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Antioxidant Activities of Some Thai and Exotic Fruits Cultivated in Thailand.

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ABSTRACT

The twenty-one methanolic extracts of meat or peel of native and exotic fruits cultivated in Thailand were determined their total phenolic content and antioxidant activities by Folin-Ciocalteu reagent and Trolox equivalent antioxidant capacity (TEAC) assay. The ripe fruit of *Carissa carandas* and the raw fruit of *Ficus hirta* presented the highest and lowest %yield of extract as 67.20% and 12.86%, respectively. The *Terminalia chebula* and *Baccauea motleyana* extracts contained the highest and lowest amount of total phenols, 6.96 and 0.05 g/100g dried fruit, respectively. The *T. chebula* and *Syzygium malaccense* extracts contained the highest and lowest amount of total phenols, 16.12 and 0.22 g/100g extract, respectively. The range of TEAC and IC_{50} values of the fruit extracts were 0.35–0.003 and 21.33 µg/mL –5.10 mg/mL, respectively. The *T. chebula* and *Diospyros peregrina* extracts showed the highest antioxidant activities, while *B. motleyana* extracts indicated the lowest. The extracts of *T. Chebula* and *D. peregrina* (raw fruit) showed equal TEAC value, however the total phenolics content of *D. peregrina* raw fruit was quite low. Finally almost methanolic extracts of Thai and exotic fruits in this study presented their antioxidant activities in direct proportion to the amount of total phenolics.
Keywords: fruits, total phenolics, antioxidant activity, TEAC

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INTRODUCTION

Thailand has a lot of kinds of fruits that are different in shape, size, taste, smell and texture. Some of them are exotic fruits that have been cultivated in Thai territory for a long time. Most of the fruits are sweet and delicious and some are sour and astringent taste. They are sources of carbohydrate, vitamins, minerals and fiber. In Thailand, fruits are not only giving high nutritional values but the fruits and other parts of their trees also beneficial as traditional medicines as shown in Table 1 [1-6]. These pharmacological activities were interesting. Therefore several researchers have been investigated and reported useful scientific information about their activities as follow. The *Annona muricata* shows cytotoxic [7], antileishmanial [8], and molluscicides activities [9]. The *Averrhoa carambola* L indicates hypotensive [10], muscle relaxant [11], and HIV-1 reverse transcriptase inhibitors acitivies [12]. The *Baccaurea sapida* presents hypotensive activity [13]. The *Carissa carandas* Linn is mentioned histamine releasing [14] and cadiotonic activities [15]. The *Coccinia grandis* indicates hypoglycaemic [16], antiHIV-1 [17] and increasing movement of intestine acitivities [18]. The *Dillenia indica* is reported anticonvulsion, antidiabetes [19], antibacterial and antiviral activities [20]. The *Diospyros peregrina* has spermicidal [21], antiameobic, antiviral and hypoglyceamic activities [22]. The *Ficus hirta* presents cytotoxic and antiviral activities [23]. The *Ficus racemosa* has been reported on hypoglyceamic [24], hypotension [25], cytotoxic [26], antiviral [27], antibacterial [28], antipyretic and anti-inflammatory activities. The *Garcinia schomburgkiana* has anti-tumor promoting activity [29]. The *Lansium domesticum* indicates antimalarial [30-31], larvicidal [32], and insecticidal acitivies [33]. The *Mimusops elengi* shows diuretic [34], antiviral [35], antifungal [36] and spermicidal [37] activities. The *Terminalia chebula* has been reported on its antibacterial [38], antiviral [39], and antioxidant activities [40-41]. However there has no report about total phenolic contents and antioxidant activities of these fruits. The aim of this study is to determine the phenolic contents and antioxidant activities of these fruits.

Table 1 The common name, Thai name, scientific name, family and traditional used of fruits in this experiment.

Scientific name	Common name	Family	Used for
Angle Marmelos	Bael, Bengal Quince, Bilak	Rutaceae	Antipyretic form malaria, flatulence (bark, root bark), influenza, bronchitis (watery from fresh leaf), drinking juice for tonic (slide of grill raw fruit), laxative (ripe fruit)
***Annona muricata* L.**	Soursop	Annonaceae	Scurvy (ripe fruit), antiameobic (seed of raw fruit), vomit stimulant & haemostatic (seed), pesticide and poison to fish (seed)
Ardisia elliptica* Thunb. *S/ST	shoebutton	Myrisnaceae	Treat gonorrhoea (root)
***Averrhoa carambola* L.**	Corambola, Star Fruit	Averrhoaceae, Oxalidaceae	Antipyretic (root, leaf), diarrheoa & antiameobic (stem bark), scurvy & laxative (fruit), Anthelmintic (flower)
Baccaurea motleyana	Lamai	Euphorbiaceae	Fruit can ate
***Baccaurea sapida* Muell. Arg.**	Lotka	Euphorbiaceae	Vitamin C supplement, anti-thirst (fruit), antipyretic (all part), antidiarrheoa, anti-TB (root), anthelmintic & antifungal (leaf),

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***Carissa carandas* Linn**	Karanda, Carunda, Christ's Thorn	Apocynaceae	Anthelmintic & appetite stimulant (root), diarrhoea & antipyretic & ear ach & sore throat (leaf), tonic(heartwood), scurvy & diarrhea (raw& ripe fruit)
***Coccinia grandis* Voigt**	Ivy gourd	Cucurbitaceae	Vitamin A supplement (leaf), antipyretic & anti-diabetic (leaf, root), expectorant(leaf), anti-iching (leaf, flower), antivomitic (root), laxative (root bark),
***Dillenia indica* L.**	Matat, chulta	Dilleniaceae	Mouth & throat astringent (leaf, bark), eat fruit, de-poisoning(root)
***Diospyros peregrina* Guerke**	River ebony	Ebenaceae	Astringent & antipyretic & anti-amoebic (stem bark, raw fruit), heal lesions of oral mucosa, (bark, ripe fruit) antidiarrhoea (bark, raw fruit, seed)
***Ficus hirta* Vahl.**	Hairy mountain fig	Moraceae	Antiiarrhoea, liver-,lung- and cardiotonic (bark),
***Ficus racemosa* L.**	Cluster fig, Country fig tree	Moraceae	Antidiarrhoea & anti-vomiting & wound healing (bark), antipyretic (root), flatulence (fruit)
***Garcinia duleis* Kurz**	Mapood	Guttiferae	Expectorant, anticough, sore throat (fruit juice); astringent (bark); antipyretic (root)
***Garcinia schomburgkiana* Pierre**	Madan	Clusiaceae, (Guttiferae)	Constipation, anti-cough, abnormal uterine bleeding
***Lansium domesticum* Correa** **Aglaia dookko Griff**	Longkong	Meliaceae	Vitamin B & phosphorus supplement, heal lesions of oral mucosa, reduced body heat
***Lepisanthes fruticosa* (Roxb) Leenh.**	Luna Nut	Sapindaceae	Antipyretic (root), antidiarrhoea (ripe fruit)
***Mimusops elengi* L.**	Bullet wood	Sapotaceae	Gingival disorder (bark), cardiotonic & antipyretic, headach (flower)
***Pouteria campechiana* Baehni**	Canistel	Sapotaceae	Febrifuge**,** skin eruptions (bark); ulcers (seed)
***Spondias pinnata* (L.f.) Kurz**	Hog plum	Anacardiaceae	Eat as vegetable (young shoot), vitamin C supplement (fruit), antithirst (fruit, root), diarrhoea (bark), anti- vomit, diuretic (bark)
***Syzygium malaccense* (L.) Merr. & L.M. Perry**	Malay apple, Pomerac	Myrtaceae	Flatulent, antithirst
***Terminalia chebula* Retz**	Myrobalan Wood	Combretaceae	Antipuretic, antiameobic antidiarrhoea, expectorant, antipyretic

MATERIALS AND METHODS

Plant

The fruits were bought from local market or collected from orchard in Nakorn-Pathom province by the researchers of Faculty of Pharmacy, Silpakorn University in June, 2008. The voucher specimens were deposited in the Department of Pharmacognosy, Silpakorn University in Nakhon-Pathom, Thailand.

Chemicals

$ABTS^{2-}$, 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfo-nate), was obtained as sulfonic acid from Sigma (St. Louis, USA). Trolox (or (+/-)–6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid) was purchased from Aldrich (Steinheim, Germany). Potassium persulfate, $FeSO_4$ x $7H_2O$, and sodium acetate were purchased from Asia Pacific Specialty Chemicals Limited (Seven Hills, Australia). Folin-Ciocalteu reagent, $FeCl_3$ x $6H_2O$ and NaCl were purchased from CarLo ErbaReagenti (Milano, Italy). 2,4,6-tri-pyridyl-s-triazine (TPTZ) was obtained from Fluka Chemie GmbH (Switzerland) and methanol was purchased from Merck (Darmstadt, Germany).

Methanolic extract of fruits

The meat or peel of the fresh fruits were dried at 55 °C for 72 hrs before grinding and passing through sieve size 30 mesh The methanolic extraction was performed by maceration of the dried samples in methanol in a ratio of sample to methanol of 1:4 for 72 hrs. The filtrates were evaporated using a rotary evaporator (Buechi R205, Switzerland). The dried extracts were kept at 4 °C until used.

Quantification of total polyphenols [42]

The 0.5 mL of methanolic fruit extracts (10 μg/mL) were mixed with 0.5 mL of Folin-Ciocalteu reagent and 0.5 mL 10% Na_2CO_3. The mixture was shaked and placed at room temperature for 1 hr before measuring the absorbance at 760 nm. The calibration curve was prepared using gallic acid with a concentration range of 2 - 8 mg/L. The total polyphenols was calculated and reported as gallic acid equivalent (GEA), g of gallic acid in 100 g of dried fruits and in 100 g of methanolic extracts.

Determination of antioxidant activity [43]

An antioxidant activity was performed by determination of scavenging effect on $ABTS^{\bullet+}$ radical. The $ABTS^{\bullet+}$ solution was prepared by mixing equal volume of 7 mM $ABTS^{2-}$ in water with 4.9 mM potassium persulfate in water. The solution was protected from light and stored at room temperature for 12 – 16 hrs. $ABTS^{\bullet+}$ formation was checked for its absorbance (A) at 734 nm using UV-Vis-Spectrophotometer, (Agilent 8453E UV-Visible Spectro-scopy System, Agilant Technology, USA.). The absorbance of $ABTS^{\bullet+}$ was equilibrated to 0.7 ($\pm$ 0.02) by diluting with water at room temperature.

All samples were prepared in a concentration range of 0 - 10 μg/μL, except for that of *D. indica* (0-20 μg/μL), and of *C. carandas* and *A. elliptica* (0-50μg/μL). A portion of each dilution (50 μL) was mixed with 3 mL of $ABTS^{\bullet+}$ solution. After the mixture had been allowed to stand for 6 minutes at room temperature, its absorbance was measured at 734 nm using a spectrophotometer. Trolox was used as a standard. The antioxidant capacity was calculated as an average of four replicate absorbance measurements, and reported as %inhibition along with IC_{50} and also as Trolox equivalent antioxidant capacity (TEAC).

Calculation of antioxidant capacity

Percent inhibition could be calculation as follows;

$$\% \text{ inhibition} = \frac{\text{A (solvent)} - \text{A (compound)}}{\text{A (solvent)}} \times 100$$

where A (solvent) was an absorbance of the sole solvent (no extract added) and A (compound) was an absorbance of the mixture of samples (or Trolox) and $ABTS^{\bullet +}$ solution at 734.

The curve was plotted between % inhibition and concentration of sample or Trolox solutions. The regression coefficient (r^2) was calculated from the linear curve. The IC_{50} was determined from the concentration that resulted in 50% inhibition. TEAC was the ratio of % inhibition of the sample to % inhibition of Trolox at the same concentration.

RESULT AND DISCUSSION

The %yield of methanolic extract of fruit in this experiment was shown in Table 2. The ripe fruit of *C. carandas* gave the highest %yield of extract (67.20%), while the raw fruit of *F. hirta* gave the lowest (12.86%). The ripe fruits of *A. elliptica*, *C. carandas, F. racemosa* and *M. elengi*and gave higher amount of %yield of extract than their raw fruits, except that of *D. peregrine*. The amount of total phenols of dried fruits and crude extracts of most fruits in this experiment were rather low. The meat of *T. chebula* fruit had moderate % yield (43.17%), but contained the highest amount of total phenols, 6.96 $\pm$ 0.12 g/100 g of dried fruit and 16.12 $\pm$ 0. 29 g/100 g extract. The *B. motleyana* contained the lowest amount of total phenols, 0.05 $\pm$ 0.00 g/100 g dried fruit, while the *S. malaccense* contained the lowest amount of total phenols, 0.22 $\pm$ 0.01 g/100g extract. The raw fruit of *D. peregrine* and *M. elengi* contained higher amount of total phenols in their dried fruit than that of their ripe fruits and the ripe fruit of *A. elliptica, C. carandas* and *D. peregrine* presented higher amount of total phenols in their crude extract than that of their raw fruits. Most of crude extracts and dried fruits in this experiment contained rather low amount of total phenols.

Table 2 The percent yield and amount of total-phenols (GAE) in dried fruits and extracts.

Type of fruit extracts	Part of plant	%yield	Amount of total-phenols(GAE)	
			g of gallic acid / 100g dried fruit	g of gallic acid / 100g extract
Angle Marmelos	fruit	14.41	0.79 ± 0.02	5.51 ± 0.11
***Annona muricata* L.**	fruit	50.86	0.65 ±0.02	1.28 ± 0.04
***Ardisia elliptica* Thunb. S/ST**	raw fruit	26.36	1.26± 0.05	3.04 ± 0.11
	ripe fruit	41.46	1.48 ± 0.10	5.64 ± 0.37
***Averrhoa carambola* L.**	fruit meat	38.31	0.68 ± 0.01	1.77 ± 0.04
Baccaurea motleyana	fruit	18.51	0.05 ± 0.00	0.29 ± 0.01
***Baccaurea sapida* Muell. Arg.**	fruit meat	59.36	0.22 ± 0.01	0.37 ± 0.02
	fruit peel	21.48	0.10 ± 0.02	0.47 ± 0.08
	pacenta	65.11	0.23 ± 0.01	0.71 ± 0.04
***Carissa carandas* Linn**	raw fruit	44.66	0.24 ± 0.00	0.53 ± 0.01
	ripe fruit	67.20	0.82 ± 0.02	1.22 ± 0.02
Coccinia grandis	ripe fruit	25.31	0.23 ± 0.03	0.90 ± 0.14
***Dillenia indica* L.**	fruit	32.95	0.80 ± 0.02	2.43 ± 0.07
***Diospyros peregrina* Guerke**	raw fruit	35.580	3.34 ± 0. 41	1.73 ± 1.16
	ripe fruit	19.76	1.07 ± 0.05	5.44 ± 0.24
***Ficus hirta* Vahl.**	fruit	12.86	0.26 ± 0.01	1.98± 0.08
***Ficus racemosa* L.**	raw fruit	13.61	0.20 ± 0.01	1.50 ± 0.08
	ripe fruit	18.85	0.21 ± 0.03	1.13± 0.15
***Garcinia duleis* Kurz**	fruit	50.32	0.47 ± 0.05	0.94 ± 0.09
Garcinia schomburgkiana	fruit meat	42.13	0.56 ± 0.03	1.32 ± 0.08
	leafless branch	21.17	2.62 ± 0.22	12.37 ± 1.05
***Lansium domesticum* Correa**	fruit peel	16.51	0.13 ± 0.03	0.81 ± 0.19
***Lepisanthes fruticosa* (Roxb)**	ripe fruit	62.65	0.48 ± 0.02	0.77 ± 0.03
***Mimusops elengi* L.**	raw fruit	32.97	4.58 ± 0.21	1.51 ± 0.07
	ripe fruit	38.61	3.26 ±0.06	1.26 ± 0.02
***Pouteria campechiana* Baehni**	fruit	31.93	0.16 ± 0.00	0.52 ± 0.01
Spondias pinnata	fruit meat	26.30	0.12 ± 0.01	0.46 ± 0.05
	fruit peel	28.74	0.31 ± 0.01	1.08 ± 0.03
***Syzygium malaccense* (L.)**	fruit	42.92	0.09 ± 0.00	0.22 ± 0.01
***Terminalia chebula* Retz**	fruit meat	43.17	6.96 ± 0.12	16.12 ± 0.29

Table 3 The slope, intercept and r^2 of % inhibition equations, IC_{50} and TEAC values of the fruit extracts.

Type of fruit extracts	Part of plant	Equations of methanol extract			IC_{50}	TEAC
		slope [a]	intercept	r^2	(μg/mL)	
Trolox		5.0248	-0.9708	0.9985	10.14	1
Angle Marmelos	fruit	0.0991	4.1147	0.9759	463.02	0.03
***Annona muricata* L.**	fruit	0.0213	2.0219	0.8828	2252.49	0.008

Ardisia elliptica **Thunb. *S/ST***	raw fruit	0.0266	2.9073	0.9906	1770.40	0.01
	ripe fruit	0.3572	2.2732	0.9874	133.61	0.076
Averrhoa carambola **L.**	fruit meat	0.0515	1.1201	0.9659	949.12	0.012
Baccaurea motleyana	fruit	0.0097	0.6259	0.9110	5090.11	0.003
Baccaurea sapida **Muell. Arg.**	fruit meat	0.0164	1.2639	0.9272	2971.71	0.005
	fruit peel	0.0968	7.0874	0.9293	443.31	0.03
	pacenta	0.0584	0.9355	0.9955	840.15	0.013
Carissa carandas **Linn**	raw fruit	0.0219*	12.4910	0.8444	-**	-**
	ripe fruit	0.3622	1.6861	0.9940	133.39	0.08
Coccinia grandis **Voigt**	ripe fruit	0.0369	1.1844	0.9780	1322.92	0.01
Dillenia indica **L.**	fruit	0.0748	5.0707	0.9825	600.66	0.025
Diospyros peregrina **Guerke**	raw fruit	1.6991	3.1351	0.9933	27.58	0.35
	ripe fruit	0.7468	2.6712	0.9937	63.38	0.15
Ficus hirta **Vahl.**	raw fruit	0.1048	0.7298	0.9973	470.14	0.02
Ficus racemosa **L.**	raw fruit	0.0506	0.0890	0.9758	986.38	0.01
	ripe fruit	0.0429	0.7464	0.9941	1148.10	0.01
Garcinia schomburgkiana	fruit meat	0.0701	3.8099	0.9430	658.92	0.02
	leafless branch	1.2576	4.0073	0.9874	36.57	0.26
Garcinia duleis **Kurz**	fruit	0.0629	3.1221	0.9614	745.28	0.02
Lansium domesticum **Correa**	fruit peel	0.0462	1.5003	0.9869	1049.78	0.012
Lepisanthes fruticosa **(Roxb)**	ripe fruit	0.0711	1.0821	0.9967	688.02	0.02
Mimusops elengi **L.**	raw fruit	1.2009	7.7204	0.9462	35.21	0.26
	ripe fruit	0.2810	3.6359	0.9841	165.00	0.06
Pouteria campechiana **Baehni**	fruit	0.0198	1.1197	0.9526	2468.70	0.006
Spondias pinnata **(L.f.) Kurz**	fruit meat	0.0131	0.6237	0.9610	3769.18	0.004

	fruit peel	0.0532	1.4589	0.9904	912.43	0.014
Syzygium malaccense (L.)	fruit	0.0186	0.5595	0.9830	2658.09	0.005
***Terminalia chebula* Retz**	fruit meat	1.7182	5.3856	0.9825	25.97	0.35

The antioxidant of all extracts of fruits in this experiment was rather low as shown in Table 3. The highest antioxidant activity (TEAC = 0.35) were shown in *T. Chebula* and *D. peregrine*. The *T. Chebula* indicated the highest amount of total phenolics, and antioxidant activity. These results were according to the report of Bajpai. *et.al*., 2005 that *T. Chebula* fruit was a source of gallic acid and had good antioxidant activities [44]. The extract that showed inverse proportion between its amount of total phenolics contents and its TEAC values was *D. peregrine*. The TEAC of *D. peregrine* raw fruit extract was 0.35 equal to *T. Chebula.* However, the total phenolics content in *D. peregrine* extract was 1.73 g/100 g crude extract comparing to 16.12 g/100 g crude extract of *T. Chebula*. The other inverse result was the total phenolic content of *D. peregrine* raw fruit that was lower than ripe fruit, but the raw fruit presented higher TEAC value. For comparing between fruit meat and leafless branch of *G. schomburgkiana,* the total phenolic content of the leafless branch was not only higher than the fruit meat, but also the antioxidant activity. For *B. motleyana* fruit extract, it gave the lowest antioxidant activity which according to low amount of total phenolic content in its extract. In addition, the *S. malaccense* extract presented the lowest total phenolic content which related to its low antioxidant activity. In this study the *A. carambola* fruit extract contained rather low antioxidant activity (TEAC = 0.012). However Shui and Leong reported that *A. carambola* fruit juice was good source of antioxidant activities, which its antioxidant activities were attributed to L-ascorbic, (-)-epicatechin and gallic acid in gallotannin forms [45].

CONCLUSION

The total phenolic contents and antioxidant activities of fruit extracts in this experiment were rather low. The *T. Chebula* fruit meat extracts showed highest total phenolic content and antioxidant activity. Most fruits, that more than one type of crude fruit of each fruit (eg. meat, peel, raw fruit and ripe fruit) was extracted, indicated their antioxidant activities direct proportion to their amount of total phenols. The correlation between amount of total phenolics and antioxidant activities in raw and ripe fruit could not make a conclusion. However, if the raw fruit presented higher antioxidant activity than the ripe fruit, it showed distinctive higher. But when the ripe fruit gave higher antioxidant activity than the raw fruit, it showed small higher, except *F. rcemosa* that raw and ripe fruit had equal antioxidant activities. The rather low antioxidant activities of most extracts in this study may correlate to the low amount of total phenolic content in the extracts.

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