cosmetics

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Formulation of Botanical Shampoo Infused with Standardised Mangosteen Peel Extract for Healthy Hair and Scalp

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Abstract: In recent decades, there has been a growing demand for shampoos derived from botanical sources due to their avoidance of synthetic and highly allergenic chemicals used as bioactives and excipients. These hair care products are free from sulfates, parabens, silicones, synthetic fragrances, and artificial colours. Natural shampoos are sustainable, skin-friendly, and eco-friendly to the environment. *Garcinia mangostana* (Mangosteen) peel is usually discarded as agricultural waste. It consists of numerous bioactives which exhibit promising activities for hair care and scalp maintenance. This study aimed to formulate and evaluate a novel hair shampoo containing standardised mangosteen peel extract. The formulation of the mangosteen shampoo utilised botanical ingredients and naturally derived components. It underwent an evaluation to assess its physicochemical properties, including visual inspection, pH, surface tension, percentage solid content, wetting time, foam ability and stability, as well as dirt dispersion. These properties were then compared to those of two commercially available hair shampoos. Its antimicrobial activity towards *Malassezia furfur* ATCC 14521 and *Staphylococcus aureus* ATCC 25923 was also examined and compared with the commercial shampoo using the microbroth dilution method. Its antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay. It was noticed that all formulations (F1–F4) had acceptable physicochemical properties, and they fell within the standard range. F2 had the best antifungal activity (MIC 0.039 mg/mL, MFC 0.156 mg/mL), and moderate antibacterial (MIC 2.50 mg/mL, MBC 5.00 mg/mL) and antioxidant activities (IC_{50} 21.9 ± 3.27 mg/mL; AEAC 26.3 ± 4.06 mg AA/100 g sample). A microscopic examination of hair strands after washing revealed the successful removal of artificial sebum, signifying a good detergency effect. The physical and chemical properties of the hair shampoo formula remained stable without phase separation. In conclusion, the formulated clean hair shampoo with standardised mangosteen peel extract has good cleansing properties, and it is effective in inhibiting dandruff-causing microbial and scavenging free radicals.

Keywords: antioxidant; antimicrobial; *Garcinia mangostana*; hair care; physicochemical properties; shampoo

Citation: Sang, S.-H.; Liew, K.B.; Lee, S.-K.; Keng, J.-W.; Lee, S.-K.; Akowuah, G.A.; Tan, C.S.; Chew, Y.-L. Formulation of Botanical Shampoo Infused with Standardised Mangosteen Peel Extract for Healthy Hair and Scalp. *Cosmetics* **2023**, *10*, 109. https://doi.org/10.3390/cosmetics10040109

Academic Editor: Adeyemi Oladapo Aremu

Received: 20 June 2023
Revised: 6 July 2023
Accepted: 12 July 2023
Published: 25 July 2023

1. Introduction

Hair has emerged as a symbol of attractiveness, and it is becoming a sign of beauty in contemporary society. Consequently, there is an increasing demand for hair and scalp

care products, such as hair shampoo, conditioner, serum, creams, masks, and more. Shampooing has gained significant importance as a regular practice in our daily lives. Hair shampoos serve the dual purpose of maintaining personal hygiene and cleansing leading to the promotion of beautiful hair and a healthy scalp [1]. More consumers prefer to use plant-based hair shampoos, which are natural and sustainable. Godeto et al. [2] recently reported that formulated herbal shampoos could also be on par with commercially available shampoos. The shampoo formulations were pleasant, producing good foaming and cleansing abilities. The pH values were within an acceptable range, they had low surface tensions, and acceptable viscosities and shearing properties. They also possessed good antimicrobial properties.

Plant-based shampoos are made using natural and naturally derived ingredients as bioactives and excipients. The plant ingredients could exhibit prominent properties such as antioxidant, antimicrobial, anti-inflammatory, moisturising, and cleansing effects. These properties could nourish the scalp, relieve dryness, protect the hair and scalp from oxidative damage, reduce breakage, prevent microbial growth on the scalp, and many more. It is believed that healthy hair strands and scalp could promote hair growth, reducing strand breakage and hair fall [1].

Garcinia mangostana (Mangosteen) peel is discarded as waste in the agricultural industry. The peel is rich in phytochemicals including anthocyanins, xanthones, phenolic acids, flavonoids, and flavonols [1,3]. These phytochemicals could exhibit numerous pharmacological activities related to the maintenance of healthy hair and scalp, namely antioxidant, anti-inflammatory, and antimicrobial activities [1,4]. Recently, the selected biological properties exhibited by the peel and its α-mangostin in hair care were reviewed [1]. Its antimicrobial activity could inhibit dandruff fungal growth; its antioxidants could protect the hair strand from oxidation and degradation; its anti-inflammatory activity could inhibit hair folliculitis; its hair follicle rejuvenation could encourage new hair growth; and its upregulation of the melanin synthesis in hair dermal papilla cells could promote hair tanning [1]. The objectives of this study were to formulate the hair shampoo with mangosteen peel extract (standardised with 10% α-mangostin), evaluate its physicochemical properties, determine the stability of the formula, and evaluate its antimicrobial and antioxidant activities.

2. Materials and Methods

2.1. Raw Materials

The following ingredients were obtained from Personal Formula Resources (M) Sdn Bhd, Malaysia, for the formulation of the hair shampoo: sodium lauryl sarcosinate, sodium cocoyl glutamate, disodium cocoamphodiacetate, cocamidopropyl betaine, lauryl glucoside, guar hydroxypropyl trimonium chloride (GHTC), aloe vera extract, rosemary extract, and rose essential oil. Ethylenediaminetetraacetic acid (EDTA), glycerin, allantoin, provitamin B-5, tween/polysorbate 80, and citric acid monoanhydrous were purchased from YKL Multi Sdn. Bhd., Malaysia. Menthol, hydrolysed silk protein, and Verstatil SL were supplied by Take It Global Sdn Bhd, Malaysia. Hydrolysed collagen was purchased from Iko Nature Sdn Bhd. Lastly, standardised mangosteen peel extract (10% α-mangostin) was procured from Chemtron Biotechnology, Malaysia. 2,2-diphenyl-1-picrylhydrazyl (DPPH), ketoconazole, ampicillin and cholesterol were obtained from Sigma–Aldrich, United States of America.

2.2. Physical Compatibility Test

Physical compatibility between the mangosteen peel extract and excipients was determined during the pre-formulation of shampoo. Mangosteen peel extract was blended with each excipient in an equal amount (1:1 ratio). The mixtures were then transferred into universal bottles, respectively. The resulting mixtures were divided equally into two sets: control to be stored at room temperature, and test samples to be placed in the stability chamber at 40 °C and 75% relative humidity (RH). The test samples were evaluated and

compared with the control samples to identify physical changes, such as colour, odour, texture, homogeneity, and phase separation weekly for a period of four weeks [5,6].

2.3. Formulation of Mangosteen Shampoo

The shampoo base was formed by mixing the following ingredients as listed below. Four formulations were prepared using the ingredients as follows.

- Citric acid (20%)
- Deionised Water
- Sodium lauroyl sarcosinate
- Cocamidopropyl betaine
- Sodium cocoyl glutamate
- Disodium cocoamphodiacetate
- Lauryl glucoside
- Glycerine
- Polysorbate 80
- Guar hydroxypropyl trimonium chloride (GHTC)
- Hydrolysed collagen
- Hydrolysed silk protein
- Verstatil SL (ECOCERT)
- Rosa Damascena (Rose) Flower Oil
- D-panthenol
- Grinded mangosteen pericarp powder (with standardised 10% α-mangostin)
- *Aloe barbadensis* leaf juice
- *Rosmarinus officinalis* (Rosemary) extract
- Allantoin
- Menthol
- Ethylene diaminetetraacetic acid tetrasodium salt (EDTA)

Three mangosteen peel hair shampoo formulas were prepared in this study (F1, F2 and F3). Hair shampoo base (F4), and 2 commercial hair shampoos (MS1 and MS2) were used for comparison (Table 1).

Table 1. Formulated hair shampoo samples and the controls.

Sample	Shampoo Formulation
F1	Shampoo containing 0.125% mangosteen peel extract
F2	Shampoo containing 0.25% mangosteen peel extract
F3	Shampoo containing 0.50% mangosteen peel extract
F4	Shampoo base, without mangosteen peel extract
MS1	Herbal Essence Natural Shampoo
MS2	Good Virtues Co Anti-Dandruff Care shampoo

2.4. Physicochemical Evaluation of Shampoo Formulation

The shampoo formulations were evaluated for their visual inspection, and physicochemical properties such as pH, surface tension, total solid contents, foaming ability and stability, wetting time, dirt dispersion, detergency ability, and stability. The physicochemical measurements were repeated in triplicates, and the mean and standard deviation (SD) were determined. The results were compared with two marketed shampoos (Herbal Essence Natural Shampoo and Good Virtues Co Anti-Dandruff Care Shampoo).

2.4.1. Physical Appearance/Visual Inspection

The prepared shampoo was evaluated based on its colour, clarity, texture, and odour at room temperature [7,8].

2.4.2. pH Measurement

The pH of 10% v/v shampoo solution in distilled water was determined using a pH meter (pH/conductivity/TDS Meter, CyberScan PC 300, EUTECH instruments, Singapore) at room temperature [9]. The measurements were repeated in triplicates and mean values and standard deviation (SD) were used for analysis [10]. The pH meter was calibrated using standard buffers solution of pH 4, 7, and 10 before each use.

2.4.3. Surface Tension

The surface tension of the diluted shampoos (10% w/v in distilled water) was evaluated using tensiometer (Sigma 700, attention, Finland) [11]. The measurements were repeated in triplicates and mean values and standard deviation (SD) were calculated.

2.4.4. Percentage of Solid Contents

The solid content percentage was determined according to the method described by AlQuadeib et al. [7] and Kinjuit et al. [12]. In brief, a clean dry evaporating dish was weighed, and 4 g of shampoo samples were added. The dish and the sample were weighed again, and the exact weight of the sample was determined. The samples were then oven-dried at 105 °C for 16 h or until a constant dry weight was achieved. The dried samples were cooled in a desiccator and were then weighed again to determine the total solid content using the following formula [13,14]. The measurements were repeated in triplicates and mean values and standard deviation (SD) were determined:

$$\text{Solid contents } (\%) = \frac{Wo - W1}{Wo} \times 100$$

where Wo is the initial weight of the shampoo sample and $W1$ is the weight of solid contents.

2.4.5. Wetting Ability

The wetting ability of the shampoo formulation was assessed using the combined canvas disc method adopted from Sbhatu et al. [8] and Mainkar et al. [15]. A canvas paper was cut into 1-inch diameter with a mean weight of 0.44 g. A total of 400 mL of 1% v/v shampoo solution was prepared in a 500 mL graduated measuring cylinder. The smooth surface of canvas disc was then positioned on top of the surface of 1% v/v shampoo solution. The duration required for the disc to begin sinking was recorded as the wetting time. The measurements were repeated in triplicates and mean values and standard deviation (SD) were determined.

2.4.6. Foaming Ability and Stability

The cylinder shake method was used to measure the foaming ability of the shampoo [13]. An amount of 50 mL of the 1% (v/v) formulated shampoo solution was poured into a 250 mL graduated cylinder. Then, the graduated cylinder was covered with stopper and was shaken 10 times. The total volume of foam produced after 1 min of shaking was recorded and denoted as foam ability. Foam stability was determined by recording the foam volume after 1 min and after 4 min of shaking. The measurements were repeated in triplicates and mean values and standard deviation (SD) were calculated.

2.4.7. Dirt Dispersion Test

Dirt dispersion test was performed as described by Al Badi et al. [13]. Briefly, a large test tube was filled with 10 mL of distilled water, as were two drops of shampoo. One drop of Indian ink was then added, and the test tube was stoppered and shaken for 10 times. The amount of ink staining in the foam was observed and categorised as none, light, moderate or heavy. The measurements were repeated in triplicates and mean values and standard deviation (SD) were determined.

2.4.8. Detergency Test

The detergency evaluation was performed using a modified Thompson method [9,15,16]. In brief, a sample of hair was cleansed with solution containing 5% sodium lauryl sulfate (SLS), and then dried, cut into 10 inches, and separated into 3 g weight groups.

The following composition was used to prepare the artificial sebum formula [15]: olive oil (20%), coconut oil (15%), stearic acid (15%), oleic acid (15%), paraffin wax (15%), and cholesterol (20%). These hair samples were then immersed in a solution of n-hexane (Full-time, China) containing 10% artificial sebum and agitated for 15 min at room temperature. After removing the samples, the solvent was evaporated at room temperature, and the amount of sebum present was measured. The hair sample was then split into two equal portions. The first portion was subjected to washing with 0.1 mL of the 10% test shampoo using the finger method [16]. The other portion served as the control without shampoo treatment. The washed samples were then oven-dried at 60 °C for 4 h to ensure complete drying [15]. The remaining sebum residing in the hair samples was extracted using 20 mL of n-hexane. The n-hexane was evaporated and the extracted sebum from both portions was quantified based on its weight. The detergency percentage was determined using the following equation [9]:

$$\text{Detergency percentage (\%)} = 100\ (1 - T/C)$$

where T represents the weight of sebum in the experimental hair portion and C is the weight of sebum in the control hair portion.

2.5. In Vitro Assays Evaluation

The bioactivities of the formulated hair shampoo, namely its antimicrobial and antioxidant activities, were evaluated.

2.5.1. Antimicrobial Activity

Two microorganisms were selected for the antimicrobial evaluation: *Malassezia furfur* ATCC 14521 and *Staphylococcus aureus* ATCC 25923. *M. furfur* was grown in the Sabouraud Dextrose agar (SDA) supplemented with 10% olive oil while *S. aureus* was maintained in nutrient agar (NA).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the samples, including mangosteen peel extract, mangosteen shampoo (F1–F3), shampoo base (F4), marketed natural shampoo (MS1) and anti-dandruff shampoo (MS2) were assessed using microbroth dilution method adopted from Chew et al. [17].

In brief, 1.6 g of each sample was first dissolved in 1 mL dimethyl sulfoxide (DMSO) and water to obtain 1.6 g/mL sample. The sample was then serially diluted two-fold with broth. A total of 100 µL of the resulting dilutions was then added into 96-well microtiter plates to yield a final concentration of 0.039–5 mg/mL. An amount of 100 µL of microbes suspension (1×10^7 cfu/mL *S. aureus* and 1×10^5 cfu/mL *M. furfur*) was added into the well. The positive controls used were ketoconazole for *M. furfur*, and ampicillin for *S. aureus*. A total of 0.25% DMSO was used as the negative control. The microtiter plate was incubated for 24–48 h. MIC was recorded as the lowest concentration which completely inhibited microbial growth. MBC was determined when no visible growth was seen on the first streak on the agar of the clear wells. The test was repeated three times.

2.5.2. Antioxidant Activity

The antioxidant activity of the mangosteen peel extracts, and mangosteen-peel-incorporated shampoo, was determined using the method of Chew et al. [18]. The antioxidant activity of the samples was determined by measuring their capability to scavenge 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and was compared with a control, L-ascorbic acid. The concentration required to scavenge 50% of free radical (IC_{50}) and antioxidant

capacity equivalent to ascorbic acid (AEAC) was calculated from spectrophotometric measures and expressed in mg/mL as follows [18,19]:

$$\text{AEAC (mg AA/100 g)} = IC_{50}\text{ (ascorbate)}/IC_{50}\text{(sample)} \times 10^{5.}$$

IC_{50} (ascorbate) of 0.00567 mg/mL was used in AEAC calculation.

2.6. Stability Study

The hair shampoo formulation with best overall satisfying of antimicrobial activity and physicochemical characteristics was selected and subjected to an accelerated stability testing with conditions of 45 °C and 75% RH in a stability chamber (Capromax M200, Malaysia) [7,20]. The selected shampoo formulation was filled into amber glass containers. The accelerated stability test was conducted to predict the product's shelf life, physical appearance, and physicochemical stability. The organoleptic and physiochemical characteristics were inspected at intervals of one month for a period of two months [20]. The mechanical stability of the shampoo formulation was evaluated using a centrifugation test. The hair shampoo was centrifuged at 2400 rpm for 3 min [11]. The phase separation was observed after the centrifugation.

2.7. Statistical Analysis

The collected data was processed using SPSS v.20. All tests were carried out in triplicates and data were presented as mean ± standard deviation (SD) (n = 3) [13]. A significant difference $p < 0.05$ was ascertained by the one-way ANOVA factor, followed by Tukey's HSD test to detect significant differences between different formulations [21].

3. Results and Discussion

3.1. Physical Compatibility Test of Pre-Formulation

To maintain the properties and final product of the hair shampoo, the compatibility of the active ingredients and excipients was evaluated. This is to ensure that the final product produced meets the aesthetic function, and maintains the physical and chemical properties within the preparation [22]. The physical compatibility study is essential to determine any incompatibility between the extract and excipients in the final formulation. The selection of suitable excipients in a formulation could produce desirable physical and chemical properties, avoid post-formulation degradation and hence ensure the stability of the active ingredients in the product formulation.

The physical compatibility test was performed by mixing the mangosteen peel extract with various excipients (1:1) used in the product formulation. No change in physical characteristics was noticed, in comparison with the controls (Table 2). This showed that the mangosteen peel extract is compatible and remained stable with the selected excipients.

Table 2. Physical compatibility of mangosteen peel extract with various excipients.

Combination of Ingredients	Duration (Weeks)			
	1	2	3	4
MG	NCC	NCC	NCC	NCC
MG + Sodium lauryl sarcosinate	NCC	NCC	NCC	NCC
MG + Sodium cocyl glutamate	NCC	NCC	NCC	NCC
MG + Disodium cocoamphodiacetate	NCC	NCC	NCC	NCC
MG + Cocoamidopropyl betaine	NCC	NCC	NCC	NCC
MG + Lauryl glucoside	NCC	NCC	NCC	NCC
MG + EDTA	NCC	NCC	NCC	NCC

Table 2. *Cont.*

Combination of Ingredients	Duration (Weeks)			
	1	**2**	**3**	**4**
MG + Tween 80	NCC	NCC	NCC	NCC
MG + GHTC	NCC	NCC	NCC	NCC
MG + Glycerin	NCC	NCC	NCC	NCC
MG + Menthol	NCC	NCC	NCC	NCC
MG + Allantoin	NCC	NCC	NCC	NCC
MG + Hydrolysed silk protein	NCC	NCC	NCC	NCC
MG + Hydrolysed collagen	NCC	NCC	NCC	NCC
MG + Aloe vera extract	NCC	NCC	NCC	NCC
MG + Rosemary extract	NCC	NCC	NCC	NCC
MG + Provitamin B5	NCC	NCC	NCC	NCC
MG + Rose oil	NCC	NCC	NCC	NCC
MG + Citric acid (20%)	NCC	NCC	NCC	NCC
MG + Verstatil SL	NCC	NCC	NCC	NCC

Abbreviations: NCC—no characteristics changes compared to control; MG—mangosteen peel extract.

3.2. Physicochemical Evaluation of Shampoo Formulation

The physicochemical properties of the formulated hair shampoos containing mangosteen peel extract (F1, F2, and F3), shampoo base (without mangosteen peel extract), and two commercially available hair shampoos (MS1 and MS2) were evaluated. The evaluation included assessments of colour, clarity, odour, texture, pH, percentage solid content, foam ability and stability, foam type, wetting time, dirt dispersion, and detergency ability.

All formulations displayed positive attributes in terms of colour, clarity, texture, and odour (Table 3). The acceptable pH of a hair shampoo is usually neutral or slightly acidic [5,10]. Shampoos with slightly acidic pH can prevent the swelling of the hair cuticle, tightening the hair follicles and making the hair strand silky and smooth [7]. The shampoo formulations F1, F2, F3, and F4 were pH-balanced (pH ranged between 5.5 and 5.9), which is the ideal pH in maintaining the isoelectric point of hair creatine [7,20]. Shampoos which are suitable for scalp treatment should have, approximately, a pH of 5.5 [23].

Table 3. Physicochemical evaluation of mangosteen peel hair shampoo formulations and marketed shampoos.

Physicochemical Parameters	Samples					
	F1	**F2**	**F3**	**F4**	**MS1**	**MS2**
Colour	Light Brown	Brown	Dark brown	Light Yellow	White	White
Clarity	Clear	Clear	Clear	Clear	Milky opaque	Milky opaque
Odour	Good	Good	Good	Good	Good	Good
Texture	Gel	Gel	Gel	Gel	Cream	Cream
pH (10% solution)	5.60 ± 0.01^{a}	5.56 ± 0.01^{b}	5.52 ± 0.01^{c}	5.69 ± 0.02^{d}	5.15 ± 0.00^{e}	5.62 ± 0.01^{f}
Percentage solid contents (%)	20.29 ± 0.08^{a}	21.93 ± 0.53^{b}	23.59 ± 0.08^{c}	19.11 ± 0.09^{d}	22.14 ± 0.13^{b}	14.91 ± 0.02^{e}
Foam ability	131.7 ± 9.50^{a}	$102.0 \pm 2.65^{b,c,d}$	75.67 ± 6.81^{e}	$98.33 \pm 6.35^{b,c,d}$	$93.00 \pm 2.65^{b,c,d}$	143.3 ± 4.16^{a}
Foam stability	Stable	Stable	Stable	Stable	Stable	Stable
Foam type	Small, dense	Small, dense	Small, very dense	Small, dense	Small, dense	Small, airy
Wetting time (seconds)	$15.53 \pm 0.88^{a,b,c}$	$16.46 \pm 0.60^{b,c,d}$	25.65 ± 0.76^{e}	$15.48 \pm 0.08^{b,c}$	61.10 ± 0.01^{f}	$17.65 \pm 0.19^{a,b,d}$
Dirt dispersion	None	None	None	None	None	None
Detergency ability (%)	$92.10 \pm 2.44^{a,c,d,e}$	85.84 ± 0.16^{b}	83.74 ± 0.85^{b}	$93.06 \pm 0.87^{a,c,e}$	$89.53 \pm 0.52^{a,d,e}$	$91.47 \pm 0.45^{a,c,d,e}$

Results were presented in mean ± SD (n = 3). Significant difference $p < 0.05$ by one-way ANOVA factor, followed by Tukey's HSD test to detect significant differences between different formulations. For each row, values followed by the same letter (a–f) are statistically insignificant ($p > 0.05$) as determined using ANOVA followed by Tukey's HSD test.

A good shampoo with easy application and rinse-off action would have 20–30% solid content [7,9,13]. Shampoo with solid content below 20% would be too watery and would be washed-off easily. Similarly, a shampoo with a solid content above 30% would contain

an excessive amount of solids, making it difficult to apply to the hair and rinse off [7,9,13]. This study showed that the solid content of the formulated hair shampoo (F1, F2, F3) was within the acceptable range. Therefore, the shampoos are expected to be easily applied and washed-off.

While foaming ability and foam stability may not necessarily affect the product's detergency, they do satisfy the consumer's psyche [23]. The present tested samples displayed small, dense, uniform and stable foams, and the foam characteristics were comparable to the commercial samples. The foaming ability and foam stability of samples were insignificant ($p > 0.05$). This showed that our formulated samples (F1, F2, F3 and F4) possessed satisfactory foaming ability and foam stability, and were comparable to the marketed products. The foaming performance could be attributed to the combined effect of surfactants used in product formulations, such as sodium lauroyl sarcosinate, sodium cocoyl glutamate, disodium cocoamphodiacetate, cocamidopropyl betaine, and lauryl glucoside. It is interesting to notice that the foaming effects of the combined naturally derived surfactants in the formulas was comparable to the marketed products using synthetic surfactants.

In the dirt dispersion test, hair shampoo with good cleansing efficacy and good performance should have ink remaining in the water portion. Shampoo solutions with ink concentrated in the foam are deemed to be of poor quality, because the dirt that stays in the foam is tough to wash away and can be re-deposited on the hair [7]. The present study showed that the tested samples exhibited good performance in the dirt dispersion test. The ink showed no distribution in the foam, and remained in the water layer only (Table 3).

A shampoo with good sebum removal capability can ensure that the product cleans the hair scalp effectively, removing the hair sebum and ensuring the scalp remains healthy. A hair shampoo formula with detergency ability above 80% is considered to have high detergency ability and sebum removal capability; 50–60% is moderate and below 50% is considered low [9]. It was noticed that all tested samples had high detergency ability. The formulated shampoo base, F4, had the highest sebum removal capability (93.04%). The mangosteen peel extract shampoos also exhibited a relatively good sebum removal effect (Table 3). The hair observed under the microscope showed that the shampoos could remove the sebum well, resulting in clean hair strands (Figure 1). The detergency ability of the tested formulated samples was comparable to the commercial products.

3.3. Antimicrobial Activity

An overgrowth of *Malassezia* fungus on the hair scalp can result in the issue of dandruff [24]. Antifungal activity was not noticed when the mangosteen peel extract (5 mg/mL) was tested. However, this study showed that the formulated hair shampoo (F1, F2, F3, and F4) could exhibit antimicrobial activity toward *M. furfur*. It is surprising to notice that the addition of mangosteen peel extract into F4 could enhance the inhibitory and fungicidal effect against *M. furfur*, up to 16-fold and 8-fold, respectively. F2 (0.25% mangosteen pericarp extract) exhibited the best inhibitory activity against *M. furfur* (MIC 0.039 mg/mL, MFC 0.156 mg/mL). This could be due to the synergistic action between the bioactive ingredients in the mangosteen peel extract and the excipients added in the formulation, that may have significantly enhanced the antifungal activity of the samples. The antifungal activity was more potent than the commercial products tested.

Angiolella et al. [25] reported that *Malassezia* sp. has a thick multilaminar cell wall that consists of chitin and lipids. The remarkable abundance of lipids (15–20% *w*/*w*) causes the cell surface to have an inherent hydrophobicity and to be resistant to antimicrobial agents. Although the extract alone did not exhibit potent antifungal activity, the formulation of various excipients in the final formula significantly enhanced the activity, which was about 2- to 16-fold stronger. The enhanced activity could be due to the synergistic effect between the bioactive ingredients with the excipients in the formulation, especially with the essential oil. Various studies have reported that α-mangostin and mangosteen peel extract could exhibit improved fungal toxic activities when they work collaboratively and synergistically with essential oils [26–29]. α-mangostin exerts its formidable influence by disrupting fungal

cell membranes [30], influencing their structural integrity. In parallel, the application of essential oils facilitates the establishment of a membrane potential across the cell wall, further compromising its stability. This concerted assault on the cell wall induces significant damage. Furthermore, essential oils demonstrate remarkable efficacy in degrading the mitochondrial membrane, resulting in the disruption of vital cellular processes such as adenosine triphosphate (ATP) assembly and the electron transport system pathway [31]. The joint activity could lower the concentrations required to inhibit and kill the fungus.

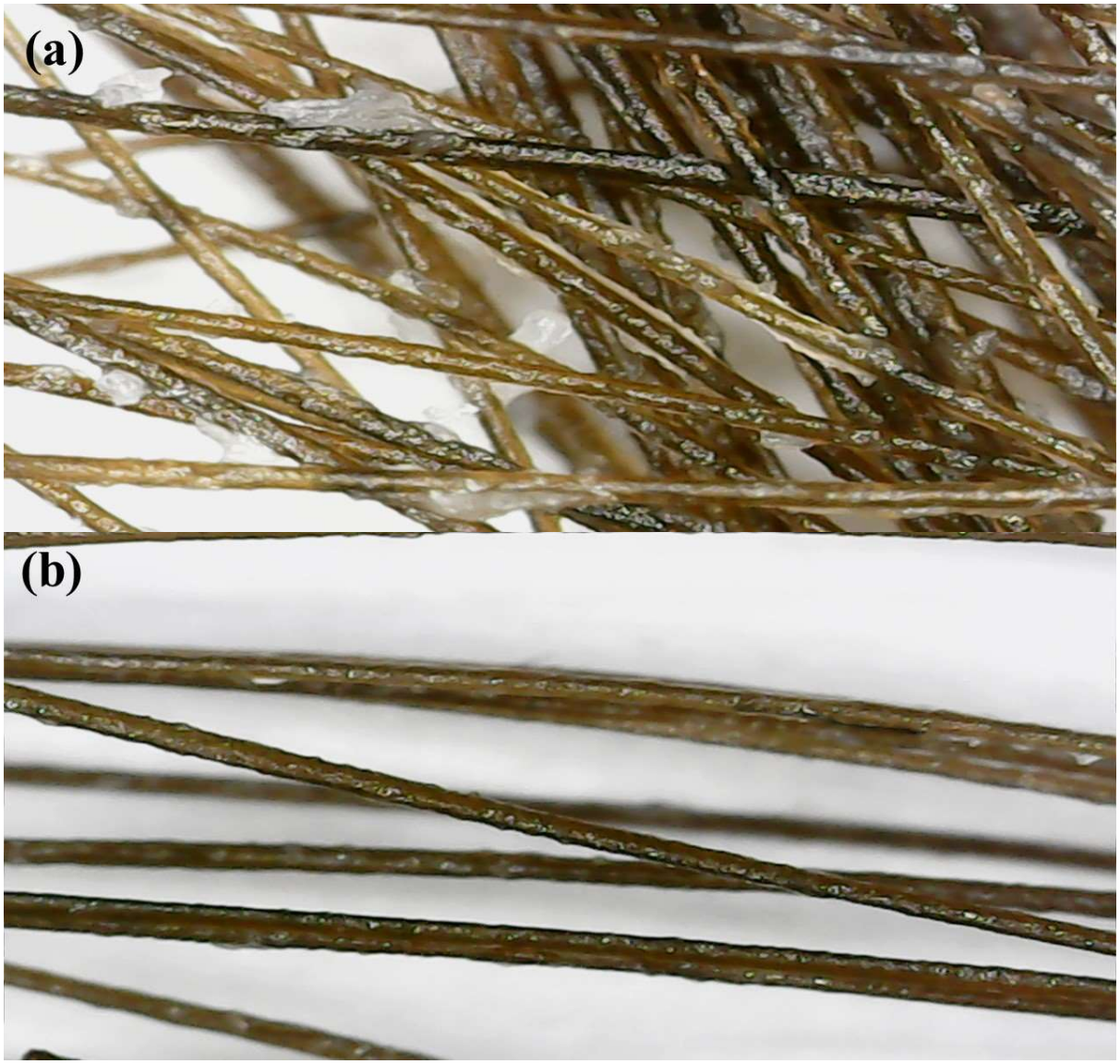

Figure 1. Hair samples examination in detergency test. Hair samples (**a**) covered with artificial sebum before washing with the shampoo formulation; (**b**) after only washing once with the F2.

S. aureus is a common microflora on healthy skin. However, an overgrowth of *S. aureus* has been positively correlated with the issue of dandruff on the scalp. It has been reported that the presence of dandruff is positively associated with the growth of *Staphylococcus* and *Propionibacterium* sp. [32]. This study showed that F2 exhibited the best antibacterial activity towards *S. aureus*, with MIC 2.5 mg/mL and MBC 5.00 mg/mL, respectively (Table 4). Although the activity was not as potent as MS2, it managed to control the overgrowth of *S. aureus* at higher concentrations, which also supported in overcoming the issue of dandruff on the scalp. A recent publication reported that α-mangostin could affect fatty acid and teichoic acid biosynthesis, and DNA replication and repair [1]. It could bind to

the lipid region of the membrane, resulting in an alteration in the membrane integrity, and translocation and leakage of the cell component. This would hence lead to rapid bactericidal activity in *S. aureus* [1].

Table 4. Antimicrobial activity of mangosteen peel hair shampoo formulations and marketed shampoos against *Malassezia furfur* and *Staphylococcus aureus*.

Samples	*Malassezia furfur*		*Staphylococcus aureus*	
	MIC (mg/mL)	**MFC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
F1	0.156	0.3125	2.50	>5.00
F2	0.039	0.156	2.50	5.00
F3	0.313	0.625	2.50	5.00
F4	0.625	1.25	5.00	>5.00
MS1	0.078	0.078	>5.00	>5.00
MS2	0.078	0.156	1.25	1.25
Mangosteen peel extract	>5.00	>5.00	5.00	5.00
Ketoconazole	6.25×10^{-5}	6.25×10^{-5}	n/a	n/a
Ampicillin	n/a	n/a	7.81×10^{-5}	7.81×10^{-5}

n/a: not applicable.

3.4. Antioxidant Activity

Sunlight could damage hair strands and result in fibres degradation. Ultra-violet rays (UV-A and UV-B) degrade the hair keratin and produce reactive oxygen species (ROS) [1,33]. Antioxidants in hair care products can disrupt the radical chain reaction, protect the hair and scalp against oxidative damage, and promote the hair repair mechanism. This will improve the integrity of hair fibres, inhibit lipid peroxidation and protein degradation, and keep the hair and scalp healthy [34].

The antioxidant activities of the shampoo samples were evaluated using DPPH free radical scavenging activity. All mangosteen peel extract shampoo samples (F1–F3) exhibited prominent free radical scavenging activities in a dose-dependent manner, in the IC_{50} 15–47 mg/mL and AEAC 12–38 mg AA/100 g samples. The present results show that the addition of standardised mangosteen peel extract significantly increased the free radical scavenging activity of shampoo base (F4), at least 2-fold (Table 5). The samples (F1–F3) exhibited stronger free radical scavenging activity than the marketed shampoos (MS1 and MS2).

Table 5. Free radical scavenging activity of mangosteen peel hair shampoo formulations and marketed shampoos.

Samples	IC_{50} (mg/mL)	AEAC (mg AA/100 g Sample)
F1	47.5 ± 1.18^{a}	12.0 ± 0.862^{a}
F2	21.9 ± 3.27^{b}	26.3 ± 4.06^{b}
F3	14.8 ± 0.928^{c}	38.4 ± 2.32^{c}
F4	$>100^{d}$	$>5.67^{d}$
MS1	$>100^{d}$	$>5.67^{d}$
MS2	47.1 ± 3.88^{a}	12.1 ± 0.984^{a}
Mangosteen peel extract	0.020 ± 0.001^{e}	28762 ± 2011^{e}

Results are mean ± SD ($n = 3$), Significant difference $p < 0.05$ by one-way ANOVA factor between three samples, followed by Tukey's HSD test to detect significant difference in between samples. For each column, values followed by the same letter (a–e) are statistically insignificant ($p > 0.05$) as determined using ANOVA and followed by Tukey's HSD test.

From the physicochemical properties, and antimicrobial and antioxidant activities evaluation, it was concluded that F2 exhibited the most desirable properties.

The continuous exposure of the hair to sunlight has the potential to diminish the tensile strength of individual hair strands [35]. This adverse effect arises from the activation

of a free radical chain reaction by ultraviolet rays, ultimately resulting in the oxidation of the protein and lipid constituents within the hair strands [1]. However, the incorporation of antioxidants in hair shampoo can effectively safeguard the hair by stabilising its proteins and amino acids, providing a protective shield against these damaging processes [1,36]. They inhibit the lipid fraction of the hair from oxidation, from keratin impairment, and maintain its natural properties [1,37]. In addition, the antioxidants could also improve the hair scalp condition, which is also directly associated with dandruff condition. The Malassezia pathogen on the scalp produces reactive oxygen species during its metabolism, leading to oxidative stress. This oxidative stress not only affects the scalp's condition, but also contributes to hair aging [1,37].

Besides mangosteen peel extract, several excipients added into the hair shampoo formulation would play an important role as antioxidants: EDTA, allantoin, menthol, provitamin B5, aloe vera and rosemary extracts, hydrolysed collagen, and rose essential oil [38–45]. They would also contribute to the antioxidant activity of the product, and hence protect the hair strands from oxidative damage and scavenge excessive free radicals on hair strands and scalp.

3.5. Stability Study

Product stability is essential to determine the quality of the products during storage. A stability study under accelerated conditions would detect the possible degradation after long-term storage [46].

Since F2 was the best formulation with the highest efficacy towards an anti-dandruff effect, the stability of this formula was tested for consistent organoleptic and physicochemical properties under accelerated conditions (45 °C, 75% RH) for 2 months. It was noticed that F2 was able to maintain its clarity, odour, colour, and texture under accelerated conditions. The physicochemical properties also remained stable (Table 6), except that there was a slight variation in the wetting time. The F2 formulation showed no phase separation after the centrifugation test. Therefore, the hair shampoo formula showed good stability, and was able to maintain its physicochemical properties during storage.

Table 6. Stability studies of shampoo formulation F2.

Physicochemical Parameters	After 1 Day	After 1 Month	After 2 Months
Physical appearance	Clear, brown	Clear, brown	Clear, brown
pH	5.57 ± 0.01	5.58 ± 0.01	5.60 ± 0.02
Surface tension (dynes/cm)	22.9 ± 0.12	22.8 ± 0.14	22.4 ± 0.05
Solid content (%)	21.4 ± 0.22	21.9 ± 0.10	22.5 ± 0.13
Foam ability and stability (mL)	102 ± 2.65 Stable	96.0 ± 5.29 Stable	103 ± 8.00 Stable
Wetting time (s)	16.5 ± 0.60 *	14.9 ± 0.10 *	13.3 ± 0.56 *
Dirt dispersion	None	None	None
Detergency ability (% sebum removal)	85.8 ± 0.16	85.9 ± 1.10	86.6 ± 1.15

Results were expressed in mean ± SD (n = 3). * Significant difference $p < 0.05$ by paired samples T-test to detect significant differences between 1 day, 1 month, and 2 months.

4. Conclusions

G. mangostana peel extract has promising bioactivities which are beneficial for hair and scalp condition. The hair shampoo formulations produced in this research used botanical and naturally derived ingredients. They possessed desirable physicochemical properties. This study showed that the F2 sample, with 0.25% mangosteen peel extract, exhibited the best antimicrobial activity and promising free radical scavenging activities. The enhanced antimicrobial activity of the F2 sample could be due to the collaborative and synergistic effects between the bioactive ingredients in mangosteen peel extract with the excipients

in the formulation, such as essential oil. The hair shampoo produced in this study was proven to have good cleansing properties. It could remove sebum effectively, exhibit a good cleansing property, be able to control dandruff, help to strengthen hair strands, and promote healthy hair and scalp. It was also proven to be stable under accelerated conditions. For further study, the efficacy of the formulated hair shampoo will be tested on patients with the issue of dandruff to further prove the efficacy of the product.

The formulation details of the product discussed in this research article are withheld due to a non-disclosure agreement (NDA) signed with a third party. As a result, specific information regarding the formulation in this publication is unable to be disclosed.

Author Contributions: Conceptualization, S.-H.S. and Y.-L.C.; investigation and formal analysis, S.-H.S.; methodology, S.-H.S. and Y.-L.C.; resources, Y.-L.C.; writing—review and editing, K.B.L., S.-K.L. (Siew-Keah Lee), J.-W.K., S.-K.L. (Sue-Kei Lee) and G.A.A.; revision and editing, C.S.T.; funding acquisition, C.S.T. and Y.-L.C. All authors have read and agreed to the published version of the manuscript.

Funding: The study was funded by the Malaysian Ministry of Higher Education through the Fundamental Research Grant Scheme (FRGS) (FRGS/1/2021/STG02/UCSI/02/1).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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