CHAPTER III

MATERIALS AND METHODS

Materials

Tea tree oil (Australian Bodycare UK Ltd.)

Carbomer (polyacrylic acid) (Sigma-Aldrich Chemie.)

Propylene glycol (Sigma-Aldrich Chemie.)

Glycerin (Sigma-Aldrich Chemie.)

Triethanolamine (Sigma-Aldrich Chemie.)

Sorbitanmonolaurate 20 (Span 20) (Sigma-Aldrich Chemie.)

Polysorbate 20 (Tween 20) (Sigma-Aldrich Chemie.)

Polyoxyethylene-10-oleyl-ether (Brij 97) (Sigma-Aldrich Chemie.)

Polysorbate 80 (Tween 80) (Sigma-Aldrich Chemie.)

Ethanol (Merck LiChrosolv®)

Snake skin (Python molurus) (Thompson Snake farm, London.)

Equipments

Gas Chromatography Hewlett Packard 5890 Series II

Gas Chromatography - Mass Spectrometry Hewlett Packard 5971 Series II MS

Mortar and Pestle

Beaker

Stirring rod

Franz diffusion cell

Magnetic bar

Magnetic stirrer

Methods

1. Formulation Procedure

The placebo gel was formulated. Tea tree oil gel formulations were prepared by using 1% tea tree oil without emulsifier and with four different emulsifiers. Four commercial emulsifiers were chosen base on the different of Hydrophile – Lipophile Balance (HLB) value, which were Sorbitanmonolaurate 20 (Span 20) (HLB 8.6), Polysorbate 20 (Tween 20) (HLB 16.7), Polyoxyethylene-10-oleyl-ether (Brij 97) (HLB 12.4), and Polysorbate 80 (Tween 80) (HLB 15), in 3 different concentrations, which were 0.5 %, 1 % and 2 %.

Table 2 Percentage of ingredients in tea tree oil gel formulations

	Tea tree oil (%)	Emulsifier (%)	Carbomer (%)	Propylene Glycol (%)	Glycerin (%)	Triethanolamine (%)
Placebo gel	-	-	0.5	10	15	0.5
Tea tree oil gel (without emulsifier)	1	-	0.5	10	15	0.5
Tea tree oil gel with 0.5% Span 20	1	0.5% Span 20	0.5	10	15	0.5
Tea tree oil gel with 1% Span 20	1	1% Span 20	0.5	10	15	0.5
Tea tree oil gel with 2% Span 20	1	2% Span 20	0.5	10	15	0.5
Tea tree oil gel with 0.5% Tween 20	1	0.5% Tween 20	0.5	10	15	0.5
Tea tree oil gel with 1% Tween 20	1	1% Tween 20	0.5	10	15	0.5
Tea tree oil gel with 2% Tween 20	1	2% Tween 20	0.5	10	15	0.5
Tea tree oil gel with 0.5% Brij 97	1	0.5% Brij 97	0.5	10	15	0.5
Tea tree oil gel with 1% Brij 97	1	1% Brij 97	0.5	10	15	0.5
Tea tree oil gel with 2% Brij 97	1	2% Brij 97	0.5	10	15	0.5
Tea tree oil gel with 0.5% Tween 80	1	0.5% Tween 80	0.5	10	15	0.5
Tea tree oil gel with 1% Tween 80	1	1% Tween 80	0.5	10	15	0.5
Tea tree oil gel with 2% Tween 80	1	2% Tween 80	0.5	10	15	0.5

To make placebo gel, disperse Carbomer in water and leave over night. After that, reblend well and add Propylene glycol and glycerin and reblend again. Stir in Triethanolamine, to adjust pH of formulation to around 5.5, and blend until homogeneous.

To make Tea tree oil gel without emulsifier, disperse Carbomer in water and leave over night. After that, reblend well and add Propylene glycol and glycerin and reblend again. Stir in Triethanolamine and blend until homogeneous. Then, stir in Tea tree oil and keep blending for 5 minutes.

To make Tea tree oil gel with emulsifier, disperse Carbomer in water and leave over night. After that, reblend well and add Propylene glycol and glycerin and reblend again. Stir in Triethanolamine and blend until homogeneous. Now, the plain gel is prepared. Blend emulsifier with Tea tree oil and keep blending for 5 minutes, then pour the mixture of emulsifier and Tea tree oil into plain gel and keep blending for 5 minutes.

2. Analysis Procedure

The analysis procedure was carried to analyze terpinen-4-ol by using Gas Chromatography and Gas Chromatography – Mass Spectrometry.

1. Components identification of tea tree oil was performed using Gas Chromatography – Mass Spectrometry. Tea tree oil 0.2% in ethanol was prepared and injected to the machine in condition as follow; injector temperature was 250°C, detector temperature was 280°C, starting temperature at 50°C and holding for 5 minutes,

increasing temperature 20°C per minute until reaching the highest temperature at 220°C, holding for 5 minutes and decreasing temperature for cooling down.

2. An analytical sampling of pure tea tree oil as the standard curve was taken under different concentrations. Standard solution of 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, 0.12%, 0.14%, 0.16%, 0.18% and 0.20% tea tree oil in ethanol were prepared by dissolved tea tree oil 1 μl, 2 μl, 3 μl, 4 μl, 5 μl, 6 μl, 7 μl, 8 μl, 9 μl and 10 μl in ethanol 5 ml, respectively.

3. Release Procedure

1. Franz diffusion cell was used for releasing procedure. Tea tree oil 1 g formulations were put on the model skin. Each formulation was applied and performed the release procedure in triplicate in order to compare accuracy and precision of the results. The receptor compartment contained ethanol as dissolvable solvent for the tea tree oil components. Snake skin was used as the skin membrane which has many properties similar to human stratum corneum. (Williams and Barry 1994:563-566) Snake skin was treated by rinsing in purified water and then rinsing in Acetone. After rinsing in water one more time, snake skin was soaking in water for 1 hour and ready to be used. The cell was adjusted to the temperature around 37°C, which is similar to human body temperature.

2. Each sample was collected every hour for 6 hours and tested by using the snake skin as a skin model, to observe its penetration. The samples were collected from the receptor compartment of franz diffusion cell by micro syringe and were injected to Gas Chromatography in condition as follow; injector temperature was 250°C, detector temperature was 280°C, starting temperature at 50°C and holding for 5 minutes, increasing temperature 20°C per minute until reaching the highest temperature at 220°C, holding for 5 minutes and decreasing temperature for cooling down.

3. Compared concentrations of main component of tea tree oil release from various gel formulations by plotting graphs between concentration (%v/v) and time by taking accumulative concentrations of main component of tea tree oil in ethanol from the receptor compartment of franz diffusion cell between 1 hour to 6 hour.