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"Study on the antioxidant capacity of the lotions with Aquilaria Baillonii Wood Oil using different surfactants and Clinical trial with scalp water"

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1. Introduction

The impact of the COVID-19 pandemic has led to the frequent use of face masks, increasing public awareness of facial skincare. Facial toners, which cleanse and hydrate the skin, continue to evolve to meet different consumer demands. As these products are not subject to seasonal limitations, they hold commercial potential. According to the *2022 Mid-Year Report on the Beauty Industry* by CMRI Beauty Marketing Research (August 2022), concerns about ageing and skin dryness have become key consumer focus areas due to the pandemic. In recent years, the use of natural essential oils has expanded significantly. However, most commercially available toners that contain essential oils primarily utilise them for fragrance enhancement rather than for their functional benefits.

Free radicals exhibit strong oxidative properties, and excessive levels in the human body can lead to diseases and ageing. The antioxidant compounds found in natural essential oils can effectively inhibit free radicals. Therefore, this study aims to evaluate the antioxidant potential of essential oils dispersed in water, assessing their feasibility for use in antioxidant facial toners in the market.

To ensure the effective dispersion of essential oils in water to create a facial toner formulation, three types of emulsifiers were employed: PEG-40 Hydrogenated Castor Oil, Polysorbate 80, and a combination of PEG-40 Hydrogenated Castor Oil/Octoxynol-10/Laureth-7/POE(20) Sorbitan Mono Laurate/Propylene Glycol. By incorporating different proportions of essential oils and encapsulating agents, the study aimed to determine the optimal conditions for maximising antioxidant efficacy, ensuring their effective application in water-based cosmetic products.

Agarwood (Aquilaria), a member of the Thymelaeaceae family, produces resin in response to injury as a self-repair mechanism. Academic studies on agarwood have identified its key marker components as 2-(2-phenylethyl) chromone derivatives and terpenes. Additionally, literature Cheng（2022） indicates that agarwood contains phenolic and flavonoid compounds with strong antioxidant properties, which contribute to the effective elimination of free radicals in the human body.

2. Materials and Methods

2.1 Experimental Materials

In this study, the antioxidant activity was measured by effectively dispersing essential oils in water to form a lotion state. The experiments include: 1. Stability test of appearance, centrifugation and temperature cycle, etc.; 2. Antioxidant capacity: DPPH free radicals ability, ABTS+ free radical scavenging ability, reducing ability and total phenolic content; 3. clinical trial of Scalp Water: 30 subjects used it once every two days. Press once on each of the 3 parts of the scalp and massage it with fingertips for 5 minutes. Instrument testing and photo recording were performed every month.

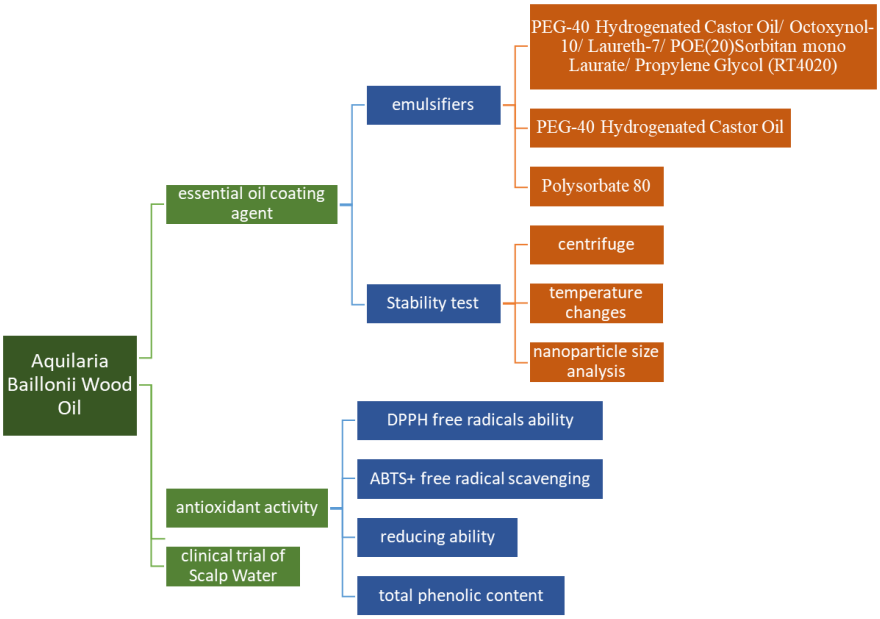

Figure 1. the raw map of experiments design

2.1.1 Instruments and Equipment

Multi-functional microplate analyser (BIO TEK ELISA READER), pH meter (EUTECH pH510), ultrasonic oscillator (DC300), vortex mixer (VORTEX-2 GENIE), centrifuge (Mini Centrifuge MINI-6K), water bath (DIGITAL WATER BATH SB-100, EYELA), electronic balance (METTLER TOLEDO), programmable constant temperature and humidity test chamber (LIAN SHEN). Cutometer® MPA 2/580 and Mexameter® MX18, Alluring Scalp/Skin Detection System (M-MK36AU) and Alldocube (iPlay 50 SE).

2.1.2 Chemicals

Table 1 Lists of Chemicals

Chemical name	Company	Chemical name	Company
Aquilaria grandiflora	SY-FORESTRY	Gallic acid monohydrate，98.0%	SIGMA
Aquilaria Baillonii Wood Oil	SY-FORESTRY	Hexamethylenetetramine，99%	SIGMA
Aquiariasinensis	SY-FORESTRY	Copper(II) sulfate pentahydrate，99%	SIGMA
2,2Diphenyl-1-picryhydrazyl(DPPH)，95%，MW=394.32	SIGMA	2,6-Bis(1,1-dimethylethyl)-4-methylphenol；BHT，99%	SIGMA
Ethanol，95%，MW=46.07	Acros Organics	Murexide，98%	SIGMA
Tris(hydroxymethyl)aminomethane，99.8%，MW=121.14	Acros Organics	Sodium carbonate，99.5%，MW=105.99	SIGMA
Hydrochloric acid，12N，MW=36.46	Acros Organics	Potassium persulfate，99.0%，MW=270.322	SIGMA
Trichloro acetic acid，99.0%，MW=163.38	SIGMA	Potassium chloride，99.5%	SIGMA
Potassium dihydrogen phosphate，KH_2PO_4，98.0%，MW=136.09	Acros Organics	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS)，98%，MW=548.68	SIGMA
Potassium hydrogen phosphate ; K_2HPO_4，98%，MW=174.2	Acros Organics	Folin& Ciocalteu's phenol reagent，2.0N，MW=94.11124	SIGMA
Potassium hexacyanoferrate(III) ; $K_3Fe(CN)_6$，99.98%，MW=329.24	SIGMA	Propylene Glycol & Diazolidinyl Urea & Methylparaben & Propylparaben	chengyi
Iron(III) chloride，97%，MW=270.29	SIGMA	PEG-40 Hydrogenated Castor Oil(PEG-40)	SIGMA
Polysorbate 80(Tween 80)	SIGMA	Ethylenediaminetetraacetic acid，98%	chengyi
PEG-40 Hydrogenated Castor Oil/ Octoxynol-10/ Laureth-7/ POE(20)Sorbitan mono Laurate/ Propylene Glycol (RT4020)			THB

2.2 Methods

2.2.1 Preparation of Toner Samples

A total of 1 gram of essential oil and surfactant was used, starting with 0.1 grams of essential oil and 0.9 grams of surfactant. Then, 49 grams of water was added, followed by 0.5 grams of compound preservative, which was thoroughly mixed. If no layering or turbidity was observed visually, the sample was subjected to centrifugation and stress testing.

Table 2 Table 2: Sample Codes Used: original text for sample compositions and pH values.

Essential oil：surfactant	**Number**	**pH value**
Aquiariasinensis：RT4020=0.1：0.9(1.98mg/mL)	A1-1	6.88

Aquiariasinensis：RT4020=0.2：0.8(3.96mg/mL)	A1-2	6.8
Aquiariasinensis：RT4020=0.3：0.7(5.94mg/mL)	A1-3	6.74
Aquiariasinensis：RT4020=0.4：0.6(7.92mg/mL)	A1-4	6.56
Aquiariasinensis：PEG-40=0.1：0.9(1.98mg/mL)	A2-1	6.9
Aquilaria grandiflora：PEG-40=0.1：0.9(1.98mg/mL)	B1-1	7.04
Aquilaria grandiflora：PEG-40=0.2：0.8(3.96mg/mL)	B1-2	6.85
Aquilaria grandiflora：PEG-40=0.3：0.7(5.94mg/mL)	B1-3	6.74
Aquilaria Baillonii Wood Oil：RT4020=0.1：0.9(1.98mg/mL)	C1-1	7.31
Aquilaria Baillonii Wood Oil：PEG-40=0.1：0.9(1.98mg/mL)	C2-1	7.37

2.2.2 Appearance Observation

The clarity and transparency of the samples were observed with the naked eye.

2.2.3 Centrifugation Test

Based on the modified method of Chou（2020）, the samples were centrifuged at 6000 rpm for three minutes, and any layering was observed with the naked eye.

2.2.4 Temperature change Testing

The samples were placed in a temperature cycling chamber with temperature settings of 50°C, 25°C, 10°C, and 25°C, each held for eight hours per cycle (24 hours per full cycle). The samples were observed weekly for five weeks to check for layering or turbidity.

2.2.5 Determination of DPPH Radical Scavenging Activity

Following the modified method of Peng（2017）, a 250 μM DPPH ethanol solution and 100 mM Tris-HCl buffer solution were prepared. A 100 μL sample was mixed with 400 μL of Tris-HCl buffer and 500 μL of DPPH ethanol solution, then shaken and left to react in the dark for 20 minutes. A 200 μL aliquot was transferred to a 96-well plate in triplicate, and its absorbance at 517 nm was measured using an ELISA reader. A standard curve was prepared using BHT ethanol solutions at concentrations of 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL.

2.2.6 Determination of $ABTS^{+}$ Radical Scavenging Activity

Using a modified method of Peng（2017）, a 7 mM ABTS aqueous solution was mixed with a 2.45 mM potassium persulphate solution and left to react for 16 hours. The solution was then diluted with ethanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. A 70 μL sample was mixed with 630 μL of diluted ABTS solution, shaken, and left to react in the dark for 10 minutes. A 200 μL aliquot was transferred to a 96-well plate in triplicate, and absorbance at 734 nm was measured. A standard curve was created using BHT ethanol solutions at varying concentrations.

2.2.7 Determination of Total Polyphenol Content

Following a modified method of Parsaei（2013）, 7.5% sodium carbonate and 10% Folin reagent solutions were prepared. A 100 µL sample was mixed with 500 µL of 10% Folin reagent, left in the dark for five minutes, then mixed with 400 µL of 7.5% sodium carbonate solution. After 30 minutes in the dark, 200 µL was transferred to a 96-well plate in triplicate, and absorbance at 765 nm was measured. A standard curve was prepared using gallic acid at concentrations of 0, 0.05, 0.1, 0.2, and 0.5 mg/mL.

2.2.8 Determination of Reducing Power

A modified method of Chiou et al. [8] was followed, using potassium phosphate buffer, potassium ferricyanide solution, TCA, and iron(III) chloride solution. Absorbance at 700 nm was measured, and a standard curve was constructed using BHT ethanol solutions.

2.2.9 clinical trial of Scalp Water

After 30 subjects used the natural scalp care group, the research tools included the test items of the scalp detector and the three-day questionnaire of the experience sampling method. The subjects used it and collected data simultaneously, and then the efficacy evaluation and analysis after the subjects used it at different time points were carried out.

Table 3 3-Day Survey Version_ Analyzing the Relationship Between Consumer Usage and Purchase Behavior

No.	Question/Coding	1	2	3/4
1	Code			
2	Did you wash your hair today?	Yes	No	
3	How many pumps of shampoo did you use?	1 pump	2 pumps	3 pumps or more
4	How many times did you rinse your hair with water today?	Once	Twice	Three times
5	How long did you rinse your hair each time?	Less than 1 minute	1–3 minutes	3–5 minutes
6	How happy did you feel after washing your hair?	Unhappy	Neutral	Happy
7	How anxious did you feel after washing your hair?	Not anxious	Neutral	Anxious
8	How much do you care about scalp health?	Do not care	Care	Care a lot
9	After blow-drying, did your scalp feel refreshed?	Felt hot and stuffy	No special feeling	Refreshing
10	After blow-drying, did your hair feel frizzy?	Not frizzy	No special feeling	Slightly frizzy
11	How did you perceive your hair loss during washing?	No change	Less hair loss	More hair loss
12	Did you observe any change in dandruff?	No change	Less dandruff	More dandruff
13	Did the frequency of scalp scratching change?	Scratched scalp 3+ times/day	Scratched 1–2 times/day	Scratched occasionally
14	Did you notice a change in the amount of white hair?	No change	Less white hair	More white hair
15	Did you notice any change in hair volume?	Felt like decreased	No noticeable difference	Felt like increased

16	Did you notice a change in the amount of black hair?	Seems decreased	No noticeable change	Seems increased

3. Results

This section may be organized into subheadings and should offer a clear and concise description of the experimental results, their interpretation, and the conclusions that can be derived from them

3.1 Stability test

3.1.1 Appearance

The cosmetic lotions produced using three types of agarwood essential oils encapsulated with different surfactants appeared transparent and free from turbidity upon visual inspection. The cosmetic lotions made from juniper essential oil and different surfactants were also visually inspected. Except for the sample containing Tween-80, which appeared more turbid, all other samples were clear and transparent. (Figure 2)

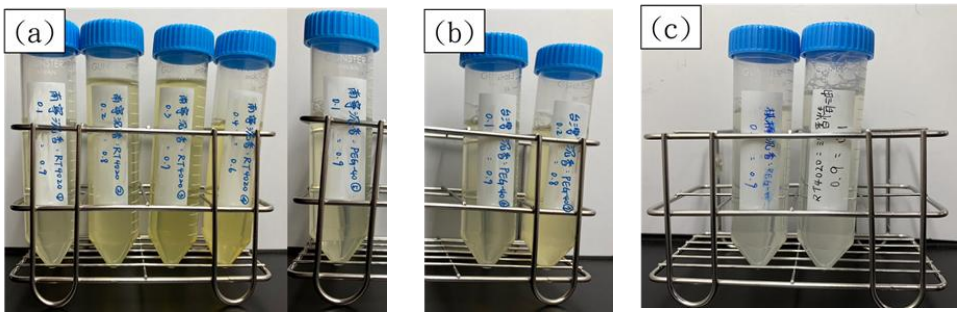

Figure 2. Agarwood samples are transparent and without turbidity when observed with naked eyes. (a) Nanning agarwood essential oil with RT4020 and PEG-40 added; (b) Taiwan agarwood essential oil with PEG-40 added; (c) Samples of agarwood with PEG-40 and RT4020 added.

3.1.2 Centrifugation Test

The samples were subjected to centrifugation at 6000 rpm for three minutes. The cosmetic lotions formulated with three types of agarwood essential oils and different surfactants, including A1-1, A1-2, A1-3, A1-4, A2-1, B1-1, B1-2, B1-3, C1-1, and C2-1, showed no phase separation after centrifugation.

3.1.3 Temperature change

The samples were placed in a temperature cycling oven and observed for five weeks. Visual inspection revealed that B1-3 became turbid and degraded, leading to their exclusion from further analysis. Other samples remained stable without degradation. (Figure 3)

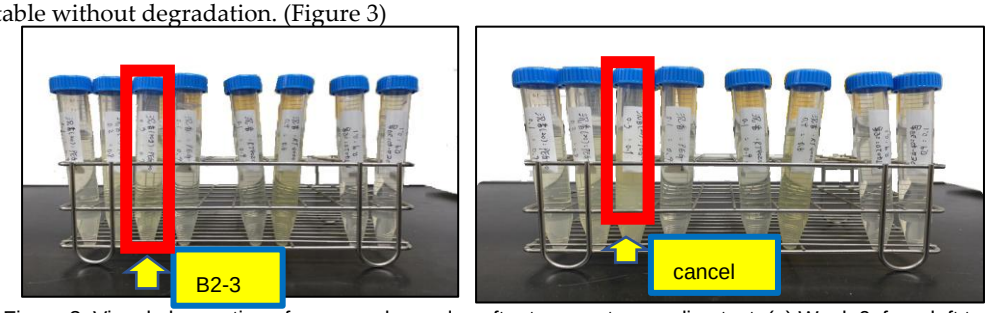

Figure 3. Visual observation of agarwood samples after temperature cycling test: (a) Week 0, from left to right: B2-1, B2-2, B2-3, A2-1, A1-1, A1-4, C1-1, C2-1; (b) Week 5, from left to right: B2-1, B2-2, B2-3, A2-1, A1-1, A1-4, C1-1, C2-1

3.1.4 nanoparticle size

The nanoparticle size measurement of agarwood samples showed that the average particle size of the stable ratio was around 10nm. (Figure 3)

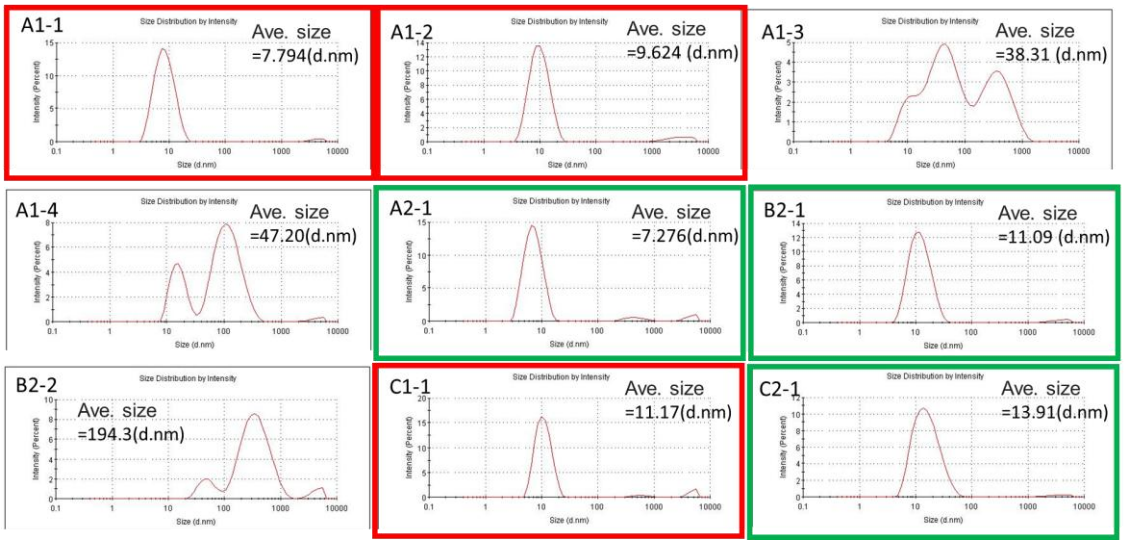

Figure3. nanoparticle size analysis of three kinds' agarwood sample lotions

3.2 antioxidant activity

3.2.1 DPPH Free Radical Scavenging Activity

Following the stability tests, the most stable ratios of essential oils and surfactants were determined, and the DPPH free radical scavenging rate was measured. The linear regression equation for the BHT standard DPPH free radical scavenging ability was y = 0.4188x + 4.3485, with an R^2 value of 0.997. The results showed that C1-1, the lotion containing *Aquilaria Baillonii Wood Oil* and RT4020, exhibited the highest DPPH free radical scavenging ability of 32.207% at a concentration of 1.98 mg/mL, while the others ranged from 5.280% to 23.589%. (Figure 4)

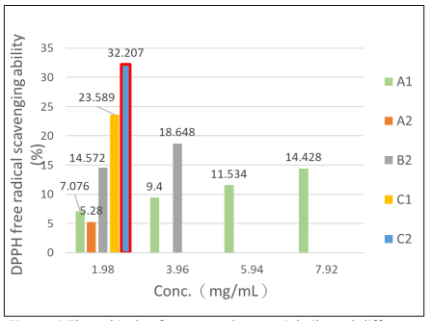

Figure 4 Three kinds of agarwood essential oils and different surfactants are used to make lotions DPPH free radical scavenging rate

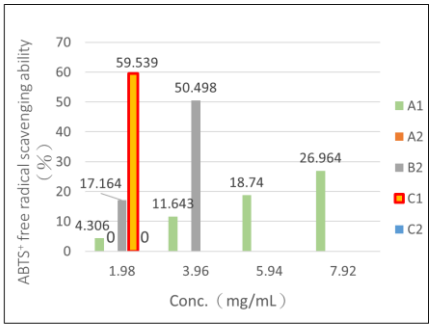

Figure 5 Three kinds of agarwood essential oils and different surfactants are used to make lotions ABTS⁺ free radical scavenging rate

3.2.2 ABTS+ Free Radical Scavenging Rate

Following the stability tests, the most stable ratios of essential oils and surfactants were determined, and the ABTS+ free radical scavenging rate was measured. The linear regression

equation for the BHT standard ABTS+ free radical scavenging ability was y = 0.8683x + 12.367, with an R^2 value of 0.9955. The results showed that C2-1, the lotion containing *Aquilaria Baillonii Wood Oil* and PEG-40, exhibited the highest ABTS+ free radical scavenging ability at 59.539%. (Figure 5)

3.2.3 Total Polyphenol Content Measurement

Following the stability tests, the most stable ratios of essential oils and surfactants were determined, and the total polyphenol content was measured. The linear regression equation for the gallic acid standard total polyphenol content was y = 0.0055x + 0.1185, with an R^2 value of 0.9975. The highest total polyphenol content was found in A2-1 (2.032), which was a lotion containing *Aquilaria crassna* agarwood oil and PEG-40. The relative concentration to the gallic acid standard was 243.364 μg/mL. (Figure 6)

3.2.4 Reducing Power Measurement

Following previous stability tests, the most stable ratios of essential oils and surfactants were determined. The linear regression equation for the BHT standard reducing power was y = 0.4434x - 2.576, with an R^2 value of 0.9996. The reducing power of the agarwood-based lotions ranged from 1.056 to 5.739, with no significant improvement observed. (Figure 7)

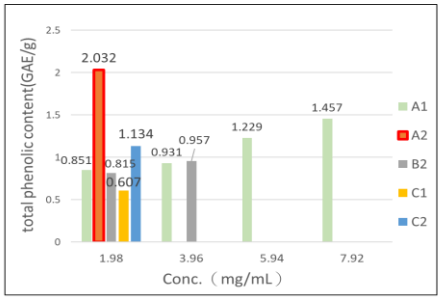

Figure 6 Three kinds of agarwood essential oils and different surfactants are used to make lotions total phenolic content

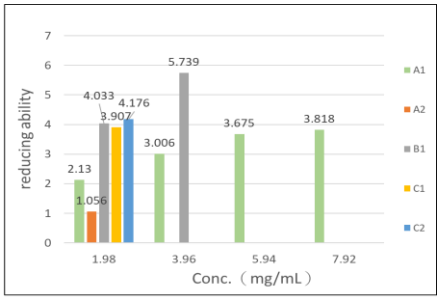

Figure 7 Three kinds of agarwood essential oils and different surfactants are used to make lotions reducing ability

3.3 clinical trial of Scalp Water

3.3.1 3-Day Survey Version

The average of question number 14 in Figure 8 moves from a time point above 2.0 to below 2.0, which means that the research subjects felt that their gray hair became less in the beginning. Later, the slope of the dotted line approaches 0, which is quite gentle. This may be because the hair growth period is long, and the initial changes are easy to see, but in the later stage, it is easier to notice the changes by oneself. The main overall trend of the chart in Question No. 15: The bar chart shows the changes in the subjects' hair volume over time, with the average number falling between 2.0 and 3.0, which means that the subjects' hair volume has changed from feeling no change to feeling it has increased, which means that the product has increased the strength of the hair and reduced the chance of hair loss. The main overall trend of the chart in Question No. 16: The bar chart shows the change in the number of black hairs of the research subjects over time. The average number is above 2.0 and below 3.0, which means that the research subjects have never felt an increase in the amount of black hair. A positive impact means that the product increases the melanin in the hair.From the Spearman correlation analysis in Table, question number sixteen and fourteen have a significant correlation <0.01, and the negative correlation coefficient value is -0.56, which is greater

than 0.3, which is a moderate correlation, indicating that the amount of black hair and white hair generated is inversely proportional; question number sixteen and fifteen have a significant correlation <0.01, and the correlation coefficient value of 0.369 is a low correlation. The amount of hair among the three is positively correlated with black hair and negatively correlated with white hair. This may be because the hair growth period is relatively long, 3 to 5 years, and the study only observed 3 months. However, the trend after using the product is that the amount of black hair and hair will increase. (Figure 8)

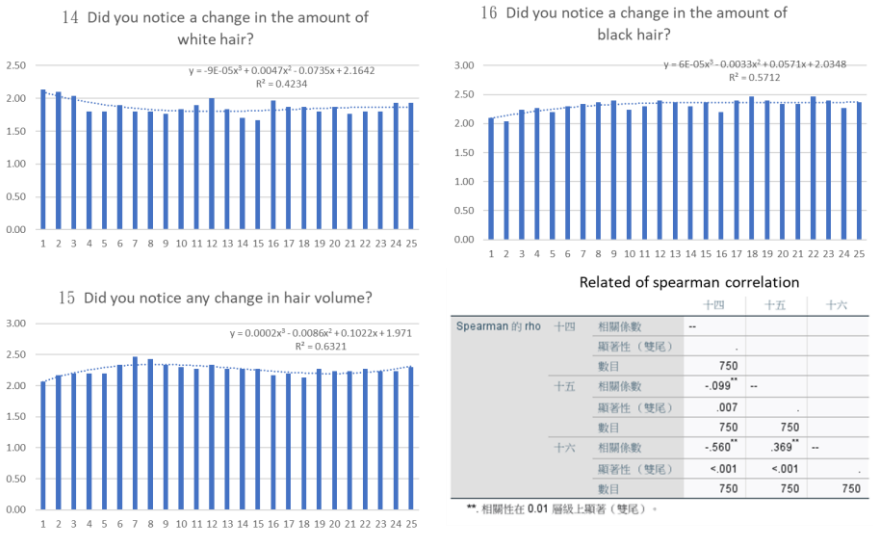

Figure 8 Questionnaire number 14-16 of scalp water and the results of spearman correlation

3.3.2 Result of Instrument

In the skin patch test, the red pigment values and photographic records of 30 subjects on day 0 and day 1 were all red, swollen and inflamed, which means that the irritation of the product is low and will not cause allergies, so continue to experience the product.

In the scalp test, from the reference point of the 0th month before the experiment and the return test in the 1st, 2nd and 3rd months after use, the research subjects were completed according to the scheduled time, and the test results in the sebum, hair volume and hair diameter part grew over time, and there was no change in the sensitive and pore parts, and the changes in dandruff gradually decreased, and remained at the lowest value of the instrument.(Figure 9/10/11)

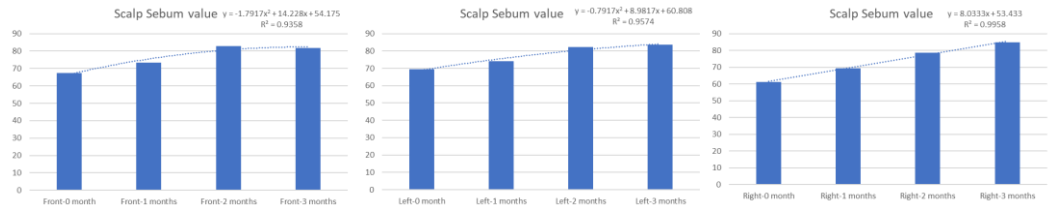

Figure 9 Changes in Scalp sebum results for 30 subjects: front, left and right

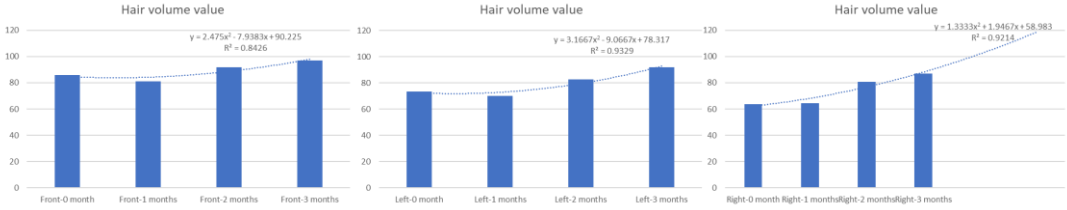

Figure 10 Changes in Hair volume value results for 30 subjects: front, left and right

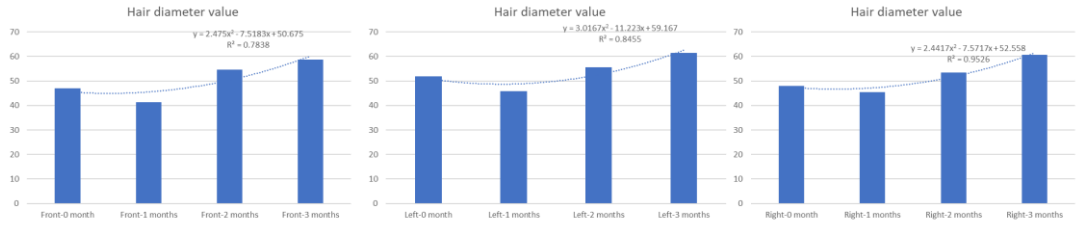

Figure 11 Changes in Hair diameter value results for 30 subjects: front, left and right

4. Discussion

The usable agarwood samples obtained after stability testing are A1-1, A1-2, A1-3, A1-4, A2-1, B1-1, B1-2, C1-1, and C1-2. According to the results, among all the agarwood samples, C1-2 showed the best DPPH free radical scavenging ability of 32.207%; C1-1 showed the best ABTS+ free radical scavenging rate of 59.539%; A2-1 measured the highest total phenol content of 2.032, which was dwarfed by the relative concentration of gallic acid of 0.345 mg/mL; the reducing power of the three agarwood samples ranged from approximately 1.056 to 5.739, with no significant improvement. Among them, C1-1 and C1-2 are samples of agarwood from *Aquilaria Baillonii Wood Oil*, and their surfactants are RT4020 and PEG-40 respectively; A2-1 is a lotion made from *Aquiariasinensis* agarwood and PEG-40.

This shows that PEG-40 has a better coating effect in the lotion made from agarwood essential oil and surfactant. Among the three types of agarwood essential oils, the lotion made from agarwood essential oil and different surfactants has better antioxidant activity than the other two. The lotion made from *Aquiariasinensis* agarwood essential oil and PEG-40 has the highest total phenol content.

After using the scalp water, instrument test results showed that dandruff was reduced to the lowest value of 1, scalp oil secretion was regulated and balanced, and the subjects' hair density (hair volume) and diameter (hair diameter) were both increased, which may improve psychological comfort. In the future, empirical sampling methods can replace instrumental experimental testing methods in a timely manner during product development, which can reduce the cost of human efficacy experiments during the development of natural cosmetics products

5. Conclusion

It was found that the antioxidant effect of *Aquilaria Baillonii Wood Oil* was best when added to PEG-40 surfactant at 0.1g. Subsequent application of scalp water on the scalp of 30 subjects has been shown to reduce dandruff, increase hair diameter and volume, and promote melanin production. The health of the scalp and hair is reflected by the ESM results, which show an increase in happiness and satisfaction, thereby increasing consumers' willingness to purchase and increasing the application of new products in the future.

6. REFERENCES / ACKNOWLEDGEMENT

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