Original Article

Antioxidant Activities of Methanol Extract from Tamarind Seeds

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Abstract : Methanol extract of tamarind (*Tamarindus indica* L.) seeds was screened for antioxidant activity. The antioxidant activities (reducing power, DPPH and lipid peroxide) showed a good antioxidant potential. The antioxidant effect of the extract indicated its potential for health benefit.

Key words : **antioxidant activity, phenolic compounds, *Tamarindus indica* L.**

Background and Rationale

Tamarind (*Tamarindus indica* L.) belongs to the family Leguminosae. It 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.[1] Tamarind seeds are also reported to contain phenolic antioxidants, such as 2-hydroxy-3′, 4′-dihydroxyacetophenone, methyl 3, 4-dihydroxybenzoate, 3, 4-dihydroxyphenyl acetate and epicatechin.[2] Phenolic compounds may have many biologic effects in terms of health promotion. From this standpoint it was of interest to evaluate the antioxidant activity of the methanol extract derived from tamarind seeds.

Methodology

Fruits studied

Fresh tamarind fruits were purchased from a local market in Bangkok, Thailand.

Chemicals used

The chemicals used were hydrochloric acid (Merck), sodium chloride (Merck), α,α-diphenyl-β-picrylhydrzyl (DPPH) (Sigma), linoleic acid (Sigma), tween 20 (Sigma), hexane (Merck), methanol (Merck), ethanol (Merck), ammonium thiocyanate (Alrich), ascorbic acid (Sigma), and (+/-)-α-tocopherol (Sigma).

Extraction protocols

The seeds were carefully separated from the fruits. Air-dried samples were homogenized by blending to a fine homogeneous powder prior to extraction.

Air-dried material (5 g) was extracted with hexane in a Soxhlet apparatus (3 h) to remove lipids. The material was dried under a stream of nitrogen and extracted further with methanol (3 h) as modified by Sudjaroen *et al.*,[3] and Owen *et al.*[4] Organic solvent was removed by rotary evaporation at 35- 40 °C (in vacuo).

Determination of antioxidant activity with DPPH radical scavenging method

The hydrogen-donating or radical-scavenging ability of seed extract from tamarind fruits was measured by using the stable radical α,α-diphenyl-β-

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picrylhydrzyl (DPPH). A methanolic solution (50 µl) of the extracts (1-20 mg/ml) was placed in a cuvette, and 2 ml of a 6×10^{-5} M methanolic solution of DPPH was added. Absorbance measurements commenced immediately at 515 nm, using spectrophotometer (Genesis 20, Thermo Fisher Scientific, USA). The decrease in absorbance was determined after 70 minutes when absorbance stabilized. The absorbance of the DPPH radical without extract, *i.e.*, control, was measured daily. The percentage inhibition of the DPPH radical in the samples was calculated according to the formula of Yen and Duh.[5]

$$\% \text{ inhibition} = [(A_{C\,(0)} - A_{A\,(t)}) / A_{C\,(0)}] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control at t = 0 and $A_{A(t)}$ is the absorbance of the antioxidant at t = 70 minutes. Vitamin C and E were used as positive controls.

Antioxidant activities in linoleic acid emulsion

The total antioxidant activity was determined according to the method of Yen and Hsieh[6]. Each extract in 0.5 ml of distilled water was mixed with linoleic acid emulsion (2.5 ml, 0.02 M, pH 7.0) and phosphate buffer (2 ml, 0.2 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of tween 20 as emulsifier, and 50 ml of phosphate buffer, and then the mixture was homogenized. The reaction mixture was incubated at 37°C. Aliquots of 0.1 ml were taken at 24 hours during incubation. The degree of oxidation was measured according to thiocyanate method by sequentially adding ethanol (4.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), sample solution (0.1 ml), and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl). The mixture was allowed to stand for 3 minutes, then the peroxide value was determined by reading the absorbance at 500 nm (Genesis 20, Thermo Fisher Scientific, USA). A control was performed with linoleic acid but without the extracts. Vitamin E was used as positive control.

Results and Discussion

The 5 g of air-dried seed powder yielded 234.8 mg of methanol extract. The methanol extract of tamarind seeds displayed appreciable antioxidant capacity. The extract showed antioxidant activities at all concentrations investigated, as shown in Table 1. At the concentration of 20 mg/ml, the extract showed DPPH radical-scavenging activity of 93.13 percent, while those of vitamin C and E were 95.29 percent and 93.21 percent, respectively. Total antioxidant activity in linoleic acid emulsion of extract was 95-97 percent at all concentrations, which was similar to that of vitamin E (94.45% at 0.1 mg/ml).

There are a few previous studies on the antioxidant activities of tamarind seeds.[2,3,8] The oxidation of low-density lipoprotein (LDL) cholesterol has been proposed as an important step in the formation of atherosclerotic lesions. The role of antioxidants as potential antiatherogenic compounds has been recognized. Many studies have demonstrated that polyphenolic flavonoids derived from plants used medicinally as chemopreventive agents have antioxidant activities[9].

Conclusions

Tamarind is an important source of food in tropi-

Table 1 Antioxidant activity of methanol extract of tamarind seeds

Extract	Concentration (mg/ml)	DPPH radical scavenging activity (%)[a]	Total antioxidant activity (%)[a,b]
Seed	1	20.49 ± 3.90	95.66 ± 0.51
	2.5	26.08 ± 7.37	95.76 ± 0.22
	5	86.03 ± 1.89	96.12 ± 0.00
	10	86.40 ± 5.75	96.38 ± 0.36
	20	93.14 ± 0.18	96.68 ± 0.65
Control: Vitamin C	0.1	95.29 ± 0.05	-
Vitamin E	0.1	93.21 ± 0.2	94.45 ± 2.2

[a] Values are means of triplicate determination ± S.D.; antioxidant activity is expressed as relative activity compared with negative control.

[b] Inhibition % (capacity to inhibit the peroxide formation in linoleic acid) = [1- (absorbance of sample at 500 nm)/(absorbance of control at 500 nm)] × 100.

cal regions, but currently the waste products of the canning industry, for example the pericarp and seeds, are discarded in Thailand. With regard to these waste products, the seeds especially appear to have real potential as safe and low-cost sources of chemopreventive natural products. Furthermore, they may have utility for increasing the shelf-life of canned foods by preventing lipid peroxi-dation. Studies are in progress to obtain a more complete profile of their anticancer potential, via a range of *in vitro* bioassays.[10]

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บทคัดย่อ : **ฤทธิ์ต้านอนุมูลอิสระของสารสกัดเมล็ดมะขามด้วยเมธานอล**

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**ภาควิชาเทคนิคการแพทย์, มหาวิทยาลัยรังสิต, จังหวัดปทุมธานี ๑๒๐๐๐*

การทดสอบฤทธิ์ต้านอนุมูลอิสระของสารสกัดเมทานอลจากเมล็ดมะขาม ในการลดอนุมูลอิสระชนิด DPPH และ Lipid peroxide พบว่ามีฤทธิ์ ดังนั้นฤทธิ์ต้านอนุมูลอิสระของสารสกัดเมทานอลจากเมล็ดมะขามอาจมีประโยชน์ต่อสุขภาพได้.

คำสำคัญ : **ฤทธิ์ต้านอนุมูลอิสระ, สารประกอบฟีนอล, เมล็ดมะขาม**