They varied in the amount of ingredients in the basic formula, as shown in Table 1. The active mixed extract was dissolved in a humectant, then mixed with nutrients dissolved in purified water. The solution of chelating agent in water was added to the previous mixture, followed by alcohol and mixed thoroughly. Hair tonic for adults should have acidic pH.

Stability test The formulated hair tonic was tested using heating and cooling cycle, at 4°C for 24 hrs and 45°C for 24 hrs, for 6 cycles. The physical stability of samples was evaluated based on their turbidity, precipitation and appearance.

In vitro anti-microbial assessment of the product Agar diffusion assay was performed against *Propionibacterium acnes, Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes and Candida albicans.* Stock of microorganism was prepared by cultivation on agar, when microorganism reveals good, it was separated to sterile water and adjusted to the concentration of 0.5 McFarland. Twenty

milliliters of nutrient agar was added and allowed to set in a Petri dish. The microorganism was added and distributed evenly over the agar surface, and let dry in aseptic condition. Twenty µl of sample were added directly onto the agar, which was then incubated at 37°C for 18-24 hrs. The clear zone of inhibition was observed and compared to that of the hair tonic base which was used as control.

Table 1 The basic formula of Hair tonic

Ingredients	Function	%w/w
95%Ethanol	Vehicle	A
Menthol	Flavor/anti-inflammatory	0.1-0.5
Propylene glycol	Humectant	10-30
Glycerin	Humectant	B
Panthenol (Pro Vitamin B5)	Moisturizer, promote blood flow	1-2
Tocopheryl Acetate	Promote blood flow	0.2-0.5
Zinc pyrithione	Anti-fungal	0.2-0.5
Crude extracts	Active ingredient	C
Disodium EDTA	Chelating agent	q.s.
Fragrance	Fragrance	q.s.
Water	Vehicle	q.s. to 100

Preparation of sample Control: Hair tonic without crude extract of *P. emblica* and *Z. limonella*. Sample: Hair tonic with crude extract of *P. emblica* and *Z. limonella* in varied concentrations.

RESULTS AND DISCUSSION

Development of hair tonic Four formulas of hair tonic containing the patented mixed extract from the fruits of *P. emblica* and *Z. limonella* were formulated. Formula 1 gave opaque brown liquid and precipitated. Alcohol was added to formula 2, producing clearer liquid but still opaque and separated when vitamin E was added. Zinc pyrithione was added to formula 3, resulting in turbid liquid and precipitated. Formula 4 was formulated without vitamin E and zinc pyrithione, but more humectants were added to eliminate problem with opaqueness. Formula 4 was stable under heating and cooling stability test.

In vitro anti-microbial assessment of the product The agar diffusion assay of the hair tonic showed that the products, with varied concentration of the active extract, were active against 7 strains of *Staphylococcus aureus* (DMST 8013 and DMST 8840), *Streptococcus pyogenes* (DMST 17020), *Staphylococcus epidermidis* (DMST 12228), *Candida albicans* (DMST 10231 and DMST 90028) and *Propionibacterium acnes* (DMST 14916) as shown in Table 2.

Table 2 Anti-microbial inhibition of hair tonic with varying concentrations of active ingredient

Conc.	*S. aureus* DMST 8013	*S. aureus* DMST 8840	*S. pyogenes* DMST 17020	*S. epidermidis* DMST 12228	*C. albicans* DMST 10231	*C. albicans* DMST 90028	*P. acnes* DMST 14916
1.5%	+1.0	+1.0	+1.1	+1.2	+1.0	+0.9	+1.5
2.5%	+1.0	+1.0	+1.3	+1.2	+1.0	+1.1	+1.5
3.5%	+1.2	+1.6	+1.5	+1.3	+1.0	+1.2	+1.6
4.5%	+1.4	+1.8	+1.8	+1.6	+1.1	+1.2	+1.8
base	-/+1.0	-/+0.9	-/+1.1	+1.1	+0.9	+1.0	+1.0

\+ = visible
-/+ = unclear
\- = invisible

CONCLUSION

The patented mixed extract from the fruits of *P. emblica* and *Z. limonella* could be used as an active extract for hair tonic product. The final product comprised of purified water, propylene glycol, alcohol, glycerin, pro vitamin B5, menthol, disodium EDTA and the active extract. The *P. emblica* and *Z. limonella* mixed extract could significantly promote the anti-microbial efficacy of the base.

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