The 4th International Conference on Agriculture and Agro-Industry
ICAAI2022
Transforming Value Chains for Global Security
Mae Fah Luang University, Chiang Rai, Thailand
November 10 -11, 2022

Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

Original Research Article

Bioactive compounds and antioxidant properties of *Houttuynia cordata* for hair cosmetic application

***Natthawut Thitipramote*[1,2,3]*, *Pichayawee Piwngern*[1], *Sariya Koontemsap*[1] and *Prinyaporn Pradmeeteekul*[2]**

[1]*School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand*
[2]*Center of Excellence in Natural Products Innovation (CENPi), Mae Fah Luang University, Chiang Rai, 57100, Thailand*
[3]*Green Cosmetic Technology Research Group, School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand*

ARTICLE INFO

Article history:
Received 11 November 2022
Received in revised form 17 July 2023
Accepted 31 July 2023

Keywords:
Antioxidant activity
Hair tonic
Houttuynia cordata
Proanthocyanidin

ABSTRACT

Houttuynia cordata or "Plu Kaow" in Thai, a perennial herb, has been used as an edible vegetable and traditional medicine plant due to its health benefits such as anti-inflammatory, antiviral, antioxidants, and skin disease. The aims of this study were to evaluate bioactive compounds (total phenolic (TPC), flavonoid (TFC), proanthocyanidins (TPAC) contents and antioxidant properties (ABTS, DPPH and FRAP activities) of Plu Kaow for used in hair cosmetic applications. Plu Kaow powder was extracted with three different solvents including DI water, 70% methanol and 70% ethanol, using an incubator shaker at 150 rpm for 6 h. The results showed that the highest yield was found in the methanolic Plu Kaow extract (8.73%). The methanolic of Plu Kaow extract had statistically the highest TPC and TPAC (128.27±8.45 mg GAE/g extract, 206.67±21.80 mg CE/g extract, $p<0.05$, respectively). Although, TFC showed no statistical difference between these three extracts, the methanolic Plu Kaow extract exhibited the highest TFC (70.74±17.31 mg QE/g extract). In the antioxidant study, the significantly highest antioxidant activities in DPPH and ABTS were found in the methanolic Plu Kaow extract (0.54±0.04 and 0.88±0.01 mg TEAC/g extract, $p<0.05$, respectively). In the FRAP assay, the result showed that the statistically highest was found in the ethanolic Plu Kaow extract (0.95±0.00 mg TEAC/g extract, $p<0.05$). According to the results, the methanolic extract Plu Kaow exhibited higher contents in bioactive compounds and antioxidant activities than other solvents. Thus, the methanolic Plu Kaow extract should be used as an active ingredient in a hair tonic formulation. Hair tonic were made by 1% and 5% methanolic Plu Kaow extract (concentration 1 mg/mL) and other ingredients such as 95% ethanol, propylene glycol, menthol, DMDM hydantoin, sodium metabisulfite. Moreover, these hair tonics were stable through Freeze-thaw cycle testing and suitable to use on scalp due to pH of products (pH 5.5). Hair tonic with 1% methanolic Plu Kaow extract was satisfied in terms of good characteristic and good feeling after use with a light texture. Results suggested that Plu Kaow extract (especially the methanolic extract) can be used as natural antioxidant for preventing anti-aging including hair aging (hair loss, or gray hair).

**Corresponding author*. Tel.: 053-916837; fax: +053-917548.
E-mail address: natthawut.thi@mfu.ac.th

ISSN: 2408-1736

INTRODUCTION

Hair is essential for self-confidence and also reflects personality. Additionally, it can easily change such as length, color, and shape for modification to create a different style (Bolduc & Shapiro, 2001). However, hair loss could occur in both men and women of all ages as the result of physical conditions, emotional stress or physical stress, nutrient deficiency, hormonal disturbances, or drug (Houschyar *et al*., 2020). Thus, this problem is a critical issue among men and women due to the increase in hair loss in the population. Various hair cosmetic has been developed with both synthetic and natural ingredients for solving the hair loss problem. For example, Minoxidil is commonly known as a medication for stopping hair loss. However, Rossi *et al.* (2012) reported the side effects of Minoxidil are itching, erythema, and dryness on the scalps which are the sign often defined as irritation signs and allergic events. Thus, natural extract was discovered as a substitution to prevent hair loss by replacing chemical substances that have the side effect.

Proanthocyanidin also known as condensed tannin, is defined as polymerized flavan-3-ols, composed of catechin and epicatechin (Fine, 2000). Proanthocyanidin is naturally found in plants such as grape seed (Takahashi *et al.,* 1998), apple (Takahashi *et al.,* 2005), red pigmented rice (Thitipramote 2016; 2022), and most berries as chokeberry and rowanberry (Hellström et al., 2009). Takahashi *et al.* (1998) assumed that proanthocyanidin extracted from grape seed stimulates the conversion of telogen phase to anagen phase. Procyanidins (subclass of proanthocyanidin) from apples exhibit potential hair growth activity, which might be related to antioxidants and anti-inflammatory (Takahashi *et al.,* 2005).

Houttuynia cordata Thunberg (*H. cordata*, Saururaceae), 'Plu Kaow' in Thai, is an interesting plant and is a medical herb widely distributed in Eastern Asia. In Japan, it is used to remedy several illnesses as a traditional medicine with anti- cancer, immune stimulation, anti- inflammatory, antiviral, anti- bacterial, anti- fungal, and anti-leukemic (Latha and Jeevaratanm, 2012). In Thailand, it is a traditional plant that occurs especially in the Northern region, favorably consumed as a side dish vegetable due to its abundant nutrients. Besides, the aerial parts of *H. cordata* contain bioactive compounds such as phenolic, flavonoid, and condensed tannin (Tuyen *et al.,* 2018). In addition, Nuengchamnong *et al.* (2009) found catechin and procyanidin B in the aqueous *H. cordata* tea extract. Thus, the objectives of this study were to investigate the bioactive compound and to determine the antioxidant activities of *H. cordata* from three different solvents (DI water, 70% methanol, and 70% ethanol) extractions as well as to apply these extracts as active ingredients on hair tonic for preventing hair loss treatment.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and solvents were analytical grade. Cosmetic ingredients were cosmetic grade. ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), aluminum chloride ($AlCl_3$), catechin, dibasic phosphate, dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 95% ethanol, ferric chloride, folin-ciocalteu, gallic acid, glydent DMDM hydantoin, hydrochloric acid (37%), methanol, menthol, monobasic phosphate, potassium acetate (CH_3COOK), potassium persulphate ($K_2S_2O_8$), propylene glycol (PG), quercetin, sodium carbonate (Na_2CO_3), sulfuric acid, vanillin, 2,4,6-tris(2-pyridyl)-1,3,5-triazin (TPTZ), trolox, sodium metabisulfite.

Sample preparation

Dry aerial parts of *H. cordata* were obtained from Chiang Rai province. The samples were blended as powder and kept in a dry place until used.

Extraction of bioactive compound

H. cordata powder was extracted with three different solvents including DI water, 70% methanol, and 70% ethanol, at a ratio of 1:10 (%w/v) using an incubator shaker at 150 rpm for 6 h. The extracts were filtrated by Whatman filter paper No.1 and organic solvents (methanol and ethanol) were removed by rotary evaporator (Eyela, USA) at 50°. The extract samples were lyophilized and kept at -4° until used.

Determination of total phenolic content (TPC)

TPC was measured according to the method of Thitipramote *et al*. (2022) with a slight modification. Gallic acid was used as a standard compound and prepared by dilute 1 mg/mL of Gallic acid with DI water to obtain 0-50 µg/mL stock. 20 µL of Gallic acid stock with DI water or 20 µL of 1 mg/mL *H. cordata* extracts were added to 100 µL of diluted 10 times Folin-Ciocalteu reagent and left to stand for 1 min. Then, added 80 µL of 7.5 % sodium carbonate into the reaction mixture and incubated at room temperature for 30 min. After that, the absorbance of the mixture was measured at 765 nm by a microplate reader (Biotek, Epoch, USA). The results were reported as milligram gallic equivalent (mg GAE/g extract).

Determination of total flavonoid content (TFC)

TFC was measured by slightly modified from Thitipramote *et al*. (2022). Quercetin was used as a standard compound and stock solution (0-50 µg/mL) was prepared by diluting 1 mg/mL of Quercetin with 95% ethanol. A volume of 25 µL of quercetin stock with 95% Ethanol or 25 µL of 1 mg/mL *H. cordata* extracts was mixed with 75 µL of 95% ethanol, then added 5 µL of 10% $AlCl_3$, 5 µL of 1 M potassium acetate and 140 µL of DI water. After the mixture was incubated at room temperature for 30 min. The absorbance of the mixture was measured at 415 nm by a microplate reader (Biotek, Epoch, USA). The results were reported as milligram quercetin equivalent (mg QE/g extract).

Determination of total proanthocyanidin content (TPAC)

TPAC was determined by the method modified from Thitipramote *et al*. (2022). 20 µl of 1 mg/mL *H. cordata* extracts were mixed with 50 µL of vanillin (1%v/v in methanol) and 50 µL of 25%v/v H_2SO_4 in methanol and DMSO was used as control and blank-control. The mixtures were incubated for 15 min in the dark at room temperature. The absorbance was measured at 500 nm by using a microplate reader (Biotek, Epoch, USA). TPAC was calculated based on the calibration curves of catechin equivalents and expressed as milligram catechin equivalents (mg CE/g extract).

Determination of DPPH radical scavenging activity

The radical scavenging activity of the *H. cordata* extract in three solvents was tested against 2,2-Diphenyl-1-picrylhydrazyl radical following the method described by Thitipramote *et al*. (2016) with slight modification. A 190 µL of DPPH (0.1 mM) in ethanol was added to 10 µL of each sample (1 mg/mL). The reaction was incubated in the dark at room temperature for 30 min and the absorbance was measured at 515 nm. Trolox was used as standard. The absorbance of samples was calculated to be % inhibition as shown below. DPPH radical scavenging activity of samples were reported as mg Trolox equivalent antioxidant capacity (mg TEAC)/g extract

$$\% \text{ inhibition} = [A\text{-}B/A] \times 100$$

Where, A = the absorbance of the control solution without antioxidant agent,
B = the absorbance of sample to be tested.

Determination of ABTS radical scavenging activity

The ABTS radical assay was adapted from Thitipramote *et al*., (2016). ABTS radical solution was prepared by mixing 7 mM ABTS

with 2.45 mM $K_2S_2O_8$ at a 1:1(v/v) ratio. Then, the mixing reagent was incubated in the dark at room temperature for 15 h. After that ABTS working solution was diluted with 50 mM phosphate buffer (pH7) at a ratio of 1:20 (v/v) to obtain an absorbance of less than 1.000. Then, 10 µL of the sample was added to 190 µL of the ABTS working solution. The reaction was performed in the dark at room temperature for 15 min and the absorbance was measured at 734 nm. Trolox was used as standard. The absorbance of sample was calculated to be % inhibition as mentioned above. ABTS radical scavenging activity of samples was reported as mg Trolox equivalent antioxidant capacity (mg TEAC/g extract).

Determination of ferric reducing antioxidant power *(*FRAP*)*

FRAP measurement of extract was slightly modified from Thitipramote *et al*. (2016). FRAP solution was prepared by mixing 1 mL of 10 mM TPTZ (in 40 mM HCl), 1 mL of 20 mM $FeCl_3$ solution, and 10 mL of 0.3M sodium acetate buffer (pH 3.6). Then 10 µL of the sample was added to 190 µL of FRAP solution. The reaction was performed in the dark at room temperature for 15 min and the absorbance was measured at 593 nm. Trolox was used as standard. FRAP was calculated as mg Trolox equivalent antioxidant capacity (mg TEAC)/g extract.

Development of Base Formula for Hair Tonic

Hair tonic was developed with a target base formula that was characterized by easy to apply, good absorption, quick evaporation, and no residual on the scalp after use. The base formula of hair tonic for hair growth was developed with slight modifications from Luliana *et al*. (2018) (Table 1). The base formula was observed for characteristics, color, and smell and measured pH. Base formulas were examined by stability test.

Table 1 Base formula of hair tonic (% w/w)

Ingredients	F1	F2	F3	F4	Function
DI water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	Diluent
95% Ethanol	30	30	30	28	Solvent
Propylene glycol	2	2	4	4	Humectant
Menthol	1	0.2	0.2	0.2	Anti-inflammation
DMDM hydantoin	0.5	0.5	0.5	0.5	Preservative
Sodium metabisulfite	0.1	0.1	0.1	0.1	Preservative

Development Hair Tonic containing *H. cordata* extract

H. cordata extract that had the highest bioactive compounds (TPC, TFC, TPAC) and/or antioxidant activities was chosen as an active ingredient in hair tonic. Then, the products were examined by the stability test.

Stability test

The stability of the product was examined by Freeze-thaw cycles. Freeze-thaw cycles were determined by storing the product at 45 °C for 24 h in a hot air oven (Memmert, Modell 100-800, Germany). Observe and record the general appearance, color, odor, and separation results and record the results every 24 h. Then stored in a refrigerator at 4°C for 24 h, equivalent to 1 cycle, proceeding until 3 cycles.

Statistical analysis

All obtained data were expressed as mean ± standard deviation. The data were statistically analyzed by analysis of variance (ANOVA) and Tukey's HSD test for post-hoc analysis using IBM SPSS 21 for Windows. The comparison was considered at the significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Extraction yield and solubility of crude extract

The extraction yield of crude extracts from *H. cordata* was shown in Table 2. Results showed that a methanolic *H. cordata* extract had the highest yield (8.73%), while an ethanolic *H. cordata* extract exhibited the lowest yield (7.68%). The characteristic of *H. cordata* extracts was brown powder with different shades.

Phenolic compound of *H. cordata* extract

The total phenolic contents (TPC) of *H. cordata* extracts were shown in Table 3. The results showed that *H. cordata* extracts had a significant difference between three different solvents ($p<0.05$). The methanolic extraction of *H. cordata* exhibited the highest TPC (128.27±8.45 mg GAE/g extract, $p<0.05$) followed by the ethanolic extract and aqueous extract (113.43±9.60 and 77.99±5.78 mg GAE/g extract, respectively, $p<0.05$). According to the previous study, the methanolic *H cordata* extract had higher TPC than the ethanolic and aqueous extract (Tuyen *et al*., 2018, and Tok *et al*., 2017). In addition, Do *et al*. (2014) reported that phenolic content was less soluble in aqueous, therefore the aqueous extract may contain more non-phenolic or phenolic compounds which contain fewer active groups than other solvents.

However, total flavonoid content showed no significant difference between *H. cordata* extracts from three different solvents. The highest TFC was found in the methanolic *H. cordata* extract (70.74±17.31 mg QE/g extract) (Table 3).

The tendency of TPAC was similar to TPC, the methanolic *H. cordata* extract had the significantly highest TPAC (206.67±21.80 mg CE/g extract. While the lowest TPAC was shown in aqueous extract (57.33±28.83 mg CE/g extract) ($p<0.05$), as shown in Table 3. According to Tuyen *et al.* (2018), a methanolic extract was found in the highest TPAC followed by an ethanolic and an aqueous extract. Proanthocyanidins content may be related to the difference of degree polymerization of proanthocyanidins due to different extraction solvents (Naima *et al*., 2015).

Phenolic compounds are the main class of secondary metabolites in plants. These compounds contain at least one phenol group which was comprised of an aromatic ring with one or more hydroxyl groups. Flavonoids are classified as phenolic compounds, according to their chemical structure, flavonoids were divided into flavanone, flavone, flavonol, anthocyanin, and dihydrochalcones (Rupasinghe *et al*., 2014). Flavanols or flavan-3-ols exist in both the monomer form, i.e., catechin and the polymer form, i.e. proanthocyanidin, also known as procyanidin. Takahashi *et al*. (1998) found that proanthocyanidins from grape seeds could promote hair growth proliferating. Similar to Esmaeilzadeh *et al*. (2021) showed that procyanidin from grape sap decreased apoptosis in hair follicle cells consequently stimulating hair growth. Furthermore, procyanidin B2 and procyanidin B3 from some plants could promote hair growth *via* some mechanism such as induction of cell proliferation or inhibited hair follicles apoptosis (Park and Lee, 2021, Kim *et al*., 2019). In this study, *H. cordata* extract showed higher TPAC than TFC as it could support hair growth by stimulating dermal papilla cell proliferation or by inhibiting apoptosis. Consequently, *H. cordata* extract may promote hair growth and prevent excessive hair loss.

Table 2 Extraction yield (dry basis) and its characteristic of *H. cordata* extracts

***H. cordata* Extracts**	**% yield**	**Characteristics**	**Photo**
Water	8.13	Brown color powder	
Methanol	8.73	Dark red brown color powder	
Ethanol	7.68	Brown color powder	

Antioxidant activities of *H. Cordata* extract

DPPH assay was based on the ability of a compound that can donate a hydrogen atom from 2,2'-diphenyl-1-picrylhydrazyl converted into 2,2'-diphenyl-1-picrylhydrazine. (Pyrzynska and Pękal, 2013). DPPH radical scavenging activity of *H. cordata* were shown in Table 4. The result was significantly different between three various solvents (p<0.05). The methanolic extract of *H. cordata* exhibited the highest DPPH radical scavenging activity (0.54±0.04 mg TEAC/g extract) followed by ethanolic extract and aqueous extract (0.44±0.02 and 0.31±0.03 mg TEAC/g extract), respectively. Previous study also showed that organic solvents had higher DPPH radical scavenging activity than aqueous extracts (Tok *et al*., 2017). This result demonstrated that the methanolic *H. Cordata* extract was a greater hydrogen donor than ethanolic and aqueous extract.

ABTS assay is tested on the capability of hydrogen donor of antioxidant, from free radical (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation or ABTS•+) to ABTS (Sharopov *et al*., 2015). The tendency of ABTS activity was similar to DPPH radical scavenging activity as shown in Table 4. The methanolic extract of *H. cordata* expressed the highest ABTS radical scavenging activity (0.88±0.01 mg TEAC/g extract) followed by the ethanolic extract and the aqueous extract (0.80±0.01 and 0.70±0.01 mg TEAC/g extract), respectively.

While the mechanism of FRAP assay is different from DPPH and ABTS assay, FRAP method is used to examine the antioxidant competency by the redox reaction of ferric ion ($Fe3^{+}$) to ferrous ($Fe2^{+}$) (Sharopov *et al*., 2015). FRAP activity results were shown in Table 4. The results indicated that the ethanolic extract of *H. cordata* (0.95±0.00 mg TEAC/g extract) had the greatest ability to donate electrons apart from other extracts. The ethanolic extract of *H. cordata* had a more potent redox competency than the capability of a hydrogen donor. Similar to Tok *et al*. (2017) demonstrated that a methanolic extract of *H. cordata* had higher FRAP activity than aqueous and ethanolic extract. The methanolic extracts of *H. cordata* might have higher amounts of TPC and TPAC than other extracts as shown in previous studies in different plants (Park and Lee, 2021)

Table 3 Total phenolic, flavonoid, and proanthocyanidin contents of *H. Cordata* extracts

Extracts	**TPC (mg GAE/g extract)**	**TFC (mg QE/g extract)**	**TPAC (mg CE/g extract)**
Water	77.99±5.78^{c}	51.16±15.17^{a}	57.33±28.83^{c}
Methanol	128.27±8.45^{a}	70.74±17.31^{a}	206.67±21.80^{a}
Ethanol	113.43±9.60^{b}	67.77±15.30^{a}	112.00±25.99^{b}

Values are expressed as means ± SD (n=3).
Different letters in the same column indicate significant differences at p< 0.05. (ANOVA, Turkey's HSD test)

Table 4 Antioxidant activities of *H. cordata* extract

Extracts	**DPPH (mg TEAC/g extract)**	**ABTS (mg TEAC/g extract)**	**FRAP (mg TEAC/g extract)**
DI Water	0.31±0.03^{c}	0.70±0.01^{c}	0.49±0.00^{c}
Methanol	0.54±0.04^{a}	0.88±0.01^{a}	0.77±0.02^{b}
Ethanol	0.44±0.02^{b}	0.80±0.01^{b}	0.95±0.00^{a}

Values are given as mean ± S.D. from quintuplicate (n=3).
Different letters in the same column indicate significant differences at p<0.05 (ANOVA, Turkey's HSD test).

Base Formula for Hair Tonic

The characteristics of the base hair tonic formula were shown in Table 5. The essential ingredient was 95% ethanol. The first formula (F1) was an opaque solution with a strong odor, cool sensation, and dryness due to menthol amount. Hence, F2 formula was decreased an amount of menthol resulting in clear solution appearance and low cooling effect. The dry feeling in F1 and F2 formulas were solved by increasing propylene glycol in F3. In order to decrease strong odor, F4 was decreased ethanol amount. In addition, F4 formula was stable through the stability test.

Hair Tonic containing *H. cordata* extract

From the results of phenolic compound and antioxidant activity, the methanolic extract of *H. cordata* exhibited higher activities than other solvent extracts. Therefore, it was chosen as an active ingredient in hair tonic formulation. Two concentrations of *H. cordata* extracts (1% and 5% of the extract solution) were used by adding to the base formula as shown in Table 6. The result showed that the characteristics of hair tonic containing the methanolic *H. cordata* extract had a clear colorless solution in the formula containing 1% of the active ingredient. On the other hand, the formula containing 5% was a clear solution with a slight yellow color. Moreover, the formula containing 1% of the active ingredient had a lower evaporation time than the one containing 5%. These formulas provided a cooling sensation feeling and comfortable when apply to the skin. The pH of two formulas were 5.78 and 5.52, respectively, which were proper to use on the scalp. In the study of stability test, both F4A and F4B showed stability. The freeze-thaw test was tested under various conditions by using extreme and rapid temperature changes. This test was used to simulate event such as encounters during transportation.

Table 5 Characteristics of base formula hair tonic

Parameter	F1	F2	F3	F4
Characteristics	Opaque solution, feel very dry and very cool after use	Clear solution, feel dry after use and low cooling effect	Clear solution, moist and low cooling effect	Clear solution, more moist and low cooling effect
pH	5	5	5	5
Color	Colorless	Colorless	Colorless	Colorless
Smell	Strong menthol, strong ethanol	Menthol, strong ethanol	Menthol, strong ethanol	Menthol
Stability test (Freeze-thaw 3 cycles)	No stable	No stable	No stable	Stable

Table 6 Characteristic of hair tonic containing 1% (F4A), and 5% (F4B) of *H. cordata* extracts

Parameter	F4A	F4B
Characteristic	Clear colorless solution	Clear solution with a slight yellow color
pH	5.78	5.52
Color	L^*= 36.44	L^*= 34.04
	a^*= -0.17	a^*= -0.23
	b^*= 0.40	b^*= 0.51
Evaporation time (min)	1.16±0.13	2.07±0.2
Stability test (Freeze-thaw 3 cycles)	Stable	Stable

Values are given as mean ± S.D (n=3).

CONCLUSIONS

In comparison of methanolic, ethanolic and aqueous extractions of Plu Kaow in antioxidant activity and phenolic compound, the methanolic extract showed the highest yield. The highest TPC, TFC and TPAC values were also found in the methanolic extract. The highest antioxidant activities in DPPH and ABTS were found in the methanolic extract, while the highest FRAP values was found in the ethanolic extract. This study asserted that among three solvent extracts, the methanolic extract was a potential solvent for extracting bioactive compounds of Plu Kaow, as it exhibited excellent values in most of the parameters. Therefore, it was selected to be used as an active ingredient in a hair tonic formulation. The hair tonic was stable through Freeze-thaw test and suitable to use on scalp due to pH of products Hair tonic with 1% methanolic Plu Kaow extract was satisfied in terms of good characteristic and good feeling after use with a light texture. Results suggested that Plu Kaow extract (especially the methanolic extract) has potential to be used as natural antioxidant for preventing anti-aging including hair aging (hair loss, or gray hair).

ACKNOWLEDGEMENTS

The authors were thankful the Scientific & Technological Instrument Center and Center of Excellence in Natural Products Innovation (CENPi), Mae Fah Luang University for all the devices and guidelines throughout the study. This work was financially supported by Mae Fah Luang University.

REFERENCES

Bolduc, C., and Shapiro, J. 2001. Hair care products: waving, straightening, conditioning, and coloring. Clinics in Dermatology, 19(4), 431–436.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., and Ju, Y.H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of food and drug analysis, 22, 296–302.

Dorkbuakaew, N., Ruengnet, P., Pradmeeteekul, P., Nimkamnerd, J., Nantitanon, W., Thitipramote, N. 2016. Bioactive compounds and antioxidant activities of *Camellia sinensis* var. assamica in different leave maturity from Northern Thailand. International Food Research Journal, 23(5), 2291-2295.

Esmaeilzadeh, Z., Shabanizadeh, A., Taghipour, Z., Vazirinejad, R., Mohammad, R.S., and Mohammad, M.T. 2021. Effect of Topical Grape Sap on Apoptosis in Hair Follicle Among Male Rats. Gene, Cell and Tissue. In Press.

Hellström, J.K., Törrönen, A.R., and Mattila, P.H. 2009. Proanthocyanidins in common food products of plant origin. Journal of agricultural and food chemistry, 57(17), 7899–7906.

Houschyar, K.S., Borrelli, M.R., Tapking, C., Popp, D., Puladi, B., Ooms, M., Chelliah, M.P., Rein, S., Pförringer, D., Thor, D., Reumuth, G., Wallner, C., Branski, L.K., Siemers, F., Grieb, G., Lehnhardt, M., Yazdi, A.S., Maan, Z.N., and Duscher, D. 2020. Molecular Mechanisms of Hair Growth and Regeneration: Current Understanding and Novel Paradigms. Dermatology. 236(4),271-280.

Kim, J., Shin J.Y., Choi, Y.H., Jang, M., Nam, Y.J., Lee, S.Y., Jeon, J., Jin, M.H and Lee, S. 2019. Hair Growth Promoting Effect of *Houttuynia cordata* Extract in Cultured Human Hair Follicle Dermal Papilla Cells, Biological and Pharmaceutical Bulletin, 42(10), 1665–1667.

Latha, B.V., and Jeevaratanm, K. 2012. Thirteen-week oral toxicity study of carotenoid pigment from Rhodotorula glutinis DFR-PDY in rats. Indian journal of experimental biology, 50, 645–651.

Luliana, S., Desnita, R., and Rawinda, R. 2018. Formulation of Hair Tonic of Meniran (*Phyllanthus niruri* L.) Ethanol Extract as Hair Grower in Male White Rat (Rattus norvegicus) Wistar Strain. Journal of Pharmaceutical Research, 7(3), 136-145.

Naima, R., Oumam, M., Hannache, H., Sesbou, A., Charrier, B., Pizzi, A. and Charrier-El Bouhtoury, F. 2015. Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan *Acacia mollisima* barks. Industrial Crops and Products, 70, 245-252.

Nuengchamnong, N., Krittasilp, K., and Ingkaninan, K. 2009. Rapid screening and identification of antioxidants in aqueous extracts of *Houttuynia cordata* using LC–ESI–MS coupled with DPPH assay. Food Chemistry, 117(4), 750–756.

Park S, and Lee J. 2021. Modulation of Hair Growth Promoting Effect by Natural Products. Pharmaceutics, 13(12), 2163.

Pyrzynska, K., and Pękal, A. 2013. Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. Analytical Methods, 5, 4288-4295.

Rossi, A., Cantisani, C., Melis, L., Iorio, A., Scali, E. and Calvieri, S. 2012. Minoxidil use in dermatology, side effects and recent patents. Recent Patent Inflammation and Allergy Drug Discovery, 6(2), 130-136.

Rupasinghe, H.P.V., Nair, S. and Robinson, A.R. 2014. Chemopreventive Properties of Fruit Phenolic Compounds and

Their Possible Mode of Actions. Studies in Natural Products Chemistry, 42, 229-266.

Sharopov, F.S., Wink, M., and Setzer, W.N. 2015. Radical scavenging and antioxidant activities of essential oil components--an experimental and computational investigation. Natural product communications, 10(1), 153–156.

Takahashi, T., Kamimura, A., Kagoura, M., Toyoda, M., and Morohashi, M. 2005. Investigation of the topical application of procyanidin oligomers from apples to identify their potential use as a hair growing agent. Journal of Cosmetic Dermatology, 4(4), 245–249.

Takahashi, T., Kamiya, T., and Yokoo, Y. 1998. Proanthocyanidins from grape seeds promote proliferation of mouse hair follicle cells in vitro and convert hair cycle in vivo. Acta dermato venereologica, 78(6), 428–432.

Tok, N.C., Jain, K.K., Sahu, N.P., Varghese, T. and Daniel N. 2017. Evaluation of antioxidative and biological activity of *Houttuynia cordata* extracts. International Journal of Scientific and Research Publications, 7(4).

Thitipramote, N., Pradmeeteekul, P., Nimkamnerd, J., Chaiwut, P., Pintathong, P., Thitilerdecha, N. 2016. Bioactive compounds and antioxidant activities of red (Brown Red Jasmine) and black (Kam Leum Pua) native pigmented rice. International Food Research Journal. 23(1),410-414.

Thitipramote, N., Imsonpang, S., Sukphopetch, P., Pradmeeteekul, P., Nimkamnerd, J., Nantitanon, W., and Chaiyana, W. 2022. Health Benefits and Safety of Red Pigmented Rice (*Oryza sativa* L.): In Vitro, Cellular, and In Vivo Activities for Hair Growth Promoting Treatment. *Cosmetics*. 9(6):111.

Tuyen, T.P., Khang, D.T., Anh, T.T., Trang, P.T. and Xuan, T.D. 2018. Antioxidant properties and total phenolic contents of various extracts from *Houttuynia cordata* Thunb. Tap Chi Sinh Hoc, 40(2se), 149-154.