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## DEVELOPMENT OF SHAMPOO COMPRISING A MIXED EXTRACT FROM FRUITS OF *PHYLLANTHUS EMBLICA* AND *ZANTHOXYLUM LIMONELLA* FOR ITCHY SCALP

Buppachart Potduang<sup>1</sup>\*, Itsara Keeta<sup>1</sup>, Suchipha Wannaphatchaiyong<sup>2</sup>, Wanlapha Saisin<sup>2</sup> and Bundit Fungsin<sup>3</sup>

<sup>1</sup>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

<sup>2</sup>Department of Pharmaceutical Sciences, Prince of Songkla University, Hatyai, Songkhla, 90110, Thailand.

<sup>3</sup>Biosciences Department, Thailand Institute of Scientific and Technological Research (TISTR), Technopolis, Klong 5, Klong Luang, Pathumthani, 12120, Thailand.

**KEYWORDS:** Shampoo, *Phyllanthus emblica*, *Zanthoxylum limonella*, Anti-microbial, Anti-inflammation

### INTRODUCTION

Shampoo is a hair care product used for the removal of oils, dirt, skin particles, environmental pollutants and other contaminant particles that gradually build up in hair. The goal is to remove the unwanted build-up without stripping out so much sebum as to make hair unmanageable<sup>1</sup>.

This research aimed to develop an anti-microbial, anti-inflammatory and antioxidant benefits shampoo for itchy scalp comprising a patented mixed extract from fruits of *Phyllanthus emblica* and *Zanthoxylum limonella*. The anti-inflammatory and anti-microbial effects of the active extract promote itchy scalp reduction. The antioxidant effect of the extract is beneficial for damaged hair by reducing harmful free radicals causing by pollutants and UV rays.

*Phyllanthus emblica* L. (EUPHORBIACEAE), known as Ma-khampom, is an herbal plant commonly used in Asian traditional medicine. Its edible fruits were reported to be used as an alternative treatment of diarrhea, jaundice, skin disorders, inflammations and premature graying. The fruit extract is used as a skin-lightening agent, benefits from its anti-tyrosinase effect. Its antioxidant activity provides protection against free radicals induced by UV. Phenolic compounds from *P. emblica* exhibited anti-inflammatory effect<sup>2-6</sup>.

*Zanthoxylum limonella* Alston (RUTACEAE), known as Ma-khwaen, has been extensively used in folk medicines for different medical purposes. The edible fruit has been traditionally used as food flavor in the northern part of Thailand. Its essential oil exhibits the anti-oxidative potential according to Sabinene, which is a potent bactericidal against the multi-drug resistant bacteria<sup>7-9</sup>. The extract is effective as anti-microbial (MIC 2.5-10 mg/ml) and anti-oxidant (EC<sub>50</sub> 5.94 µg/ml)<sup>10</sup>.

The patented mixed extract from fruits of *P. emblica* and *Z. limonella* is effective as *in vitro* anti-microbial (MIC 4.5 mg/ml) against *Propionibacterium acnes*, *Staphylococcus aureus*, *S. epidermidis* and *Streptococcus pyogenes*. It is an anti-oxidant (EC<sub>50</sub> 7.9 µg/ml) and a potent anti-inflammatory on croton oil-induced rat ear edema better than std. Diclofenac<sup>11-12</sup>. The mixed extract was used as an active extract to develop a patented moisturizing shampoo for preventing and reducing dry and itchy scalp. The shampoo formula composes of the active ingredients in an appropriate solvent. Humectants are responsible to increase water content of the top layers of scalp, and affect the scalp moisture contain. Surfactants wash dirt on hair and scalp. A flavoring agent was added for good smell with refresh and relaxes feeling. A chelating agent stabilizes the product not to precipitate. The preservative is essential to prevent product damage caused by microorganisms and to protect the product from inadvertent contamination by the consumer during use. The mixed extract from fruits of *P. emblica* and *Z. limonella* benefits the product for its antimicrobial and anti-inflammation.

### MATERIALS AND METHODS

**Plant material** The dry fruit powder 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 the mixed extract** The water-ethanol crude extracts from dry fruits of *P. emblica* and *Z. limonella* were mixed in a patented proportion for the best anti-microbial activity.

**Formulation of shampoo** Shampoo consisting of the *P. emblica* and *Z. limonella* mixed extract was formulated in 10 formulas. They were varied in amount of ingredients in the basic formula as shown in Table 1. The active mixed extract was dissolved in a humectant with small amount of water; and then added other surfactants, foam booster, conditioning agents, thickening agents. The chelating agent solution in water was added to the previous mixture, added purified water and mixed thoroughly. The

preservative and fragrance were then added while stirring. 50% citric acid solution was used to adjust pH. Shampoo for adults should have pH 5.0 – 8.5.

**Stability test** The formulated shampoo was tested under heating and cooling test (H&C) at 4 °C 24 hrs and 45 °C 24 hrs for 6 cycles. The physical stability of samples was evaluated on turbidity, precipitation and appearance.

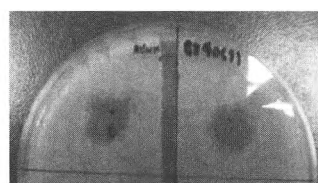
**In vitro anti-microbial assessment of shampoo** Tissue diffusion assay was performed against *P. acnes*, *S. aureus*, *S. epidermidis*, *S. pyogenes* and *C. albicans*. Stock of microorganism was prepared by cultivation on agar, when microorganism revealed good it was separated to sterile water and 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. 20 µl of 50% shampoo solution was added directly onto 1 cm diameter tissue paper on nutrient agar. When the time completed at 1 minute, took the tissue out of the agar cultures. Closed the lid and incubated at 37 °C for 18-48 hrs. The clear zone of inhibition was observed compared to shampoo base. The shampoo base was used as control.

Table 1 The basic formula of shampoo.

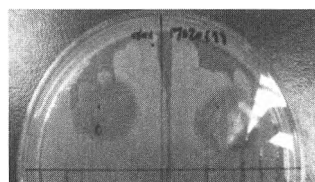
Ingredients	function	%w/w
Sodium laureth sulfate (70%)	Anionic surfactant	30-50
Coamidopropyl betaine	Amphoteric surfactant	1-5
Cocamide DEA	Foam boosters & Stabilizer	X
A	Conditioning agent	1-5
Lanolin	Conditioning agent	1-5
Dimethicone	Conditioning agent	1-5
PEG 400	Thickening agent	X
Glycerine	Humectants	1-5
Crude extracts	Active ingredient	X
Propylene glycol	Humectants & Co-solvent	1-5
Tetrasodium EDTA	Chelating agent	0-0.02
Citric acid solution (50%)	Acidifying agent	q.s.
B	Preservative	0.1-1
Fragrance	Odorant	q.s.
Water	Vehicle	q.s. to 100



*Staphylococcus aureus* DMST 8013



*Staphylococcus aureus* DMST 8840

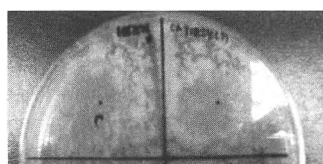


*Streptococcus pyogenes* DMST 17020



*Staphylococcus epidermidis* DMST 12228

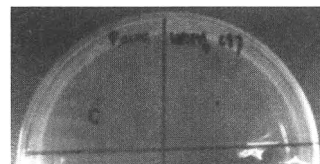
**Figure 1** Microbial inhibition of the shampoo Formula 10 consisting of the patented mixed extract from fruits of *P. emblica* and *Z. limonella* against 4 strains of *S. aureus*, *S. pyogenes* and *S. epidermidis*. The results in a Petri dish compared between shampoo base (left) and Formula 10 shampoo (right).



Candida albicans DMST 10231



Candida albicans DMST 90028



Propionibacterium acnes DMST 14916

**Figure 2** Microbial semi-inhibition of the shampoo Formula 10 consisting of the patented mixed extract from fruits of *P. emblica* and *Z. limonella* against 3 strains of *C. albicans* and *P. acnes*. The results in a Petri dish compared between shampoo base (left) and Formula 10 shampoo (right).

## RESULTS AND DISCUSSION

The shampoo containing the patented mixed extract from fruits of *P. emblica* and *Z. limonella* was formulated in 10 formulas. Formula 1 gave clear brown and pearlescent solution, but it was not viscous and the opacifying agent separated. More thickening agent was added in Formula 2 and 3. Formula 2 was thicker than Formula 1, but Formula 3 was not viscous. Viscosity adjusted with surfactant was performed in Formula 4 and 5. Formula 5 was thicker than 4. Viscosity and color were further adjusted in Formula 6 to 8. Formula 8 was better than Formula 6 and 7. Formula 9 was derived from Formula 8 by adding surfactant, different conditioning agent and opacifying agent which resulted that it was not stable under stability test. The opacifying agent was not used in Formula 10. Formula 10 was selected for their acceptable viscosity and stability.

*In vitro* anti-microbial assessment of Formula 10 revealed that the shampoo was active against 4 strains of *S. aureus* (DMST 8013 and DMST 8840), *S. pyogenes* (DMST 17020) and *S. epidermidis* (DMST 12228) as shown in Figure 1. It was semi-active against 3 strains of *C. albicans* (DMST 10231 and DMST 90028) and *P. acnes* (DMST 14916) as shown in Figure 2. The anti-microbial activity was stable after 6 cycles of heating and cooling test.

## CONCLUSION

The patented mixed extract from fruits of *P. emblica* and *Z. limonella* could be used as an active extract for shampoo products. The best shampoo was Formula 10 with acceptable moisture, pH and viscosity. It comprised appropriate amount of chelating agent and solubilizer to give stable clear brown solution. An appropriate amount of a flavoring agent was added to the Formula 10. The product benefits from the anti-inflammatory, anti-microbial and antioxidant effects of the active extract for reducing itchy scalp as well as harmful free radicals causing by pollutants and UV rays.

## ACKNOWLEDGMENTS

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