Research Article

Coffee (*Coffea arabica* L.) Pulp Extracts as a Potential Source of Whitening Agents

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ABSTRACT

The aims of this study were to determine antioxidants and anti-tyrosinase activities of coffee pulp extract, then stability evaluation of lotion from the extract so that coffee pulp may be finally used in cosmetic products. Potential antioxidant activity was evaluated by DPPH, whereas tyrosinase inhibitory activity was investigated against L-DOPA. Furthermore, the physicochemical properties and tyrosinase inhibitory activity of lotions under freezing and thawing conditions were determined. Coffee pulp extracts showed antioxidant activity and expressed anti-tyrosinase activities 40 % inhibition. Besides, their active compounds, caffeine and caffeic acid, revealed strongly inhibit tyrosinase activity with 45- 47 % inhibition. Among lotion formulas tested, Coffee pulp lotion 1 (CPL1) formulas more stable of tyrosinase inhibitory activity than CPL2 formulas under freezing and thawing conditions. Coffee pulp lotion extract at 0.6 % showed effective to inhibitory tyrosinase activity. The physicochemical properties of lotion demonstrated that CPL1 and CPL2 formulas are stable in pH, color, and total polyphenol content (TPC) under freeze-thaw cycles conditions. However, CPL1 demonstrated a higher of TPC and more stable of viscosity properties than CPL2 formulas. These results suggest that anti-tyrosinase properties of the extract might be the action of caffeine and caffeic acid. The CPL1.1 containg the extract at 0.6 % exhibited a greater stability of physicochemical properties and tyrosinase inhibitory activity under freezing and thawing conditions. These finding provided the potential of coffee pulp extracts as new sources of whitening agents and could be applied in cosmetics industry.

Keywords: Coffee pulp, antioxidant activity, tyrosinase inhibitory activity, whitening lotion.

INTRODUCTION

Because over-exposure of the skin to ultraviolet (UV) radiation is the main cause of sunburns, wrinkles, degenerative aging, pigmentary changes, and cancer [1]. UV radiation induced DNA damage in keratinocytes, which triggers of melanin production in melanocytes[2]. Nowadays, sunscreen or skin lightening products were interested to protect or against from UV radiation. Natural plants gained more attention in cosmetics products for their functional properties and less side effects. Several natural plants are a source of biologically active compounds which reveal antioxidant, antibacterial, anti-aging, and anti-melasma properties [3,4]. Additionally, natural plants rich polyphenols may be a potential used as skin protection from UV damage and sunburn [5].

Coffee is a member of the Rubiaceae family, which abundant a powerful nutrient and contains several biological active compounds, such as caffeine, chlorogenic acids, diterpenes, and trigonelline [6]. Many studies have shown many beneficial effects of coffee including anti-diabetes [7], anti-cancer [8], a decreased risk of dementia and alzheimer's disease [9]. Recently, coffee silverskin, coffee-roasting by-product, are suggested to have a potential in cosmetic applications due to their high antioxidant compounds and active ingredients that improve skin aging, anti-inflammatory, antimicrobial, anti-cellulite, and anti-hair loss activities, and UV damage protection [10]. Besides, coffee pulp containing hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acid) also has antioxidant activity, and antibacterial properties [11].

However, there is less evidence of coffee pulp on anti-tyrosinase and developing whitening lotion. Therefore, the aim of this study was to investigate the antioxidants, anti-tyrosinase activities, and stability evaluation of lotion from coffee pulp extract so that coffee pulp may be eventually used in cosmetic products.

MATERIALS AND METHODS

Chemical and reagents

Standard D-(-)-quinic acid, L-(-)-malic acid, citric acid, chlorogenic acid, Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, and D-(-)-quinic acid and potassium hydroxide were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Cetyl alcohol, lanolin, stearic acid, glycerin, methyl paraben,

triethanolamine, citric acid, DL-alphatocopheryl acetate, grape seed oil, Coenzyme Q10, Vitamin B3 were obtained from Namsiang Company limited (Thailand). Formic acid (analytical grade) was purchased from JT Baker (Philipsburg, NJ, USA). Nylon syringe filter 0.45 µm was bought from Lubitech Technologies Ltd. (Shanghai, China). Muller-Hinton agar (MHA) and Muller-Hinton broth (MHB) were purchased from Difco Laboratories, Inc. (New Jersey, USA). Thin layer Chromatography (TLC) silica gel 60 F254 alumina sheet and Dragendorff's reagent spray solution were purchased from Merck (Damstadt, Germany). Ferric chloride was bought from Ajax finechem Pty Ltd. (Aukland, New Zealand). Iron (III) chloride hexahydrate was obtained from POCH S.A. (Sowinskiego, Poland). Ethyl acetate and methanol were purchased from RCI Labscan limited. (Bangkok, Thailand).

Plant materials

Coffee pulp were obtained from Chao-Thai-Pukao Factory. Plants collected from Baan Khun Lao, Wieng Pa Pao, and Chiang Rai, Thailand. The voucher specimen of plant (collection no. 003806) was deposited at Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand.

Sample preparation

Ripe coffee was harvested, washed, and removed coffee bean from pulp with a machine. Pulp was dried by using far infrared rays and blended by using blender machine. The coffee pulp was extracted with hot water (coffee pulp: hot water; 1:5) for 10 min. This extraction was repeated 3 times. The filtered solution was lyophilized to obtain dry powders and the powder extract was stored at -20°C before used.

Determination of total phenolic content

The amount of total phenolics in coffee pulp extracts was assessed according to the Folin-Ciocalteu procedure as modified by Kahkonen and coworkers (1999) [12]. Briefly, a mixture containing 200µL of extract, 1 ml of Folin-Ciocalteu reagent and 0.8 ml of Na_2CO_3 (7.5% w/v), the mixture was incubated at room temperature for 30 min. Absorption at 750 nm was measured by using spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram plant extract.

Determination of antioxidant activity

The scavenging activity of coffee pulp extract on 1,1-diphenyl- 2-picrylhydrazyl (DPPH), was determined according to the method of Blois (1958) [13] with a slight modification. Ten microliters of various concentrations of the extracts or standard antioxidants solution (trolox) was added to 190 µL of 80 µM DPPH in methanol. The mixture was shaken and kept in the dark at room temperature for 30 mins. The DPPH radical scavenging activity was then determined by spectrophotometrically measuring the reduction of the DPPH solution at 517 nm. The DPPH radical scavenging activity was calculated from control according to the following formula: % Inhibition = [(A_{517} control – A_{517} test sample)/ A_{517} control)] x 100 where A_{715} is the absorbance at 715 nm.

Preparation of whitening lotion

Six formulations of coffee pulp extract were prepared as skin whitening lotion. Formula of whitening lotion contains the following ingredients: cetyl alcohol, lanolin, stearic acid, vitamin E, grape seed oil, Co Q10, vitamin B3, glycerin, methyl paraben, triethanolamine, coffee pulp extract, and ascorbic acid as showed in Table 1. The lotion was prepared by the oily phase and then added to the aqueous phase with continuously mixing until homogeneous and stir until room temperature.

Table 1: The formulas of whitening lotion with coffee pulp extract.

Additives/ Extracts (%)	CPL1			CPL2		
	CPL1.0	CPL1.1	CPL1.2	CPL2.0	CPL2.1	CPL2.2
Cetyl alcohol	1	1	1	1	1	1
Lanolin	2	2	2	2	2	2
Stearic acid	10	10	10	10	10	10
Vitamin E	0.1	0.1	0.1	0.1	0.1	0.1
Grape seed oil	0.1	0.1	0.1	0.1	0.1	0.1
Co Q10	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin B3	0.1	0.1	0.1	0.1	0.1	0.1
Glycerin	4	4	4	4	4	4
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Triethanolamine	2.0	2.0	2.0	2.0	2.0	2.0
Water	79.4	78.8	78.2	80.4	79.8	79.2
Coffee pulp extract	-	0.6	1.2	-	0.6	1.2
Ascorbic acid	1	1	1	-	-	-
Total	100	100	100	100	100	100

CPL1, CPL2: Coffee pulp lotion formulation 1 and 2, respectively; CPL1.0, CPL1.1, CPL1.2: Coffee pulp lotion formulation 1.0, 1.1 and 1.2, respectively.; CPL2.0, CPL2.1, CPL2.2: Coffee pulp lotion formulation 2.0, 2.1 and 2.2, respectively.

Stability testing

Stability testing of formulations were stored under standardized test conditions and examined at periodic intervals (freeze-thaw cycles) by exposing the product to freezing temperatures (approximately 4°C) for 12 hours and then placed in a higher temperature (approximately 45°C) for 12 hours for six cycle. Color, viscosity, pH, were monitored during stability testing.

Determination of tyrosinase activity

The determination of tyrosinase activity was examined using L-DOPA as substrate according to the method of Wang Y and coworkers [14] with slight modification. Briefly, 40 µL of coffee pulp extract or lotion was mixed with 40 µL of phosphate buffer solution (0.1 M, pH 6.8), 40 µL of a 2.5 mM L-DOPA, and 80 µL tyrosinase solution (31 u/ml). After incubation at 37˚C for 30 minutes, the activity was determined by measuring the absorbance at 475 nm with reference 700 nm using spectrophotometer. Kojic acid was used as positive control. The percentage of tyrosinase inhibition was calculated as follows: % Inhibition = [(A_{475} control – A_{475} test sample)/ A_{475} control)] x 100 where A_{475} is the absorbance at 475 nm.

Statistical analysis

Data are reported as means ± SD or SEM. *Data were analyzed* by one-way *analysis of variance* (*ANOVA*). Statistical significance was considered at a *p* value of less than *0.05*.

RESULTS AND DISCUSSION

Total polyphenol content and antioxidant activity

The percent yield, total polyphenol contents, and antioxidant activity of coffee pulp extract examined herein are presented in Table 2. The percentage yield of extract expressed on dry weight basis of coffee pulp revealed 28.48 while the total polyphenol content 11.65±0.01 mg GAE/g extract. The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay showed 26.17±0.45 % inhibition. This data agreed with a previous report which exhibited the percent yield of coffee pulp extract (CPE); CPE1, CPE2, and CPE3 in range 13.89 - 28.74%, while total phenolic content with 7.61-17.40 mg/L of gallic acid and also showed antioxidant properties [11]. Phytochemical test of coffee pulp extract found phenolic compounds such as hydroxycinnamic acid and derivatives including alkaloid compounds, trigonelline, caffeine, flavonols and anthocyanidins [11,15].

Table 2: The percent yield, total polyphenol, and antioxidant activity of coffee pulp extract.

	Aqueous extract of Coffee pulp
Percentage yield (W/W %)	28.48 %
Total polyphenol content (mg gallic acid equivalent (GAE)/g. extract)	11.65±0.01
Antioxidant activity by DPPH assay (% inhibition)	26.17±0.45

Mean±SD, (N=3)

Tyrosinase inhibitory activity

To examine the potential of coffee pulp extract as anti-tyrosinase agents, the extract was incubated with L-DOPA. Tyrosinase is the rate-limiting step in melanin production which plays a role in skin protection against UV radiation [16]. Accordingly, the inhibitory effects of coffee pulp extract and their active compounds were evaluated on tyrosinase activity compared with kojic acid herein (Table 3). Kojic acid (0.02 mg/mL) displayed anti-tyrosinase activity with 45.04±1.63 % inhibition whereas the coffee pulp extract (2 mg/mL) exhibited 40.74±1.99 % inhibition which was less effective than kojic acid. Our previous study reported that coffee pulp extract contains quinic acid, ferulic acid, gluconic acid, caffeine, caffeic acid, chlorogenic acid, malic acid, and citric acid [11]. Thus, their active compounds of coffee pulp extract were also to clarify the anti-tyrosinase activity. Caffeine and caffeic acid strongly inhibit tyrosinase activity with 45- 47 % inhibition. These findings demonstrate that anti-tyrosinase properties of the extract may be the effect of caffeine and caffeic acid in the extract. Caffeine has been suggested to have a potential as skin-whitening agents due to its ability to inhibit of intracellular tyrosinase activity and melanin production in B16-F10 melanoma cells [17]. It has been reported that ferulic and caffeic acids effectively suppressed melanin production in the B16 melanoma cells by both substances inhibited casein kinase 2 (CK2)-induced phosphorylation of tyrosinase. Moreover, ferulic acid also directly bind to the enzyme [18].

Table 3: Tyrosinase inhibitory activities of coffee pulp extract and their active compounds.

Extract/Main components (2 mg/mL)	Anti-tyrosinase activity (%inhibition)
Extract	40.74±1.99
D-(-)-Quinic acid	31.85±0.265
Caffeine	45.02±8.84
Caffeic acid	47.77±7.25
Chlorogenic acid	12.55±1.84
L-(-)-Malic acid	12.83±1.33
Citric acid	25.79±4.94
Kojic acid (0.02 mg/mL)	45.04±1.63

Mean±SD, (N=3)

To obtain a suitable formulation of lotion form coffee pulp extract, this study determined the tyrosinase activity and stability of lotion. Tyrosinase inhibitory activity via the freeze-thaw cycle of lotion form coffee pulp extract as shown in Table 4. Ingredients of all formulas: coffee pulp lotion formulation 1(CPL1) and 2 (CPL2) as shown in Table 1 are similar except different concentrations of the extract and presence or absence of ascorbic acid. Our study demonstrated that lotion formulation of all CPL1 more stable of tyrosinase inhibitory activity than CPL2 under freezing and thawing conditions (Table 4). It possible that the presence of coffee extract with ascorbic acid contribute to the stability of lotion. Furthermore, not all the difference anti-tyrosinase actions between the extract at 0.6 % and 1.2 %. Therefore, the concentration of the extract at 0.6 % may be effective in anti-tyrosinase activities. Additionally, grape seed extracts were suggested to have a potential as skin-lighting agents due to more effective on anti-tyrosinase activity and its high total phenolic content [19]. Ascorbic acid and its derivatives have been reported to decrease in the tyrosinase activity and melanin content by the reduction of intracellular reactive oxygen species (ROS) [20].

Table 4: Tyrosinase inhibitory activity of lotion from coffee pulp extract in normal and freeze-thaw cycles conditions.

Conditions	Anti-tyrosinase activity (IC_{50} mg/mL)					
	CPL1			CPL2		
	CPL1.0	CPL1.1	CPL1.2	CPL2.0	CPL2.1	CPL2.2
Normal	3.15±1.12	3.30±1.12	3.16±1.10	18.22±3.29	2.92±1.52	4.62±1.33
Freeze-thaw cycle	3.56±1.09	3.42±1.17	3.87±1.10	12.06±2.31	5.54±2.63	5.73±1.39

Mean±SEM, (N=3)

Moreover, the physicochemical properties of lotion were determined including pH, viscosity, color, and total phenolic content (TPC) (Table 5). This study demonstrated that pH, color, and TPC of CPL1 and CPL2 formulas are likely to be stable when compare between normal and freeze-thaw cycles conditions except the viscosity trend to reduce in freeze-thaw cycles conditions. However, CPL1 formulas revealed a higher of TPC and more stable of viscosity properties than CPL2 formulas. These data are possible that the combination of different extract concentrations and other ingredients in lotion may lead to additive or synergistic or antagonistic effects.

Table 5: Physicochemical stability of lotion with coffee pulp extract in normal and freeze-thaw cycles conditions.

Formulations/ Testing	Conditions	CPL1			CPL2		
		CPL1.0	CPL1.1	CPL1.2	CPL2.0	CPL2.1	CPL2.2
pH	Normal	6.34	6.12	5.88	7.52	6.66	6.90
	Freeze-thaw cycle	6.90	6.68	6.40	7.31	7.25	7.04
Viscosity (10 rpm)	Normal	3522	3324	2768	3165	9297	8702
	Freeze-thaw cycle	2828	1687	2292	3008	4366	3979
Color	Normal	white	Light brown	brown	white	Light brown	brown
	Freeze-thaw cycle	white	Light brown	brown	white	Light brown	brown
Total phenolic content (Gallic acid equivalence (GAE) mg/g extract)	Normal	55.38 ± 2.53	53.39 ± 5.55	52.68 ± 4.89	23.95 ± 8.66	25.34 ± 0.32	27.58 ± 0.23
	Freeze-thaw cycle	52.05 ± 7.55	53.61 ± 2.96	54.42 ± 1.11	28.41 ± 0.59	25.93 ± 0.07	29.97 ± 1.19

Mean±SD

CONCLUSION

In the present study demonstrate that coffee pulp extracts possessed antioxidant and anti-tyrosinase activities. Our results indicate that anti-tyrosinase properties of the extract may be the action of caffeine and caffeic acid. In addition, the CPL1 formulas especially CPL1.1 exhibited more stable in physicochemical properties and tyrosinase inhibitory activity under freezing and thawing conditions. This research provided that the coffee pulp extracts can be used as a potential as new sources of whitening agents. However, a clinical trial for skin whitening lotion needs to be elucidated.

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