WALAILAK JOURNAL

Life Sciences

http://wjst.wu.ac.th https://doi.org/10.48048/wjst.2021.8943

Bioactivity Determination and Development of Oil in Water Emulsion Containing *Cassia fistula* Bark Extract

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Received: 17 September 2019, Revised: 27 March 2020, Accepted: 17 April 2020

Abstract

The determination of active contents, free radical scavenging, and tyrosinase inhibition activity of *Cassia fistula* extracts for skincare development is presented in this study. *C. fistula* was extracted by 95 % ethanol. The extracts were then determined for total phenolic content, phytochemical constituents, free radical scavenging, and tyrosinase inhibition activity. The formulation containing the selected *C. fistula* extract was prepared and examined for this formulation's bioactivities and stability. *C. fistula* extract from the bark provided the highest total phenolic content and free radical scavenging activity of 22.35±1.38 mgGAE/g and the IC_{50} value of 7.90±0.10 µg/mL, respectively. *C. fistula* extract from the bark was observed having tyrosinase inhibition activity at the IC_{50} value of 1.71×10^{3}±0.01 µg/mL and selected to be an active ingredient in oil in water emulsion. At 4±2 °C storage, the formulation containing *C. fistula* extract from bark showed the highest stability without changing the color, odor, homogeneity, while pH and viscosity were slightly changed. The formulation's bioactivities containing *C. fistula* extract from bark were slightly decreased in all temperatures at the end of the stability test. In this study, *C. fistula* extract from the bark can potentially inhibit tyrosinase in a free radical scavenging activity. Therefore, it may be an appropriate choice for skincare products that aim to provide whitening effects.

Keywords: *Cassia fistula*, Tyrosinase inhibition, Free radical scavenging, Phytochemical analysis, Formulation

Introduction

Cassia fistula, from the family Fabaceae, is found in numerous Asian countries such as Thailand, China, Myanmar, and India. This is a medicinal plant, which is rich in phenolics containing catechin, epicatechin, kaempferol, ellagic acid, and rhein [1]. The hydro-alcoholic extract from the fruit pulp of *C. fistula* displayed an in vitro free radical scavenging activity, certainly due to its relatively rich content of phenolic compounds, fatty acids, flavonoids, tannins, and glycosides [2,3]. The effect of *C. fistula* in traditional medicine is known to be involved with treating various disorders including, skin diseases, leprosy, haematemesis, pruritus, and diabetes [2,4]. In previous studies, *C. fistula* flower extract was found to possess antioxidant, anticancer, antibacterial, antifungal, and anti-diabetic properties [5,6]. The pulp has been recognized for its anti-diabetic properties, which are applied in treatments of gout and rheumatism [7]. The leaves and ripe pods have been traditionally used as a laxative [8,9]. The flower, seed, fruit, and pulp have been used to treat skin diseases, including leprosy [4]. Recently, it was shown that *C. fistula* pods extract, which was rich in certain polyphenols, exhibited the good potential to treat the skin of adult Asian patients with melasma compared to placebo, due to its capacity to significantly decrease the tyrosine activity-mediated melanin level [10]. This study aimed to determine the active

contents and bioactivities of *C. fistula* extracts from various parts. The stability and bioactivities of the formulation containing *C. fistula* extract were further investigated for the development of skincare products.

Material and methods

Preparation and extraction of *C. fistula*

The various parts of *C. fistula* were collected from the University of Phayao, Thailand. The voucher specimens of the plant samples were authenticated by a botanist and deposited at the School of Pharmaceutical Sciences, University of Phayao, Thailand. Fresh samples were air-dried and ground into powder. After that, the powder was soaked in 95 % ethanol at room temperature for 72 h following the proper ratio as shown in **Table 1**. The extract was filtered by using Whatman No.1 filter paper and evaporated under reduced pressure by a rotary evaporator. The dried extracts were kept at 4 °C until further analysis.

Table 1 The ratio of raw materials from *C. fistula* and solvent for extraction.

Parts	Weight of raw materials (g)	Volume of 95 % ethanol (L)
Flower	400	2.5
Pod	400	1.0
Bark	400	1.2
Leaves	400	1.5

Table 2 The ingredients of the formulation for incorporated with *C. fistula* extract.

Ingredient	INCI name	Function	Formulation (% w/w)
Oil phase			
SFE839	Cyclopentasiloxane (and) Dimethicone (and) Dimethicone/Vinyl Dimethicone Crosspolymer	Silicone (cross polymer)	6
Cetiol OE	Dicaprylyl Ether	Emollient	3
Tween20	Polysorbate 20 (non ionic surfactant)	Emulsifier	2
Sepiplus	Acrylamide/Ammonium Acrylate Copolymer & Polyisobutene & Polysorbate 20	Copolymer	0.85
Water phase			
C. fistula extract	-	Active ingredient	0.01
Butylene glycol	1,3 butylene glycol	Humectant	4
Glycerin	Glycerin	Co-solvent	3
Spectrastat	Caprylhydroxamic Acid (and) Caprylyl Glycol (and) Glycerin	Preservative	0.7
DI water	Deionized water	Solvent	q.s. to 100

Phytochemical analysis

The extracts were investigated through a phytochemical analysis of alkaloids, anthraquinones, anthocyanins, flavonoids, polyphenols, steroids, tannin, and terpenoids. The extract (0.05 g) was added 2 mL of 10 % H_2SO_4 and filtered for alkaloids. A volume of 1 mL of filtrate was added 5 drops of Dragendorff's reagent by the test tube's sides. Brownish-red precipitation indicated the presence of alkaloids [11]. For anthraquinones, the extract (0.05 g) was hydrolyzed with 2 mL of 10 % HCl and warmed with a water bath for 5 min. A volume of 1 mL of extract solution was added 1 mL of dichloromethane and 1 mL of 10 % NH_3 solution. The formation of rose pink coloration indicated the presence of anthraquinones [12]. For anthocyanins, the extract (0.05 g) was added 2.5 mL of 95 % ethanol. A volume of 1 mL of supernatant was added to 0.1 mL of 2N HCl and 0.1 mL of 10 % NH_3 solution. The appearance of a pink-red that turns blue-violet indicated the presence of anthocyanin [12]. For flavonoids, the extract (0.05 g) was added to 2 mL of 95 % ethanol. A volume of 1 ml of the extract solution was added magnesium ribbon and 0.5 mL of the concentrated HCl for flavonoids investigation. The pink-red color indicated the presence of flavonoids [13]. The extract (0.05 g) was added 2 mL of 80 % ethanol, 5 mL of 10 % lead acetate, and filtrated for steroids. The filtrates were dried by evaporating dish. The samples were added with 3 drops of acetic anhydrous and conc. H_2SO_4, respectively. The blue or green color indicated the presence of steroids [14]. The extract (0.05 g) was added to 2 mL distilled water and filtered for tannins and polyphenols. A volume of 1 mL of filtrate was added 10 drops of gelatin solution, 1 % $FeCl_3$ solution, $Ca(OH)_2$ solution, bromine water, then the presence of tannin indicated the formation of a white, blue, grey, and yellow precipitate, respectively [15]. For terpenoids, the extract (0.05 g) was added to 2 mL of dichloromethane. Conc. H_2SO_4 (1 mL) was carefully added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids [16].

Total phenolic content determination

Total phenolic content was measured by Folin-Ciocalteu assay using 96-well microplate with slight modification [6]. A volume of 20 µL of extracts solution dissolved in ethanol was mixed in a 96-well plate with 80 µL of 10 % Na_2CO_3 and 100 µL of 1 N Folin-Ciocalteu reagent. The plate was covered and incubated at 25±2 °C for 1 h in the dark. The absorbance at 700 nm was measured by the microplate reader (BioTek Synergy H1, VT, USA). The total phenolic content was presented as mg of gallic acid equivalents per gram of the extract (mgGAE/g).

Free radical scavenging assay

Free radical scavenging activity was evaluated using 2, 2 diphenyl-1-picrylhydrazyl (DPPH) [17]. In brief, the reaction consisting of 100 µL of 0.1 mM DPPH in ethanol and 100 µl of various sample concentrations was added in 96-well plates. The absorbance of the plate was measured at 517 nm after the incubation for 30 min in the dark at 25±2 °C. The samples were calculated for free radical scavenging activity as follows: % scavenging activity = [(ABcontrol-ABsample)/ABcontrol]×100, where ABcontrol was the absorbance of the control and ABsample was the absorbance of the sample. The IC_{50} value was obtained from the sample concentration at 50 % free scavenging activity.

Tyrosinase inhibition assay

Tyrosinase inhibition activity was evaluated by using L-DOPA and mushroom tyrosinase [18]. In 96-well plates, a volume of 20 µL of the samples, 140 µL of 20 mM phosphate buffer at pH 6.8 and 20 µL of tyrosinase solution in phosphate buffer (30 units/ml) were added. The plates were allowed to react at 25±2 °C for 10 min and then added 20 µL of 0.8 mM L-DOPA in phosphate buffer. The absorbance at 475 nm was measured after the incubation at 25±2 °C for 20 min. The samples were calculated through a tyrosinase inhibition activity as follows: % tyrosinase inhibition = [(ABcontrol-ABsample)/ABcontrol]×100, where ABcontrol was the absorbance of the control and ABsample was the absorbance of the sample. The IC_{50} value of tyrosinase inhibition was obtained from the sample concentration at 50 % tyrosinase inhibition activity.

Preparation of skin care product containing *C. fistula* extract

Oil in the water (O/W) emulsion was formulated as presented in **Table 2**. *C. fistula* extract which gave the high bioactivity was selected and incorporated in this formulation at 0.01 %. Oil phase was prepared by mixed SFE839, Cetiol OE, Tween20, and Sepiplus with constant stirring. Water phase containing *C. fistula* extract, butylene glycol, glycerin, and Spectrastat was added and mixed with constant stirring. Oil phase was added to water phase then allowed cooling with constant stirring.

Physicochemical characteristic test of the formulation

The appearances of the formulation were observed in color, odor, and homogeneity. pH of formulation was measured by pH meter which was calibrated using standard buffer solution. Viscosity of the formulation was determined by Brookfield viscometer (BioTek Synergy H1, VT, USA) at 40 rpm.

Bioactivities determination of the formulation

The formulation containing *C. fistula* extract (0.15 g) was added 1 mL of 95 % ethanol. The mixture was mixed and centrifuged at 6,000 rpm for 20 min. Free radical scavenging activity and tyrosinase inhibition were determined as previously described.

Stability test of the formulation

The stability of formulations was conducted by keeping at 4±2, 25±2 and 45±2 °C for 28 days and physicochemical characteristics were observed at the end of experiment [19]. The stability of formulation viscosity was determined by the percentage change from day 0 (% change). The values that were not greater than 10 % indicated the high stability of formulation.

Data analysis

All assays were performed in triplicate with separated experiments. The data was calculated as mean ± SD. The one-way analysis of variance (ANOVA) was used to evaluate the results at $p < 0.05$. Statistical analysis was performed using the SPSS program for Windows.

Results and discussion

Percentage yield, physical characteristics and phytochemical analysis of *C. fistula* extracts

The percentage yields, physical characteristics, and phytochemical analysis of *C. fistula* extracts are summarized in **Tables 3**. Most extracts were brown color and solid form, whereas the leave extract was green color and has a solid form. The extracts obtained from ethanolic extraction may contain both polar and non-polar compounds. The extracts gave percentage yields ranging from 5.49 to 15.99 %. The highest percentage yield was from the bark extract. The phytochemical analysis of *C. fistula* extracts showed that alkaloids, tannin, and polyphenol were observed in flower, pod, bark, and leave extracts, while flavonoid was only observed in bark extract. Terpenoids was found in flower and pod extracts. In this study, anthraquinones, anthocyanins, and steroids were not found in *C. fistula* extracts.

Total phenolic content determination

Table 4 presents the total phenolic content of *C. fistula* extracts. The total phenolic content of flower, pod, bark, and leave extracts were 5.40±0.40, 7.22±0.55, 22.35±1.38 and 7.68±0.50 mgGAE/g, respectively. The total phenolic content from the bark extract was significantly higher when compared with flower, pod, and leaves extracts ($p < 0.05$).

Bioactivities of *C. fistula* extracts

In **Table 4**, free radical scavenging activity of the extracts from flower, pod, bark, and leave of *C. fistula* exhibited the IC_{50} values of 51.50±6.40, 36.10±2.90, 7.90±0.10 and 60.50±1.70 µg/mL, respectively. The bark extract exhibited the significantly highest free radical scavenging activity as compared with other extracts ($p < 0.05$), which was 0.2658 folds of gallic acid. In addition, IC_{50} values of tyrosinase inhibition from the extracts of flower, pod, bark, and leave were 0.54×10^{3}±0.01,

$2.13\times10^3\pm0.01$, $1.71\times10^3\pm0.01$ and $2.18\times10^3\pm0.02$ µg/mL, which were 0.4074, 0.1032, 0.1286 and 0.1009 folds of kojic acid, respectively. Thus, the significant highest melanogenesis inhibition from flower extract can be anticipated as compared with the other extracts ($p < 0.05$). The bark extract was selected and considered as active ingredient for the developed skin care product based on its bioactivities, including the highest free radical scavenging activity, total phenolic content, and high tyrosinase inhibition activity. The formulation containing *C. fistula* bark extract was further investigated for the bioactivities and physicochemical stability.

Table 3 The percentage yields and phytochemical analysis of *C. fistula* extracts.

Sample	Appearance and color	Percentage yield (%)	Phytochemical analysis						
			Alkaloids	Anthraquinones	Anthocyanins	Flavonoids	Steroids	Tannin and polyphenols	Terpenoids
Flower	Solid in brown	7.69	+	-	-	-	-	+G, +F, +B	+
Pod	Solid in brown	5.49	+	-	-	-	-	+F, +L	+
Bark	Solid in brown	15.99	+	-	-	+	-	+G, +F, +L	-
Leaves	Solid in green	6.67	+	-	-	-	-	+F	-

Note: Percentage yield (%) = [Dried extract weight (g)/ Dried *C. fistula* weight (g)]×100, + represents the present of the phytochemical constituents in the extracts, +G represents the positive result by gelatin solution test, +F represents the positive result by ferric chloride test, +L represents the positive result by lime water test, +B represents the positive result by bromine water test, - represents no detection.

Table 4 Total phenolic content, free radical scavenging and tyrosinase inhibition activity of *C. fistula* extracts

Sample	Total phenolic content (mgGAE/g)	IC_{50} of free radical scavenging (µg/mL)	IC_{50} of tyrosinase inhibition (µg/mL)
Flower	5.40±0.40	51.50±6.40	$0.54\times10^3\pm0.01^{(c)}$
Pod	7.22±0.55	36.10±2.90	$2.13\times10^3\pm0.01$
Bark	$22.35\pm1.38^{(a)}$	$7.90\pm0.10^{(b)}$	$1.71\times10^3\pm0.01$
Leave	7.68±0.50	60.50±1.70	$2.18\times10^3\pm0.02$
Gallic acid	-	2.10±0.04	-
Kojic acid	-	-	$0.22 \times 10^3 \pm 0.01$

Note: (a) indicated significant difference compared with flower, pod, and leave extracts ($p < 0.05$); (b) indicated significant difference compared with flower, pod, and leave extracts ($p < 0.05$); (c) indicated significant difference compared with pod, bark, and leave extracts ($p < 0.05$).

Physicochemical stability of formulation containing *C. fistula* bark extracts

The physicochemical stability of the formulation containing *C. fistula* bark extract was kept at 4±2, 25±2, 45±2 °C was presented in **Table 5**. After 28 days storage at 4±2 °C, the formulation was observed as having no changes in color, odor, texture, and homogeneity, while pH and viscosity were slightly changed. The formulations were kept at 45±2 °C. After 5 days, the formulations were dramatically changed in texture, which decreased viscosity and could not be detected at 40 rpm. These results indicated that the formulation containing *C. fistula* bark extract was highly stable at 4±2 °C storage.

Free radical scavenging activity determination of formulation containing *C. fistula* bark extract

At initial time (D0), the formulation containing *C. fistula* bark extract exhibited the percentage of free radical scavenging activity at 79.81±0.58 %. After 14 days storage (D14), the formulation kept at 4±2, 25±2 and 45±2 °C gave the percentage of free radical scavenging activity at 79.55±0.25, 77.69±0.45 and 69.64±0.21 %, respectively. After 28 days storage (D28), the formulation kept at 4±2, 25±2 and 45±2 °C gave the percentage of free radical scavenging activity at 74.67±0.39, 70.68±0.55 and 64.25±0.26 %, respectively. In the end of the experiment, the formulations containing *C. fistula* bark extract had a decreased on free radical scavenging activity at all temperatures. However, the formulation kept at 4±2 °C was observed to have the highest free radical scavenging as presented in **Table 6**.

Tyrosinase inhibition activity determination of formulation containing *C. fistula* bark extract

For tyrosinase inhibition, the formulation containing *C. fistula* bark extract exhibited the percentage of tyrosinase inhibition activity of 12.67±1.65 % at initial time (D0). After 14 days storage (D14), the formulation kept at 4±2, 25±2 and 45±2 °C gave the percentage of tyrosinase inhibition activity at 11.00±0.27, 9.49±0.25 and 6.99±0.11 %, respectively. After 28 days storage (D28), the formulation kept at 4±2, 25±2 and 45±2 °C gave the percentage of tyrosinase inhibition activity at 7.51±0.18, 6.48±0.11 and 4.49±0.19%, respectively. At the end of the experiment, the formulations containing *C. fistula* bark extract decreased in tyrosinase inhibition activity at all temperatures. The formulation kept at 4±2 °C was observed the highest tyrosinase inhibition activity as presented in **Table 6**.

Table 5 Physicochemical stability of the formulation containing *C. fistula* bark extracts at 0.01 % kept at 4±2, 25±2 and 45±2 °C for 28 days.

Temperature (°C)	Day	Physical characteristics					
		Color	Odor	Texture	Homogeneity	pH	Viscosity at 40 RPM (%change)
4±2	0	0	0	0	0	6.07	cp 417.8
	3	0	0	0	0	6.08	cp 404.8 (3.11 %)
	5	0	0	0	0	6.11	cp 381.5 (8.69 %)
	7	0	0	0	0	6.13	cp 396.9 (5.00 %)
	14	0	0	0	0	6.12	cp 386.2 (7.56 %)
	21	0	0	0	0	6.14	cp 383.1 (8.31 %)
	28	0	0	0	0	6.12	cp 388.1 (7.11 %)
25±2	0	0	0	0	0	6.07	cp 417.8
	3	0	0	0	0	6.19	cp 293.5 (29.75 %)
	5	+	+	+	+	6.27	cp 207.5 (50.33 %)
	7	+	+	+	+	6.35	-
	14	+	+	+	+	6.68	-
	21	+	+	+	+	6.67	-
	28	+	+	+	+	6.68	-
45±2	0	0	0	0	0	6.07	cp 417.8
	3	++	++	++	++	6.20	cp 76.0 (81.81 %)
	5	++	++	++	++	6.53	-
	7	++	++	++	++	6.61	-
	14	++	++	++	++	6.63	-
	21	++	++	++	++	6.69	-
	28	++	++	++	++	6.68	-

Note: - = Not detected; 0 = The formulation containing *C. fistula* extracts is stable; + and ++ = The intensity of instability of the formulation containing *C. fistula* extracts.

Table 6 Free radical scavenging and tyrosinase inhibition activity of the formulation containing *C. fistula* extracts at 0.01 % kept at 4±2, 25±2 and 45±2 °C for 28 days.

Day	% Free radical scavenging			% Tyrosinase inhibition		
	4±2 °C	25±2 °C	45±2 °C	4±2 °C	25±2 °C	45±2 °C
0 (D0)	-	79.80±0.58	-	-	12.67 ± 1.65	-
14 (D14)	79.55±0.25	77.69±0.45	69.64±0.21	11.01±0.27	9.49 ± 0.25	6.99±0.11
28 (D28)	74.67±0.39	70.68±0.55	64.25±0.26	7.51±0.18	6.48 ± 0.11	4.49±0.19

Discussion

For the extraction process, since the physical characteristics from various parts of *C. fistula* were different, the proper volumes of 95 % ethanol were adjusted depending on plant materials to obtain high percentage yields of the extracts. Previous research showed that major phytochemicals represented in the *C. fistula* extract were phenolic compounds and flavonoids. The main phenolic compounds in the *C. fistula* flower extract were protocatechuic acid, vanillic acid, chlorogenic acid, and ferulic acid [20]. Moreover, the various types of flavonoids, including kaempferol, rhein, fistulin, alkaloids, and triterpenes, were also found in the flower extracts [21]. A pulp consisted of anthraquinone glycosides, sennosides A and B, rhein and its glucoside, barbaloin, aloin, formic acid, pectin, and tannin. Leaves and flowers contained anthraquinone, tannin, oxyanthraquinone, rhein, and volatile oils. The *C. fistula* extracts, which contained a rich source of tannins, flavanoids, and glycosides might be medicinally important and nutritionally valuable [22]. Siddhuraju et al. showed the yield percentage and total phenolic content of the extracts from different parts of *C. fistula*. Among the different extracts, the highest and lowest extraction yields were observed in the fruit pulp and leave extract, respectively [23]. For anthraquinone, it was not detected in these extracts. The collection of raw materials from various places may affect the degradation of anthraquinones, which could not be detected by a preliminary phytochemical screening test. In this study, the bark extract of *C. fistula* gave the highest percentage yield, total phenolic contents, and free radical scavenging activity. The antioxidant mechanism of the phenolic compounds was the scavenging activity, which is done by transferring the single electron [24]. The presence of phenolic compounds, anthraquinones, xanthones, proanthocyanidins, and flavonols could be the reason for its reasonably free radical scavenging activity in the extracts of the stem bark. The elevated free radical scavenging activity of the stem bark extract might be due to the presence of tannins and flavonoid [23]. The previous study showed that the aqueous and methanolic extracts of *C. fistula* bark at the concentration of 250 µg/mL showed promising free radical scavenging activity by DPPH assay percentage inhibition of 50.13±0.01 and 52.12±0.01 %, respectively [25]. The *C. fistula* fruit pulp's hydro-alcoholic extracts at various concentrations (5 - 60 µg/mL) exhibited free radical scavenging activity of DPPH in a dose-dependent manner. The extract at a 10 and 60 µg/mL concentration showed a percentage inhibition of 9.67±0.28 and 17.03±0.27 %, respectively [26]. Tyrosinase was an enzyme that involved in the rate-limiting step of the melanin production, including the hydroxylation of tyrosine to β-3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to DOPA quinone [27]. This enzyme was able to convert tyrosine to melanin [28]. Overproduction of the melanin and melanin precursors due to the increased of free radical generation may induce hyperpigmentation. It was most likely that the antioxidant played an important role in regulating melanin biosynthesis [29]. From the previous study, *C. fistula* flower extract incubated with tyrosinase could successfully inhibit enzyme activity in a dose-dependent manner (35 - 42%) at a concentration of 50 - 200 µg/mL [20]. Plant extracts contained polyphenolic compounds, such as tannin and flavonoids, reacted with reactive oxygen species and inhibited tyrosinase activity [30]. The hydroxyl group of the condensed tannins was also involved in tyrosinase inhibition activity. This substitution pattern was also reported to be responsible for the tyrosinase inhibition activity of flavonoids by binding to the enzyme's active site. Additionally, some tannins could decrease melanin production by directly

scavenged o-quinones [31]. Therefore, the inhibition of tyrosinase activity from phytochemical compounds tended to induce skin whitening due to reduced melanin synthesis. Although the *C. fistula* formulation seemed to be stable at 4 °C, the tyrosinase inhibition activity of the formulation was slightly decreased at the end of the study. The bioactivities of this formulation were slightly decreased due to the instability of phytochemicals. This formulation prepared by oil-in-water (o/w) emulsions may induce oxidative and hydrolysis reaction to tyrosinase inhibition's active compounds. From a previous study, chamomile extracts stored at room temperature and at 4 °C demonstrated degradation of flavonoids in a stability test [32]. Tannins were also oxidized to produce quinones that were highly reactive electrophilic and toxic molecules [33]. However, emulsions could be formulated with no aqueous phase to produce an anhydrous emulsion system that could offer a stable vehicle for delayed degradation of active compounds sensitive to hydrolysis or oxidation. Moreover, adding a stabilizer or antioxidant into the formulation might enhance the stability of active contents [34].

Conclusions

This study aimed to investigate active contents, bioactivities, and stability study of *C. fistula* extracts from various parts for the development of a skincare product. In this study, *C. fistula* bark extract exhibited the highest total phenolic content and free radical scavenging activity of 22.35±1.38 mgGAE/g and the IC_{50} value of 7.90±0.10 µg/mL, respectively, while tyrosinase inhibition activity gave the IC_{50} value of 1.71×10^{3}±0.01 µg/mL. The bark extract, which gave the cosmetic application the potential, was selected to prepare the skincare product. At the stability test's initial time, the formulation containing *C. fistula* bark extract gave the percentage of free radical scavenging and tyrosinase inhibition activity at 79.80±0.58 and 12.68±1.65 %, respectively. For stability study, the formulation containing *C. fistula* extract from bark kept at 4±2 °C for 28 days were slightly decreased the bioactivities. This result has suggested the cosmetic potential of anti-oxidation and tyrosinase inhibition of *C. fistula* bark extract for further developments as the whitening products.

Acknowledgements

The authors would like to thank the School of Pharmaceutical Sciences, University of Phayao for facilities in this research.

References

[1] Y Kashiwada, H Iizuka, K Yoshioka, RF Chen, GI Nonaka and I Nishioka. Tannins and related compounds. XCIII: Occurrence of enantiomeric proanthocyanidins in the leguminosae plants, *Cassia fistula* L. and *C. javanica* L. *Chem. Pharm. Bull.* 1990; **38**, 888-93.
[2] G Manonmani, V Bhavapriya, S Kalpana, S Govindasamy and T Apparanantham. Antioxidant activity of *Cassia fistula* (Linn.) flowers in alloxan induced diabetic rats. *J. Ethnopharmacol.* 2005; **97**, 39-42.
[3] NR Bhalodia, PB Nariya, R Acharya and VJ Shukla. *In vitro* antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn. *Ayu* 2013; **34**, 209-14.
[4] N Bhalodia, P Nariya, R Acharya and VJ Shukla. *In vitro* antibacterial and antifungal activities of *Cassia fistula* Linn. fruit pulp extracts. *Ayu* 2012; **33**, 123-9.
[5] V Duraipandiyan and S Ignacimuthu. Antibacterial and antifungal activity of *Cassia fistula* L.: An ethnomedicinal plant. *J. Ethnopharmacol.* 2007; **112**, 590-4.
[6] A Luximon-Ramma, T Bahorun, MA Soobrattee and OI Aruoma. Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of *Cassia fistula*. *J. Agr. Food Chem.* 2002; **50**, 5042-7.
[7] O Hänninen, K Kaartinen, AL Rauma, M Nenonen, R Törrönen, S Häkkinen and H Adlercreutz. Antioxidants in vegan diet and rheumatic disorders. *Toxicology* 2000; **155**, 45-53.

[8] P Mangmeesri, K Wonsuphasawad, W Viseshsindh and W Gritsanapan. The comparison between the laxative effectiveness of *Cassia fistula* pod pulp extract and *Cassia angustifolia* in Thai constipated patients. *Plant Med.* 2014; **80**, 1-12.
[9] A Sakulpanich and W Gritsanapan. Determination of anthraquinone contents in *Cassia fistula* leaves for alternative source of laxative drugs. *Planta Med.* 2009; **75**, 11.
[10] BA Khan, N Akhtar, I Hussain, KA Abbas, A Rasul and A Alergologii. Whitening efficacy of plant extracts including Hippophae rhamnoides and *Cassia fistula* extracts on the skin of Asian patients with melasma. *Adv. Dermatol. Allergol.* 2013; **30**, 226-32.
[11] M Shyam Krishnan, P Dhanalakshmi, G Yamini Sudhalakshmi, S Gopalakrishnan, A Manimaran, S Sindhu, E Sagadevan and P Arumugam. Evaluation of phytochemical constituents and antioxidant activity of Indian medicinal plant *Hydnocarpus pentandra. Int. J. Pharm. Pharm. Sci.* 2013; **5**, 453-8.
[12] R Sawant and AG Godghate. Qualitative phytochemical screening of rhizomes of *Curcuma longa* Linn. *Int. J. Sci. Environ. Technol.* 2013; **2**, 634-41.
[13] SE Allen, HM Grimshaw, JA Parkinson and C Quarmby. *Chemical analysis of ecological materials*. Blackwell Scientific Publications, Oxford, 1974, p. 565.
[14] HO Edeoga, D Okwu and BO Mbaebie. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 2005; **4**, 685-8.
[15] GE Trease and WC Evans. *Pharmacognosy*. 13th eds. Bailliere Tindall, London, 1989, p. 176-80.
[16] G Ayoola, H Coker, S Adesegun, A Adepoju-Bello, K Obaweya, E Ezennia and T Atangbayila. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop. J. Pharm. Res.* 2008; **7**, 1019-24.
[17] K Singsai, T Akaravichien, V Kukongviriyapan and J Sattayasai. Protective effects of streblus asper leaf extract on H_2O_2-induced ROS in SK-N-SH cells and MPTP-induced Parkinson's disease-like symptoms in C57BL/6 mouse. *Evid. Based Complement. Alternat. Med.* 2015; **2015**, 1-6.
[18] K Tantrakarnsakul. 2010, Bioactive compounds from the root bark of artocarpus lakoocha. Ph. D. Dissertation. Chulalongkorn University, Bangkok, Thailand.
[19] A Aswal, M Kalra and A Rout. Preparation and evaluation of polyherbal cosmetic cream. *Pharm. Lett.* 2013; **5**, 83-8.
[20] P Limtrakul, S Yodkeeree, P Thippraphan, W Punfa and J Srisomboon. Anti-aging and tyrosinase inhibition effects of *Cassia fistula* flower butanolic extract. *BMC Complem. Altern. M.* 2016; **16**, 497.
[21] T Bahorun, VS Neergheen and OI Aruoma. Phytochemical constituents of *Cassia fistula. Afr. J. Biotechnol.* 2005; **4**, 1530-40.
[22] M Danish, P Singh, G Mishra, S Srivastava, K Jha and RL Khosa. *Cassia fistula* Linn. (Amulthus)-an important medicinal plant: A review of its traditional uses, phytochemistry and pharmacological properties. *J. Nat. Prod. Plant Resour.* 2011; **1**, 101-18.
[23] P Siddhuraju, P Mohan and K Becker. Studies on the antioxidant activity of Indian laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem.* 2002; **79**, 61-7.
[24] CW Choi, SC Kim, SS Hwang, BK Choi, HJ Ahn, MY Lee and SK Kim. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Sci.* 2002; **163**, 1161-8.
[25] R Ilavarasan, M Malika and SVenkataraman. Anti-inflammatory and antioxidant activities of *Cassia fistula* Linn bark extracts. *Afr. J. Tradit. Complement Altern. Med.* 2005; **2**, 70-85.
[26] NR Bhalodia and VJ Shukla. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. *J. Adv. Pharm. Technol. Res.* 2011; **2**, 104.
[27] GE Costin and VJ Hearing. Human skin pigmentation: Melanocytes modulate skin color in response to stress. *FASEB J.* 2007; **21**, 976-94.
[28] YSC Bae-Harboe and HY Park. Tyrosinase: A central regulatory protein for cutaneous pigmentation. *J. Invest. Dermatol.* 2012; **132**, 2678-80.
[29] JM Wood and KU Schallreuter. Studies on the reactions between human tyrosinase, superoxide anion, hydrogen peroxide and thiols. *Biochim. Biophys. Acta* 1991; **1074**, 378-85.

[30] P Maisuthisakul and MH Gordon. Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. *Food Chem.* 2009; **117**, 332-41.
[31] XX Chen, Y Shi, WM Chai, HL Feng, JX Zhuang and QX Chen. Condensed tannins from *Ficus virens* as tyrosinase inhibitors: Structure, inhibitory activity and molecular mechanism. *PLoS One* 2014; **9**, 1-12.
[32] JK Srivastava and S Gupta. Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. *Mol. Cell Pharmacol.* 2009; **1**, 138.
[33] A Tuominen and T Sundman. Stability and oxidation products of hydrolysable tannins in basic conditions detected by HPLC/DAD-ESI/QTOF/MS. *Phytochem. Anal.* 2013; **24**, 424-35.
[34] CC Lin, CH Yang, NF Chang, PS Wu, YS Chen, SM Lee and CW Chen. Study on the stability of deoxyarbutin in an anhydrous emulsion system. *Int. J. Mol. Sci.* 2011; **12**, 5946-54.