Formulation and Evaluation of Hair Shampoo Containing Tea Tree (Melaleuca alternifolia) Oil and Virgin Coconut (Cocos nucifera) Oil

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Abstract. Tea Tree oil (TTO) contains beneficial properties such as antibacterial, antimicrobial, antiviral and anti-fungal. Whereas, the medium chain fatty acids in Virgin Coconut oil (VCO) able to protect hair follicles from heat, restoring hair’s moisture and other damage. This paper describes the physical properties of seven hair shampoo formulations containing differing amount of TTO and VCO. The essential oils (TTO) applied in these formulations were extracted from fresh tea trees using steam distillation method and the VCO was produced from fermentation of fresh mature kernel coconut. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted to determine the essential oil components of TTO and fatty acid composition of VCO. The shampoo formulations were subjected to evaluation of several parameters namely organoleptic, pH, viscosity, total solid content, foam stability, and dirt dispersion. The results show that the TTO was composed of terpene hydrocarbons with terpinene-4-ol as the major component; meanwhile lauric acid is major component of VCO. All the shampoo formulations were acid-balanced with pH range between 6.23 – 6.43; total solid contents were between 29.92 – 35.61%; stable foaming with the same foam volume for 4 minutes and no dirt was observed. Rheological test showed formulation with 6% TTO (0% VCO) has pseudo-plastic behavior and relatively lower total solid content which are desirable attributes in hair shampoo. Overall, TTO- and VCO-containing shampoo formulations showed ideal physicochemical properties for hair cleansing and treatments.

1. Introduction
The concept of incorporating natural-based ingredients in cosmetic industries is widely grown in the market due to the major concern and consciousness on producing safe and environmentally friendly products [1]. Active ingredients from natural sources (plants, animals, microorganisms) have been broadly used for centuries as main substances in cosmetics preparations [2]. Natural sources may contain compound such as polyphenols, fatty acids, terpenes and vitamin which promote several bioactivities and can be incorporated in cosmetic preparation [3]. In this paper, TTO and VCO were incorporated in haircare preparations due to variety of benefits offered from both oils. TTO has been used largely in various industries for its beneficial properties [4], whereas virgin coconut oil has been used since ancient time as multipurpose oils and nowadays it’s become a trend in cosmetic application [5].

Tea tree oil has been used for many years as a component in medicinal products especially in treating cutaneous infections due to its antimicrobial and anti-inflammatory properties [6]. The essential oil of tea tree is obtained by using steam distillation process, originated from Australia and also known as Melaleuca alternifolia [7]. The chemical composition contains approximately 100 components, which are mostly consists of monoterpenes, sesquiterpenes and related alcohols. Major compound of
tea tree oil is Terpinen-4-ol, which has long been considered as the main anti-microbial properties to TTO [8].

Virgin coconut oil was extracted from fresh and mature kernel without the use of heat and also without undergoing chemical refining [9]. It was previously demonstrated by protein loss and water retention measurements, that coconut oil can prevent cuticular damage which usually occur during combing process [10]. The hydrophobicity properties of coconut oil, reduces the water penetration into the fiber which give beneficial effect by providing a lubricating film on the hair [11]. Ruetsch et al. [12] have shown that coconut oil penetrates into the hair cortex and reduces the swelling of the hair fiber, a factor that can cause hair damaged. VCO also acts by filling the gap between the cuticle and prevent the penetration of aggressive substances such as surfactants into the hair follicle [13].

As TTO and VCO were proven to have beneficial properties, incorporating these two oils in a formulation of shampoo will benefit in overcoming hair and scalp problem. Therefore, this research was designed to formulate and to study the characteristics by evaluating the physicochemical properties of shampoo containing TTO and VCO.

2. Materials and Methods
2.1 Preparation of Raw Material
Tea tree leaves were collected from tea tree field at the Demonstration Plot located at Mile 30 Kimanis, Papar, Sabah, Malaysia. Approximately 1.0 kg of tea tree leaves were subjected to steam distillation system to obtain the essential oil [8]. VCO was produced using fermentation method [14]. Sodium lauryl ether sulfate (SLES), natrium chloride, ethylenediaminetetraacetic acid (EDTA), cocamidopropyl betaine, polysorbate-20, DMDM hydantoin, citric acid, potassium hydroxide, sulfuric acid, methanol and hexane were United States Pharmacopeia (USP) grade which purchased from Sigma Aldrich (USA).

2.2 GC-MS analysis of TTO and VCO
The resulted TTO was subjected to methanol dilution of 1:10 for analysis. GC-MS analysis were carried out on Agilent 5997A. Helium was used as a carrier gas and the column used was an HP-5MS UI (5% diphenyl 95% dimethylpolysiloxane), 30m × 0.25mm × 0.25 um. Temperature program was from 50°C to 280°C at 3°C/min. The temperatures of the transfer line and the injector were 280oC and 250oC, respectively, with MS parameters were as follows: ion source filament voltage 70eV and the mass range was 40–400 m/z [15].

Similarly, the obtained TTO was subjected to two-step methylation process according to the method described by Wang et al. [16]. Exactly 40 μL of VCO was placed into 10 mL centrifuge tubes to which 0.7 mL of 10 M potassium hydroxide solution and 5.3 mL of methanol were added. The reaction was performed at 55°C for 1.5 h with mixing for 5 s every 20 min. After cooling to room temperature, 0.58 mL of sulfuric acid (10 M) solution was added and the reaction was continued at 55°C for 1.5 h with mixing for 5 s every 20 min. After cooling to room temperature, 3 mL of n-hexane was added and mixed for 5 min. Finally, the tubes were centrifuged for 5 min and subjected to GC-MS analysis. Helium was used as a carrier gas and the column used was an HP-5MS UI (5% diphenyl 95% dimethylpolysiloxane), 30m × 0.25mm × 0.25 um. The temperature program was held at 180°C for 40mins. The temperatures of the transfer line and the injector were 230oC and 200oC, respectively. MS parameters were operated with the electron impact mode at 70eV and in the scan range 35 – 600 m/z [17]. The identification is based upon the mass spectra matching with libraries (NIST and WILEY) and mass spectra from literatures.

2.3 Formulation of Hair Shampoo
Seven shampoo formulation were prepared (w/v) and tested in this study. As can be seen in Table 1, formulations F1 to F7 are containing increasing amount of TTO (0 – 6 %, w/v) and vice versa for the amount of VCO. The rest of the ingredients are the same for all the seven formulations
Table 1: The composition of ingredients used for the formulation (%w/w)

| Ingredient                  | F1   | F2   | F3   | F4   | F5   | F6   | F7   |
|-----------------------------|------|------|------|------|------|------|------|
| Distilled water             | 55.5 | 55.5 | 55.5 | 55.5 | 55.5 | 55.5 | 55.5 |
| SLES                        | 20   | 20   | 20   | 20   | 20   | 20   | 20   |
| Natrium Chloride            | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  |
| EDTA                        | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  |
| Cocamidopropyl Betaine      | 10   | 10   | 10   | 10   | 10   | 10   | 10   |
| Polysorbate-20              | 6    | 6    | 6    | 6    | 6    | 6    | 6    |
| Virgin coconut oil          | 6    | 5    | 4    | 3    | 2    | 1    | 0    |
| Tea tree oil                | 0    | 1    | 2    | 3    | 4    | 5    | 6    |
| DMDM Hydantoin              | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  | 0.5  |
| Citric Acid                 | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |

2.4 Evaluation of Shampoo Formulation

The prepared shampoo formulations were evaluated for its organoleptic characteristic, pH, foaming ability, total solid contents, viscosity and dirt dispersion.

2.4.1. Organoleptic characteristic

The formulations prepared were evaluated for the organoleptic characteristics, such as color, smell and texture as described by Krunali et al. [18].

2.4.2. pH measurement

Each formulation was diluted with distilled water to 10% (v/v) shampoo solution [19]. Readings of pH were taken using Mettler Toledo FE20-ATC Kit FiveEasy™ Benchtop pH Meter.

2.4.3. Foaming ability

Foaming ability was evaluated by using cylinder shake method as reported by Badi & Khan [20]. At room temperature, 1% of 50 ml shampoo solution was put into a 250 ml graduated cylinder, which was then covered by hand and shaken 10 times. The total volume of the content after 1 and 4 minutes of shaking were recorded.

2.4.4. Total solid contents

Percentage of solid content was carried out based on SM 2540 C standard [21]. The samples were placed into an oven at 105°C for 16 hours or until a constant oven dry weight was achieved and then was placed into desiccator to cool. The weight of samples were recorded for total solid calculation.

2.4.5. Viscosity measurement

Rheology of product determine the requisite stability of formulation in terms of consistency and flow characteristics of each formulation. The viscosity measurement was performed at room temperature using Brookfield DV3T Rheometer by varying the rotational speeds (100 rpm, 150rpm and 200rpm) [22].

2.4.6. Dirt dispersion

For this test, 10 ml of distilled water was placed into a large test tube. Two drops of the shampoo were added into the test tube followed by one drop of Indian ink. Test tube was shaken for ten times. The amount of ink stained in the foam was recorded as none, light, moderate or heavy [19].
2.5 **Statistical analysis**

Data were analyzed using SPSS general linear model and one way ANOVA with \( p \) values <0.05 were considered as significant.

3. **RESULTS AND DISCUSSION**

3.1 **GC-MS Analysis on TTO and VCO**

Figures 1 and 2 show the GC-MS chromatograms of TTO and VCO, respectively. Meanwhile, Tables 2 and 3 show the components of TTO and VCO, respectively, identified by GC-MS analysis. The major component of TTO is terpinene-4-ol (42.54%); an organic compound possesses strong antimicrobial and anti-inflammatory properties [22]. From previous study, it is suggested that TTO treatment can treat fungal infections of the skin and mucous membranes as well as in the treatment of dandruff [23].

![Figure 1: GC-MS chromatogram for chemical composition of TTO](image)

![Figure 2: GC-MS chromatogram of VCO](image)
The composition of fatty acid showed that the coconut oil is rich in saturated fatty acids, with high proportion of lauric acid (43.83%). Lauric acid is the triglyceride component in coconut oil with short chain fatty acid that has a high affinity for hair proteins and its straight linear chain along with low molecular weight makes them easily to penetrate and absorb deeper into the hair shaft [24]. When VCO penetrates the hair it reduces the amount of water absorbs in the hair and leading to lowering of swelling propensity of the cuticle, which limits the upward curving of the surface cuticle [13]. This reduces the chipping away of the cuticle cells, which leads to protein loss prevention [25].

### Table 2: Chemical composition for TTO

| Components identified     | Molecular weight (g/mol) | Retention time (min) | Peak area (%) | MS Matching quality (%) |
|---------------------------|--------------------------|----------------------|---------------|-------------------------|
| Alpha-phellandrene        | 136.238                  | 6.70                 | 1.17          | 91                      |
| Alpha-pinene              | 136.23                   | 6.89                 | 2.83          | 95                      |
| Alpha terpinene           | 136.238                  | 9.46                 | 10.89         | 96                      |
| p-cymene                  | 134.21                   | 9.70                 | 2.49          | 95                      |
| Sabinene                  | 136.23                   | 9.84                 | 2.19          | 87                      |
| Eucalyptol                | 154.249                  | 9.91                 | 3.07          | 96                      |
| Gamma-terpinene           | 136.238                  | 10.91                | 19.60         | 95                      |
| Terpinolene               | 136.23                   | 11.87                | 4.24          | 97                      |
| Terpinene-4-ol            | 154.253                  | 15.09                | 42.54         | 95                      |
| Alpha-terpineol           | 154.253                  | 15.44                | 4.19          | 86                      |
| Valencene                 | 204.357                  | 23.61                | 1.65          | 90                      |
| Bicylogermacrene          | 204.357                  | 25.38                | 2.78          | 98                      |
| Delta-cadinene            | 204.357                  | 26.18                | 2.36          | 99                      |

### Table 3: Fatty Acid Composition of VCO from GC-MS Analysis

| Components identified    | Molecular weight (g/mol) | Retention time (min) | Peak area (%) | MS Matching quality (%) |
|--------------------------|--------------------------|----------------------|---------------|-------------------------|
| Methyl caprylate         | 158.241                  | 13.11                | 2.54          | 95                      |
| Methyl caprinate         | 186.295                  | 19.92                | 2.39          | 96                      |
| Capric acid              | 172.268                  | 21.55                | 1.88          | 97                      |
| Methyl laurate           | 214.349                  | 26.19                | 10.56         | 97                      |
| Lauric acid              | 200.322                  | 27.98                | 43.83         | 98                      |
| Methyl myristate         | 242.403                  | 31.84                | 4.26          | 98                      |
| Myristic acid            | 228.376                  | 33.09                | 14.07         | 99                      |
| Methyl isopalmitate      | 298.511                  | 36.98                | 5.32          | 98                      |
| Methyl oleate            | 296.495                  | 41.07                | 4.40          | 99                      |
| Methyl stearate          | 298.511                  | 41.67                | 3.54          | 98                      |

3.2 Evaluation of Shampoo Formulation

From the parameters evaluated (Table 4), the pH level for all formulations were acid-balanced which range between 6.23 to 6.43 and it was observed there was a significance differences among the formulations ($p<0.05$). The higher pH level of shampoo the harsher the shampoo on human’s hair. As previous study [26] shown that lower pH of shampoos may cause less frizzing for generating less negative static electricity on the fiber surface.
Table 4: Physical properties of the hair shampoo formulations

| Formulation | Organoleptic characteristics | pH         | Foam Height (ml) | Total Solid content (%) | Dirt Dispersion |
|-------------|------------------------------|------------|------------------|-------------------------|----------------|
|             |                              |            | 1min 2mins 3mins 4mins |                          |                |
| F1          | Clear light-yellow liquid, VCO odour | 6.43±0.02  | 90 90 90 88       | 34.87±0.39              | None           |
| F2          | Clear light-yellow liquid, VCO and TTO odour | 6.26±0.01  | 102 102 102 102   | 35.61±0.43              | None           |
| F3          | Clear light-yellow liquid, VCO and TTO odour | 6.33±0.03  | 100 100 100 98    | 35.49±0.25              | None           |
| F4          | Clear light-yellow liquid, strong TTO odour | 6.39±0.05  | 94 94 94 93       | 32.29±0.34              | None           |
| F5          | Clear light-yellow liquid, strong TTO odour | 6.23±0.02  | 110 110 108 108   | 33.42±0.58              | None           |
| F6          | Clear light-yellow liquid, strong TTO odour | 6.28±0.03  | 96 96 96 95       | 31.59±0.35              | None           |
| F7          | Cloudy white liquid, strong TTO odour | 6.36±0.01  | 102 102 102 102   | 29.92±0.22              | None           |

From the results, all formulations have good foaming ability and there was no significance difference observed among the different formulations. Height of foam produced only shows slight changes for 4 minutes observation which showed the stability of foaming for the shampoos. Total solid content in shampoo indicates their capability in cleaning action. Study by Badi & Khan [20], revealed the best range are between 20 – 30% as it is easy to apply and rinse out from hair. Statistically, there were significance difference between all formulations (p<0.05). The results showed F7 (6% of TTO) has the capability to be washed away on human hair more easily than the other formulations.

Table 5 shows rheological evaluations for the shampoo formulations, with only F7 (6% TTO 0% VCO) showed pseudo plastic behavior. Pseudo-plastic behavior is a desirable attribute for rheological properties in shampoos. Pseudoplastic behavior is when the rheological properties will show high viscosity at low rotational speed and decrease in viscosity at high rotational speed. Low viscosities at high shear rates (e.g. spreading the product on the hair) make the product easier to apply. High viscosities at low shear rates may also help suspend materials in the product.

Table 5: Rheological evaluations by measuring viscosity at different rotational speed

| Formulation | Viscosity (cP) |
|-------------|----------------|
|             | 100rpm 150rpm 200rpm |
| F1          | 55.60±0.80 63.73±0.27 70.27±0.31 |
| F2          | 58.37±1.10 69.23±0.59 76.60±0.20 |
| F3          | 86.40±1.20 89.45±0.39 92.73±0.50 |
| F4          | 58.83±0.85 70.76±0.15 80.47±0.12 |
| F5          | 57.20±1.04 70.53±0.23 81.67±0.42 |
| F6          | 90.33±0.70 111.23±0.25 130.87±0.12 |
| F7          | 815.33±3.06 796.00±4.16 774.67±2.00 |
It is considered as poor quality shampoo if the ink were concentrated in the foam, as the dirt should stay in water. As reported by Saad et al. [19], dirt that remains in the foam will be difficult to rinse away and will be redeposited on the hair. From the observations of dirt dispersion test (Figure 3), the ink was not dispersed into the foam for all the formulations, therefore prepared formulations are satisfactory. This might be due to same amount of surfactants which acts as a cleaning agents in all of the seven formulations.

| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|----|----|----|----|----|----|----|
| ![Image](image1.jpg) | ![Image](image2.jpg) | ![Image](image3.jpg) | ![Image](image4.jpg) | ![Image](image5.jpg) | ![Image](image6.jpg) | ![Image](image7.jpg) |

**Figure 3**: Dirt dispersion test on shampoo formulation

From the physical properties (pH, foaming ability and dirt dispersion), all seven formulations show results which indicate good quality shampoos. The results show high solid content and non-pseudoplastic behavior on formulations containing 6 %VCO which are not desirable quality for shampoo. The combination between surfactants and VCO might not suitable in this formulation and affecting the stability of the emulsion. Yani et al. [20] suggested that to improve the stability of VCO emulsions in cleansing formulations, more suitable emulsifier and additives, better operations and conditions are required.

4. Conclusion
This research aimed to prepare and evaluate shampoo formulation containing TTO and VCO. These oils were analyzed for its major component and the results obtained were in accordance with other previous study. Terpinene-4-ol from TTO benefits against bacteria which can cause scalp infection and fatty acids of VCO helps in reducing swelling and protein loss from hair fiber. TTO and VCO plays an important role and benefits in the treatment of human hair and scalp due to its own important characteristics and beneficial properties. However, further research and development are still required to improve and enhance the physical properties of the shampoo in order to produce more quality products.

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6. References
[1] Aburjai T and Natsheh F M 2003 Plants used in cosmetics Phytotherapy Research 17(9) 987-1000
[2] Fowler J J, Woolery-Lloyd H, Waldorf H and Saini R 2010 Innovations in natural ingredients and their use in skin care Journal of Drugs in Dermatology 9 72-81
[3] Mohd Setapar H M 2018 Natural ingredients in cosmetics from Malaysian plants: A Review Sains Malaysiana 47(5) 951-9
[4] Carson C F and Riley T V 1995 Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia Journal of Applied Bacteriology 78(3) 264-9
[5] Rele A S and Mohile R B 2003 Effect of mineral oil, sunflower oil, and coconut oil on prevention of hair damage Journal of Cosmetic Science 54(2) 175-192
[6] May J, Chan C H, King A, Williams L and French G L 2000 Time–kill studies of tea tree oils on clinical isolates Journal of Antimicrobial Chemotherapy 45(5) 639-643
[7] Hammer K A, Carson C F and Riley T V 2004 Antifungal effects of Melaleuca alternifolia (tea tree) oil and its components on Candida albicans, Candida glabrata and Saccharomyces cerevisiae Journal of Antimicrobial Chemotherapy 53(6) 1081-5
[8] Carson C F, Hammer K A and Riley T V 2006 Melaleuca alternifolia (tea tree) oil: A review of antimicrobial and other medicinal properties Clinical Microbiology Reviews 19(1) 50-62

[9] Villarino B J, Dy L M and Lizada M C 2007 Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil LWT-Food Science and Technology 40(2) 193-9

[10] Rele A S and Mohile R B 1999 Effect of coconut oil on prevention of hair damage Journal of Cosmetic Science 50(6) 327-340

[11] Keis K, Persaud D, Kamath Y K and Rele A S 2005 Investigation of penetration abilities of various oils into human hair fibers Journal of Cosmetic Science 56(5) 283-295

[12] Ruetsch S B, Kamath Y K and Rele A S 2001 Secondary ion mass spectrometric investigation of penetration of coconut and mineral oils into human hair Journal of Cosmetic Science 52 169-184

[13] Gode V, Bhalla N, Shirhatti V, Mhaskar S and Kamath Y 2012 Quantitative measurement of the penetration of coconut oil into human hair using radio labeled coconut oil J. Cosmet. Sci. 63(1) 27-31

[14] Marina A M, Man Y C and Amin I 2009 Virgin coconut oil: emerging functional food oil Trends in Food Science & Technology 20(10) 481-7

[15] Tranchida P Q, Shellie R A, Purcaro G, Conte L S, Dugo P, Dugo G and Mondello L 2010 Analysis of fresh and aged tea tree essential oils by using GC×GC-qMS Journal of chromatographic science 48(4) 262-6

[16] Wang J, Wu W, Wang X, Wang M and Wu F 2015 An effective GC method for the determination of the fatty acid composition in silkworm pupae oil using a two-step methylation process Journal of the Serbian Chemical Society 80(1) 9

[17] Kurata S, Yamaguchi K and Nagai M 2005 Rapid discrimination of fatty acid composition in fats and oils by electrospray ionization mass spectrometry Analytical Sciences 21(12) 1457-65

[18] Krnunci T, Dhara P, Meshram D B and Mitesh P 2013 Evaluation of standards of some selected shampoo preparation. World Journal of Pharmacy Pharmaceutical Science 2 3622-30

[19] Kumar A and Mali R R 2010 Evaluation of prepared shampoo formulations and to compare formulated shampoo with marketed shampoos International Journal of Pharmaceutical Sciences Review and Research 3(1) 120-126.

[20] Badi K A and Khan S A 2014 Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoo Beni-Suef University Journal of Basic Applied Science 3 301-305.

[21] AlQuadeib B T, Eltahir E K, Banafa R A and Al-Hadhaier L A 2018 Pharmaceutical evaluation of different shampoo brands in local Saudi market Saudi Pharmaceutical Journal 26(1) 98-106.

[22] Cox S D, Mann C M and Markham J L 2001 Interactions between components of the essential oil of Melaleuca alternifolia Journal of Applied Microbiology 91(3) 492-7

[23] Nenoff P, Haustein U F and Brandt W 1996 Antifungal activity of the essential oil of Melaleuca alternifolia (tea tree oil) against pathogenic fungi in vitro Skin Pharmacology and Physiology 9(6) 388-394

[24] Rele A S and Mohile R B 2003 Effect of mineral oil, sunflower oil, and coconut oil on prevention of hair damage Journal of Cosmetic Science 54(2) 175-192

[25] Dias M F R G 2015 Hair cosmetics: An overview International Journal of Trichology 7(1) 2-15

[26] Dias M F R G, Almeida A M, Cecato P M R, Adriano A R and Pichler J 2014 The shampoo pH can affect the hair: myth or reality? International journal of trichology 6(3) 95-99

[27] Saad A H and Kadhim R B 2011 Formulation and evaluation of herbal shampoo from Ziziphus spina leaves extract International Journal of Research in Ayurveda and Pharmacy 2(6) 1802-6

[28] Yani S, Aladin A, Wiyani L and Modding B 2018 Evaluation of viscosity and pH on emulsions of virgin coconut oil beverages IOP Conference Series: Earth and Environmental Science 175(1) 1-8