Original Article

Antihypertensive and Antioxidant Activities of Methanol Extract from Malva Nut *(Scaphium scaphigerum)*
ฤทธิ์ต้านอนุมูลอิสระของสารสกัดเมทานอลจากเมล็ดสำรอง

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Abstract

Malva nut is the seed of *Scaphium scaphigerum* and has long been used as a traditional medicine in South East Asia. This paper reports on the antihypertensive activity by Angiotensin Converting Enzyme (ACE) inhibition and antioxidant activities of methanol extract from Malva nut by scavenging DPPH radical and lipid peroxide. The results show that methanol extract of Malva nut possess only antioxidant activity in linoleic emulsion and no ACE inbition activity.

Keywords **:** Antioxidant activity, Antihypertensive activity, *Scaphium scaphigerum*, Malva nut.

บทคัดย่อ

การทดสอบฤทธิ์ต้านอนุมูลอิสระของสารสกัดเมทานอลจากเมล็ดสำรองในการลดอนุมูลอิสระชนิด DPPH และ lipid peroxide และฤทธิ์ลดความดันโดยการยับยั้งเอนไซม์ Angiotensin Converting Enzyme (ACE) พบว่ามีสารสกัดเมทานอลจากเมล็ดสำรองฤทธิ์ต้านอนุมูลอิสระที่ดีเฉพาะต่อ lipid peroxide เท่านั้น และไม่มีฤทธิ์ยับยั้งเอนไซม์ ACE

คำสำคัญ **:** ฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ลดความดัน เมล็ดสำรอง

Introduction

Malva nut fruit [*Scaphium scaphigerum* (G. Don) Guib and Planch] is known in Thailand as Pungtalay or Sumrong. The plant belongs to the Sterculiaceae family, which includes other species such as *Scaphium macropodum* Beumee and *Sterculia lychnophora* Hance. *Scaphium scaphigerum* is growing in Vietnam, China, Malaysia, Indonesia as well as the east part of Thailand, especially in the drier regions (1).

Malva nuts are harvested from a native tall tree (20-40 m tall) and the dry fruit is about 25x15 mm, ellipsoid in shape and glabrous (Fig. 1). The seeds are known to contain a large amount of mucilaginous substance and have been used as a traditional medicine in South East Asia. The jelly made from Malva nuts is consumed, when sweetened, as dessert, but its principal use is for relief of cancer sores and cough.

Fig.1 Dry seeds of Malva nut fruit.

Many studies have shown that water- soluble polysaccharide gums may have beneficial effects on human health, including reducing serum cholesterol levels. Foods that include soluble fiber have received an official approval by the US Food and Drug Administration and permit a claim to be made about their potential use in reducing the risk of heart disease (2). Likewise, foods fortified with Malva nut gum may be well accepted by the consumer since there is well-established knowledge of medicinal uses of Malva nuts. However, there had sparely reports of Malva nut on biological properties in terms of health promotion. The objective of the present study was to evaluate antioxidant and antihypertensive properties of methanol extract from Malva nut.

Materials and methods

Materials

Mature Malva nut fruits were collected in Eastern Thailand, transported to the laboratory, dried and stored at room temperature.

Chemicals

Hydrochloric acid (Merck), sodium chloride (Merck), α, α-diphenyl-ß-picrylhydrzyl (DPPH) (Sigma), linoleic acid (Sigma), tween 20 (Sigma), hexane (Merck), methanol (Merck), ethanol (Merck), ammonium thiocyanate (Aldrich), ascorbic acid (Sigma), (+/-)-α-tocopherol (Sigma), hippuryl-L-histidyl-L-leucine; HHL(Sigma), Angiotensin I Converting Enzyme (ACE) prepared from rabbit lung acetone powder (Sigma), ethyl acetate (Merck), Hippuric acid (Merck), benzenesulfonyl chloride; BSC (Fulka), pyridine (Fulka), Captopril (Sigma). All solutions were made up in double distilled water.

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 (20 g) was extracted with hexane in a Soxhlet apparatus (3 h) to remove lipid. The material was dried under a stream of nitrogen and extracted further with methanol (3 h) as modified from Sudjaroen *et al.*, 2005 and Owen *et al.*, 2000 (3, 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 Malva nut fruits were measured by using the stable radical α, α - diphenyl-ß-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 x 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 min when absorbance stabilized. The absorbance of the DPPH radical without extract, *i.e.*, control, was measured daily. The percent inhibition of the DPPH radical in the samples was calculated according to the formula of Yen and Duh (5).

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

Where $A_{C(o)}$ is the absorbance of the control at

t = 0 and $A_{A(t)}$ is the absorbance of the antioxidant at t = 70 min. 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 hr. 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 min, then 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.

Antihypertensive effect by Angiotensin Converting Enzyme (ACE) Inhibition Activity

The inhibitory activity against ACE was assayed using the method of Cushman and Cheung (7). The assay mixture contained potassium phosphate buffer 100mM (pH 8.3), sodium chloride 300mM, hippuryl-L-histidyl-L-leucine (HHL) 5mM, and enzyme prepared from rabbit lung acetone powder (Sigma), 0.10 mU per 0.25 ml of assay volume. An appropriate amount of the extracts was added to the assay mixture, incubated for 30 min at 37˚C, and then terminated by adding 1 N HCl 0.25 ml. The hippuric acid liberated was extracted with 1.5 ml of ethyl acetate, and 1.0 ml of the extract was evaporated by heating at 120˚C for 30 min in an oil bath. The residue was then dissolved in 1.0 ml of distilled water. The detection of hippuric acid substrate was using the method of Tomokuni and Ogata(8). 0.5 ml of suspension was pipetted into a glass test tube, and 0.5 ml of pyridine added and mixed. Then 0.2 ml of BSC was added and mixed for about 5 sec with a vibration mixer. The colored solution was allowed to stand for 30 min at room temperature, diluted to 5 ml with ethanol and well mixed, and the solution was centrifuged at 1,500-2,000 g for 5 min to remove a little turbidity. The absorbance was determined at 410 nm vs. ethanol (1-cm light path). The HA standard and blank (water) were treated in the same way.

Results and discussion

The methanol extract of Malva nut displayed appreciable antioxidant capacity only in linoleic emulsion. The extract showed antioxidant activities at all concentrations investigated, as shown in Table 1. At the concentration of 1 to 20 mg/ml, the extract had not DPPH radical-scavenging activity when those of vitamin C and E were 95.29% and 93.21%, respectively. Total antioxidant activity in linoleic acid emulsion of extract was 87-91% at all concentration, which was nearly similar to that of vitamin E (94.45% at 0.1 mg/ml). This different antioxidant effect of extract on DPPH radical and lipid peroxide may caused by solubility and pH of methanol extract in different environment of two antioxidant assays. However, no ACE inhibition activity of methanol extract of Malva nut was seen at any concentration.

Table 1 Antioxidant activity of methanol extract of Malva nut

Extract	Concenttation (mg/m)	DPPH radical scavenging activity (%)[a]	Total antioxdant activity (%)[a, b]
Seed	1	-	87.51 ± 9.91
	2.5	-	87.64 ± 10.95
	5	-	89.28 ± 8.20
	10	-	91.24 ± 7.22
	20	-	89.93 ± 4.70
Control:			
Vitamin C	0.1	95.29 ± 0.05	-
Vitamin E	0.1	93.21 ± 0.20	94.45 ± 2.20

[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)] x 100.

There are no previous studies on the antioxidant activities and antihypertensive of Malva nut. Malva nut contains water-soluble polysaccharide gums (9) which may have beneficial effects on human health, including reducing serum cholesterol levels. In addition it may reduce risk of cardiovascular diseases by its antioxidant potential, however, it cannot reduce hypertension by ACE inhibition activity. 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 been demonstrated that polyphenolic flavonoids derived from plants used as chemopreventive agent that is medicinally have antioxidant activities (10).

Conclusion

Malva nut is an important source of food in tropical regions, and the jelly made from Malva nuts is consumed in Thailand as foods fortified may be well accepted by the consumer since there is well-established knowledge of medicinal uses of Malva nuts. It also appears to have real potential as safe and low-cost source are of chemopreventive natural products. Studies are in progress to obtain a more chemopreventive profile such as anticancer potential, via a range of *in vitro* bioassays (11).

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