Tropical Journal of Pharmaceutical Research September 2014; 13 (9): 1477-1485
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i9.14

Original Research Article

Evaluation of the Antioxidant Activities of Ya-hom Intajak, a Thai Herbal Formulation, and its Component Plants

Jantanarak Tuekaew[1], Nisarat Siriwatanametanon[1], Yuvadee Wongkrajang[2], Rungravi Temsiririrkkul[1] and Ibrahim Jantan[3]*

[1]Department of Pharmaceutical Botany, [2]Department of Physiology, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Road, Rajathevi, Bangkok, 10400, Thailand, [3]Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

For correspondence: **Email: profibj@gmail.com; **Tel:** +603 92897315; **Fax:** +60326983271*

Received: 25 February 2014 *Revised accepted: 3 August 2014*

Abstract

Purpose: *To evaluate the antioxidant effect of 80 % ethanol extract and its n-hexane and dichloromethane fractions of Ya-hom Intajak, and its 47 medicinal plants components.*
Methods: *The 80 % ethanol extract and its n-hexane and dichloromethane fractions were investigated on human low-density lipoprotein (LDL) peroxidation by thiobarbituric acid reactive substances (TBARS) assay. Antioxidant effect was also determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and ferric reducing antioxidant power (FRAP) assays.*
Results: *The extract of Ya-hom Intajak exhibited moderate DPPH scavenging activity with an IC_{50} value of 99.08 µg/ml and a FRAP value of 1.12 mmol FeSO4/g. Among the individual plants, Terminalia chebula Retz., Caesalpinia sappan L., Cinnamomum bejolghota (Buch.-Ham.) and Ci. verum J. Presl, showed strong antioxidant activities. T. chebula was the most potent plant in the DPPH assay while Ca. sappan had the highest FRAP value. Among the extracts, the dichloromethane fraction showed the highest inhibitory effect on LDL peroxidation with a half maximal inhibitory concentration (IC_{50}) value comparable to that of probucol (0.82 µg/ml). Pearson correlation analysis revealed that total phenolic content (TPC) showed high positive correlations with FRAP ($r = 0.908$, $p < 0.01$) and DPPH activities ($r = 0.648$, $p < 0.01$).*
Conclusion: *Ya-hom Intajak is a valuable source of natural antioxidants and has a potential application as a cardiovascular protective formulation.*

Keywords: *Ya-hom Intajak, Antioxidant activity, Terminalia chebula Retz., Caesalpinia sappan L., Cinnamomum bejolghota (Buch.-Ham.), Ci. verum J. Presl , Phenolic content*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The initiation and progression of early stage of atherosclerosis and the development of cardiovascular diseases have been attributed to the oxidation of low-density lipoproteins (LDL). Reduction of LDL oxidation may be one of the most important therapeutic approaches to prevent the development of atherosclerosis. Many antioxidants have been developed to delay or inhibit the oxidation of the biomolecules by terminating the initiation or propagation of the oxidizing chain reactions by free radicals and inhibiting the foam cell formation [1]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet

oxygen or decomposing peroxides [2]. Many studies have indicated that there was a high correlation between the antioxidant activity of some plants and their phenolic contents [3-5]. The bioactive components can effectively inhibit LDL oxidation and may prevent atherosclerosis by reducing and slowing down the progression to advance stage [5].

Ya-hom is a traditional herbal formulation used widely in Thailand for the treatment of nausea, vomiting, dizziness and fainting, especially among the aged population. It is also used as a cardiotonic agent and tonic for longevity. There are many preparations of Ya-hom with most formula contain similar major ingredients but different total composition and amounts of medicinal plants [6]. There have been reports on the effects of Ya-hom on both animal and human cardiovascular function. The effect of Ya-hom on the pulse rate and pulse pressure in human has been reported [7]. The effect of the herbal preparation on blood pressure in rats and in isolated rat aortic ring and atrium have also been investigated [8-10]. However, the results obtained by the different studies were not in agreement and remain inconclusive, most probably due to the use of different Ya-hom formula, extraction methods and experimental models. Among those Ya-hom preparations, Ya-hom Intajak was listed by the National Drug Committee (Thailand) in the List of Herbal Medicine Products A.D. 2011 and declared as over the counter herbal drug. Ya-hom Inthajak is also accepted to be used as cardiotonic agent and also improving blood circulation [11]. The aim of this study was to evaluate the antioxidant effect of Ya-hom Intajak against human LDL oxidation, radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The relationships between these activities and their total phenolic contents and total flavonoid content were also established.

EXPERIMENTAL

Preparation of extracts

Yahum Intajak used in this study contained 47 single component plants as listed in Tables 1(a) and 1(b). The single medicinal plant components of the herbal formulation were purchased from Jaokrompur, a herbal drug store in Bangkok, Thailand and their identities were confirmed by Dr Wongsatit Chuakul of Mahidol University, Thailand. Ya-hom Intajak formula was prepared by mixing and grinding equal amount of each of the 47 component herbs, according to the formulatory provided by the List of Herbal Medicines Products A.D. 2011 [11]. The powdered mixture (100 g) was extracted with 2,000 ml of 80 % ethanol using a percolator and then the ethanol extract was successfully fractionated with n-hexane, dichloromethane and 80 % ethanol. Each plant was dried in a hot air oven at 50 °C, ground and then 30 g of the powder was macerated with 600 ml of 80 % ethanol. The extract was filtered through Whatman filter paper No.1 and the entire extraction process was repeated thrice on the marc. The extracts and filtrates were concentrated under reduced pressure.

Determination of DPPH radical scavenging activity

DPPH scavenging activity of the extracts was carried out according to the method described by Kordali *et al* with slight modification [12]. Briefly, 2 ml of each extract at various concentrations (100, 80, 60, 40 and 20 µg/µl) were respectively added to 2 ml of freshly prepared DPPH methanol solution (4 mg/100 ml). The mixtures were vortexed vigorously with a votex mixer and allowed to stand in the dark for 30 min at room temperature for any radical-antioxidant reaction to occur. Finally, the absorbance of these mixtures was measured by using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 517 nm. The difference in absorbance between the sample and the control was calculated as percentage (%) inhibition of DPPH activity. The IC_{50} values, i.e. concentration of sample providing 50 % of radical scavenging activity was obtained through interpolation of linear regression analysis. Ascorbic acid in methanol was used as a positive control and the assay was conducted in triplicate for each sample concentration.

Ferric reducing ability power assay (FRAP)

The FRAP assay was performed according to the procedure described by Benzie and Szeto [13]. The FRAP reagent was prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ dissolved by 40 mM HCl and 20 mM ferric chloride in the volume ratio of 10:1:1. The different concentrations (400-800 µg/ml) of sample was dissolved using methanol as a solvent. 25 µl of each concentration of the sample was added to 175 µl of FRAP reagent. Blank experiment contained 25 µl of sample and 175 µl of sodium acetate buffer. The absorbance of the mixtures was measured at 593 nm using UV– spectrophotometer (DKSH). Ascorbic acid in methanol was used as a positive control and the

experiment was conducted in triplicate. The standard curve was plotted using $FeSO_4$ solutions and the result is expressed as mM of $FeSO_4$ equivalents.

Evaluation of inhibitory effect on human low density lipoprotein peroxidation

The assay was performed following the method of Dillon with slight modification [14]. The procedure included LDL isolation, LDL oxidation and TBARS assay. The use of human whole blood in this study was approved by the Ethics Committee of the Universiti Kebangsaan Malaysia (approval no. FF-120-2007), following the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS and WHO) [15]. Whole blood was drawn from the vein of healthy volunteers aged between 24 and 70 years who were normolipidemic, non-smoking, had not taken any medications and supplements within the last two weeks and also claimed they fasted for 8 h, prior to blood withdrawal.

For LDL isolation, 9 ml of the blood was added into 1 ml of 3.8 % (w/v) sodium citrate (Merck, Darmstadt, Germany) solution as an anticoagulant, then centrifuged at 2,000 g for 20 min to separate plasma. The plasma (3.2 ml) was mixed well with 0.8 ml of OptiprepTM (60 % iodixanol) (Sigma Chemical Co.) as the density gradient medium to give a final iodixanol concentration of 12 % (v/v). This mixture was added in to 8.9 ml OptisealTM tube, then carefully added 4 ml of 6 % iodixanol in saline, after that topped up with 0.9 ml of saline. The tube was ultracentrifuged at 402,000 times gravity (g), 16 °C for 3 h 10 min using the Ti.70.1 rotor. The subfractions of lipoprotein: high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) was obtained. LDL was characterized by measuring the amount of protein by using Bovine serum albumin (Protein kit Sigma Chemical Co.) as a standard. The purity of LDL was measured by using electrophoresis. Agarose gel (Bio-Rad, USA) was used as solid support in electrophoresis. Samples were electrophoresed at a constant 45 mA/gel for 45 min, then oven dried at 85 °C for 20 min and stained with Sudan Black (Sigma,USA). The band was visually observed under UV detector. Then, LDL was diluted with phosphate buffer saline (PBS) (pH 7.4) to a final concentration of 200 µg protein/ml before oxidation testing.

For oxidation of LDL, the reaction was initiated by adding freshly prepared 10 µM $CuSO_4$ (Sigma, USA) solution. The samples were dissolved in DMSO to obtain serial concentrations of 1.25, 0.63, 0.31, 0.16 and 0.08 µg/µl. Five µl of the samples were added to a cuvete containing 945 µl of LDL, 50 µl of 10 µM $CuSO_4$ and incubated at 37 °C for 5 h. The final concentrations of the extracts in the mixture were 6.25, 3.13, 1.56, 0.78 and 0.39 µg/ml. 0.5 % DMSO was used as control and blank while probucol (Sigma, USA) was used as the positive control. The oxidation of LDL was terminated by rapid freezing and kept at -20 °C with no longer than 24 h.

For thiobarbituric reactive substances (TBARS) assay, 100 µl of sodium dodecyl sulphate (SDS) (Zeptometrix Co., USA) and 2.5 ml of thiobarbituric acid (TBA) (Zeptometrix Co., USA) were added to the mixture. Then, it was incubated at 95 °C for 1 h to promote peroxidation. The mixture was put on ice for 10 min to cool down and stop the peroxidation process. The precipitate formed was removed by centrifugation at 3000 rpm, 15 min. The absorbance was measured at 532 nm. Then, the malondialdehyde (MDA) in the supernatant was calculated and expressed as nmoles of MDA/mg LDL protein. Standard MDA was used as a reference and plotted the standard curve.

Determination of total phenolic content

The total phenolic content (TPC) of extract was determined using the Folin-Ciocalteau method with some modification [16]. Briefly, 33 µl of each sample was mixed with 83 µl of Folin-Ciocalteau reagent (Sigma, USA) which was diluted (1:10) with water. Then 133 µl of 7.5 % w/v sodium bicarbonate was added in to the mixture. The absorbance of the mixture was measured at 765 nm after for 30 min standing at room temperature. Gallic acid (5-35 µg/ml) (Sigma, USA) was used as standard and plotted standard curve. The total phenolic content was reported as gallic acid equivalents (GAE) per gram of sample.

Determination of total flavonoid content

The total flavonoid content (TFC) was determined using the Dowd method [17]. Briefly, 100 µl of 2 % $AlCl_3$ in methanol was mixed with 100 µl of the sample solution. The absorbance of the mixture was measured at 415 nm using Pelkin Elmer UV-VIS lambda 25 spectrophotometer after 10 min against a blank sample consisting of methanol. The TFC was determined using a standard curve of quercetin (5 - 30 µg/ml). The mean of three readings was used and expressed as milligrams of quercetin equivalents (QE)/l g of extract.

Statistical analysis

All the data are presented as standard error of the mean ± (SEM) from triplicate experiments and were analysed using Statistically Package for Social Sciences (SPSS) software version 17.0. A one-way analysis of variance (ANOVA) was used for multiple comparison. The concentration of the compounds required to inhibit 50 % oxidation (IC_{50}) for active extract was determined using probit programme. The correlation between TPC and TFC in the extracts and antioxidant properties was described by the Pearson product-movement correlation coefficient (r). $P < 0.05$ was considered to be statistically significant.

RESULTS

In this study, DPPH radical scavenging capacity and FRAP have been used to evaluate antioxidant activity of the ethanol extract of Ya-hom Intajak and its fraction together with 47 medicinal plants contained in the Ya-hom formula. The samples were evaluated for their radical scavenging capacity using DPPH assay which is based on the scavenging of the stable DPPH• by an antioxidant. Tables 1(a) and 1(b) show the IC_{50} values of DPPH scavenging activity of the ethanol extract of Ya-hom and its fractions and the single plants. The FRAP assay was used to investigate antioxidant activity which is based on the capacity of antioxidants to reduce ferric ions to ferrous ions. The FRAP values (mmol Fe (II)/1g dried extract) of the ethanol extract of Ya-hom and its fractions and the single plants are shown in Table 1(a) and (b).

The TPC and TFC of the extracts were determined and their values varied considerably, ranging from 6.73 to 268.11 mg gallic acid equivalents per gram (mg GAE/g) and from 0.80 to 87.33 mg QE/g, respectively [Tables 1(a) and (b)]. Statistical correlations have been studied between TPC and TFC of the extracts of the 47 plants and their DPPH scavenging capacity and FRAP. To access the degree and the direction of the linear relationship between TPC, TFC and antioxidant activity, a bivariate Pearson's product-movement correlation coefficient (r) was calculated. The Pearson correlation analysis indicates that the DPPH assay showed positive correlation with TPC ($r = 0.647$, $p < 0.01$) and TFC ($r = 0.285$, $p < 0.05$) and the FRAP assay showed strong positive correlation with both phenolic contents ($r = 0.908$, $p < 0.01$) and flavonoid contents ($r = 0.508$, $p < 0.01$). The high correlation between the antioxidant activity and their TPC and TFC indicated that phenolic compounds might be a major contributor of antioxidant activities of these plants.

The ethanol extract of Ya-hom Intajak and its fractions were also investigated for their ability to inhibit copper-mediated oxidation on isolated human LDL. The human LDL was isolated by ultracentrifugation method and its purity was evaluated by using UV spectrophotometer and agarose gel electrophoresis. LDL was incubated with copper ions which catalyzed a lipid peroxidation process, in the presence or absence (negative control) of each of the extract. The level of in vitro oxidative modification of LDL oxidation was measured quantitatively by the TBARS method based on malondialdehyde (MDA) production. Probucol was used as the positive control. The inhibitory effect (IC_{50}) of the extracts on LDL oxidation are shown in Table 2.

DISCUSSION

The antioxidant activity of botanical materials has been measured by various methods such as DPPH radical scavenging activity assay, ABTS radical cation scavenging activity assay, superoxide anion radical scavenging activity assay, oxygen radical absorbance capacity (ORAC) assay, ferric reducing/antioxidant power (FRAP) assay and metal chelating activity assay. The need to use different methods of antioxidant capacity measurement is due to these various mechanisms of antioxidant action. Determination of the antioxidant activity of plant extracts and compounds often gave different results as the methods used are based on different reaction mechanisms [18].

The ethanol extract of Ya-hom showed moderate DPPH scavenging activity (99.08 µg/ml). The IC_{50} values of the 47 single plants ranged from 15.35 to 1261.84 µg/ml with a difference of 83-fold between the least and most active extract. Of all the plants tested, ten plants showed greater DPPH scavenging activity than the whole Ya-hom formula, and *Terminalia chebula* was the most potent with IC_{50} value (15.35 µg/ml), comparable to that of ascorbic acid, the positive control used. In a previous study, gallic acid from the gall of *T. chebula* has showed *in vitro* anti-aging activities on DPPH radical scavenging and stimulation index on normal human fibroblast proliferation [19].

Table 1: FRAP values (mmol FeSO4/g dried extract) and IC_{50} values (µg/ml) of the ethanol extract of Ya-hom Intajak and its fractions and 47 of its plant constituents on DPPH radical scavenging activity, their total contents of phenolic and flavonoid (mg QE/g) and their percentage (%) yields of extracts

Sample	**DPPH radical scavenging activity IC_{50} value (µg/ml)**	**FRAP value (mmol $FeSO_4$/g)**	**Total phenolic content (mg GAE/g)**	**Total flavonoid content (mg QE/g)**	**Extract yield (%w/w)**
Ya-hom Formula extract	99.08 ± 8.77	0.93 ± 0.12	51.67 ± 1.87	8.41 ± 0.36	22.50
Ethanol Fraction	145.5 ± 6.71	0.81 ± 0.12	112.35 ± 6.08	13.18 ± 1.00	13.95
Dichloromethane Fraction	154.25 ± 7.15	0.76 ± 0.08	112.61 ± 5.39	1.37 ± 0.09	2.88
Hexane Fraction	303.73 ± 31.34	0.65 ± 0.09	48.75 ± 4.41	10.13 ± 0.90	4.06
Alyxia reinwardtii Blume	>500	0.49 ± 0.02	27.33 ± 1.21	1.37 ± 0.09	9.17
Amomum testaceum Ridl.	263.93 ± 11.72	0.53 ± 0.09	49.44 ± 7.16	3.93 ± 1.00	2.50
Anacyclus pyrethrum (L.) Lagasca	300.45 ± 7.51	0.48 ± 0.02	42.21 ± 1.59	7.74 ± 0.18	4.34
Angelica dahurica Benth.	>500	0.29 ± 0.02	24.31 ± 0.38	1.24 ± 0.15	9.46
Angelica sinensis (Oliv.) Diels	>500	0.22 ± 0.01	13.34 ± 1.05	1.20 ± 0.07	30.93
Aquilaria gallocha Roxb.	318.24 ± 14.10	0.69 ± 0.16	49.92 ± 4.75	13.06 ± 0.71	3.08
Aristolochia pierrei	453.48 ± 29.38	0.79 ± 0.02	32.59 ± 1.47	16.20 ± 0.92	2.21
Artemisia annua L.	462.52 ± 24.15	0.32 ± 0.01	36.93 ± 0.78	36.73 ± 0.16	14.41
Atractylodes lancea (Thung.) DC.	>500	0.32 ± 0.02	19.63 ± 10.00	2.77 ± 0.08	10.89
Bixa orellana L.	61.63 ± 5.19	1.08 ± 0.05	128.29 ± 5.45	24.75 ± 1.90	14.59
Caesalpinia sappan L.	32.37 ± 1.25	2.77 ± 0.10	247.83 ± 7.98	75.18 ± 2.39	7.34
Cananga odorata (Lam.) Hook. f & Thomson var. *odorata*	327.39 ± 20.68	0.40 ± 0.04	30.67 ± 0.83	17.79 ± 1.48	28.02
	-	-	-	-	-
Cinnamomum bejolghota (Buch.-Ham.)	20.04 ± 0.75	2.30 ± 0.21	247.21 ± 6.46	8.46 ± 0.44	27.96
Cinnamomum verum J. Presl	38.5 ± 2.24	1.93 ± 0.24	144.65 ± 5.86	13.26 ± 1.98	23.39
*Coriandrum sativum*L.	>500	0.23 ± 0.03	16.82 ± 1.45	1.86 ± 0.10	7.11
Cuminum cyminum L.	419.86 ± 18.88	0.25 ± 0.04	24.97 ± 1.28	40.37 ± 3.68	13.85
Dracaena loureiri Gagnep.	149.85 ± 10.90	0.36 ± 0.38	68.17 ± 2.7	25.17 ± 0.66	12.93
Enhalus acoroides (L.f.) Royle	484.09 ± 14.20	0.35 ± 0.06	22.61 ± 0.15	14.43 ± 1.17	5.57
Euphorbia antiquorum L.	>500	0.36 ± 0.03	29.59 ± 2.55	2.09 ± 0.07	7.52
Foeniculum vulgare Mill. var *dulce* Alef	>500	0.18 ± 0.05	24.02 ± 3.1	10.57 ± 0.23	8.57
Gymnopetalum chinense (Lour.) Merr.	>500	0.21 ± 0.03	6.87 ± 0.96	3.10 ± 0.54	12.20
Jasminum sambac (L.) Aiton	>500	0.25 ± 0.03	21.51 ± 1.3	22.71 ± 0.58	20.69
Lepidium sativum L.	355.73 ± 12.72	0.62 ± 0.02	39.34 ± 2.17	13.30 ± 0.87	12.06
Mammea siamensis	220.89 ± 6.85	0.55 ± 0.01	50.58 ± 5.3	13.90 ± 0.30	20.59
Mesua ferrea L.	121.611 ± 11.62	0.65 ± 0.03	46.78 ± 2.2	13.58 ± 0.34	19.63
Michelia champaca L.	139.75 ± 3.10	0.77 ± 0.08	77.39 ± 0.75	13.45 ±0.60	11.51
Mimusops elengi L. (Flower)	146.56 ± 4.32	0.53 ± 0.05	54.51 ± 1.54	6.07 ± 0.25	19.04

Table 1 (continued): FRAP values (mmol FeSO4/g dried extract) and IC_{50} values (µg/ml) of the ethanol extract of Ya-hom Intajak and its fractions and 47 of its plant constituents on DPPH radical scavenging activity, their total contents of phenolic and flavonoid (mg QE/g) and their percentage (%) yields of extracts

Sample	DPPH radical scavenging activity IC_{50} value (µg/ml)	FRAP value (mmol $FeSO_4$/g)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	Extract yield (%w/w)
Mimusops elengi L. (Infected wood)	>500	0.37 ± 0.05	27.84 ± 0.22	6.13 ± 0.48	4.95
*Myristica fragrans*Houtt. (Aril)	49.78 ± 2.76	0.73 ± 1.03	81.26 ± 2.41	0.80 ± 1.07	17.47
*Myristica fragrans*Houtt. (Seed)	115.13 ± 0.53	0.50 ± 0.63	67.03 ± 9.3	4.12 ± 0.09	11.78
Nigella sativa L.	>500	0.25 ± 0.05	6.73 ± 0.51	1.97 ± 0.05	15.65
Picrorhiza kurrooa Royle ex Benth.	348.77 ± 13.37	0.44 ± 0.03	49.72 ± 4.5	25.35 ± 0.53	23.55
Piper retrofractum Vahl	>500	0.26 ± 0.01	18.18 ± 1.09	4.26 ± 0.22	15.89
Piper ribesoides Wall.	143.46 ± 10.07	1.05 ± 0.06	59.22 ± 1.51	3.38 ±0.30	6.59
Piper sarmentosum Roxb.	>500	0.27 ± 0.01	22.01 ± 0.78	15.17 ± 0.77	12.36
Plumbago indica L.	273.01 ± 20.69	0.47 ± 0.05	29.77 ± 1.59	3.64 ± 0.21	23.57
Rheum palmatum L.	81.82 ± 5.54	1.60 ± 0.11	151.17 ± 5.05	10.78 ± 2.3	31.42
Santalum album L.	240.38 ± 3.91	0.35 ± 0.36	48.79 ± 1.18	7.29 ± 0.66	5.56
Saussurea lappa C.B.Clarke	499.48 ± 22.29	0.38 ± 0.06	31.17 ± 1.78	4.73 ± 0.26	28.59
Syzygium aromaticum (L.)	37.36 ± 3.55	2.66 ± 0.18	201.38 ± 5.65	15.00 ± 0.22	26.11
Terminalia chebula Retz.	15.35 ± 0.8	2.72 ± 0.08	268.11± 0.58	87.33 ± 2.57	10.39
Tiliacora triandra (Colebr.) Diels	141.56 ± 12.2	1.07 ± 0.09	75.86 ± 5.46	6.91 ± 0.38	8.93
Tinospora crispa (L.) Miers	383.16 ± 17.03	0.51 ± 0.06	29.83 ± 2.14	4.52 ± 0.49	12.81
Trachyspermum ammi (L.) Sprague	221.48 ± 18.58	0.43 ± 0.41	69.74 ± 1.25	30.42 ± 0.80	10.68
Urceola minutiflora (Pierre) D.J. Middleton	134.91 ± 4.75	0.38 ± 0.42	59.20 ± 1.67	4.36 ± 0.34	12.06
Urceola rosea (H. & Arn.) D.J. Middleton	-	-	-	-	-
Zingiber officinale Roscoe	37.16 ± 1.14	0.78 ± 1.11	198.26 ± 5.51	18.29 ± 1.70	12.65
Ascobic acid	88.00 ± 2.54	1.54 ± 0.18	72.20 ± 0.30	13.44 ± 0.77	11.69
Trolox	12.94 ± 0.20	2.93 ± 0.03	-	-	-
	18.80 ± 0.56	2.99 ± 0.11	-	-	-

Table 2: Inhibition (%) and IC_{50} values (µg/ml) of ethanol extract of Ya-hom Intajak and its n-hexane, dichloromethane and 80 % ethanol fractions on LDL oxidation

Sample	Concentration (µg/ml)	Inhibition (%)	IC_{50}
Yahom formula extract	6.25	72.85 ± 4.16	2.43
	3.13	67.37 ± 3.13	
	1.56	51.73 ± 5.78	
	0.78	5.57 ± 1.50	
	0.39	2.30 ± 0.81	
Ethanol fraction	6.25	72.92 ± 1.80	2.76
	3.13	57.16 ± 4.51	
	1.56	38.51 ± 4.42	
	0.78	5.57 ± 1.50	
	0.39	4.93 ± 0.37	
Dichloromethane fraction	6.25	77.61 ± 3.10	0.82
	3.13	77.56 ± 0.07	
	1.56	67.82 ± 0.04	
	0.78	55.23 ± 0.40	
	0.39	27.61 ± 0.40	
n-Hexane fraction	6.25	72.86 ± 3.56	1.47
	3.13	71.92 ± 0.80	
	1.56	57.30 ± 0.24	
	0.78	36.96 ± 2.94	
	0.39	17.25 ± 0.81	
Probucol	6.25	76.32 ± 0.01	0.75
	3.13	70.18 ± 0.80	
	1.56	59.53 ± 1.81	
	0.78	51.93 ± 1.73	
	0.39	39.88 ± 2.57	

Other plants exhibiting strong DPPH scavenging activity were *Caesalpinia sappan, Cinnamomum bejolghota* and *C. verum*. The results indicate that the extracts of these plants contained compounds that were relatively strong scavengers of free radicals. The antioxidant effect is due to the ability of the compounds in the plants extracts to transfer electron or hydrogen atom to neutralize radicals of DPPH to form neutral DPPH molecules [20].

The ethanol extract of Ya-hom showed the highest antioxidant capacity (1.12 mmol Fe(II)/g) while its fractions exhibited lower activity. The FRAP values of the 47 plants varied from 0.18 to 2.77 mmol Fe(II)/g with a difference of 15-fold between the lowest and highest activity. Among the plants tested, seven plants showed higher FRAP value than the whole Ya-hom formula and *Caesalpinia sappan* had the highest FRAP value (2.77 mmol Fe(II)/g) which was comparable to that of the positive control, ascorbic acid. *C. sappan* alcohol extract has been reported to exhibit IC_{50} values on DPPH and nitric oxide method comparable to those of ascorbic acid and rutin. Moreover, hematein, a compound isolated from *C. sappan* wood was able to reduce fatty streak lesion of cholesterol-fed rabbits and has a potential to be used as an anti-atherogenic agent [21]. Other plants with strong FRAP activity were *Terminalia chebula, Cinnamomum bejolghota* and *C. verum*.

The results showed that the methanol extracts of these plants contained high levels of phenolic contents. Among all the plant extracts, the highest TPC and TFC was observed in the extract of *Terminalia chebula* while the lowest TPC was in the extract of *Nigella sativa* and the lowest TF was in the extract of *Myristica fragrans*. Phenolic compounds have been reported to be the major contributor to the antioxidant activities of grain, vegetables and other botanical materials [18]. Flavonoid is one of the most important natural phenolics which some of them became to be widely known as potent antioxidants such as quercetin and rutin.

The extracts and its fractions showed significant antioxidant activity on LDL oxidation as at 6.25 µg/ml, all samples exhibited greater than 70 % inhibition of LDL oxidation. The most active sample was dichloromethane fraction which showed an IC_{50} value (0.82 µg/ml) comparable to that of probucol, a potent inhibitor of copper-catalysed LDL peroxidation (Table 2). The results

demonstrated that the extracts inhibited the copper-mediated oxidation of LDL in a dose-dependent manner, as the concentration of the extract increased the percentage inhibition increased. The results indicate that the extract of Ya-hom contained compounds that are relatively strong inhibitors of LDL peroxidation. The antioxidant effect is due to the ability of the compounds in the plants extracts to chelate Cu^{2+} ion and thus may inhibit the initiation of LDL oxidation and free radical formation at the lipoprotein. The extracts may also form chelating complexes with transition metals to reduce their availability as catalysts for free radical generation [20].

CONCLUSION

This study indicates that the two types of antioxidant capacity measurements, DPPH and FRAP, of the plant extracts provide broadly similar order of antioxidant activity. The results imply that the antioxidants in these plants are capable of scavenging free radicals and reducing oxidants. The high correlation between the antioxidant and activities and their TPC and TF indicates that phenolic compounds are the major contributor of antioxidant capacities of these plants. The extract of Ya-hom Intajak and its fractions possesses significant antioxidant activity on LDL oxidation, indicating that the extract contained compounds that were relatively strong inhibitors of LDL peroxidation. The plants are valuable sources of natural antioxidants.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Graduate Studies of Mahidol University Alumni Association, Thailand and the Ministry of Agriculture Malaysia for financial support (grant no. 05-01-02-SF1008).

REFERENCES

1. *Heinecke JW. Lipoprotein oxidation in cardiovascular disease: chief culprit or innocent bystander. J Exp Med. 2006; 203: 813-816.*
2. *Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997; 2: 152-159.*
3. *Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem. 1998; 46: 4113-4117.*
4. *Odabasoglu F, Aslan A, Cakir A, Suleyman H, Karaqoz Y, Halici M, Bayir Y. Comparison of antioxidant activity and phenolic content of three lichen species. Phytother Res. 2004; 11: 938-941.*
5. *Hodzic Z, Pasalic H, Memisevic A, Srabovic M, Saletovic M, Poljakovic M. The Influence of total phenols content on antioxidant capacity in the whole grain extract. Eur J Sci Res. 2009; 3: 471-477.*
6. *Sirisangtrakul W, Sripanidkulchai B. Interference of Thai Traditional Medicine (Ya-hom Ampanthong) on hepatic cytochrome P-450 enzymes and pentobarbital-induced sleeping in mice. Pakistan J Biol Sci. 2011; 14: 91-98.*
7. *Matangkasombat O. Pharmacological effects of Thai folk medicine (Ya-hom) on the blood pressure and cardiac function in man and experimental animal. Complete report submitted to the National Research Council of Thailand. 1974; 1-22.*
8. *Suvitayavat W, Tunglert S, Thirawarapan SS, Bunyapraphatsara N. Effects of Ya-hom on blood pressure in rats. J Ethnopharmacol. 2005; 97: 3-508.*
9. *Suvitayavat W, Tunglert S, Thirawarapan SS, Kitpati C, Bunyapraphatsara N. Actions of Ya-hom, a herbal drug combination, on isolated rat aortic ring and atrial contractions. Phytomedicine. 2005; 12: 561-569.*
10. *Na Pattaloong P, Sawasdimongkol K. Action of Thai traditional cardiotonic preparations (Ya-hom) on isolated rat atrium. Bull Depart Med Sc. 1995; 37: 271-288.*
11. *National Drug Committee. List of Herbal Medicine Product A.D. 2011, Agricultural Cooperative Federation Printing, Thailand.*
12. *Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A. Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish Artemisia species. J Agri Food Chem. 2005; 53: 1408-1416.*
13. *Benzie IFF, Szeto YT. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J Agri Food Chem. 1999; 47: 633-636.*
14. *Dillon SA, Burmi RS, Lowe GM, Billington D, Rahman K. Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density lipoprotein. Life Sci. 2003; 72: 1583-1594*
15. *International Ethical Guidelines for Biomedical Research Involving Human Subjects. Prepared by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO). Geneva, 2002. 64 pp. ISBN 92 9036 075 5*
16. *Singleton VL, Orthofer R, Raventos RML. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol. 1999; 299: 152-178.*
17. *Meda A, Lamien CE, Romito M, Millogo J. Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina fasan honey, as well as their radical scavenging activity. Food Chem. 2005; 91: 571-577.*

18. Wong SP, Leong LP, Koh JHW. Antioxidant activities of aqueous extracts of selected plants. Food Chem. 2006; 99: 775-783.
19. Manosroi A, Jantrawut P, Akihisa T, Manosri W, Manosri J. In vitro anti-aging activities of Terminalia chebula gall extract. Pharm Biol. 2010; 48: 469-481.
20. Yu L, Zhou K, Parry JW. Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. LWT-Food Sci Technol. 2005; 38: 463-470.
21. Badami S, Moorkoth S, Rai SR, Kanna E, Bhojraj S. Antioxidant Activity of Caesalpinia sappan Heartwood. Biol Pharm Bull. 2003; 26: 1534-1537.