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Development of Rice By-Products Based Hair Tonic Mixed with Traditional Thai Herbal Extracts: A Sustainable Approach for Hair Care

Issaraporn Somboonwatthanakul[1], Sirirat Deeseenthum[1], Worachot Saengha[1] and Vijitra Luang-In[1]*

Natural Antioxidant Innovation Research Unit, Department of Biotechnology, Faculty of Technology, Mahasarakham University, Khamriang, Kantarawichai, Mahasarakham, Thailand.
*Corresponding Author E-mail:vijitra.l@msu.ac.th

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Previously, byproducts from Thai rice mill industry including crude riceberry oil (CRO) and broken riceberry (BRB) showed antioxidant and tyrosinase-stimulating activities. This work aimed to develop 5 hair tonic formulae by mixing CRO (0.2-1.0%) and BRB (0.4-2.0%) with three Thai traditional herbal ethanolic extracts (2.0%) of *Catunaregam tomentosa* (CT), *Acacia concinna* (AC) and *Tinospora crispa* (TC) in 4:3:3 (v/v). The results showed that formula 5 (F5) containing 2% BRB, 1.0% CRO and 2% herbal extracts exhibited the highest antioxidant activity (DPPH scavenging capacity at 16.6 TEAC mg/g, FRAP value at 1.18 $FeSO_4$ mg/g, and ABTS value at 14.9 TEAC mg/g) and tyrosinase-stimulating activity of 38%. This hair tonic was safe to use based on skin irritation test, and stable over 8-week storage at room temperature. Hence, F5 hair tonic (coded (R)) was tested on 20 subjects divided in 2 groups (older and younger age groups) with 10 individuals each in a randomized, double-blind, placebo-controlled experimental design with split-head method where treatments were conducted as intra-individual trial for 10, 20 and 30 days. The hair tonic coded C (Control without BRB, CRO or herbal extracts) and the commercial hair tonic coded G were tested for comparison. Adverse reactions were not noticed during the study. The hair tonic R was able to reduce hair loss by 48-71% in 2 groups with no significant difference from the commercial hair tonic. In comparison with the efficacy in the reduction of gray hair of the 2 groups at each time interval, the experimental (R) hair tonic product was found to be more effective in group 2 (younger age). However, the efficacy in the reduction of gray hair was lower than that in the reduction of hair loss. The experimental hair tonic R was more effective in dandruff reduction in group 1 (older age). The R hair tonic product received a satisfactory score of 6.79±0.32 out of 9.0 (moderate-to-high likeness) with the highest score from the aroma and the lowest score from the opaque color resulting from mixing aqueous herbal extract and oil. This is a sustainable way to add value to byproducts from rice mill industry and Thai traditional herbs as cosmetic elements in the formulation of hair products.

Keywords: Antioxidant; Broken riceberry; Flavonoid; Hair tonic; Thai herbs; Tyrosinase.

The market for hair loss prevention products had a value of US$ 23,599.87 million in 2021. It is projected to increase at a compound annual growth rate (CAGR) of 4.2% from 2021 to 2028, reaching US$ 31,524.52 million by 2028.[1] This tends to expand as the population increases. In Thailand, hair loss prevention products are products that come from both domestic production bases and imported from abroad which can be divided into 2 groups: medicines and cosmetics.

There are only two approved drugs, namely Minoxidil and Finasteride, that can only be used to treat people with hereditary hair loss problems, but the disadvantage is that they cause unwanted side effects. [2] In addition, premature graying of hair (premature canities) is considered a health problem that has a negative effect on mental health and personality.[3] The cause of it may be due to genetics, aging, reduction in the number of melanocytes responsible for producing the pigment melanin, deficiency in tyrosinase enzyme activity responsible for converting the precursor tyrosine into melanin at the hair roots, oxidative stress resulting in the body creating the free radicals such as hydrogen peroxide in large quantities and causes melanocyte cells to decrease and die quickly (apoptosis).[4]

Dandruff is a scalp condition that primarily affects males. Topical agents and synthetic drugs utilized in the treatment of dandruff exhibit distinct adverse effects, such as localized burning sensation, depressive symptoms, dizziness, headaches, pruritus or dermatitis, nausea, abdominal discomfort, alterations in vision, vomiting, hair discoloration, scalp dryness or oiliness, and heightened hair loss.[5] Hence, the utilization of plant extracts holds significant potential in both the management and prophylaxis of dandruff.

Prior research demonstrated that broken riceberry (BRB) and crude riceberry oil (CRO), byproducts of the Thai rice mill industry, had the potential to stimulate growth of hair and hair melanogenesis. It is known that CRO and BRB contained gamma-oryzanol and fatty acids (oleic acid, linoleic acid, stearic acid). [6] These compounds were proved to have potential as a treatment for hair loss.[7] The antioxidant activity of the BRB extract was determined to be 6.36 mg Trolox equivalent antioxidant capacity (TEAC)/g (DPPH assay), 1.33 mg $FeSO_4$/g (FRAP assay), and 5.82 mg TEAC/g (ABTS assay), with respective total phenolic content and total flavonoid content values of 9.94 mg gallic acid equivalent (GAE)/g and 67.2 mg catechin equivalent (CE)/g, respectively. It is noteworthy that the ethanolic CRO and aqueous BRB extract demonstrated tyrosinase-stimulating activities of 26% and 45%, respectively.[6] Additionally, it has been reported that some Thai traditional herbs can stimulate cells in the hair roots causing new hair to be created and reducing hair loss. *Acacia concinna* (AC) in the Fabaceae family has pods containing 20.8% saponin, including acacinin A, B, C, D and E. It acts as a surfactant, foaming agent, hair conditioner, hair volumizer, hair fortifier, hair stimulant with antibacterial, and anti-dandruff properties.[8,9] *Catunaregam tomentosa* (CT) in the Rubiaceae family contains tannins and cardiac glycosides. The total amount of phenolic content from methanolic extract of fruits was 423.70+3.73 mg GAE/g extract with DPPH antioxidant activity (IC_{50} = 5.12+0.34 mg/mL).[10] Its ripe fruits are used for hair washing. [11] *Tinospora crispa* (TC) in the Menispermaceae family contains alkaloids, flavonoids, flavone glycosides, triterpenes, which had high antioxidant activity.[12,13] The extract has a FRAP value of 11,011.11 ± 1,145.42 μmol Fe^{+2}/g and a DPPH inhibition of 55.79 ± 7.90%, with an IC_{50} of 22 μg/mL. [14] In this context, CRO, BRB, CT, AC, and TC emerge as promising candidates, each known for its unique phytochemical composition and traditional applications. Hair care has long been a focal point in the pursuit of well-being. The quest for effective and natural solutions has led researchers to explore the potential of natural or botanical extracts in developing innovative hair tonics. This research aimed to develop five hair tonic formulae by mixing CRO (0.2-1.0%) and BRB (0.4-2.0%) with three Thai traditional herbal ethanolic extracts (2.0%) of CT, AC and TC in 4:3:3 (v/v) and tested them on twenty subjects for anti-hair loss, anti-grey hair and anti-dandruff evaluation of the products. This could pave the way for advancements in the field of hair tonic development and reinforce the significance of nature-inspired solutions in contemporary cosmetology.

MATERIALS AND METHODS

Agricultural Materials

Organic BRB and CRO were obtained from the E-san Community Enterprise Agrarian Network, Roi Et Province, Thailand.[6] Fruits of CT, pods of AC, and stems of TC were collected in September 2018 (rainy season) from Ku Thong subdistrict, Chiang Yuen District, Maha Sarakham Province, Thailand.

Extraction of BRB, CRO and Thai Local Herbs

Extraction of BRB and CRO was

conducted as a previous report.[6] Each herb was extracted using maceration method [14] by taking 200 g of dried fruits (CT), pods (AC), stems (TC) and extracting them with 1 L of ethanol for 3 days in a shaker at 200 rpm at 40 °C. Ethanol from the extract was evaporated using a rotary evaporator at 42 °C. The herbal extract was dissolved in distilled water to make a concentration of 20 mg/mL crude plant extract and stored at -20 °C.

Hair Tonic Formulations

Five formulae (F1-F5)(Figure 1) of hair tonic products were shown in Table 1. The preparation of each formula is as follows: (1) Dissolve glycerin in 10 mL ddH_2O. (2) Add propylene glycol to Step 1 and mix well. (3) Add PEG40 Hydrogenated castor oil to Step 2 and mix well. (4) Add BRB extract and herbal extracts into Step 3 and mix well. (5) Add CRO along with Emulgen (emulsifier). The concentration of BRB in a range of 0.4–2.0%, CRO at 0.2–1.0% and herbal extracts at 2.0% were implemented based on a range of concentrations used in the previous studies that formulated effective hair tonics for with bioactive oil ingredient at 0.1–0.9% [15] or rice bran extract at 0.5% for hair growth in human subjects. [7] (6) Make a homogeneous emulsion using an ultrasonic bath for 15 min at room temperature (37 °C in Thailand). (7) Adjust pH using Triethanolamine to 5.5. (8) Filter the mixture through Whatman filter paper no. 1 (0.45 µm), then filter the clear part through a membrane filter (0.2 µm). (9) Package in a 200 mL plastic bottle pre-sterilized by UV for 45 min, and (10) Store at room temperature. The chemical compositions e.g. propylene glycol, emulgen, PEG40 hydrogenated castor oil, glycerin, tea tree essential oil, rosemary essential oil, rice milk essential oil, phenoxyethanol and coffee extract in Table 1 were purchased from a chemical store (www.chemipan.com). After that, the above five formulae of hair tonics were tested for their stimulating effect on melanin synthesis using tyrosinase activity assay and for their antioxidant activities. The formula with the highest antioxidant activity and the highest tyrosinase stimulating activity was chosen to test with subjects next.

Antioxidant Activities of Hair Tonic Formulae

Scavenging of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay, ferric reducing antioxidant power assay (FRAP), 2,2'-azino-di(3-Ethyl-benzthiazoline sulfonic (ABTS)•+ scavenging assay were conducted as per a previous work. [6]

Tyrosinase Stimulating Activity of Hair Tonic Formulae

This experiment was conducted as per a previous work. [6]

Hair Tonic Stability Test

The stability after preparing the hair tonic products was determined by observing the following properties: (1) Color, observed with eyes. (2) Fragrance, observed by smelling. (3) Viscosity (cP) measured by a viscometer. (4) Segregation, observed by centrifugation at 5,000 rpm for 30 min. (5) pH measured by a pH meter. The product stability was tested using alternating hot and cold temperature conditions (heating/cooling) for 6 cycles, alternating at 4 °C and 45 °C every 24 h, and the above five properties were observed. The product stability was also tested upon storage of the products at room temperature for 8 weeks, and the above properties were observed.

Irritation Test Using a Single Patch Test

One mL of hair tonic was pipetted onto a patch test strip using a 0.5 inchwide gauze cloth. The skin was wiped clean with 70% ethanol. The prepared test strip was placed on the front of the volunteer's arm and covered it with gauze tape to prevent the gauze from slipping and left for 48 h. Afterwards, the test strip was peeled off and the symptoms of irritation on the skin were scored on the level of 0-5 (Table 2) within 30 min.

Efficacy of Reducing Hair Loss, Gray Hair and Dandruff Reduction of Hair Tonic on Subjects

The efficacy study mentioned below comprised 20 healthy Thai subjects, aged between 20 and 60, who were experiencing dandruff, premature canities, or hair loss (Table 3).

Prior to enrollment, all recruited participants were provided with comprehensive information about the study by written and verbal means. They subsequently signed a written consent form, which had been authorized by the Ethical Council of Mahasarakham University, Thailand (Approval no. 018/2560). The study involving human participants adhered to the latest Declaration of Helsinki in all aspects.

The experiment was conducted according to the method of Chaisripipat et al. (2015)[17] using a total of 20 subjects. A randomized, double-blind,

placebo-controlled experiment with a split-head method was used to compare treatments in the same individuals (intra-individual trial) divided into 2 experimental groups: Group 1: 10 subjects tested a control hair tonic (C) (not mixed with rice byproducts or herbs)(Figure 2A) on the left side of the scalp and at the same time tested F5 hair tonic (R)(Figure 2B) on the right side of the scalp. Group 2: Another 10 subjects tested the commercial hair tonic Giffarine Herbita (G) (Figure 2C) on the left side of the scalp and at the same time, tested F5 hair tonic (R) on the right side of the scalp. The subjects were randomly assigned to apply 5 drops (0.13 g) of hair tonic on one side of their head and equal amounts of another hair tonic formulation on the other. The chemical was applied to the scalp with the same hand and head location throughout the research. The control cleansing shampoo, No More Tears (Johnson & Johnson, Thailand), was used to wash hair every two days at 10 g each wash.

To test the efficacy of reducing hair loss, each subject used a comb to detangle each side of their hair. The hair that fell from each side of the scalp (was then collected on white paper in the morning and evening on days 0, 10, 20, and 30. The amount of hair lost was recorded to find the average % reduction in hair loss on days 0, 10, 20, and 30 after using hair tonics.

% hair loss reduced = [(no. of hair loss day 0 – no. of hair loss day 10, 20, 30) / No. of hair loss day 0] ×100%

To test the efficacy of preventing gray hair, each volunteer's gray hair was counted from 4 head areas (an area of 1 cm^2) on each side of their head on days 0, 10, 20, and 30 after using the hair tonic. [18] The number of gray hair in each of the 4 areas on each side of the head added together was recorded to find the average value of gray hair on each side.

% gray hair reduced = [(no. of gray hair day 0 – no. of gray hair day 10, 20, 30) / No. of gray hair day 0] ×100%

To evaluate the anti-dandruff treatment, a sticky disc was used to lift the dandruff on the hair of each volunteer on days 0, 10, 20, and 30. Chaisripipat et al. (2015) scores were recorded on either side of the head in triplicate. $R = (D_t\text{-}D_0)/D_0 \times 100$, where R reflects decreased efficacy, D_t represents the dandruff scale at days 10, 20, and 30, and D_0 represents the dandruff scale at day 0.

Satisfaction Survey

All subjects took a satisfaction survey using the interview questionnaire on a 9-point likeness scale (1 = significant dislike; 9 = greatest likeness).

Statistical Analysis

The measurements in triplicate were expressed as means ± standard deviation (SD). Significant differences were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test at $p < 0.05$ using the SPSS package (demo version).

Fig. 1. Hair tonic products formulae 1-5 (F1-F5) in a volume of 20 mL

RESULTS AND DISCUSSION

Antioxidant Activity and Tyrosinase Stimulation of the Hair Tonic Formulae

The results showed that F5, amongst hair tonic formulae 1-5, gave the highest antioxidant activity of all 3 methods, including DPPH scavenging capacity at 16.6 TEAC mg/g, FRAP value at 1.18 $FeSO_4$ mg/g, and ABTS value at 14.9 TEAC mg/g (Figure 3A-C). This is probably due to the highest ingredients from BRB extract at 2% and agricultural by-products from CRO at 2%. The stimulation of the tyrosinase enzyme is also the highest at 38% tyrosinase stimulation for F5 product (Figure 3D). It was discovered that gamma-oryzanol can induce hair follicle formation, stimulate melanogenesis, and promote hair growth [19]; therefore, our BRB and CRO extracts containing gamma-oryzanol and fatty acids have the potential to be incorporated into hair treatment products.[6] Antioxidants from herbal extracts in hair function by safeguarding the hair keratin, which is responsible for causing damage to the hair structure; preventing the degradation of integral lipids in the hair fiber, which weakens the cell membranes and leads to hair brittleness and loss; preventing the oxidation of melanin, which is responsible for hair whitening; and repairing and protecting damage by counteracting free radicals and decelerating lipid oxidation. [19]

Hair Tonic Product Stability Test

When stored at room temperature for 8 weeks, F5 hair tonic product was found to be stable, did not separate into layers, had no viscosity (< 5 cP), did not change color, had constant pH of 5.5 and had no reduction in the fragrance of rice milk (Table 5). However, when testing the stability of the hair tonic formula where low temperature conditions alternated with high temperature (at 4 °C 24 h alternating with 45 °C 24 h) for 6 cycles, it was found that the fragrance of the hair tonic product decreased. When the experiment was carried out to the 9th - 12th day, no separation of layers, no viscosity, and no color change (Table 5) indicating that the F5 hair tonic formula was stable upon storage at room temperature.

Regarding hair tonic lotion preparations intended for application on the scalp, SNI standard number 16-4955-1998 specifies that the pH of hair tonic preparations must fall within the range of 3.0 to 7.0. [20] Excessive acidity can lead to skin

Table 1. Compositions for 5 formulae (F1-F5) of hair tonic products

Composition (mL)	F1	F2	F3	F4	F5
BRB extract 20 mg/mL (aq)	2.0 (0.4%)	4.0 (0.8%)	6.0 (1.2%)	8.0 (1.6%)	10.0 (2.0%)
Herbal extracts 20 mg/mL (aq) (CT:AC:TC in 4:3:3)	10.0 (2.0%)	10.0 (2.0%)	10.0 (2.0%)	10.0 (2.0%)	10.0 (2.0%)
CRO 20 mg/mL (aq)	1.0 (0.2%)	2.0 (0.4%)	3.0 (0.6%)	4.0 (0.8%)	5.0(1.0%)
Propylene glycol	2.0	2.0	2.0	2.0	2.0
Emulgen	2.0	2.0	2.0	2.0	2.0
PEG40 Hydrogenated castor oil	0.3	0.3	0.3	0.3	0.3
Glycerin	3.0	3.0	3.0	3.0	3.0
Tea tree essential oil	1.0	1.0	1.0	1.0	1.0
Rosemary essential oil	1.0	1.0	1.0	1.0	1.0
Rice milk essential oil	1.0	1.0	1.0	1.0	1.0
Phenoxyethanol (0.1% v/v)	0.1	0.1	0.1	0.1	0.1
Coffee extract	0.1	0.1	0.1	0.1	0.1
ddH_2O	adjust the volume to 100 mL				

aq = aqueous

Table 2. Irritation scores

Symptoms of irritation	Score
No swelling or redness	0
Slightly red	1
Very red, but the skin on the arm is still smooth	2
Red and bumps appear	3
Red, blistered, swollen, congested with blood	4

irritation, while excessive alkalinity can result in parched and scaly skin. It can be inferred that the pH value of this formula is satisfactory. In addition, SNI indicates that a good hair tonic has a viscosity of <5 cPs [20] which was accordant with our results (Table 5).

Efficacy in Reducing Hair Loss, Gray Hair and Dandruff of Hair Tonic on Subjects

Adverse reactions i.e. irritations were not noticed during the study. The results of hair loss reduction showed that in group 1 with the average age of adulthood and elderhood, a reduction in hair loss from using the control (C) and experimental (R) hair tonic product was found in the range of 64-78% and 60-71%, respectively, over 30 days with no significance between C and R, and with increasing duration time (Table 6). Group 2 (a younger age range) found that the reduction in hair loss from using commercial hair tonic product (G) and experimental (R) hair tonic products were in the range of 31-67% and 20-48%, respectively over 30 days with no significance between G and R (Table 6). However, the increasing efficacy in the reduction of hair loss was observed from day 10 to 30 in these 2 products. In comparison with the efficacy in the reduction of hair loss of the 2 groups at each interval (10, 20 and 30 days), the experimental (R) hair tonic product was found to be more effective in group 1 (older age) than group 2 (younger age).

Table 3. General information of 20 subjects in 2 groups

Age (years)	No. of subjects	%
18-30	7	35
31-40	4	20
41-50	4	20
51-60	5	25
Gender	No. of subjects	%
Male	2	10
Female	18	90

Group 1. Use of C and R hair tonics; $n = 10$

Age (years)	No. of subjects	%
18-30	0	0
31-40	3	30
41-50	2	20
51-60	5	50
Gender	No. of subjects	%
Male	1	10
Female	9	90

Group 2. Use of G and R hair tonics; $n = 10$

Age (years)	No. of subjects	%
18-30	7	70
31-40	1	10
41-50	2	20
51-60	0	0
Gender	No. of subjects	%
Male	1	10
Female	9	90

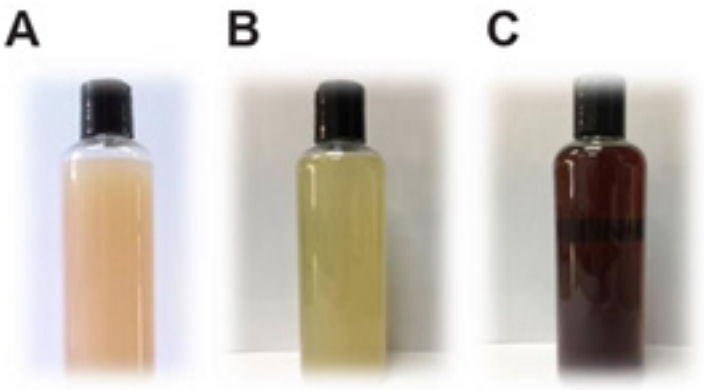

Fig. 2. Hair tonic products in 200 mL were used in testing with subjects (A) Control hair tonic, coded C (without BRB, CRO or herbal extracts). (B) F5 hair tonic, coded R. (C) Commercial hair tonic, coded G

As for the test results of the efficacy of hair tonic in reducing gray hair, it was found that in group 1, no impact was noticed. The control set (C) and the experimental set (R) gave the reduction of gray hair in the range of 0.5-14.3% and 0.5-20%, respectively, over a period of 30 days with no significance between C and R (Table 6). However, the increasing efficacy in reduction of gray hair was observed from day 10 to 30 in these 2 products indicating that if used continuously, it would reduce gray hair better. In group 2, it was found that the reduction of gray hair was more effective. The use of commercial hair tonic product (G) and trial set (R) was found in the range of 7-26% and

Table 4. Stability test of F5 hair tonic with alternating low temperature and high temperature conditions for 6 cycles

Temp. (°C)	Day	Color	Fragrance	Separation	Viscosity (cP)	pH
4	1	Light yellow	Strong	No	< 5	5.5
45	2	Light yellow	Strong	No	< 5	5.5
4	3	Light yellow	Strong	No	< 5	5.5
45	4	Light yellow	Strong	No	< 5	5.5
4	5	Light yellow	Strong	No	< 5	5.5
45	6	Light yellow	Strong	No	< 5	5.5
4	7	Light yellow	Strong	No	< 5	5.5
45	8	Light yellow	Strong	No	< 5	5.5
4	9	Light yellow	Moderate	No	< 5	5.5
45	10	Light yellow	Moderate	No	< 5	5.5
4	11	Light yellow	Light	No	< 5	5.5
45	12	Light yellow	Light	No	< 5	5.5

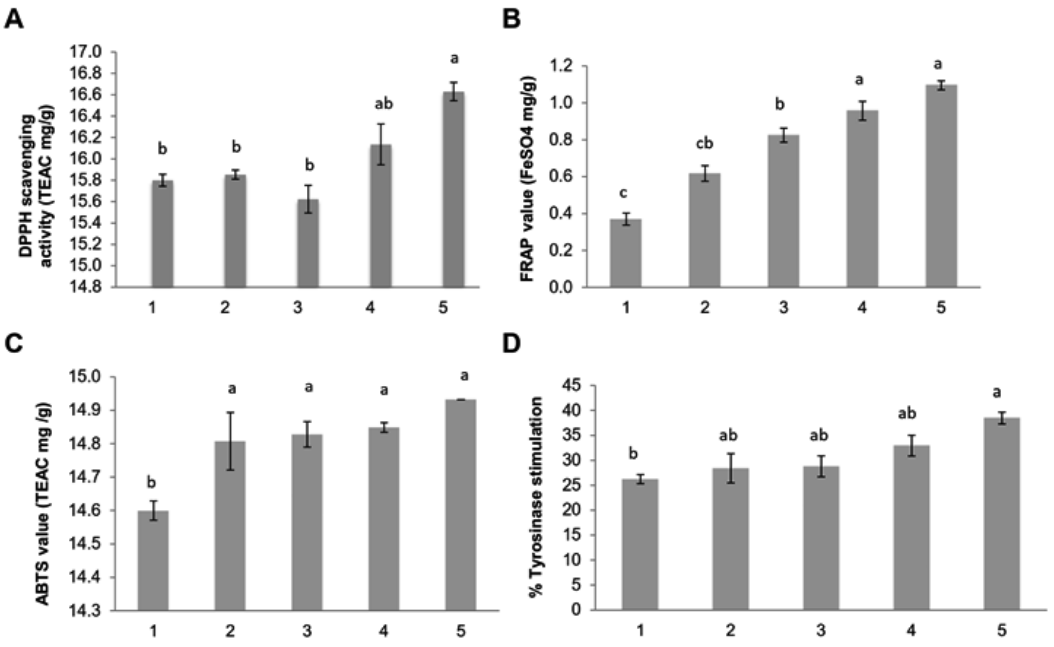

Fig. 3. Antioxidant activity of 5 hair tonic formulae (1-5) and tyrosinase stimulating property (A) DPPH scavenging activity assay. (B) FRAP assay. (C) ABTS antioxidant activity. (D) Tyrosinase stimulating activity. Different lowercase letters displayed significant differences in the bars ($p < 0.05$)

Table 5. Efficacy of hair tonic product against hair loss, gray hair and dandruff

G	Hair tonic	Hair loss reduction (%)			Gray hair reduction (%)			Dandruff reduction (%)		
		Day 10	Day 20	Day 30	Day 10	Day 20	Day 30	Day 10	Day 20	Day 30
1	C	63.9 ± 9.5^{Aa}	70.2 ± 8.8^{Aa}	77.5 ± 8.2^{Aa}	0.5 ± 0.1^{Bc}	2.5 ± 0.2^{Bb}	14.3 ± 1.1^{Aa}	40.0 ± 16.3^{Ab}	55.0 ± 15.7^{Ab}	88.9 ± 7.4^{Aa}
	R	59.4 ± 9.0^{Aa}	69.0 ± 7.2^{Aa}	70.9 ± 10.5^{Aa}	0.5 ± 0.1^{Bc}	2.5 ± 0.2^{Bb}	20.0 ± 1.2^{Aa}	55.0 ± 15.7^{Ab}	67.5 ± 14.9^{Ab}	91.7 ± 5.9^{Aa}
2	G	30.8 ± 7.7^{Bb}	40.5 ± 6.9^{Bb}	66.5 ± 6.2^{Ba}	7.0 ± 5.2^{Aa}	17.5 ± 7.5^{Aa}	26.0 ± 10.8^{Aa}	13.5 ± 6.6^{Ba}	31.7 ± 8.8^{Ba}	28.3 ± 10.0^{Ba}
	R	20.2 ± 6.1^{Bb}	46.2 ± 10.2^{Ba}	48.0 ± 10.9^{Ba}	8.6 ± 5.3^{Aa}	24.7 ± 10.5^{Aa}	26.9 ± 10.7^{Aa}	16.67 ± 8.6^{Ba}	23.0 ± 10.0^{Ba}	23.0 ± 10.0^{Ba}

Values shown are mean ± SD from experiments on 10 subjects in each group (G1 and G2). Uppercase letters indicate statistically significant differences in columns and lowercase letters in rows ($p < 0.05$) for each efficacy. R = F5 hair tonic, C = Controlled hair tonic and G = Giffarine commercial hair tonic.

9-27% reduction of gray hair, respectively over a period of 30 days with no significance between G and R (Table 6). In comparison with the efficacy in reduction of gray hair of the 2 groups at each interval (10, 20 and 30 days), experimental (R) hair tonic product was found to be more effective in group 2 (younger age) than group 1 (older age). However, the efficacy in reduction of gray hair was lower than that in reduction of hair loss.

As for the efficacy of the hair tonic in reducing dandruff, it was found that in group 1, a very high reduction in dandruff was found. The control set (C) and the experimental set (R) were in the range of 40-89% and 55-92% reduction in dandruff efficacy, respectively, with over a period of 30 days with no significance between C and R (Table 6). However, the increasing efficacy in reduction of dandruff was observed from day 10 to 30 in these 2 products indicating that if used continuously, it would reduce dandruff better.

In group 2, it was found that the reduction of dandruff was much less effective. The use of the commercial hair tonic product (G) and the trial kit (R) led to the efficacy of reducing dandruff in the range of 14-28% and 17-23%, respectively, with no significant difference in results. It was found that the experimental hair tonic (R) was more effective in group 1 (older age).

Previous research using local herbal extracts as an ingredient in hair tonic products to nourish the scalp found that citronella oil at concentrations of 5, 10 and 15% in hair tonic products can reduce dandruff problems. It is significantly more effective than the control (no citronella oil) formula at 33, 75, and 51%, respectively ($p < 0.005$) on the 7th day of use. The efficiency increased when used until the 14th day, it can reduce dandruff by 52, 81, and 74%, respectively. [17] These results of citronella oil in hair tonic are with higher efficacy when compared with our experimental R hair tonic product.

Traditionally, CT, AC and TC herbal extracts are found to be used in medicines. The fruit of CT was incorporated into diets. [21] Dried pods of AC are commonly used as herbal medicine such as a laxative. [22] TC was used as antidiabetic medicine. [23] Upon topical application especially in hair care, the previous work confirmed that the AC plant contains saponins, alkaloids, tannins, anthraquinone glycosides, sugar, and flavonoids.

Table 6. Comparison of herbal hair tonics in literature and in the market

No.	Hair tonic formulation	Active constituents	Hair care benefits
1	This work. Thailand.	1. Saponin[6]	1. hair cleansing[6]
	1. *Catunaregam tomentosa* (CT)	2. Saponin, including acacinin A, B, C, D and E[35]	2. hair strengthening, hair growth, anti-dandruff[35]
	2. *Acacia concinna* (AC)	3. Alkaloids, flavonoids, flavone glycosides, triterpenes[11]	3. anti-hair loss[27]
	3. *Tinospora crispa* (TC)	4-5. γ-oryzanol, tocopherols, γ-linolenic, linoleic, and oleic acids[6,7]	4-5. hair growth[7],
	4. Broken riceberry (BRB)		hair melanogenesis [28]
	5. Crude riceberry oil (CRO)		
2	Meduri et al. (2021). [30] India.	1. amino acids and vitamin C	1. hair growth
	1. *Hibiscusrosa sinensis*	2. tannins, mosaic acid, amino acids, alkaloids, flavonoid glycosides, phenolic glycosides, and terpenoids[36]	2. hair pigmentation, hair growth
	2. *Phyllanthus emblica*	3. proteolytic enzymes	3. hair soothing
	3. *Aloe barbadensis*	4. iron, protein, flavonoids and saponins	4. hair growth
	4. *Trigonella foenum graceum*	5. inositol	5. anti-hair loss
	5. Fermented rice water		
3	Yu et al. (2017) [31]	1. epigallocatechin-3-gallate	1. hair growth
	Hebro herbal hair oil	2. vitamin C, amino acids, and minerals	2. hair growth and pigmentation
	DA-5512 formulation. Korea.	3. vitamins A and C	3. anti-hair loss and dandruff
	1. *Thea sinensis* L.	4. isoflavones puerarin, daidzein, and genistein[37]	4. hair pigmentation
	2. *Emblica officinalis*	5. saponins, polyphenols, tannins flavonoids, glycosides, and alkaloids[38]	5. hair pigmentation
	3. *Pinus densiflora*	6. alkaloid, phlobotannins, tannins flavanoids, glycosides, and saponins[39]	6. anti-dandruff
	4. *Pueraria thunbergiana*		
	5. *Tribulus terrestris*		
	6. *Zingiber officinale*		
4	Begum *et al*. (2023)[32] India.	1. proteins, amino acids, fats, oils, steroids, glycosides, phenolic compounds, flavonoids, volatile oil, and vitamins	1. hair growth
	1. *Rosmarinus officinalis* (rosemary 1%)		
5	Goswami & Mukhopadhyay (2022).[33] India.	1. ellagic acid, chebulinic acid, gallic acid, chebulagic acid, leutolin, fatty acids	1.Anti-dandruff and anti-hair fall
	The EnQ ultimate hair care tonic	2. wedelolactone, wedelic acid, apigenin, luteolin, bamyrin	2. anti-hair fall and anti-gray hair
	1. *Emblica Officinalis*	3. hersaponin, apigenin, cucurbitacin, D-mannitol	3. anti-alllergic
	2. *Eclipta prostrata*		
	3. *Bacopa monnieri*		

6	Goswami & Mukhopadhyay (2022).[33] India. Blue nectar hair tonic 1. *Aloe barbadensis* 2. *Rosmarinus officinalis* 3. *Hibiscus rosasinesis* 4. *Eclipta prostrata*	1. vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids 2. *p*-cymene, linalool, γ-terpinene, thymol, and betapinene 3. flavonoids, anthocyanins and cyaniding -3, 5- diglucoside 4. wedelolactone, wedelic acid, apigenin, luteolin, and bamyrin	1. hair smoothing 2. hair growth 3. hair boom and anti-gray hair 4. anti-hair fall and anti-gray hair
7	Almas et al. (2022).[34] Indonesia. 1. *Aleurites moluccana* (Candlenut oil 7%)	1. palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid	1. hair growth in mice

Fig. 4. The hair tonic product is in a container with a label

Pod extract cleanses and controls dandruff. The fruits of this plant are used to wash hair, improve hair development, emetic, expectorant, and purgative. [24] Saponin is the principal foaming agent. [25] It naturally lowers pH and preserves hair oils, keeping them glossy and healthy. It also conditions and strengthens hair. [26] In addition, AC and TC plants showed some effects on dermal papilla cells that preventing balding. [27]

Previously, rice bran extract exhibited comparable hair growth-promoting potential to 3% minoxidil in C57BL/6 mice, indicating that it induced hair follicles to enter the anagen phase. Significant growth was observed in the quantity of hair follicles.[7] Rice bran extract, specifically linoleic acid and 'gamma-oryzanol constituent, stimulates hair growth. This implies that rice by-products may have potential as a treatment for hair loss. [7] It was also shown that rice bran extract promotes melanin biosynthesis in hair follicle like tissue model and organ culture model. [28] Additionally, when applied topically, rice bran enhances the production of growth factors and molecular signals that stimulate cell growth during the anagen phase, such as beta-catenin. It also suppresses enzymes that trigger the transition from anagen to catagen/telogen phases, like TGF-beta and Type I 5 alpha-reductase. Rice bran is safe for human usage in cosmetics as it is not genotoxic or cytotoxic. The Amazon.com search produced 119 hair products containing rice, indicating its popularity for over-the-counter purchase. RB promotes hair development by enhancing the expression of growth factors and molecular signals linked to sustaining the anagen phase, reducing inflammation, blocking 5 alpha-reductase, and stimulating melanogenesis. [29]

Our hair tonic was compared to other herbal hair tonics in literature/the market in terms of formulations and hair care benefits (Table 6). Meduri et al. (2021) formulated a herbal shampoo with fermented rice water for hair growth and hair melanogenesis purposes using different herb extract from our work. [30] They highlighted that fermented rice water, which contains inositol, was effective in reducing hair loss. The herbal shampoo was formulated with traditional Indian plants including *Hibiscusrosa sinensis*, *Phyllanthus emblica*, *Aloe vera*, *Trigonella foenum graceum*,

Table 7. Satisfaction results for the hair tonic product

Satisfaction attribute	Mean score
1. Product	
1.1 Appearance	6.65 ± 1.14
1.2 Differences from those in the market	6.80 ± 1.20
1.3 Volume/content per bottle	6.80 ± 1.06
1.4 Color	6.40 ± 1.67
1.5 Fragrance	7.20 ± 1.28
1.6 Texture	6.80 ± 0.89
1.7 Branding and label	6.70 ± 1.53
1.8 Label complete detail	6.85 ± 1.53
1.9 Reliability	6.60 ± 0.75
1.10 Strength of bottle	7.35 ± 1.30
2. Price	
2.1 Appropriate ($5/200 mL)	6.45 ± 1.70
2.2 Compared with prices in the market	6.85 ± 0.88
Total	6.79 ± 0.32
	Moderate-to-High

making it a suitable, safe, and efficient remedy for hair loss. [30] In addition, Hebro herbal hair oil was commercially in the market based on patented DA-5512 formulation comprising 6 herbal extracts including *Thea sinensis* L.*, Emblica officinalis, Pinus densiflora, Pueraria thunbergiana, Tribulus terrestris,* and *Zingiber officinale*. [31] Preclinical and clinical research in Korea showed that this DA-5512 formulated extract effectively boosted hair growth and enhanced hair health.

From another research work in India, *Rosmarinus officinalis* (rosemary 1%) was used in a hair lotion and the hair growth stimulating action was tested on C57BL/6 mice, with water as the control and 2% minoxidil hair lotion as the standard. It was found that a herbal lotion demonstrated a more pronounced hair growth promotion effect compared to animals treated with the standard medication. [32] A few marketed hair tonics in India showed different herbal ingredients with similar hair care benefits. [33] Another work using candlenut oil showed that it could promote hair growth in mice. [34] It can be seen that herbal hair tonics from different countries (Table 6) tend to use different herbal ingredients for formulations with similar hair care benefits.

Natural bioactive ingredients are more receiving attention as alternatives for hair care application nowadays since oral finasteride, topical minoxidil, and approved medications for the treatment of alopecia may occasionally cause undesirable side effects. The results of our research demonstrated that the bioactivity of Thai herbal plants in combination with rice by-products could serve as a functional hair care component.

Satisfaction Survey

From a survey of satisfaction with hair tonic products (Figure 4) in terms of product and price. The results showed that 20 subjects expressed satisfaction with the hair tonic product at a total mean of 6.79 ± 0.32 (out of 9)(Table 8), equivalent to moderate-to-high liking. The highest score was the fragrance of the product (7.20 ± 1.28). The lowest score is the color of the product (6.40 ± 1.67) because buyers do not like the cloudy color of mixing natural extracts with oils from by-products.

CONCLUSION

BRB, CRO by-products with Thai herbal extracts (CT, AC and TC) can be formulated into hair tonics for hair care with similar reduction efficacy in hair loss, gray hair and dandruff as the commercial hair tonic in Thailand. This novel hair tonic may be a safe way to treat hair loss, gray hair and dandruff. It is paraben-, formaldehyde-, and synthetic dye-free, making it scalp-friendly. Positive response on product satisfaction was received. The trial product had no adverse events

to healthy male and female volunteers for 1 month, indicating that it is safe to use topically. This work also presented a new light in adding value to by-products from the agricultural production process and Thai local herbs and also reducing chemical use in hair care products for a sustainable future.

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Conflict of Interest

All authors disclose that there is no conflict of interest among them.

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Ethics of Human Experimentation

All procedures performed in studies involving human participants were under the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by Human Ethics Committee at Mahasarakham University, Mahasarakham, Thailand (Approval no. 018/2560).

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