Free Radical Scavenging and Lipid Peroxidation of Thai Medicinal Plants Used for Diabetic Treatment

Lerluck Steinrut PhD*,
Arunporn Itharat PhD**, Srisopa Ruangnoo MPharm**

** Graduate School, Thammasat University, Klongluang, Pathumthani ,Thailand*
*** Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Klongluang, Pathumthani, Thailand*

Objective: *To investigate antioxidant activities of some selected plants used to treat diabetic patients by Thai folk doctors.*
Material and Method*: Ten Thai plants were selected, macerated and extracted with 95% ethanol. DPPH scavenging and lipid peroxidation assays were used to test the antioxidant activities of the extracts.*
Result*s: Mammea siamensis (Saaraphee), Tacca chantrieri (Narapusri) and Albizia myriophylla (Cha Em Thai) showed the highest antioxidant activity on DPPH radical assay (EC_{50} = 10.17, 10.24 and 14.45 µg/ml, respectively). Mimussops elengi (Pikun), M. siamensis (Saaraphee) and A. myriophylla (Cha Em Thai) exhibited the highest antioxidant activity by lipid peroxidation assay (IC_{50} 0.39, 0.43 and 0.70 µg/ml, respectively). Six of ten plant extracts showed antioxidant activity on both assays (IC_{50} < 20 µg/ml).*
Conclusion: *These plant extracts containing in Thai folklore antidiabetic medicine, which showed high antioxidant activity, may be used effectively for the treatment of diabetic patients.*

Keywords*: Antioxidant, DPPH, Lipid peroxidation, Diabetic, Thai medicinal plants*

J Med Assoc Thai 2011; 94 (Suppl. 7): S178-S182
Full text. e-Journal: *http://www.jmat.mat.or.th*

Oxidative stress is a cause of many diseases such as diabetes mellitus. It is a consequence of hyperglycemia, change in energy metabolism and inflammatory mediators which plays an important role in the pathology of diabetes. It can also deplete cellular antioxidant defense system and enhance production of reactive oxygen species (ROS)[1]. The normal levels of oxidative stress markers, such as serum malonaldehyde, will be increased by an oxidizing of low density lipoproteins (LDL), so LDL level in serum shows high value in diabetes[2]. In type I diabetes, a redox imbalance shows effect on apoptosis of pancreatic islets cell[3] in which islet cells are particularly susceptible to free radical damage. In type 2 diabetes, hyperglycemia may induce free radical production in plasma which will be associated with increased intracellular concentration of free radical and depleted antioxidant defenses. Thus, the roles of antioxidant compounds or plant extract on diabetes are prevention of diabetes occurrence and protection of diabetes progression.

The aim of this research was to investigate the antioxidant activity of 10 selected Thai medicinal plants which have been used to treat diabetes by Thai folk doctors using two antioxidant assays, *i.e.* DPPH scavenging and lipid peroxidation assay.

Correspondence to:
Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Rungsit Campus, Klongluang, Pathumtani, 12120, Thailand.
Phone & Fax: 0-2926-9749
E-mail: iarunporn@yahoo.com

Material and Method

Reagents

Methanol, ethanol, sodium hydroxide, butanol, ferric chloride and ascorbic acid as analytical grade and butylate hydroxyl toluene (BHT) were purchased from Merck (Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), propyl gallate and thiobarbituric acid were products Fluka (USA). Liposome which was extracted from bovine brain (B3635) was purchased from Sigma, USA.

Plant materials

The parts of plants which were reported to be used as antihyperglycemia, antiinflammation and tonic by folk doctors in Thailand, were collected and purchased from several regions of Thailand. The places of collection, plant parts, voucher specimens and

traditional uses are shown in Table 1. Authentication of plant materials was carried out and kept at the herbarium of the Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkla, Thailand.

Preparations for plant extraction

The plant samples were washed with water to remove the remaining sand and to reduce the microbial contamination. The cleaned plant materials were cut into small pieces, dried at 50°C and ground. The dried ground plant material (100 g) was macerated with 95% ethanol for 3 days, filtered and concentrated to dryness under vacuum. The marc was macerated again twice, filtered and dried in a vacuum evaporator. All extracts of each plant were combined and the percentage of yield was calculated as shown in Table 2. The extracts were then kept at -20°C before reaction testing.

DPPH radical scavenging assay

The scavenging effect of the extracts on DPPH radical was examined based on the method of Yamasaki[4]. This method is based on chemical reaction. Butylated hydroxy toluene (BHT) was used as reference standard and positive control. Samples for testing were dissolved in absolute ethanol to obtain a concentration of 1,000 µg/ml. Each dissolved sample was diluted several times to the final concentrations of 1, 10, 50 and 100 µg/ml. Each concentration was tested in triplicate. A portion of sample solution (500 µl) was mixed with an equal volume of 6 x 10^{-5} M DPPH (in ethanol) and allowed to stand at room temperature for 30 min. The absorbance (A) was measured at 520 nm. BHT as a positive standard was tested in the same procedure. The scavenging activity of the sample corresponds to the intensity of quenching DPPH. The results are expressed as percentage of inhibition.

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Effective concentrations of the samples required to scavenge DPPH radical by 50% (EC_{50}) were obtained by linear regression analysis of dose-response curve plots of % inhibition versus plant extract concentration and EC_{50} calculated by the prism program.

Lipid peroxidation by liposome assay

Determination of the antioxidative activity using the Thiobarbituric acid (TBA) assay is based on the prevention of the formation of malondialdehyde, a degradation product of lipid peroxidation. This assay follows the method of Uchiyama and Mihara[5]. It involves the reaction between liposomes, prepared from a bovine brain extract was suspended in phosphate buffered saline (PBS) (5 mg/ml), 0.1 ml $FeCl_3$, 0.1 ml ascorbic acid (1 mM) and 0.5 ml PBS and 0.1 ml sample of ethanolic extract to be assessed. Propyl gallate (1 x 10^{-4}M) is used as positive control. All test tubes were incubated at 37°C for 20 min. The lipid peroxidation of liposomes should occur within that incubation period, unless the test substance exerted a protective antioxidant effect. The extract was prepared as 0.1, 1, 10, 50 and 100 µg/ml solutions. Four replicates were carried out for each mixture. The TBA assay [0.1 ml 2% BHT, 0.5 ml in 1% TBA (in 50 mM NaOH), 0.5 ml 1% HCl] was performed after the 30 min incubation at 85°C and allowed to cool to room temperature. Thiobarbituric acid should form a coloured adduct with malonaldehyde and 2.5 ml of butanol was added for the extraction of the complex to all tubes, mixed and centrifuged at 3,500 rpm for 10-20 min. The butanol fraction was removed and placed in 96 well plate and the extinction was determined at 532 nm on a spectrophotometer. Propyl gallate was used as positive control of every experiment as more than 80% inhibition, and percent inhibition was calculated.

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

The results were presented as means of triplicates, and the amounts required for a 50% reduction (IC_{50}) were determined by dose-response curve plots of % inhibition versus concentration and EC_{50} calculated by the prism program.

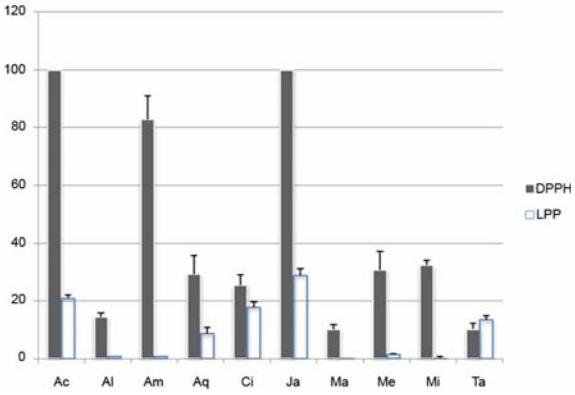

Fig. 1 Antioxidant activity [EC_{50} (µg/ml)] of the medicinal plants comparing results between DPPH assay (black bar) and lipid peroxidation by liposome assay(blank bar) (Ac = Wan Num, Al = Cha Em Thai, Am = Krawaan, Aq = Kritsanaa, Ci = Op Choei, Ja = Mali Laa, Ma = Saaraphee, Me = Bunnaak, Mi = Pikun, Ta = Narapusri)

Table 1. Ethnobotanical data and biological activity of plants

Scientific name and family of plant	Thai name of plant	Place of specimen collection	Voucher specimen number	Part used	Biological activity	Thai Traditional use[6]
Acorus calamus; Araceae	Wan Num	Chanthaburi	SKP015010301	rhizome	Anti-inflammatory[7]	Antiflatulence Lung infection
Albizia myriophylla; Ceasalpiniaceae	Cha Em Thai	Chanthaburi	SKP115011301	stem	n/a	Tonic and flavor
Amomum krervanh; Zingiberaceae	Krawaan	Chanthaburi	SKP206011101	fruit	Antioxidant[8]	Antiflatulant
Aquilaria crassna; Thymelaeaceae	Kritsanaa	Chanthaburi	SKP193010301	stem	n/a	Heart tonic
Cinnamomum zeylanicum; Lauraceae	Op Choei	Chanthaburi	SKP 096031901	bark	Antimicrobial activity[9] Antioxidant[10]	Antidiabetes Increase cerebral blood flow
Jasminum sambac; Oleaceae	Mali Laa	Nakorn-pathom	SKP12911901	flower	n/a	Heart tonic Eye infection
Mammea siamensis; Sapotaceae	Saaraphee	Ratchaburi	SKP083131901	flower	Antioxidant[11]	Heart tonic
Mesua ferrea; Sapotaceae	Bunnaak	Ratchaburi	SKP083130601	flower	n/a	Heart tonic Blood tonic
Mimussops elengi; Sapotaceae	Pikun	Ratchaburi	SKP171130501	flower	Antioxidant[12]	Heart tonic Blood tonic
Tacca chantrieri; Polypodiaceae	Narapusri	Nakonrat-chasima	SKP152200301	rhizome	Cytotoxic activity[13]	Antipyretic Wound healing

n/a = not applicable

Table 2. Antioxidant activity of the ethanolic plant extracts by DPPH and lipid peroxidation assays

Scientific name of plant and control used	% yield	DPPH		Lipid peroxidation	
		% inhibition at concentration of 100 µg/ml	EC_{50} (µg/ml)	% inhibition at concentration of 100 µg/ml	EC_{50} (µg/ml)
Acorus calamus.	9.25	37.7 ± 1.1	> 100	92.0 ± 3.7	20.99 ± 1.1
Albizia myriophylla	22.38	89.4 ± 0.3	14.45 ± 1.5	107.0 ± 3.6	0.70 ± 0.5
Amomum krervanh	2.96	54.0 ± 1.5	82.79 ± 8.1	92.9 ± 3.0	0.77 ± 0.71
Aquilaria crassna	4.30	80.8 ± 1.0	29.36 ± 6.3	100.3 ± 4.6	8.59 ± 2.3
Cinnamomum zeylanicum	15.65	98.8 ± 1.7	25.39 ± 3.6	91.5 ± 3.4	17.82 ± 1.8
Jasminum sambac	13.36	14.7 ± 0.8	> 100	106.0 ± 1.0	28.84 ± 2.3
Mammea siamensis	31.99	97.1 ± 0.8	10.17 ± 1.5	107.4 ± 3.0	0.43 ± 0.2
Mesua ferrea	30.27	92.7 ± 0.9	30.65 ± 6.6	103.3 ± 3.5	1.40 ± 0.4
Mimussops elengi	19.86	92.3 ± 1.4	32.28 ± 1.7	107.5 ± 3.7	0.39 ± 0.3
Tacca chantrieri	3.61	92.8 ± 0.8	10.24 ± 1.9	88.1 ± 3.8	13.40 ± 1.6
BHT		86.4 ± 0.5	12.35 ± 0.2	-	-
Propyl gallate				-	12.01 ± 2.1

Results and discussion

The ethnobotanical data of the investigated plant species are shown in Table 1 and the percentage of yield of plant extracts are shown in Table 2. The highest yield was obtained from *M. siamensis* (Saaraphee), followed by *M. ferrea* (Bunnaak) and *A. myriophylla* (Cha Em Thai) (32%, 30% and 22%, respectively). The results of antioxidant activity by DPPH and Lipid peroxidation are shown in Table 2.

The ethanolic extracts of *M. siamensis*

(Saaraphee) and *T. chantrieri* (Narapusri) exhibited the highest antioxidant activity by DPPH assay (EC_{50} = 10.17 and 10.24 μg/ml, respectively). Their activities were higher than that of the positive antioxidant compound, BHT, (EC_{50} = 12.23 μg/ml). These results support that of the previous report which showed the DPPH EC_{50} of the absolute ethanol extract of *M. siamensis* to be 32.71 μg/ml[11]. *M. siamensis* extract also showed antioxidant activity by lipid peroxidation assay (EC_{50} = 0.43 ± 0.2 μg/ml). Additional research should be conducted on this plant to isolate its antioxidant compounds and test their efficacy in the prevention of diabetes. The ethanolic extract from *T. chantrieri* also showed antioxidant activity by both assays. This has never been previously reported. This plant has been used for wound healing[6] in diabetes preparations, therefore, its high antioxidant activity will strongly support its use for diabetic treatment. Of ten plant extracts, six showed higher antioxidant activity by lipid peroxidation than propyl gallate. All ten plants showed high anti-lipid peroxidation. These results indicate that these plants can be used to deplete malonaldehyde which increases the levels of oxidative stress markers such as thiobarbituric acid reactive substances (TBARS). This would lead to the oxidization of low density lipoprotein (LDL) in diabetes[2,14]. The ethanolic extract of *M. elengi* showed the highest antioxidant activity by lipid peroxidation (EC_{50} = 0.39 μg/ml) which corroborates a previous report in which a different assay was used showing its EC_{50} of 0.23-0.55 μg/ml[12]. These results should support its use as a heart and blood tonic in Thai traditional medicine since it can affect fat metabolism in the cell. The *M. elengi* extract showed less free radical scavenging by DPPH, which is based on chemical reaction, but showed high anti-lipid peroxidation based on enzymatic reaction. Therefore, it should be studied further to isolate and test its anti-lipid peroxidation compounds. *A. krervanh* also showed high anti-lipid peroxidation activity but it was less active on DPPH assay. This result could be suggested that *A. krervanh* and *M. elengi* contain high amounts of volatile oils so they showed anti-lipid peroxidation by liposome assay better than chemical scavenging assay by DPPH. *A. myriophylla* (Cha Em Thai) which has been used for its sweet flavor in Thai traditional medicine showed both antioxidant activities. Thus, it should be promoted as a sweetening agent for diabetic patients because it can protect free radicals from both chemical reactions, *e.g.* reactive oxygen species, and from lipid peroxidation which are the processes of diabetic progression.

Conclusion

Results from the present study showed similar trend of the two antioxidant assays on six plant extracts. They appear to support the notion that the plants used for diabetic treatment in the Thai traditional medicine can decrease free radical production from hyperglycemic condition of diabetic patients. Especially, these plants showed inhibition of MDA production which can oxidize low density lipoprotein (LDL) that occurs in diabetes.

Acknowledgement

This work was supported by the National Research University Project of Thailand Office of Higher Education Commission and Thammasat University.

Potential conflicts of interest

None.

References

1. Tesfamariam B. Free radicals in diabetic endothelial cell dysfunction. Free Radic Biol Med 1994; 16: 383-91.
2. Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci (Lond) 1996; 90: 255-60.
3. Hamaoka R, Fujii J, Miyagawa J, Takahashi M, Kishimoto M, Moriwaki M, et al. Overexpression of the aldose reductase gene induces apoptosis in pancreatic beta-cells by causing a redox imbalance. J Biochem 1999; 126: 41-7.
4. Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the antioxidative effects of methanol extracts of crude drugs. Chem Pharm Bull (Tokyo) 1994; 42: 1663-5.
5. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86: 271-8.
6. Foundation of Resuscitate and Encourage Thai Traditional Medicine. Thai pharmaceutical book. Bangkok: Pikanate Printing Center Cooporation; 2005.
7. Kim H, Han TH, Lee SG. Anti-inflammatory activity of a water extract of Acorus calamus L. leaves on keratinocyte HaCaT cells. J Ethnopharmacol 2009; 122: 149-56.

8. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of edible plants of Thailand. Food Chem 2005; 92: 491-7.
9. Unlu M, Ergene E, Unlu GV, Zeytinoglu HS, Vural N. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae). Food Chem Toxicol 2010; 48: 3274-80.
10. Jayaprakasha GK, Negi PS, Jena BS, Jagan LM. Antioxidant and antimutagenic activities of Cinnamomum zeylanicum fruit extracts. J Food Compost Anal 2007; 20: 330-6.
11. Leelapornpsid P, Chansakaow S, Chiyasut C, Wongwattananukul N. Antioxidant activity of some volatile oils and absolutes from Thai aromatic plants. Acta Horticulturae 2008; 786: 61-66.
12. Aromdee C, Vorarat S, Benjamapriyagoon S. Physicochemical properties of "Phikul", Scientific Program and Abstract Supplement The 22nd Annual Research Meeting in Pharmaceutical Sciences; December 2, 2005. Thai J Pharm Sci 2005; 29 (Suppl): 29.
13. Yokosuka A, Mimaki Y, Sashida Y. Spirostanol saponins from the rhizomes of Tacca chantrieri and their cytotoxic activity. Phytochemistry 2002; 61: 73-8.
14. Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C, Knoebl P, et al. Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. Am J Med 1995; 98: 469-75.

การศึกษาฤทธิ์ต้านอนุมูลอิสระด้วยวิธี free radical scavenging และ lipid peroxidation ของ สมุนไพรไทยที่ใช้รักษาเบาหวาน

เลอลักษณ์ เสถียรรัตน์, อรุณพร อิฐรัตน์, ศรีโสภา เรืองหนู

วัตถุประสงค์*: เพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระของสมุนไพรที่หมอพื้นบ้านใช้รักษาเบาหวาน*
วัสดุและวิธีการ*: สมุนไพร 10 ชนิด หมักด้วย เอทธานอล 95% นำมาทดสอบฤทธิ์ต้านอนุมูลอิสระ ด้วยวิธี DPPH scavenging และ lipid peroxidation*
ผลการศึกษา*: สารภี เนระพูสี ชะเอมไทย แสดงฤทธิ์ต้านอนุมูลอิสระ ที่ใช้วิธี DPPH (EC_{50}=10.17,10.24 and 14.45 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ) พิกุล สารภี และชะเอมไทย ออกฤทธิ์ต้านอนุมูลอิสระด้วยวิธี lipid peroxidation สูงที่สุด (IC_{50} 0.39, 0.43 และ0.70 ไมโครกรัมต่อมิลลิลิตรตามลำดับ) สารสกัดสมุนไพร 6 ใน 10 ชนิด มีฤทธิ์ต้านอนุมูลอิสระทั้งสองวิธี (IC_{50} น้อยกว่า 20 ไมโครกรัมต่อมิลลิลิตร)*
สรุป*: สมุนไพรที่หมอพื้นบานใช้รักษาเบาหวานส่วนใหญ่มีฤทธิ์ต้านอนุมูลอิสระ*