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The Tyrosinase and Cyclooxygenase Inhibitory Activities and Cytotoxicity Screening of *Tamarindus indica* Seeds

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Abstract**—**The methanolic extracts from seeds of tamarind (*Tamarindus indica*) was prepared by Soxhlet apparatus extraction and evaluated for total phenolic content by Folin-Ciocalteu method. Then, methanolic extract was screened biological activities (*In vitro*) for anti-melanogenic activity by tyrosinase inhibition test, anti-inflammation activity by cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) inhibition test, and cytotoxic screening test with Vero cells. The results showed that total phenolic content, which contained in extract, was contained 27.72 mg of gallic acid equivalent per g of dry weight. The ability to inhibit tyrosinase enzyme, which exerted by Tamarind seed extracts (1 mg/ml) was 52.13 ± 0.42 %. The extract was not possessed inhibitory effect to COX-1 and COX-2 enzymes and cytotoxic effect to Vero cells. The finding is concludes that tested seed extract was possessed antimelanogenic activity with non-toxic effects. However, there was not exhibited anti-inflammatory activity. Further studies include the use of advance biological models to confirm this biological activity, as well as, the isolation and characterization of the purified compounds that it was contained.

Keywords**—***Tamarindus indica*, anti-melanogenic, anti-inflammatotion, cytotoxicity, seed, phenolic compounds.

I. Introduction

THE increase in production and accumulation of melanins are cause of increase skin alignments e.g. acquired hyperpigmentation such as melisma, proinflammatory melanoderma, solar lentigo etc. The hyperpigmentation of epidermis and dermis depend on either increased numbers of melanocytes or the activity of enzyme [1]. Tyosinase is the key enzyme responsible for the physiological process which plays crucial protective role against sunburn, i.e. melanogenesis. The melanocyte are responsible for the biosynthesis of melanin through enzymatic conversion of L-tyrosine which leads to the sun tanning effect. However, over production of melanin pigment is not desirable, especially on face [2]. The reduction of hyperpigmentation is, therefore, the goal of cosmetic industries which can be commercialized worldwide. A number of natural ingredients are currently being used in cosmetics based on their properties as antioxidant, anti-inflammatory or anti tyrosinase activity. These natural products are, for example, the grape seed extract, ascorbic acid licorice extract, arbutin, kojic acid, resveratrol, berry extract, etc. [1]-[3]. Arbutin and kojic acid are used for their anti-tyrosiase activities but do not satisfy by physicians due irritability and side effects in human use [4].

Special attention is focused on extraction and biological tests of inexpensive residues from agricultures and food industries. The previous study speculates on increasing the values of the waste by-products of fruit industries [5]. Tamarind (*Tamarindus indica* L.), belongs to the family Leguminosae and grows naturally in many tropical and sub-tropical regions. In Thailand, two types of Tamarind are found in abundance, the so-called sweet and sour varieties. Tamarind is an important food resource for the Thai population. The flower and leaf are eaten as vegetables, while the germ obtained from the seed is used for manufacturing Tamarind gum which is well-known as a component of jelly [6]. Tamarind seeds are also reported to contain phenolic antioxidants, such as 2-hydroxy-30, 40-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin [7]. Extracts exhibit antioxidant potential by reducing lipid peroxidation in vitro [7], [8] and anti-microbial activity [9]. The content of Tamarind seeds comprised only procyanidins, represented (%) mainly by oligomeric procyanidin tetramer (30.2), procyanidin hexamer (23.8), procyanidin trimer (18.1), procyanidin pentamer (17.6) with lower amounts of procyanidin B2 (5.5) and (-)-epicatechin (4.8).and possesses antioxidant activity [10]. Aims of our study were conduct to test for other biological activities of tamarind seed extract by determining of antityrosinase activity and anti-inflammatory activity or cyclooxygenase inhibitory activity.

II. Materials and Methods

A. Preparation of Tamarind Seed Extract

Tamarind (*Tamarindus indica* L. cv. Thailand Sweet) were collected from local markets in Thailand. Fruit pericarps and seeds were separated and air-dried. Dried materials were blended to power before extraction. Extraction of fruit materials was modified previously [10]. Air-dried material (50 g) was extracted with hexane in a soxhlet apparatus (3 h) to remove lipid. After drying, the solid was extracted with methanol (3x3h). Organic solvent was removed by rotary evaporation at 35°C in vacuo. The extracts of methanolic fraction were dissolved in DMSO and kept at -20 °C until use.

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B. Measurement of Total Phenolic Content

Total phenolic content was measured using Folin-Ciocalteau assay modifying by the method of Singleton VL, 1999 [11]. Folin-Ciocalteau reagent (phosphomolybdic phosphotunstic acid reagent) together with fruit extracts (1.0 mg/ml) were added into 96-well plate. The mixture was incubated at room temperature for 6 min. After incubation, 7% sodium carbonate were then added and further incubated at 45 °C for 45 min. The absorbance was taken to 765 nm by using microtiter plate reader. The standard curve was linear between 50-250 mg/L gallic acid. Total phenolic content was expressed as milligrams of gallic acid equivalent (mg GEA) per 1 g of dried extract.

C. Mushroom Tyrosinase Activity

To evaluate the inhibitory action of methanolic extract of tamarind seed on tyrosinase, tyrosinase isolated from mushrooms was utilized as described previously with a minor modification [12], [13]. In brief, 20 ml of aqueous solution of mushroom tyrosinase (1000 units) was added to a 96-well microplate, in a total volume of 200 ml assay mixture containing 1mM L-tyrosine solution, 50mM phosphate buffer (pH 6.5). The assay mixture was incubated at 25 °C for 30 min. Following incubation, the amount of dopachrome produced in the reaction mixture was determined spectrophotometrically at 492 nm (OD492) in a microplate reader. IC50 is the concentration of a drug that inhibits a standard response by 50% and gallic acid 3.59×10^{-6} M was used as positive control.

D. Anti-Inflammatory Activity

Mouse COX-1 and COX-2 null cell lines were used for this test. Sample was tested for anti-COX-2 and anti-COX-1 activities at 10-5 g/ml using radioimmunoassay (RIA). The RIA method used for measuring PGE2 concentrations in the culture supernatant is based on the competition between PGE2 in the samples and 3H labled PGE2 for anti-PGE2 antibody binding sites. Aspirin and DMSO were used as positive and negative control, respectively [14], [15].

E. In Vitro Cytotoxicity Test

African green monkey kidney fibroblast (Vero) cells were performed employing the calorimetric method as described by Skehan and co-workers. The sulforhodamine B (SRB) assay was performed to assess growth inhibition using a colorimetric assay, which estimates cell number indirectly by staining total cellular protein with the dye SRB [16]. Briefly, 100 µl/each well of cell suspensions ($0.5\text{-}2.0 \times 10^5$ cells/ml) were seeded in 96-well microtiter plates and incubated at 37ºC to allow for cell attachment. After 24 h, the cells were treated with the extract by adding 100 µl/well of each concentration in triplicate to obtain a final concentration of 50.0, 25.0, 12.5, 6.25, 3.125, 1.5625 and 0.7813 µg/well for the extracts (two-fold serial dilution). The Ellipticine 0.603 µg/ml and 0.5% DMSO were used as positive and negative control, respectively.

III. Results and Discussion

The results showed that Total phenolic content, which contained in methanolic extracts of Tamarind and Longan seed, were contained 27.72 mg of gallic acid equivalent per g of dry weight. The total phenolic content was independent /unrelated to weight of extract. The phenolic content may depend on several factors, normal condition or stress condition i.e. infection, high UV explosion, trauma, harvesting period, harvesting method [17].

Tamarind extract was not possessed any cytotoxic effect to Vero cells at 50 µg/ml, which was evaluated by Sulforhodamine B (SRB) colorimetric assay (Table I)

TABLE I
CYTOTOXIC ACTIVITY OF TAMARIND SEED EXTRACT DETERMINED BY SULFORHODAMINE B (SRP) ASSAY [A, B]

Methanol extract	Final conc. (µg/ml)	% Growth	Cytotoxicity activity	IC_{50} (µl/ml)
Tamarind seed	50.0	92.49	Non-toxic	-
	25.0	91.27	Non-toxic	-
	12.5	92.38	Non-toxic	-
	6.25	97.56	Non-toxic	-
	3.125	99.40	Non-toxic	-

[a] IC_{50} of positive control: Ellipticine = 0.603 µg/ml
[b] Negative control: 0.5% Dimethylsulfoxide (DMSO)

TABLE II
TYROSINASE INHIBITION OF TAMARIND SEED EXTRACT [A, B]

Methanol extract	% Tyrosinase Inhibition [a]
Tamarind seed	52.13 ± 0.42
Gallic acid (3.6 µM) [b]	51.02 ± 0.45

[a] %Mean ± SD of Tyrosinase inhibition calculated from triplicate independent tests
[b] Gallic acid may as positive control and can use replace kojic acid (Kim, 2007)

The ability of inhibit tyrosinase enzyme, which exerted by Tamarind seed extract (1 mg/ml), was 52.13 ± 0.42 % (Table II). Methanolic extract of tamarind seed cannot inhibit COX-1 and COX-2 enzymes at concentration = 100 µg/ml (Table III).

TABLE III
RESULTR OF CYCOOXYGENASE (COX) INHIBITION TEST [A, B]

Methanol extract	Anti-inflammatory [a]	
	COX-1	COX-2
Tamarind seed	inactive [a]	inactive [a]
Aspirin (10 µg/ml)	active [b]	active [b]

[a] 0.5% Dimethylsulfoxide (DMSO) was diluent and negative control
[b] Aspirin (10 µg/ml) was positive control as non-selective COX inhibitor (Kim, 2007)

The finding showed that tamarind extract moderately inhibited enzyme tyrosinase and rate of activity similarly to gallic acid as previous study (Kim, 2007). However, this test was direct enzyme inhibition test and phenolic compounds are antioxidants, which are stronger than vitamin C and vitamin E, may reduce free radical and indirectly decrease melanin production. The further study needs to conduct to test the extract with melanoma cell line. No any COX-1 and COX-2 inhibitory effect of tamarind extract was evaluated by

screening test. Most of phenolic and other secondary metabolises were inhibit COX enzyme via induce COX down regulation gene expression especially COX-2 [18]. We should be conduct next study on tamarind seed extract to down regulate COX gene expression.

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