

FREE RADICAL SCAVENGING EFFICACY OF TAMARIND SEED COAT AND ITS COSMETICS APPLICATION

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ABSTRACT: Free radical scavenging activity and total phenolics content of Thai Tamarind seed coat extracts from the partition and maceration were compared. Tamarind seed coat suspended in 70% EtOH and then partitioned with CH₂Cl₂ and EtOAc, respectively. The EtOAc fraction posed the strongest antioxidant activity with highest total phenolics content (IC₅₀ = 1.791 ppm; 63,691.00 mg GAE/100 g) followed by 70% EtOH (IC₅₀ = 3.002 ppm; 21,425.78 mg GAE/100 g), Aq. (IC₅₀ = 3.024 ppm; 16,105.30 mg GAE/100 g) and CH₂Cl₂ (IC₅₀ = 5.122 ppm; 6,848.31 mg GAE/100 g) fractions, respectively. Maceration of seed coat powder in *n*-hexane, EtOAc and 95% EtOH, respectively, was conducted. The EtOAc extract had greater antioxidant activity and total phenolics content (IC₅₀ = 2.164 ppm; 5,205.05 mg GAE/100 g) than the 95% EtOH extract (IC₅₀ = 5.145 ppm; 713.24 mg GAE/100 g). Free radical scavenging activity was found related with the total phenolics content (R² = 0.6507). The EtOAc from partition was developed in the stable with most preference milky base lotion which preliminary prepared at its IC₅₀, two and three folds of IC₅₀, individually. All formulations were found physically and chemically stables.

Keywords: *Tamarindus indica*, Tamarind, antioxidant, total phenolics content, cosmetics

INTRODUCTION: Tamarind or *Tamarindus indica* L. is widely growth in tropical regions including Thailand and has long been supplied as an important nutrition source and traditional medications¹⁾. In Thailand, the flower, fruit and leaf are consumed as food materials of which there are two species commonly cultivated particularly the sweet Tamarind²⁾ which has been developed in several cultivars that bring variety of tastes and accounted as one of an economic plant. There were several attempts searching for possible potential utilization of Tamarind seed which is waste from the consumption. Biological activity assessment of Tamarind seed was reported on the radical scavenging¹⁾, lipid peroxidation reducing³⁾ and anti-microbial activities⁴⁾ including anti-inflammatory potential⁵⁾. However, the reported preparation methods are complicated and relied on advanced techniques.

Therefore, development of the ease and practical extraction was conducted. The antioxidant activity of each extract was evaluated with the assessment of total phenolics content to relate the active principle compound with biological activity. Furthermore, applications of Tamarind seed coat extract based on antioxidant activity appropriate for anti-wrinkle cosmetics were performed.

MATERIALS AND METHODS:

Sample preparation

All of solvents used are reagent grade unless otherwise stated. Those for cosmetic formulation are cosmetic grade. Tamarind seed coat cultivated in Chiang Rai was prepared by removing of the edible part and heat under 140°C for 45 min⁵⁾. The seed coat was further ground into powder. The powder (50 g.) was extracted by the modified method from the literature⁵⁾ by suspended the powder in 70% EtOH (1,000 ml) followings vigorous shaking for 30 min in separatory funnel and filtered (× 3). The filtrates were combined and concentrated *in vacuo*. The crude extract was dissolved in 100 ml of 70% EtOH and partitioned with CH₂Cl₂ (150 ml × 3). The organic layers were combined and washed by saturated brine and concentrated to afford CH₂Cl₂ fraction. The aqueous part was further partitioned with EtOAc (150 ml × 3) and worked up as usual to give EtOAc fraction. The remaining aqueous layer was concentrated by azeotropic distillation with MeOH yielded Aq. fraction. Separately, the seed coat powder (100 g) was macerated in *n*-hexane, EtOAc and 95% EtOH, respectively, with shaking at 150 rpm for 24 hr under ambient temperature. The whole was individually filtered and concentrated to obtain mac. *n*-hexane, mac.

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EtOAc and mac. 95% EtOH, respectively.

Free radical scavenging activity assay

The antioxidant activities were measured using the free stable radical, 1, 1-diphenyl-2-picrylhydrazyl (DPPH)⁶. DPPH was prepared in absolute EtOH at a concentration of 6×10^{-5} M. Samples were prepared in absolute EtOH at the concentration of 1.0-10.0 ppm as well as ascorbic acid (1.0-8.0 ppm) which was used to prepare a calibration curve ($R^2 > 0.999$). Butylated hydroxytoluene (BHT) was additionally used as a second positive control. A portion of the sample solution was mixed with equal volume of DPPH and was allowed to stand in the dark at room temperature for 30 min. The absorbance was then measured at 517 nm by using of microplate reader (ASYS/UVM340, UK). The absorbance obtained was converted into free radical scavenging activity by using the following formula;

$$\% \text{ free radical scavenging activity}$$

$$= [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}]$$

where A is the absorbance.

The assays were done in triplicate. An IC_{50} values was obtained by plotting means of % inhibitions (with SD) of each assay versus concentrations prepared by the serial dilution.

Determination of total phenolics content

The total phenolics content of all extracts were determined as previously described⁷ with the using of Folin-Ciocalteu reagent and gallic acid as a standard. The standard curve with the linear correlation (R^2) of more than 0.999 was generated by using of gallic acid concentrated 5-30 ppm. The total phenolic content was measured in each with 4 μ l of the Folin-Ciocalteu reagent in a 96

wells plate and shook for 3 min, thereafter Na_2CO_3 (0.577 M, 80 μ l) was added. The absorbance was measured at 750 nm following 1 hr incubation under ambient temperature. All of tests were done in triplicate and analyzed similar to those of antioxidant activity. The measurement was reported as mg of gallic acid equivalents per 100 g of fraction (mg GAE/100 g).

Cosmetics formulation

Four milky base lotions were preliminary prepared with the ingredients listed in Table 1. Physicochemical characters were determined by pH meter (Mettler Toledo/S20, Switzerland) and Viscometer RVDV-II+Pro (Brookfield, USA). All of the base lotions were preliminary tested on physical stability by centrifugation assays at 3,000 ppm for 30 min under ambient temperature⁸ prior to the accelerated test of 6 freeze-thaw cycles at 45 and 4 °C for 24 hr at each temperature⁹. Base milky lotions which passed the stability test were further evaluated on preference tests which were carried out by 26 female and 24 male volunteers and the base lotions were scored by the hedonic system from 0 – 4 (dislike – most prefer) using the interview questionnaires. Base lotion with the best preference was chosen for further experiment. Separate bases were incorporated by the Tamarind seed coat extract (EtOAc fraction) at the concentrations of its IC_{50} , two and three folds of IC_{50} (0.0017%, 0.0034% and 0.0051% w/w, respectively). Physical stability test was performed as previously described. For chemical stability, each formula (0.3 g) was dissolved in absolute EtOH (5 ml) and centrifuged at 3,500 ppm for 5 min under room temperature and the supernatants were collected¹⁰ for the total phenolics content determination.

RESULTS AND DISCUSSION: The highest yield was found in the 70% EtOH extract as it was the starting crude extract for further liquid-liquid extraction as shown in Table 2.

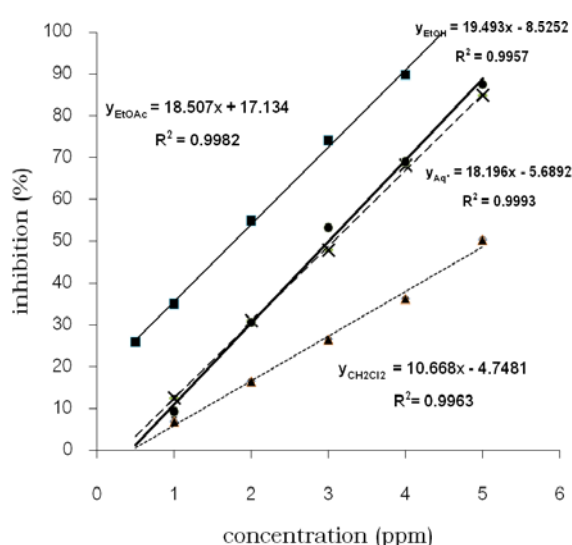
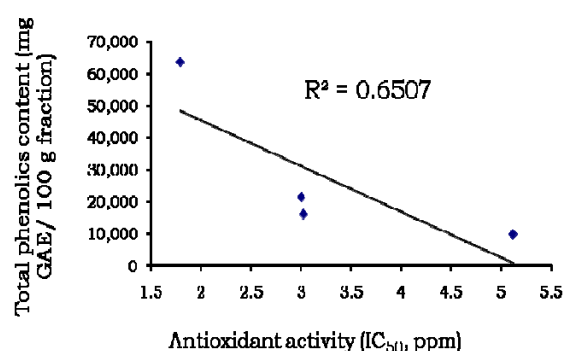
Radical scavenging activity of each extract was evaluated to obtain the IC_{50} as shown in Fig. 1. The EtOAc fraction posed the most potent

Table 1 Milky base lotion formulation

Ingredient	Formula			
	1	2	3	4
1% Carbopol 941	14.8	13.2	12	14.8
Propylene glycol	2	3.6	4.8	2
Emulsifier	1	1	1	1
DI water	58.7	58.7	58.7	58.7
Cyclomethicone	5	5	5	5
Rice bran oil	2	2	2	2
Stearic acid	3.5	3.5	3.5	2.4
Isopropyl myristate	10	10	10	10
Triethanolamine	1	1	1	1
Preservative	2	2	2	2
Perfume	-	qs	qs	qs

Table 2 Extractive yields, antioxidant activity and total phenolics content

Samples	Yield (%)	DPPH scavenging activity (IC ₅₀ , ppm)	Total phenolics content (mg GAE/100 g)
70% EtOH	10.46	3.002	21,425.78
CH ₂ Cl ₂	0.06	5.122	9,848.31
EtOAc	1.03	1.791	63,691.00
Aq.	10.23	3.024	16,105.30
mac. EtOAc	0.16	2.164	5,205.05
mac. 95% EtOH	5.24	5.145	713.24
BHT	-	22.227	-
Ascorbic acid	-	2.276	-

**Fig. 1.** DPPH scavenging activity of 70% EtOH (σ), CH₂Cl₂ (●), EtOAc (■) and Aq. (×) fractions**Fig. 2.** Correlation between antioxidant activity and total phenolics content by partition

antioxidant activity and particularly stronger than ascorbic acid and BHT which were used as positive controls (Table 2). The crude 70% EtOH was slightly better on radical scavenging activity comparing to the Aq. and CH₂Cl₂ fractions, respectively. According to the EtOAc fraction posed the strongest antioxidant activity, maceration of the seed coat was conducted in an attempt to shorten the antioxidant extraction.

Although stepwise maceration based on polarity of the solvents was more convenience, the obtaining yield was lower than that from stepwise partition owing to more chemical constituents were extracted by 70% EtOH respecting to its higher polarity. Free radical scavenging activity and total phenolics content were evaluated in each sample except mac. *n*-hexane as most of active compounds have moderate to high polarity extracted by EtOAc and EtOH. Despite the extractive yield of mac. EtOAc was less, the biological activity was more potent than that of 70% EtOH, Aq. and CH₂Cl₂ fractions from the partition. Contrary, mac. 95% EtOH extract was less potent on free radical scavenging activity. However, considering on the antioxidant activity, maceration with EtOAc was more feasible and practical.

Total phenolics content was determined in an attempt to relate antioxidant activity with the active principles. Furthermore, it will be more convenience and practical in quality control of Tamarind seed extract for applications. In the partition method, the EtOAc fraction had the highest phenolics content followed by 70% EtOH, Aq. and CH₂Cl₂ fractions, respectively. Moreover, the presenting antioxidant activity and phenolics contents of this modified extraction method were greater than the previous study¹⁾. In addition, the antioxidant activity was related with the phenolics content ($R^2 = 0.6507$) as shown in Fig. 2. Thus, higher phenolics content fraction possesses a stronger antioxidant activity. Furthermore, development of cosmetics containing EtOAc fraction from partition which posed the best antioxidant activity and total phenolics content was done. Four milky base lotions with white and smooth opaque appearance were preliminary

Table 3 Physical stability and preference towards bases

Condition	Parameter	Formula			
		1	2	3	4
Initial	pH	6.35	6.28	6.60	6.65
	Viscosity (cP)	1657	1613	680	910
Freeze-Thaw 6 cycles	pH	6.24	6.27	6.54	6.46
	Viscosity (cP)	1643	1604	675	309
Overall preference		2.31±	2.54±	2.62±	2.94±
		1.01	0.88	0.85	0.87

Table 4 Chemical and physical stability of the anti-wrinkle cosmetics

Condition	Parameter	Formula		
		A	B	C
Initial	pH	6.61	6.65	6.59
	Viscosity (cP)	925	927	919
	Total phenolics (µg GAE/ml)	0.0129	0.0260	0.0389
Freeze-Thaw 6 cycles	pH	6.60	6.60	6.57
	Viscosity (cP)	909	911	904
	Total phenolics (µg GAE/ml)	0.0128±	0.0253±	0.0387±0.002

prepared and evaluated on their stability and preference.

All of the base lotions retained their homogeneity followings centrifugation assays. Thus, all of them were evaluated under accelerated tests. All of the base lotions were homogeneous white with opaque and smooth texture as appeared in the initial state with the consistence of pH and viscosity as shown in Table 3. They were further evaluated on performance. It was found that base no. 4 was the most preference. Therefore, base lotion no. 4 was chosen for incorporation of the extract at IC₅₀, two and three folds of IC₅₀ of the best antioxidant fraction and labeled as formula A, B and C respectively.

All of the developed formulas passed centrifugation assays and further evaluated by accelerated test. Chemical stability was tracked by the total phenolics content and all of the 3 developed formulations were found chemically and physically stables (Table 4).

CONCLUSION: Tamarind seed coated extracts posed high antioxidant activity as well as total phenolics content particularly the EtOAc extracts from either partition or maceration. This practical and economical extraction is ease for operation and applications. The finding relationship of the active principle compounds and biological activity is available for quality control of the extracts and

exhibits potential sustainable utilization of this agricultural waste. In addition, applications of cosmetics by using of Tamarind seed coat extract were chemically and physically stables. Efficacy evaluation of the developed anti-wrinkle cosmetics will be further conducted.

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