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Antioxidants in Thai Herb, Vegetable and Fruit Inhibit Hemolysis and Heinz Body Formation in Human Erythrocytes

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1. Introduction

Over centuries, Thai traditional herbal beverage and herbal tea have played an important role not only in quenching the thirst but also in providing therapy for common ailments. Recently, ready-to-drink beverages; tea, herbal-tea and fruit juice, are become very popular for Thai people, especially the teenagers. Because of the media presents results of promoting health and preventing many diseases by intake fruit, vegetable and herb which contained high level of antioxidant activities. These plants are rich of antioxidants those are capable to terminate free radical reactions and prevent oxidative damage. Vegetables and fruits are important sources of antioxidant in diet. Scientists have been searching for more than 2 decades to identify the specific ingredients in fruit vegetable and herb that account for their many health promotion benefits. That search points to plant metabolites, many of which are antioxidants, phytochemicals, mixtures of vitamins and fiber content. Thousands experimental studies have examined the role of specifics flavonoids or phenolics in disease prevention(Sen et al., 2010; Virgili & Marino, 2008). Different phytochemicals in herbal products are safer than synthetic medicine and beneficial in the treatment of diseases caused by free radicals. Multiple biological effects of them have been described, among them; antioxidants, cellular signals, cardioprotective effects, antibiotics, antiinflammation, antiallergic, anticoagulation, antineoplastic, anti-mutagenesis, anti-carcinogenesis (Ames et al.,1993; Lin and Liang, 2000; Ziegler, 1991; Packer and Colman,1999; Halliwell and Gutteridge, 2001; Nakamura, 1997; Cook & Samma, 1996; Chen and Yen, 1997; Lin & Liang, 2000).

The major fraction of those plants is nutraceuticals, flavonoids, for neutralizing stress induced free radicals. Flavonoids are effective antioxidants and may protect against several chronic diseases. Flavonoids are divided into six different classes (flavanols, flavanones, flavones, isoflavones, flavonols and anthocyanins) depended on different molecular structure. The flavonol quercetin and the flavone apigenin are found in many fruits and vegetables such as onions, apples, broccoli, and berries. Catechins are main flavonoids in green tea. Cyanidin and anthocyanidins are the pigment of black rice, berries and grapes. Genistein is an isoflavone found in legumes. Hesperitin, Naringenin and Eriodictyol are

flavanones in citrus fruits. The group of flavanol catechins is derived from green tea, red grapes, red wine and chocolate (Beecher, 2003). The antioxidant activities of phenolics are mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition they have a metal chelation potential (Halliwell and Gutteridge, 2001). Flavonoids may suppress LDL oxidation and inflammatory progression in the artery wall (Hertog et al, 1995; Peluso, 2006). It was found that catechins promote many biological functions, including prevention of cardiovascular diseases (Langley-Evans, 2001) and cancer (Kohlmeier et al, 1997; Steinmetz & Potter, 1991). Polyphenolic phytochemicals such as epigallocatechin from tea, the flavonoids quercetin and genistein from onions and soya, curcumin in curry spice and resveratrol from red grapes are diet constituents with high efficacy in preclinical carcinogenesis of colorectum, breast and prostate (Thomasset et al., 2006; Lambert et al., 2005; Surh, 2003). Low cerebrovascular disease was associated with high intake of the flavonol kaempferol and of the flavonones naringenin and hesperitin and there was a trend of reduction in type 2 diabetes was associated with higher quercetin and myricetin intake (Knekt et al., 2002). The other reported a strong inverse association between the sum of quercetin, myricetin, luteolin, and apigenin intaked and stroke (Keli et al.,1996) and low risk of lung cancer at high flavonoids intake (Knekt et al.,1997). Black rice anthocyanins reduced oxidative damage (Sangkitikomol et al., 2010a), anti-inflammation (Wang et al., 2007), enhanced LDL-receptor (Sangkitikomol et al., 2010b) and promoted cardiovascular health status (Ling et al., 2002; Wang et al., 2007). Aging is the major risk factor for neurodegenerative diseases such as Parkinson's diseases and Alzheimer's. Polyphenolic compounds could affect on cells not only due to their antioxidant activities but also due to their modulation of different pathways including signaling cascades, antiapoptotic processes and the synthesis of the amyloid β peptide (Ramassamy, 2006).

CytochromeP450 isoenzymes (CYP450s) are major enzymes in phase I of biotransformation system which involved in the metabolism of various endogenous chemicals such as; fatty acids, steroids, hormones, bile acids, eicosanoids and exogenous chemicals such as; xenobiotics, carcinogens, mutagens and environmental pollutants. A major function of CYP450s is catalyzed the reactions then conversion these nonpolar compounds into polar metabolites which can be conjugated by phase II enzymes. The oxygen activation by CYP450s' catalytic function generate the reactive oxygen species (ROS) (Guengerich, 2008). Flavonoids may be beneficial for health protection by reducing oxidative damage and minimizing toxicity by regulating mRNA CYP450s expression for suitable production or inhibition of CYP450s isoenzymes, thereby maintaining xenobiotic biotransformation balance. Flavonoids from St. John's wort can selectively inhibit CYP1 enzymes may be useful as chemoprotective agents in prostate cancer (Chaudhary & Willett, 2006). Inhibition of PAH-induced carcinogenesis using cancer chemoprevention; methoxylated flavones and stilbene resveratrol, could effectively inhibit the benzo[*a*]pyrene-DNA binding and CYP 1A induction which were the early step in molecular levels of cencer prevention (Tsuji & Walle, 2007). Emerging evidence indicates that transcriptional activation of the antioxidant response element (ARE) plays a crucial role in modulating oxidative stress and providing cytoprotection against prooxidant stimuli (Nguyen et al., 2003). Several chemopreventive agents, such as curcumin, caffeic acid phenethyl ester, rectinoic acid, (–)-epigallocatechin-3-gallate and (–)-epicatechin-3-gallate from tea, directed to protect DNA and other important

cellular molecules by inducing the synthesis of phase II detoxifying genes and antioxidant genes via the Nrf2–ARE signaling pathway. Thereby enhancing those genes transcription and stimulating carcinogen detoxification/inactivation. The Nrf2 (nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2), is a regulator of genes encoding antioxidants and phase II enzymes such as glutathione S-transferase, NAD(P)H:quinine oxidoreductase 1, UDP-glucuronosyl-transferase, γ-glutamate cysteine ligase, and hemeoxygenase-1. The Nrf2 is known to mediate detoxification and/or to exert antioxidant functions thereby protecting cells from genotoxic damage (Lee & Surh, 2005; Zhang & Gordon, 2004; Hayes & McMahon, 2001). NF-κB regulated genes such as inducible nitric oxide synthase (iNOS) and cyclooxgenase-2 (COX-2) which are the inflammatory mediators and may promote cancinogenesis (Greten & Karin, 2004; Surh, 2003). Nrf2 encodes for antioxidant and general cytoprotection genes, while NF-κB regulates the expression of proinflammatory genes. A variety of antiinflammatory or anticarcinogenic phytochemicals suppress NF-κB signalling and activate the Nrf2-ARE pathway (Bellezza et al., 2010)

There is no evidence that different types of tea, herbal tea, fruit and vegetable currently available in the markets of Thailand have any significant antioxidant contents. The aim of this study was to carry out a survey of relative levels of total antioxidant activities by TEAC assay (Re et al., 1999) with reference to their total phenolic contents (Singleton et al., 1999). In order to find the natural sources of antioxidants, thereafter some of them were selected to study the inhibition effect on hemolysis (Sangkitikomol et al.,2010a) as a marker of lipid peroxidation, induced by 2, 2´-azobis (2-amidinopropane) hydrochloride and the inhibition effect on Heinz body formation, as a marker of protein oxidation, induced by N-acethylphenylhydrazine (Sangkitikomol et al., 2001; 2010a) by using normal fresh human red blood cells.

2. Measuring antioxidant activities of tea, herbal tea, fruit and vegetable

2.1 Chemicals

ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] diammonium salt, TPTZ (2,4,6-tripyridyl-s-triazine), gallic acid, Trolox(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), AAPH[2,2'-azobis(2 amidinopropane) dihydrochloride], Folin Ciocalteu's phenol reagent and APHZ (N-acethylphenylhydrazine) were purchased from Sigma-Aldrich, St. Louis, MO,USA. All other basic reagents were of analytical grade.

2.2 Preparation of plant extracts

Different types of Thai plants were purchased from Thai-herb shops, health food shops, and local markets. These samples were crushed into small pieces and some were ready-made, they were 152 samples of fruit, vegetable and herb, 33 brands of tea. A hundred milligram of dry material was extracted with 2 x 10 ml of solvent using ultrasonic bath for 5 minutes sonication, centrifuged at 3000 rpm for 10 minutes and the combined extracts were kept in deep freezer at -80°C until used. Using 2 kinds of solvent for extraction, one is 80 % aqueous methanol and the other is deionized reverse osmosis water. The plant water-extracts were used to determine lipid and protein oxidation in human red blood cells, and the other were used to analyze the antioxidant activity. Fourteen samples of ready-to-drink beverages were purchased from supper markets and directly used for analysis.

2.3 Preparation of fresh whole blood

Human blood samples were obtained from The National Blood Centre, Thai Red Cross Society, Bangkok, Thailand. Fresh blood was collected in heparinised tubes and centrifuged at 252 × g for 3 min. Plasma was carefully removed by aspiration in order to obtain a hematocrit of approximately 50% (packed red blood cells; RBCs) for hemolysis test and Heinz body formation. The blood was stored at 4°C and used within 3 h.

2.4 Determination of antioxidants in plant extracts

The total antioxidant determination and total phenolic contents were performed on the Shimadzu spectrophotometer model UV160A (Tokyo, Japan). All determinations were carried out at least three times of the standards and samples. And the correlation analysis had been done by using program Microsolf Excel 2007.

2.4.1 Trolox Equivalent Antioxidant Capacity (TEAC) Assay

Total antioxidant activity is measured by TEAC assay of Re et al. (1999). It is a screening of lipophilic and hydrophilic antioxidants activity by decolorization assay. The radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•) is generated by oxidation of ABTS with potassium persulfate. Then the radical ABTS• is reduced in the presence of hydrogen-donating antioxidants from the plant extracts. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of concentration of antioxidants and of Trolox for the standards. The results were expressed as Trolox equivalent in mM per kilogram of dry weight (TE mM/kg.dw).

2.4.2 Folin cioculteau phenol assay (Folin assay)

Modified Folin Cioculteau Phenol assay (Singleton et al., 1999) is used to determine reducing properties of phenolics contents. Briefly, 500 µl of samples or standards was mixed with 500 µl of 10% Folin reagent, let stand for 20 min, added 10 mM Na_2CO_3 350 µl, let stand for 20 min for the solution turned blue color and then measured the absorbance at 750 nm by using gallic acid as the standards. The results were expressed as Gallic acid equivalent (GE) mM/kg.dw.

2.4.3 Determination of AAPH-Induced oxidative damage of erythrocyte membrane

The erythrocyte susceptibility to oxidative destruction was evaluated *in vitro* by subjecting the cells to oxidative stress. Since a peroxyl radical initiator, AAPH, has been proved to cause an oxidation of cell membrane proteins and lipids resulting in lysis of red blood cells (RBCs), oxidative hemolysis induced by AAPH (Sangkitikomol et al.,2010a) was used as a tool in the present study. Briefly, positive control was added 0.100 ml of fresh whole blood into pre-incubated medium [1.00 ml of phosphate buffer saline (PBS) pH 7.4], then incubated at 37 °C for 5 min. One ml of PBS solution with 200 mM AAPH was added to the whole blood suspension. The reaction mixture was shaken gently at 37 °C under aerobic condition for three and a half hours. Reaction mixture (0.100 ml) was withdrawn to 1.5 ml of ice cold PBS at 120, 150, 180 and 210 minutes after added AAPH and centrifuged at 1006 × g for 10 min. The extent of hemolysis was determined by measuring the absorbance of

hemolysate at 540 nm. Negative control was done the same as positive control without AAPH presented. And the plant extracts was done the same as positive control excepted only using the extract 0.5 ml mixed with 0.5 ml of double strength of PBS in stead of 1.00 ml pre-incubated medium. Similarly, 0.100 ml of reaction mixture of negative control was treated with 1.5 ml of distilled water to yield complete hemolysis. Percentage hemolysis was calculated according to the equation. % hemolysis = absorbance of the sample aliquot divided by absorbance of the complete hemolysis x 100. Data was represented as the time required to achieve 50 % hemolysis (T_{50} min).

2.4.4 Determination of APHZ-Induced Heinz body formation in cytosol of erythrocytes

APHZ was used as a free radical initiator inside RBCs to oxidize proteins mostly hemoglobin based on the modification of protocol described previously (Sangkitikomol et al. 2001). Using Heinz body formation was a marker of protein oxidation for testing the antioxidants properties of plant extracts. Briefly, positive control was added 0.1 ml of fresh whole blood into 2.0 ml of reaction medium [contained 1.0 g/L of APHZ, 2.0 g/L of glucose in phosphate buffer (1.3 parts of 1/15 M of KH_2PO_4 and 8.7 parts of 1/15 M of Na_2HPO_4, pH 7.4)] and incubated for 2 hours at 37^{o}C under aerobic condition. Heinz bodies are precipitates of oxidized or denatured hemoglobin that adhere strongly to the red blood cell membrane. Negative control was done without APHZ added. And the plant extracts were done the same as positive control excepted only using the plant extract 1.0 ml mixed with 1.0 ml of double strength of reaction medium. Staining Heinz bodies in RBC with 3 drops of crystal violet solution (10 g/L in 0.73 % of normal saline) and 3 drops of RBC from reaction medium for 5 minutes at room temperature. Made blood smear on glass slides and counted RBC with Heinz body inside/1,000 of RBC using light microscope. The results were reported in % inhibition of Heinz body formation.

2.4.5 Results

Antioxidant activities of 152 herbs and 33 brands of tea using TEAC assay varied considerably with the types of plants and the types of tea.The range of antioxidant activities and total phenolic contents were several hundred-fold. The plants which are very good sources of antioxidants are *Quercus infectoria*, *Areca catechu Linn., Terminalia spp., Phyllanthus emblica Linn., Punica granutum Linn., Eugenia caryophyllus* (Table 1) and tea (*Camellia sinensis*) (Table 2). Correlation analysis (R) between the antioxidant activity measurements of plant-extracts and tea-extracts with the measured total phenol concentration were calculated to be 0.988 and 0.902, respectively (Figure 1-2). Antioxidant activities varied considerably with the types of tea (Table 2). In the TEAC assay tea-extracts with the highest to the lowest activity were green tea, oolong tea and black tea, respectively. The range of antioxidant activity was 15.6-fold for the TEAC analysis and 6.6-fold for Folin assay. Fourteen samples of ready-to-drink beverages were found small amount of polyphenolics and antioxidant contents (Table 3). Selected 30 samples with high level of total antioxidant activities, within the range 141-9490 mM TE/kg.dw., were used to study the inhibition effect of lipid peroxidation and protein oxidation. The results showed that 13 out of 30 samples showed inhibition effect on Heinz body formation and the other had no effect may be caused by antioxidant contents in the extracts was not enough to inhibit Heinz body formation inside red blood cells. All

samples showed prolong T_{50} min. of hemolysis assay, and most of them had very strong inhibition effect which showed the T_{50} min. were longer than 3 and a half hours. Time required to get T_{50} min. of control positive was between 120-150 min. (Table 4).

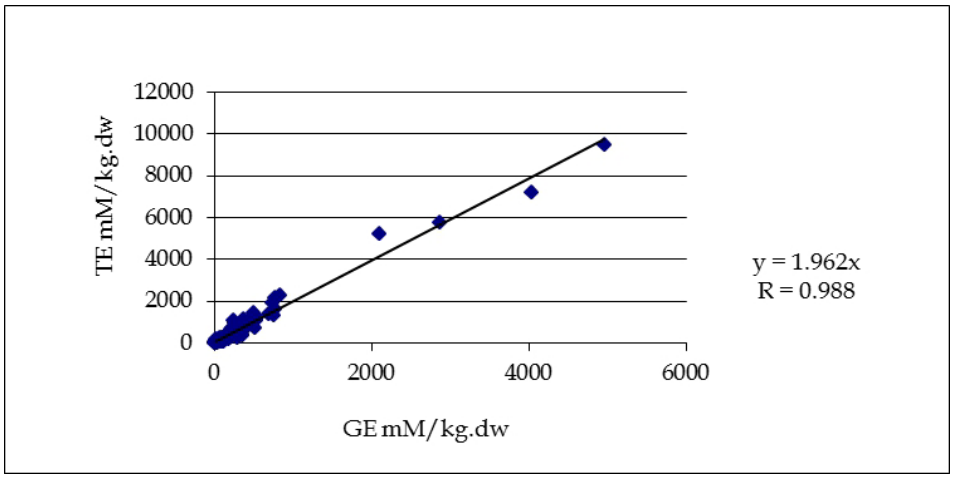

Fig. 1. Correlation between polyphenolics using Folin assay with total antioxidant activities using Trolox equivalent antioxidant capacity in 152 plant-extracts. Both assays are expressed in dry weight.

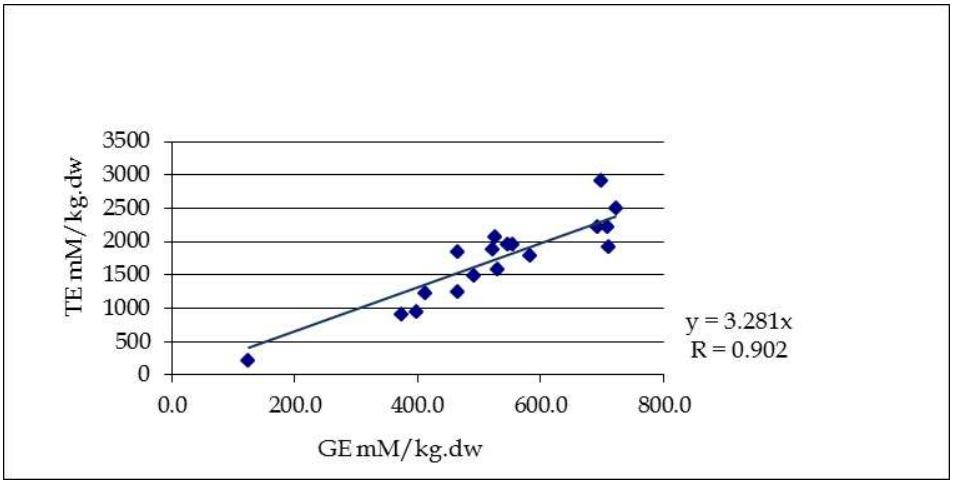

Fig. 2. Correlation between 33 polyphenolics using Folin assay with total antioxidant activities using Trolox equivalent antioxidant capacity in 33 tea-extracts. Both assays are expressed in dry weight.

No	Scientific Name of plants	Folin Assay GE mM/kg.dw.	TEAC Assay TE mM/kg.dw.
1	*Abroma augusta Linn.*	63 ± 3	40 ± 3
2	*Acacia catechu Willd.*	4038 ± 106	7183 ± 204
3	*Acacia rugata Merr.*	94 ± 10	52 ± 1
4	*Adhatoda vasica (L.) Nees*	47 ± 3	56 ± 10
5	*Aiiium ascalonicum Linn.*	221 ± 30	357 ± 20
6	*Albizia myriophylla Benth.*	61 ± 8	144± 7
7	*Allium tuberosum Roxb.*	36 ± 4	80.8 ± 3.5
8	*Amomum cadamomum*	5 ± 1	12.0 ± 1.0
9	*Anethum graveolens Linn.*	47 ± 4	55 ± 4
10	*Angelina sinensis*	25 ± 2	46 ± 4
11	*Areca catechu Linn.*	2876 ± 68	5771 ± 46
12	*Artimisia pallens Wall. Ex Bess.*	35 ± 5	44 ± 5
13	*Artocapus heterophyllus Lamk.*	116 ± 10	29 ± 4
14	*Artocarpus lakoocha Roxb.*	744 ± 32	1923 ± 201
15	*Atractylodes lyrata Sieb.et Zucc.*	19 ± 3	31 ± 6
16	*Azodirachta indica A Juss.* (stem)	237 ± 10	467± 39
17	*Azodirachta indica A Juss.* (flower)	53 ± 2	99 ± 8
18	Azo*dirachta indica A Juss.* (leaf)	188 ± 10	322 ± 35
19	*Baliospermum montanum Willd.*	38 ± 2	64 ± 6
20	*Baliospermum montanum Willd.*	157 ± 4	212 ± 15
21	*Bambusa spp.*	42 ± 3	74± 10
22	*Boesenbergia pandurate Holtt.*	107 ± 6	221 ± 15
23	*Bougainvillea spectabilis Willd.*	157 ± 10	218 ± 12
24	*Brassica camprestris L ssp.*	59 ± 2	144 ± 11
25	*Bridelia siamensis Craib.*	98 ± 8	129 ± 10
26	*Caesalpinia sappan Linn.*	520 ± 15	1292 ± 102
27	*Cantharanthus roseus* (flower)	95 ± 10	134 ± 12
28	*Cantharanthus roseus* (leaf)	107 ± 7	151 ± 23
29	*Carica papaya Linn.* (leaf)	47 ± 2	90 ± 3
30	*Carum carvi Linn.*	53 ± 5	66 ± 10
31	*Casicum frutecens Linn.* (fruit)	58 + 3	89 ± 4
32	*Casicum frutecens Linn.* (seed)	40 ± 5	15 ± 3
33	*Cassia sophera Linn.*	59 ± 7	88 ± 7
34	*Cinnamomum cassia*	47 ± 4	38 ± 6
35	*Cinnamomum zeylanicum Linn.*	219 ± 22	426 ± 31
36	*Citrus hystrix DC.* (leaf)	56 ± 10	88 ± 6
37	*Citrus hystrix DC.* (peel)	90 ± 9	254 ± 29
38	*Citrus medica Linn. Var.*	53 ± 6	102 ± 10
39	*Cladogynos orientalis Zipp. Ex Span*	27 ± 3	32 ± 5
40	*Combretum extensum*	61 ± 7	158 ± 15
41	*Combretum quadrangulare Kurz.*	164 ± 10	399 ± 29
42	*Conioselinum univitatum*	49 ± 8	73 ± 10
43	*Connarus ferugineus*	417 ± 31	767 ± 60
44	*Cuminumc cyminum Linn.*	60 ± 5	94 ± 10
45	*Curcuma longa Linn.*	352 ± 20	395 ± 41

No	Scientific Name of plants	Folin Assay GE mM/kg.dw.	TEAC Assay TE mM/kg.dw.
46	*Cymbopogon citratus stapf.*	50 ± 4	64 ± 5
47	*Cyperus rotundus Linn.*	22 ± 1	189 ± 15
48	*Diospyros decandra Lour.*	33 ± 4	66 ± 9
49	*Diosyrros mollis Griff*	38 ± 2	49 ± 4
50	*Dracaena loureiri Gagnep.*	169 ± 9	292 ± 27
51	*Emita sonchifolia DC.*	32 ± 3	49 ± 7
52	*Erythrina suberosa Roxb.*	105 ± 9	165 ± 20
53	*Eugenia caryophyllus*	687 ± 15	1400 ± 302
54	*Eurycoma longifolia Jack*	23.4 ±1.6	34.5 ± 4.1
55	*Garcinia mangostana* (peel)	243 ± 12	1095 ± 206
56	*Glycyrrhiza glabra Linn.*	295 ± 20	242 ± 30
57	*Hamisonia perforata (Lour.) Merr.*	405 ± 31	887 ± 41
58	*Heracleumsiamnicum Craib.*	42 ± 4	55 ± 5
59	*Hibiscus abelmoschus Linn.*	22 ± 2	30 ± 2
60	*Illicium verum Hook*	100 ± 9	171 ± 16
61	*Impormoca reptans Poir.*	377 ± 25	751 ± 32
62	*Ixora cibdela craib*	84 ± 7	134 ± 10
63	*Jatropha gossypifolia Linn.*	41± 3	69 ± 6
64	*Jusminum sambac Ait.*	49 ± 3	71 ± 9
65	*Leucaena leucocephala de Wit*	143 ± 10	316 ± 25
66	*Levisticum officinale Koch*	52 ± 3	64 ± 5
67	*Lycium chinensis*	62 ± 9	66 ± 8
68	*Mammea siamensis*	133 ± 15	291 ± 25
69	*Mangifera indica Linn.* (leaf)	539 ± 32	1118 ± 90
70	*Mesua ferrea Linn.*	222 ± 38	357 ± 30
71	*Millingtonia hortensis Linn.*	100 ± 15	133 ± 12
72	*Mimusops elengi Linn.*	52 ± 9	114 ± 11
73	*Molindia citrifolia Linn.* (leaf)	41 ± 7	79 ± 9
74	*Momordica charantia Linn.*	28 ± 3	29 ± 3
75	*Murdandia lorifomis (Hassk.)*	36 ± 3	56 ± 4
76	*Musa sapientum Linn.* (leaf)	28 ± 2	38 ± 4
77	*Myristica fragrans Houtt.*	77 ± 6	247 ± 15
78	*Myristica fragrans Houtt.*	8 ± 1	21 ± 4
79	*Nelumbo Mucifera Gaertn* (flower)	500 ± 3	1014 ± 101
80	*Nelumbo Mucifera Gaertn* (leaf)	308 ± 24	714 ± 35
81	*Nicotiana tabacum*	97 ± 12	106 ± 14
82	*Nigella sativa Linn.*	83 ± 5	118 ± 11.
83	*Ocimum basilicum*	56 ± 4	76 ± 8
84	*Ocimum sanctum Linn.* (flower)	95 ± 7	130 ± 10
85	*Ocimum sanctum Linn.* (leaf)	53 ± 4	64 ± 6
86	*Pandanus odoratissimus Linn.*	29 ± 3	36 ± 4
87	*Panicum repens Linn.*	21 ± 4	21 ± 2
88	*Petroselium crispum (Mill.)*	107 ± 10	200 ± 14
89	*Pimpinella anisum Linn.*	59 ± 5	107 ± 10
90	*Pinus mercukusii Jungh & de Vriese*	65 ± 5	83 ± 7

No	Scientific Name of plants	Folin Assay GE mM/kg.dw.	TEAC Assay TE mM/kg.dw.
91	*Piper aurantiacum Mig*	75 ± 10	108 ± 12
92	*Piper betle Linn.*	360 ± 15	375 ± 20
93	*Piper chaba Hunt.*	31 ± 3	37 ± 4
94	*Piper nigrum Linn*(Black pepper)	24 ± 4	34 ± 4
95	*Piper sarmentosum Roxb.*	48 ± 4	71 ± 7
96	*Pithecellobium tenue Craib*	507 ± 32	1433 ± 102
97	*Plantago ovata Forskal P.*	17 ± 2	22 ± 2
98	*Plumbago indica Linn.*	66 ± 6	100 ± 11
99	*Plumeria alba Linn.*	21 ± 3	35 ± 4
100	*Polygonium odoratum Lour.*	168 ± 12	380± 20
101	*Psidium guajava*	376 ± 29	1121 ± 100
102	*Punica granutum Linn.*	778 ± 60	2157 ± 180
103	*Quercus infectoria*	4962 ± 202	9490 ± 390
104	*Rosa domescena Mill.*	428 ± 24	1035 ± 95
105	*Sidarhombifolia Linn.*	97 ± 18	113 ± 11
106	*Smilax corbulalia Kunta*	752 ± 80	1308 ± 99
107	*Smilax micro-china T. Koyama*	162 ± 11	239 ± 16
108	*Spilanthes acmella Linn.*	44 ± 3	71 ± 8
109	*Spilanthes acmella Linn.*	39 ± 5	73 ± 9
110	*Stemona toberosa Lour.*	14 ± 2	26 ± 2
111	*Streblus asper Lour.*	16 ± 1	20 ± 2
112	*Strychnos lucida R. Br.*	81 ± 3	91 ± 8
113	*Syzygium gratum*	227 ± 27	718 ± 66
114	*Tamaridus indica Linn.*	57 ± 6	104 ± 11
115	*Tamarindus indica Linn.*	255 ± 20	932 ± 46
116	*Terminalia chebula Retz.*	772 ± 65	1640 ± 112
117	*Terminalia spp.*	2100 ± 100	5216 ± 150
118	*Tiliacora triandra Diels*	83 ± 8	86 ± 10
119	*Tinospora cordifolia Miers*	65± 8	77 ± 6
120	*Urceola minutiflora Pierre*	172 ± 10	283 ± 18
121	*Xylinbaria minutiflora Pierre*	539 ± 50	1050 ± 97
122	*Zingiber cassumunar Roxb.*	97 ± 11	113 ± 11
123	*Ziziphus mauritiana*	74 ± 7	29 ± 3
124	*Andrographis paniculata Nee*	140 ± 9	164 ± 10
125	*Bulbostylis barbata Clarke*	116 ± 11	119 ± 10
126	*Carcinia atroviridis Griff.*	22 ± 2	34 ± 3
127	*Carthamus tinctorius Linn*	142 ± 10	186 ± 10
128	*Cassia alata Linn.*	124 ± 10	208 ± 14
129	*Cassia angustifilia vohl.*	84 ± 9	139 ± 11
130	*Cassia tora Linn.*	111 ± 10	203 ± 18
131	*Centella asiatica Urban*	78 ± 6	136 ± 10
132	*Chrysanthenum indicum*	96 ± 8	114 ± 10
133	*Clitorea ternatea Linn.*	86 ± 6	156 ± 10
134	*Cymbopogon citratus stapf.*	50 ± 4	64 ± 6
135	*Ganoderma lucidum* (sample 1)	21 ± 2	29 ± 2

No	Scientific Name of plants	Folin Assay GE mM/kg.dw.	TEAC Assay TE mM/kg.dw.
136	*Gonoderma lucidum* (sample 2)	19 ± 2	57 ± 3
137	*Ginkgo biloba*	216 ± 11	652 ± 40
138	*Mimosa pudica Linn.*	123 ± 10	193 ± 19
139	*Morus alba Linn.* (sample 1)	56 ± 4	126 ± 9
140	*Morus alba Linn.*(sample 2)	119 ± 10	139 ± 10
141	*Nelumbo Mucifera Gaertn* (pollen)	143 ± 12	290 ± 18
142	*Oroxylum indicum Vent.*	511 ± 20	710 ± 32
143	*Pandanus amaryllifolius Roxb*	89 ± 9	141 ± 11
144	*Phyllanthus emblica Linn.*	841 ± 40	2288 ± 91
145	*Phyllanthus urinaria Linn.*	219 ± 29	330 ± 25
146	*Rhinacanthus nasutus Kurz Sn.*	16 ± 1	41 ± 3
147	*Schefflera leucantha* (sample 1)	38 ± 4	88 ± 9
148	*Schefflera leucantha*(sample 2)	57 ± 4	82 ± 6
149	*Thunbergia iaurifolia Linn.*	189 ± 12	190 ± 11
150	*Tinospara erispa Miers*	20 ± 2	31 ± 4
151	*Zingiber officenale Roscoe*	194 ± 11	220 ± 19
152	*Zingiber officinale Roscoe* (young)	37 ± 3	60 ± 4

All determinations were carried out at least three times .

*Polyphenolics are expressed as the mean ± SD in mM of catechin equivalents per kilogram of dry weights.

**Total antioxidant activities are expressed as the mean ± SD in mM of Trolox equivalents per kilogram of dry weights.

Table 1. The polyphenolic contents and total antioxidant activities of 152 plant-extracts using Folin assay and TEAC assay, respectively.

Type of Tea	Folin Assay GE mM/kg.dw	TEAC Assay TE mM/kg.dw.
Green tea (brand 1)	818 ± 66	3307 ± 112
Green tea (brand 2)	807 ± 42	2740 ± 96
Green tea (brand 3)	752 ± 52	2541 ± 69
Green tea (brand 4)	697 ± 37	2452 ± 100
Green tea (brand 5)	633 ± 26	1848 ± 74
Green tea (brand 6)	636 ± 46	2252 ± 56
Green tea (brand 7)	622 ± 41	2369 ± 78
Green tea (brand 8)	698 + 43	2836 ± 82
Green tea (brand 9)	506± 40	1704 ± 40
Green tea (brand 10)	563 ± 52	1901 ± 70
Green tea (brand11)	466 ±12	1800 ± 36
Green tea (brand 12)	444 ±16	1650 ± 45
Green tea (brand 13)	438 ± 28	1236 ± 56
Green tea (brand 14)	422± 25	1459 ± 55
Oolong (brand 1)	722 ± 43	2500 ± 85
Oolong (brand 2)	711 ± 44	1926 ± 38
Oolong (brand 3)	709 ± 28	2214 ± 83
Oolong (brand 4)	698 ± 17	2906 ± 97

Type of Tea	Folin Assay GE mM/kg.dw	TEAC Assay TE mM/kg.dw.
Oolong (brand 5)	693 ± 36	2222 ± 80
Oolong (brand 6)	600 ± 22	1994 ± 71
Oolong (brand 7)	583 ± 22	1793 ± 38
Oolong (brand 8)	526 ± 29	2062 ± 71
Oolong (brand 9)	555 ± 26	1963 ± 24
Oolong (brand 10)	547± 35	1963 ± 67
Oolong (brand 11)	523 ±18	1878 ± 41
Oolong (brand 12)	464 ± 30	1840 ± 31
Oolong (brand 13)	412+ 20	1222 ± 38
Oolong (brand 14)	464 ± 19	1237 ± 43
Black tea (brand 1)	530 ± 21	1578 ± 38
Black tea (brand 2)	491 ± 20	1489 ± 34
Black tea (brand 3)	398 ± 30	942 ± 18
Black tea (brand4)	374 ± 21	904 ± 26
Black tea (brand 5)	125 ± 34	212 ± 6

All determinations were carried out at least three times .

*Polyphenolics are expressed as the mean ± SD in mM of catechin equivalents per kilogram dry weight.

**Total antioxidant activities are expressed as the mean ± SD in mM of Trolox equivalents per kilogram dry weight.

Table 2. The polyphenolic contents and total antioxidant activities of 33 tea-extracts using Folin assay and TEAC assay, respectively.

Kind of beverages	Folin Assay* GE μM/L	TEAC Assay** TE μM/L
1. Green tea(sample1)	7,800 ± 500	11,400 ± 1,000
2. Green tea(sample2)	4,200 ± 200	9,000 ± 600
3. Green tea(sample3)	5,400 ± 300	8,900 ± 700
4. Green tea(sample4)	3,500 ± 100	5,000 ± 300
5. Grape juice (sample1)	8,400 ± 300	12,200 ± 400
6. Grape juice (sample2)	3,500 ± 100	4,600 ± 200
7. Guava juice	4,200 ± 200	4,200 ± 100
8. Carrot mixed fruit juice	3,400 ± 100	4,200 ± 300
9. Passion fruit tea	2,800 ± 100	3,600 ± 400
10. Pine apple juice(sample1)	700 ± 200	2,700 ± 200
11. Pine apple juice(sample2)	1,000 ± 100	2,400 ± 100
12. Orange juice	3,000 ± 100	2,400 ± 200
13. Apple juice	1,900 ± 100	2,000 ± 100
14. Litchi juice	600 ± 100	900 ± 100

All determinations were carried out at least three times .

*Polyphenolics are expressed as the mean ± SD in mM of catechin equivalents per liter of beverage.

**Total antioxidant activities are expressed as the mean ± SD in mM of Trolox equivalents per liter of beverage.

Table 3. The polyphenolic contents and total antioxidant activities of 14 beverages using Folin assay and TEAC assay, respectively.

Name of plants	TEAC Assay TE mM/kg.dw	50%Hemolysis* T_{50} min.	Heinz body** % inhibition
1. *Quercus infectoria*	9,490	>210	100
2. *Areca catechu Linn.*	5,771	>210	100
3. *Terminalia spp.*	5,216	>210	100
4. *Phyllanthus emblica Linn.*	2,288	>210	100
5. *Punica granutum Linn.*	2,157	>210	100
6. *Camellia sinensis (green tea)*	2,906	>210	100
7. *Eugenia caryophyllus*	1,400	>210	100
8. *Mangifera indica Linn*	1,118	>210	100
9. *Camellia sinensis(Oolong tea)*	2,204	>210	100
10. *Rosa domescena Mill.*	1,035	>210	100
11. *Tagetes erecta Linn.*	1,129	>210	100
12. *Psidium guajava*	1,121	>210	100
13. *Syzygium gratum*	718	>210	100
14. *Impormoca reptans Poir.*	751	>210	0
15. *Nelumbo Mucifera Gaertn*	1,014	>210	0
16. *Smilax corbulalia Kunth*	1,308	>210	0
17. *Nelumbo Mucifera Gaertn*	714	>210	0
18. *Tamarindus indica Linn.*	932	180	0
19. *Garcinia mangostana*	1,095	>210	0
20. *Cinnamomum cassia*	686	>210	0
21. *Mesua ferrea Linn.*	357	>210	0
22. *Ginkgo biloba (leaf)*	652	>210	0
23. *Aiiium ascalonicum Linn.*	357	>210	0
24. *Azodirachta indica A Juss.*	467	>210	0
25. *Phyllanthus urinaria Linn.*	330	>210	0
26. *Polygonium odoratum Lour.*	380	>210	0
27. *Leucaena leucocephala*	316	>210	0
28. *Erythrina suberosa Roxb.*	165	>210	0
29. *Citrus hystrix DC.*	254	>210	0
30. *Pandanus amaryllifolius Roxb*	141	>210	0
31. Positive control		120-150	0
32. Negative control		>210	100

*T50 min. = the time required to achieve 50 % hemolysis.

** Blood smear on glass slides and counted RBC with Heinz body inside/1,000 of RBC. The results were reported in % inhibition of Heinz body formation.

Table 4.Total antioxidant activities of 30 plant-extracts with inhibition effect on hemolysis, $T_{50\ min.}$, and inhibition effect on Heinz body formation.

3. Conclusion

For our knowledge this current work is the first report of phenolic contents and total antioxidant activities of Thai herb. The value of antioxidant activities of plant polyphenols showed varied widely with the various types of herb. The range of antioxidant activities and total phenolic contents were several hundred-fold for herbal extracts and 15.6-fold using TEAC analysis and 6.5-fold using Folin assay for 33 brands of tea. Nevertheless there was a very good relationship between the results from the TEAC assay which can measure the antioxidants activities and polyphenols in herbal extracts ($r = 0.988$) and in tea extracts ($r = 0.902$). It is suggested that the process of oxidation to make black tea and oolong tea may cause decreasing their antioxidant activities, and the correlation between the antioxidant activities and total phenolic contents of tea extracts was lower than that of herbal extracts.

Tea is one of the most popular and widely consumed as daily beverage in the world. At present, tea has become an important agricultural product that more than 40 countries in the world, especially Thailand, grow tea trees within Asian countries producing 90 % of the world output. All tea trees have their origin directly or indirectly in China and come from the same plant, *Camellia sinensis*. The composition of tea varies with the age of the leaf (plunking position), climate, horticultural practices and the process of storage (Lin et al., 1996, 1998). The different types of tea result from variation in processing of the leaves after they are harvested. The difference in processing results in different types of polyphenolics profiles between oxidation and no oxidation tea. There are different methods in manufacturing tea that give green tea (no oxidation) contains catechins. (Lin et al, 1998) black tea (fully oxidation tea) contains the polymeric compounds, thearubigins and theaflavins, and oolong tea (semi-oxidation tea) contains a mixture of the monomeric polyphenols and higher molecular weight theaflavins (Graham, 1992). Several epidemiological studies have shown beneficial effects of tea in cancer, cardiovascular, and neurological diseases (Zaveri, 2006).

Selected 30 types of herb with various polyphenol levels to study antioxidant activities by the modified methods which were developed by the author. For the test of AAPH induced hemolysis, most studies used isolated erythrocyte suspension for the simplicity of the system and the data interpretation. However fresh whole blood was employed as the *ex vivo* sample for this study to ensure the erythrocytes were tested in the least modified state. To study the effect of herbal extracts against oxidative damage to lipids and proteins in human erythrocytes, free radical initiators, AAPH and APHZ, were employed for inducing oxidative stress. AAPH was source of free radical formation capable of inducing oxidation of lipid and protein structurally located on erythrocyte membranes (Chaudhuri et al., 2007). The results showed that erythrocytes were more resistant to AAPH-induced oxidation when herbal extracts were added, most of them had very strong inhibition effect. It was showed that the T_{50} mins were longer than 3 and a half hours. Plant polyphenols are membrane-active antioxidant agents (Saija et al., 1995) and are the cell metabolism regulators by modulating the fluidity of lipid bilayer, since they have been demonstrated to control cell signal pathways by targeting receptors on the cell surface or by intercalating the lipid bilayer of membranes (Tarahovsky et al., 2008). Polyphenols

interact with the surface of bilayer through hydrogen bonding have been shown to reduce the accessibility of oxidants, thus protecting the structure and function of membranes (Oteiza et al., 2005).

APHZ was a source of free radical formation inside cytosol of erythrocytes leading to induction of proteins mostly hemoglobin and lipid bilayer of membranes oxidation (Sangkitikomol et al., 2001). Phenylhydrazine in the presence of oxidase or peroxidase , it reacts with oxyhemoglobin to form phenylhydrazine radicals. Phenylhydrazine slowly oxidizes in aqueous solution to form O_2 •- and H_2O_2 and the end products of the reaction are benzene and N_2. Phenylhydrazine radical is the most damaging agent that can denature hemoglobin molecules. The oxidation of reactive sulfhydryl (S-H) groups creates disulfide bonds that may change the conformation of globin chains, resulting in precipitation of the hemoglobin molecules called Heinz bodies, then follows by membrane lipids oxidation and causing hemolysis (Winterbourn, 1985). Bioavailability differs greatly from one polyphenol to the other; therefore the most abundant polyphenols in plants are not leading to the highest concentrations of active metabolites in target cells. Gallic acid and isoflavones are the most absorbed polyphenols, followed by catechins, flavanones, and quercetin glucosides. The least absorbed polyphenols are proanthocyanidins, galloylated tea catechins, and anthocyanins (Manach et al., 2005). The results showed that some herbal extracts could inhibit Heinz body formation inside erythrocytes, but the other could not. This finding suggested that the process of proteins and lipids oxidation was still taking place as a result of insufficiency of absorbed polyphenols inside erythrocytes to inhibit oxidation.

The advantage of using erythrocytes (human living cells) as the models for screening test of antioxidant properties of herbal extracts, because it is simple and low cost to perform the analysis and the given data could be extrapolated to happen in human body.

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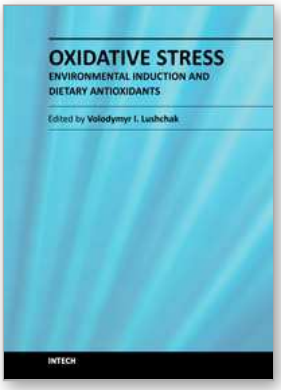

Oxidative Stress - Environmental Induction and Dietary Antioxidants

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This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

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