

Anti-oxidative and anti-inflammatory properties of Southern Thai foods extracts

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Abstract

Eight selected indigenous Southern Thai foods extracted and solubilized in water, 50% ethanol and 95% ethanol. Antioxidant activities were determined including free radicals scavenging and reducing properties. Key of phenolic compounds were found to include gallic acid, protocatechuic acid, chlorogenic acid, *p*-coumaric acid and ferulic acid. Anti-inflammatory (Nitric Oxide inhibition in macrophage RAW264.7 cells) was significant particularly in water extracts of Kaeng Som Pla Too Aor Dip, Kua Kling Kai, and Khao Yam Nham Bu Du (where IC₅₀ 7-26 times the magnitude of 95% ethanol extract). The observed anti-inflammatory effects were relatively high in the presence of key phenolics such as gallic acid. Further investigation in health implication is warranted.

Keywords

Antioxidant activity

Anti-inflammation

Southern Thai food

Oxidative stress

Nitric oxide inhibition

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Introduction

In a normal living process, continuous production of free radicals and reactive oxygen species (ROS) results in oxidative damage but, through evolution, our body develops a defense anti-oxidative mechanism against these factors, (Noori, 2012) i.e., antioxidant defense system (Rajalakshmi and Narasimhan, 1996). Free radicals exert physiological benefits as part of energy production and cell growth (Sen *et al.*, 2010). Energy imbalance or exposure to toxic substances such as heavy metals (Ercal *et al.*, 2001) and pollutants (Bano *et al.*, 2012) and over production of free radicals, can cause oxidative damage to macromolecules, e.g. protein, lipids, and DNA (Pittella *et al.*, 2009). Free radicals can be neutralized by a wide variety of antioxidants. Antioxidants are any substance delay or inhibit the process of oxidation (Tirzitis and Bartosz, 2010) that can lead to preventative and repair mechanisms against oxidative stresses. Some anti-oxidative enzymes participate in free radical neutralizing processes, such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). Non-enzymatic antioxidants that participate in oxidative stress defense include: ascorbic acid, alpha-tocopherol and glutathione (Khansari *et al.*, 2009).

Functional ingredients such as antioxidants

have been linked to prevent or counteract metabolic oxidative stresses caused by health deterioration, such as DNA damage, and cancer. These are non-communicable diseases that impact aging people and the quality of living (Birben and Sahiner, 2012). Resulting inflammatory response then triggers cascade reactions that lead to several degenerative diseases including atherosclerosis, cancer, diabetes mellitus, cardio-vascular, kidney, Parkinson's and Alzheimer's diseases. Inflammation is the primary immune system reaction to eliminate pathogens or other stimuli in order to restore stressed cells to a normal state or to replace destroyed tissue with scars (Emmendoerffer *et al.*, 2000). Prolonged (chronic) inflammation often results in changes at the inflammation site involving simultaneous destruction and tissue healing. The initial change involves adhesion of neutrophils to vascular tissue (Mantovani *et al.*, 2011). Then, neutrophils release certain molecules, namely pro-inflammatory cytokines such as interleukins (IL-1, IL-6, IL-12, and IL-18), tumor necrosis factor- α (TNF- α), interferon (IFN)- γ , and granulocyte-macrophage colony-stimulating factor (Fain *et al.*, 2006). Inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) genes are activated. Activated neutrophils migrate into the tissue and become macrophages to clear dead cells and pathogens (Butterfield *et al.*, 2006). Persisting

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presences of stimuli may give rise to a secretion of pro-inflammatory cytokines (Jungbauer and Mediakovic, 2009).

Food research on antioxidants and health is under a functional food concept that is promising and profitable in the industry. Research is needed to determine food that inhibit or delay oxidation, reduce oxidative stresses and reduce inflammatory processes (Sen *et al.*, 2010). Several plant polyphenols, including phenolic acid, exhibit antioxidant properties, namely hydrogen atom donor and/or singlet oxygen quencher. Some of these can be bioactive compounds with beneficial effects against certain chronic diseases such as diabetes, obesity, cardiovascular disease and cancer (Pandey and Rizvi, 2009).

Identification of food rich in polyphenols with biological activities have been increasing in recent food research and this serves ancient beliefs Asian culture. In Thailand, there are local recipes that contain specific plants or meats believed to have special disease prevention or maintaining health and well being. Traditional Thai (and South East Asian) herbs and spices have been shown to have antioxidant activity (Wong and Mine, 2004), anti-inflammation ability (Yang *et al.*, 2004), and anti-mutagenicity (Pimsaeng, 1993; Baba *et al.*, 2002). Anti-oxidative properties of culinary herbs and spices used in Thai cooking, such as garlic, shallots, lemongrass, and turmeric have been reported to exhibit anti-oxidative and/or anti-inflammatory properties. For example, shallot contains flavone, sulfur-containing compounds and polyphenol derivatives with anti-inflammatory activity by inhibiting vascular permeability (Yang *et al.*, 2004). Lemongrass contains citral showing anti-mutagenic activity in ethanol extract retards the growth of fibrosarcoma cells transplanted in mice (Pimsaeng, 1993). Garlic contains S-allylcysteine (SAC), allicin and their derivatives with anti-inflammatory (Youn *et al.*, 2008) and anti-oxidative (Ide and Lau, 1997) activities. Chili contains capsaicinoids with anti-inflammatory activity observed by the ability to inhibit COX-2 and iNOS (Tuntipopipat *et al.*, 2011). Turmeric contains curcumin, a polyphenol that can scavenge free radicals such as superoxide and hydroxyl radicals (Ach and Lokesh, 1992; Zhou *et al.*, 2011). However, these herbs and spices are rarely consumed alone or eaten raw. They are mixed, ground, and cooked in certain ways that provide distinctive taste and eating pleasure. Limited information is available on prepared Thai foods (containing mixed ingredients) and their ability to exhibit anti-oxidative, anti-inflammatory and other

functional properties.

The objective of this work was to investigate Southern Thai food extracts (known to be more rich in herbs and spices used) in terms of anti-oxidative and anti-inflammatory effects and the presence of phenolic compounds present in prepared foods. These would serve as the database and potential health implication research in the future.

Materials and Methods

Sample preparation

Eight Southern Thai Dishes of various categories (Kua Kling Kai, Kaeng Pa Kai, Nham Prik Ka Pi, Nham Bu Du, Kaeng Leang, Kaeng Tai Pla, Kaeng Som Pla Too Aor Dip, Khao Yam Nham Bu Du) are shown in Table 1 (named as Food 1, Food 2, and so on). Top 20 of the most popular Southern Thai foods were surveyed from personnel of Prince of Songkla University. Out of the 20 most popular foods, top eight highest herbs and spices were selected for the study. Food recipes of each type were obtained from 3 popular local vendors which were found to be similar to those published by Thongkam (2011). Therefore, all eight recipes from Thongkam (2011) were used (Table 1). Five hundred g of each prepared food was blended (Panasonic Blender MX 151 SP, Thailand) and freeze-dried to approximate 5% moisture (0.055 mbar, 12 h, at -40°C, Dura Dry, Dura Freeze Dryer, Canada). After milling (3 s, Super Blender, AIKO, China) and passing through 20 mesh sieves, the powders were stored in plastic bottles with screw caps and stored at -20°C until extraction and analyses.

Sample extraction

Twelve g of freeze-dried Southern Thai food were divided for fat removal by hexane or defatted powder and "as-is" (non-defatted). One hundred and twenty ml of hexane was added to make 1:10 (w/v powder/hexane), mixed (Vortex-mixer Genie 2 G560E, Scientific Industries, USA) for 30 s, and sonicated for 15 min (Digital Ultrasonic Cleaner 4820, Blazer, USA). After centrifugation (2,432×g, Hettich Zentrifugen, MIKRO 22R, Buckinghamshire, U.K.), the supernatant was removed and left dried at 30°C (ambient air). The pellets were further divided in three equal portions for further extraction by three solvents (0, 50 and 95% ethanol) at sample:solvent ratio of 1:30 (w/v). After shaken for 1 h at 120 rpm (Wise Shake®, SHO-2D, Wertheim, Germany) at room temperature each aliquot was centrifuged at 2,432×g for 15 min at 4°C. The supernatant fractions were evaporated under a vacuum (175 mmHg, 45-

Table 1. List of selected Southern Thai-dish and description of major ingredients

Food name	Description
Curry without coconut milk	
<i>Kua Kling Kai</i> (Food 1)	Curry paste (dry hot chili, fresh garlic, turmeric, lemongrass, pepper, shallot) with chicken and kaffir lime leaves
<i>Kaeng Pa Kai</i> (Food 2)	Curry paste (dry hot chili, fresh garlic, turmeric, lemongrass, pepper, shallot) with shrimp paste, baby corn, green pepper, straw mushroom and chicken
<i>Kaeng Som Pla Too Aor Dip</i> (Food 3)	Curry paste (dry hot chili, turmeric, fresh garlic, shallot) with shrimp paste, mackerel, tamarind juice and "Koon" stems
Salad	
<i>Khao yam Nham Bu Du</i> (Food 4)	Rice with mixed vegetables (Torch ginger, Wild betel leaf bush or "Cha-Plu" leaves, green (raw) mango, lemongrass, bean sprouts, toasted coconut, long bean, paprika, lime juice) and <i>Nham Bu Du</i> (sauce).
Curry with coconut milk	
<i>Kaeng Tai Pla</i> (Food 5)	Curry paste (dry hot chili, fresh garlic, turmeric, lemongrass, pepper) with shrimp paste, fermented fish stomach (liquid), grilled and deboned mackerel, bamboo, winged bean, round eggplant, kaffir lime leaves, and garcinia
Soup	
<i>Kaeng Leang</i> (Food 6)	Thin soup with dried pepper paste (shallot, pepper, shrimp paste, dried shrimp), mixed vegetables (Angled gourd or "Buab", Ivy gourd or "Tom lueng", pumpkin, sweet basil leaf) and white prawns
Dip	
<i>Nham Bu Du</i> (Food 7)	Fermented fish mixed with spices and herbs (chili, lemongrass, shallot, kaffir lime leaves), grilled and deboned mackerel, palm sugar and lime
<i>Nham Prik Ka Pi</i> (Food 8)	Shrimp paste with chili, lime, shallot, garlic, pea eggplant, and dried shrimp

50°C) for 30 min, and then freeze-dried and stored in brown bottles at -20°C until use.

Antioxidant activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was determined using a modified method from Brand-William *et al* (1995). Free radical scavenging activity was measured from a decoloration of DPPH radical of food extracts using gallic acid as standard solutions (2, 4, 6, 8 and 10 µg/ml in distilled water). Food extracts were diluted with distilled water to make 1, 2, 3, 4, and 5 mg/ml concentrations. One hundred microliter of food extract solutions and gallic acid standard solutions were individually placed in each well of a 96-well plate. One hundred microliter of DPPH solution (0.2 mM in ethanol) as added to each well and then left at room temperature for 30 min in darkness. Absorbance (517 nm) was measured under the microplate reader using a blank solution (containing 100 µl distilled water and 100 µl of 0.2 mM DPPH).

$$\% \text{ gallic acid} = [(Cs - X) - a]/b$$

Where Cs = absorbance of sample

X = absorbance of blank solution

a, b = intercept and slope of the standard curve

Analyses were performed in triplicates and the results were expressed in mg of gallic acid equivalents (GAE) per g dry crude extract.

The sample concentration providing 50% inhibition (IC₅₀) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicates.

Ferric Reducing Antioxidant Power (FRAP) assay was determined according to the method of Benzie and Strain (1996). A stock solution (FRAP) containing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 10 mM TPTZ solution in 40 mM HCl, and 2.5 ml of 20 mM FeCl₃·6H₂O was prepared. After mixing, it was incubated at 37°C for 30 min. Food extracts were diluted with distilled water to make 1, 2, 3, 4, and 5 mg/ml concentrations. Thirty microliter each of these solutions was placed in a 96-well microtiter plate. Thirty microliter of Trolox standard solutions (5, 10, 25, 50, 100 and 200 µM Trolox/ml in ethanol) were placed in standard (empty) wells. FRAP solution (270 µl) was then added to these wells and kept for 30 min in darkness. In this reaction, ferric-tripyridyltriazine (Fe³⁺-TPTZ) complex is reduced from ferric (yellow) to ferrous (blue) form where

the ferrous tripyridyltriazine complex produced was measured by absorbance at 595 nm with a microplate reader (Microplate reader, Biotek, Power wave X, Winooski, USA). Trolox treated group was used as the standard curve.

Anti-inflammatory activity in macrophage RAW264.7 cell lines

Nitric Oxide (NO) inhibition was measured by the method modified from Tewtrakul *et al.* (2009). Macrophage RAW264.7 cell line was cultured in RPMI (Roswell Park Memorial Institute) medium supplemented with 10% heat-inactivated fetal bovine serum, Penicillin (100 U/ml) and Streptomycin (100 mg/ml) and maintained in a humidified incubator. RAW264.7 cells were harvested by scraper and suspension in a fresh medium and seeded in 96-well plates (1x10⁶ cells/well) allowed to adhere for 2 h at 37°C in a humidified atmosphere containing 5% CO₂. Old medium was replaced with 0.1 ml fresh medium containing 1 µg/ml of lipopolysaccharides (LPS) from *Escherichia coli*, serotype 055:B5. Food extract (0.1 ml) of 1, 10, 100, 1000 µg/ml concentrations (in deionized water) were added and incubated for 24 h. The positive control was cells without food extract but with LPS whereas the negative control was cells without food extract and LPS. The nitrite (indicating NO production) in the culture medium was detected using a reaction with Griess reagent (Green *et al.*, 1982). One hundred microliter of each supernatant was mixed with 100 µl of Griess reagent before the absorbance at 570 nm was measured. L-Nitroarginine (L-NA) treated group was used as the standard drug for inhibition of NO production.

$$\text{NO Inhibition (\%)} = \left(\frac{A-B}{A-C} \right) \times 100$$

Where A is 570 nm absorbance of the Control (LPS (+), Sample (-)); B is 570 nm absorbance of the test sample (LPS (+), Sample (+)); C is absorbance of the Negative Control (LPS (-), Sample (-)); A-C is nitrite concentration (µg/ml);

Cell viability was measured using 3',-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent which was absorbed into the living RAW264.7 cells and converted into formazan according to the method of Yamamoto *et al.* (2002). The chemical conversion is facilitated by succinate dehydrogenase in the mitochondria of living cells. The amount of accumulated formazan indicates the activity of succinate dehydrogenase, and thus reflects the cell viability. The 96-well plates from above (at a density of 1x10⁶ cells per well) were added with 10

µl MTT solution (5 mg/ml in phosphate buffer saline or PBS) solution at 37°C for 2 h. The medium was then removed the cells and dye crystals (formazan) were dissolved by adding 100 µl of 0.04 M HCl (in isopropanol) before the absorbance at 570 nm was measured.

Total phenolic contents (TPC)

TPC was measured according to the method of Slinkard and Singleton (1997). Food extracts were diluted with distilled water to 0.1, 0.5, 1, 2, 3, 4, 5 and 10 mg/ml. Sample solutions (12.5 µl) and standard solutions (containing 5, 10, 25, 50, 100, 125, 150 and 200 µg/ml of gallic acid in distilled water) were individually placed in a 96-well plate. Reagent blank was distilled water. Folin-Ciocalteu phenol reagent (12.5 µl) was added to each well and left for 6 min. Na₂CO₃ solution (125 µl of 7% w/v in distilled water) was mixed in together with 100 µl of distilled water and the mixture allowed to stand for 90 min in darkness and absorbance was measured at 760 nm. Gallic acid treated group was used to make the standard curve. Each crude extract was analyzed in triplicates and the results were expressed in mg of gallic acid equivalents (GAE) per g dry crude extract and 100 g dry weight.

Identification of phenolic compounds by High Performance Liquid Chromatographic (HPLC)

Phenolic acid standards were consisted of gallic acid, protocatechuic acid, p-coumaric acid, chlorogenic acid and ferulic acid. Solutions were made by dissolving 0.01 g of each in 10 ml distilled water. Aliquots of 0.5 ml of each solution were put in 5.0 ml volumetric flasks, and adjust volume with distilled water. Working solutions were prepared to 1, 5, 12.5, 25 and 50 µg of gallic acid, protocatechuic acid, p-coumaric acid, chlorogenic acid and ferulic acid per milliliter.

Defatted dried crude samples (0.01 g) were solubilized in 2 ml of acid methanol (62.5% methanol: 6 M HCl, 4:1 v/v) and shaken at 70°C for 2 h (Merken and Beecher, 2000). A reversed-phase High Performance Liquid Chromatography (HPLC) method (Tian *et al.*, 2004) with modification was used to characterize the phenolic compounds in the extracts. After filtered through a 0.20-µm pore size, a 10-µL aliquot was separated using an Agilent 1200 HPLC system (Agilent 1200, Waldbronn, Germany) equipped with a diode array detector on a 250 mm x 4.6 mm i.d., 5 µm, Eclipse XDB-C18, analytical column (Agilent, Santa Clara, CA, USA). The mobile phase was purified water with 0.1% (v/v) trifluoroacetic acid (TFA) (solvent A), and acetonitrile (solvent B).

Flow rate is 0.8 ml/min. Column temperature was controlled at 40°C. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% of solvent B; from 5 to 15 min 9% solvent B; from 15 to 22 min linear gradient from 9 to 11% solvent B; from 22 to 35 min, linear gradient from 11 to 18% solvent B; and from 35 to 42 min, 18% solvent B.

Hydroxybenzoic acid compounds (gallic acid and protocatechuic acid) were detected at 290 nm and hydroxycinnamic (chlorogenic acid, ρ -coumaric acid and ferulic acid) was detected at 325 nm.

For separation of capsaicin according to the method of Othman *et al.* (2011) by HPLC using isocratic program composing of 50% solvent A (0.1% TFA in water) and 50% solvent B (acetonitrile) at flow rate of 1 ml/min, temperature set up at 30°C. A reverse phase 250 mm x 4.6 mm i.d., 5 μ m, Eclipse XDB-C18, analytical column (Agilent, Santa Clara, CA, USA) and detected at 280 nm.

Statistical analysis

All data were analyzed with the IBM statistical package for Windows (version 19.0). All data were carried out in triplicates and reported as means \pm standard deviation. Data were analyzed by one way-analysis of variance (ANOVA), and statistical significance with Duncan's multiple range test ($P < 0.05$).

Results and Discussion

Antioxidant activity – radical scavenging property by DPPH

Free radical scavenging capacity (DPPH) data for non-defatted and defatted food extracted with water, 50% ethanol and 95% ethanol are shown in Table 2. Non-defatted extracts IC_{50} were varied from 72.90 to 968.82 mg/g dry crude extract. Defatted sample showed IC_{50} of DPPH from 79.92 to 947.51 mg/g dry crude extract. Lowest IC_{50} for scavenging property was Food 2 (non-defatted and 95% ethanol extracted) at 72.90 mg/g dry crude extract. However, they are not significantly different from Food 8 and Food 6 (non-defatted and 95% ethanol extracted) with IC_{50} of 73.50 and 79.50 mg/g dry crude extract, respectively. Just show it on the Table footnote.

Curry paste is one of the main compositions of four recipes (Food 1, Food 2, Food 3, and Food 5) which are abundant in turmeric, a characteristic main ingredient for the South. Curcumin, in turmeric helps protect cells from free radical damage and helps prevent obesity-associated inflammation including diabetes in obese mice (Ahn *et al.*, 2010). Food 5

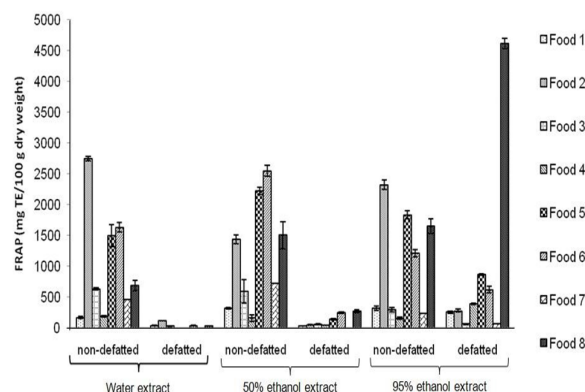


Figure 1. Antioxidant activity of non-defatted and defatted of Southern Thai foods using FRAP assay (μ M Trolox/100g dry weight)

(non-defatted, 50 and 95% ethanol extract) and Food 2 (both defatted and non-defatted, and 95% ethanol extract) showed low IC_{50} of DPPH radical scavenging activity; these are also turmeric-containing materials.

Some of defatted food extracts showed higher activity of DPPH scavenging than non-defatted ones such as water extract of Food 1, Food 2, Food 4 and Food 7 while about 60% of non-defatted food extracted by ethanol showed higher DPPH activity than defatted foods (Data not shown). It has been reported that a non-defatted methanol and ethanol extracts yielded a higher DPPH activity than defatted counterparts. Some of this might be interfered by lipid oxidation.

Anti-oxidative property by Reducing Property - FRAP

Lipids in non-defatted samples may interfere with FRAP readings (Brand-Williams *et al.*, 1996), evaluation of FRAP results is concentrated on defatted samples (Figure 1). Highest reducing power was found in defatted 95% ethanol extract of Food 8 ($4,619.70 \pm 85.51 \mu$ M TE/100 g dry food). This ascorbic acid-rich product was extremely high in reducing power followed by Food 5, Food 6, and Food 4, respectively (95% ethanol extracts).

Anti-inflammatory activity by NO inhibition in macrophage RAW264.7 cell lines

NO inhibition in LPS-activated RAW264.7 murine macrophage cell line was studied in the presence of defatted (water, 50% and 95% ethanol soluble) extracts of eight Southern Thai foods. For all cases cell viability was $>80\%$. It was found that anti-inflammatory activities reduce the production of NO. The IC_{50} results of inhibitory activity are shown for water and 95% ethanol extracts in Table 2.

The extracts of Southern Thai foods inhibited LPS-induced NO production with the highest inhibition in the case of water extracted, Food 3

Table 2. Radical scavenging activity by DPPH and NO inhibition in macrophage RAW 264.7 cells expressed by IC₅₀ (mg/ g dry crude extract) of Southern Thai food extracts

Food name	IC ₅₀ for DPPH scavenging activity (mg/g dry crude extract)						IC ₅₀ for NO inhibition in macrophage RAW 264.7 cells (mg/g dry crude extract)		
	Non-defatted			Defatted			Defatted		
	Water extract	50% ethanol extract	95% ethanol extract	Water extract	50% ethanol extract	95% ethanol extract	Water extract	50% ethanol extract	95% ethanol extract
Food 1	574.00±13.53 ^d	516.33±35.19 ^c	260.79±25.47 ^c	503.25±5.16 ^d	586.41±24.03 ^d	593.50±10.60 ^e	77.99±0.81 ^b	79.12±1.10 ^a	72.93±1.50 ^a
Food 2	736.44±1.86 ^a	734.21±73.29 ^a	72.90±7.06 ^a	87.00±3.12 ^a	79.92±5.24 ^a	81.00±7.07 ^a	134.16±28.13 ^c	>1000 ^b	84.88±7.31 ^a
Food 3	250.06±7.47 ^a	441.23±19.93 ^b	968.82±104.55 ^f	866.43±39.88 ^e	527.35±15.70 ^c	522.00±18.38 ^f	25.12±5.24 ^a	>1000 ^b	838.13±41.85 ^d
Food 4	705.50±13.83 ^a	604.16±23.73 ^a	597.02±34.69 ^a	555.00±69.54 ^a	753.34±38.70 ^a	771.50±26.16 ^b	123.09±18.61 ^c	>1000 ^b	>1000 ^e
Food 5	918.79±257.97 ^f	481.50±11.14 ^b	120.72±7.84 ^b	114.00±11.13 ^b	264.63±2.56 ^b	169.50±57.27 ^b	>1000 ^e	>1000 ^b	129.74±79.14 ^b
Food 6	459.39±12.89 ^b	678.81±56.32 ^a	79.50±2.56 ^a	87.50±7.77 ^a	92.79±4.81 ^a	182.00±22.63 ^b	129.29±5.72 ^c	>1000 ^b	>1000 ^e
Food 7	483.00±11.31 ^c	498.27±22.79 ^b	535.60±18.81 ^d	369.50±23.33 ^c	947.51±11.81 ^f	368.00±14.14 ^c	259.62±32.57 ^d	>1000 ^b	193.65±36.58 ^c
Food 8	216.85±17.76 ^a	212.46±1.80 ^a	73.50±1.55 ^a	728.14±43.16 ^f	250.87±11.43 ^b	364.50±14.84 ^c	>1000 ^e	>1000 ^b	99.11±10.53 ^a

DPPH IC₅₀ of ascorbic acid as 4.33±0.37 µg/ml.

IC₅₀ of L-Nitroarginine in NO inhibition in cell line as 47.57 ± 3.58 µg/ml.

All values are expressed as mean±SD (n = 3).

Different superscript letters in same column indicate significant difference (P< 0.05) analyzed by Duncan's multiple range test

Table 3. Total phenolic contents (TPC) by Folin-ciocalteu method of Southern Thai food extracts

Food name		TPC in mg GAE (Non-defatted)			TPC in mg GAE (Defatted with hexane)		
		water extract	50% ethanol extract	95% ethanol extract	water extract	50% ethanol extract	95% ethanol extract
Food 1	/g dry crude extract	8.45±0.48 ^c	7.25±0.14 ^f	4.76±0.97 ^a	7.20±0.28 ^a	13.46±0.61 ^d	11.05±0.74 ^d
	/100 g dry food	174.30±9.70 ^F	147.35±2.76 ^F	107.67±21.91 ^G	298.47±11.81 ^B	135.68±6.39 ^G	147.32±9.85 ^E
Food 2	/g dry crude extract	32.18±4.42 ^a	23.59±1.75 ^a	15.66±2.05 ^b	9.73±0.26 ^d	31.89±0.18 ^a	17.35±0.29 ^c
	/100 g dry food	753.19±103.43 ^{BC}	436.14±32.29 ^{DE}	388.40±50.95 ^B	505.36±13.69 ^A	97.08±0.55 ^H	135.62±2.29 ^E
Food 3	/g dry crude extract	8.14±1.33 ^c	9.02 ± 0.70 ^a	10.67±0.77 ^c	7.77±0.19 ^a	8.35±1.31 ^a	16.85±1.03 ^c
	/100 g dry food	395.28±64.64 ^E	416.05±32.31 ^E	293.59±21.34 ^D	141.08±3.57 ^E	256.89±36.14 ^D	327.46±19.98 ^C
Food 4	/g dry crude extract	4.09±0.82 ^d	3.69±0.23 ^e	3.66 ± 0.41 ^a	2.17±0.37 ^f	3.33±0.75 ^e	28.69±3.40 ^a
	/100 g dry food	131.40±26.41 ^F	136.10± 8.74 ^F	108.63±45.47 ^G	22.59±3.84 ^G	119.88± 21.87 ^F	39.31±4.66 ^B
Food 5	/g dry crude extract	28.97± 0.94 ^a	19.72 ± 1.19 ^b	16.06 ± 0.30 ^b	22.58±0.94 ^a	25.33 ± 0.57 ^b	30.24±2.75 ^a
	/100 g dry food	855.82±27.80 ^B	727.26 ± 44.02 ^B	692.10 ± 13.13 ^A	135.18±5.62 ^E	530.36±12.27 ^B	200.80±0.60 ^D
Food 6	/g dry crude extract	15.31±1.83 ^b	17.38±0.45 ^c	10.31±0.86 ^c	16.10±0.70 ^b	20.57±0.58 ^c	18.53±3.37 ^c
	/100 g dry food	971.74 ±116.34 ^A	467.69±11.99 ^D	266.11±22.17 ^E	83.88±3.64 ^F	440.24±12.67 ^C	189.09±34.42 ^F
Food 7	/g dry crude extract	12.31±1.46 ^b	11.30±0.11 ^d	7.91±0.18 ^d	10.47±0.49 ^d	9.37±0.17 ^a	11.87±0.76 ^d
	/100 g dry food	706.69±84.10 ^C	773.55±7.81 ^A	221.16±4.93 ^F	198.40±9.27 ^D	232.86±4.14 ^E	201.97±13.23 ^D
Food 8	/g dry crude extract	12.24±1.36 ^b	11.02±0.61 ^d	18.99±0.86 ^a	11.89±0.35 ^c	14.09±0.95 ^d	24.58±0.76 ^b
	/100 g dry food	528.13±58.86 ^D	561.25±31.27 ^C	344.49±15.65 ^C	242.79 ±7.11 ^C	769.49±52.89 ^A	607.47±18.77 ^A

All values are expressed as mean±SD (n = 3).

Different superscript letters in same column (per gram dry crude extract and per 100 gram dry food) indicate significant difference (P< 0.05) analyzed by Duncan's multiple range test.

(IC₅₀ value of 25.12 mg/g dry crude extract) without significant effect on cell viability. Surprisingly, found that Food 3 (water extracted) show IC₅₀ lower than positive control (L-Nitrosamine 47.57±3.58 µg/ml). Moderate levels of NO inhibition were found in water, 50% and 95% ethanol extracted Food 1 with IC₅₀

values of 77.99±0.81, 79.12±1.10 and 72.93±1.50 mg/g dry crude extract, respectively. 95% ethanol extracts that showed some moderate NO inhibition were Food 2 and Food 8 at IC₅₀ of 84.88±7.31 and 99.11±10.53 mg/g dry crude extract, respectively. Note that both Food 1 and Food 2 were relatively low

Table 4. Phenolic compounds of defatted crude extracts from various foods products by HPLC method (mg/g dry crude extracts)

Food name	Solvent	Hydroxybenzoic acid		Hydrocinnamic acid			capsaicin
		gallic acid	p-catechuic acid	chlorogenic acid	coumaric acid	ferulic acid	
Food 1	water	0.014±0.001	0.037±0.001	0	0.008±0.001	0.024±0.001	0
	50% EtOH	1.013±0.078	0	0	0	0	1.171±0.00
	95% EtOH	0.106±0.000	0.026±0.000	0.029±0.001	0.093±0.001	0	0.203±0.10
Food 2	water	0.012±0.000	0.174±0.002	0	0.043±0.002	0.054±0.000	0.231±0.041
	50% EtOH	0.116±0.001	0.065±0.004	0.295±0.000	0.052±0.004	0	1.327±0.038
	95% EtOH	0.178±0.000	0	0.062±0.001	0	0.170±0.001	2.320±0.038
Food 3	water	0.030±0.000	0.119±0.002	0	0	0	0
	50% EtOH	0.136±0.001	0	0.044±0.009	0.040 ± 0.000	0	0.353±0.011
	95% EtOH	7.267±0.160	0.030±0.002	0	0.102±0.002	0.042±0.001	0.815±0.032
Food 4	water	0	0	0	0	0	0
	50% EtOH	0.034±0.001	0.084±0.000	0	0	0.029±0.000	0
	95% EtOH	0	0.062±0.003	0	0	0	0
Food 5	water	0.044±0.004	0	0.414±0.001	1.254±0.051	0.522±0.028	0
	50% EtOH	0.054±0.000	0.075±0.000	0.337±0.002	0.055 ± 0.000	0.562±0.001	0.272±0.016
	95% EtOH	10.835±0.024	0.175±0.002	0.069±0.000	0	0.305±0.013	0.086±0.000
Food 6	water	0.040±0.000	0.061±0.001	0.181±0.001	0.067±0.000	0.386±0.042	0
	50% EtOH	0.014±0.000	0.096±0.000	0.195±0.001	0.106±0.004	0.362±0.001	0
	95% EtOH	7.373±0.007	0	0	0	0.430±0.011	0
Food 7	water	0.089 ±0.014	0	0	0	0	0
	50% EtOH	0.013±0.001	0.028±0.000	0	0	0	0
	95% EtOH	11.040±0.000	0	0	0	0.322±0.015	0
Food 8	water	0.129±0.007	0	0	0	0	0
	50% EtOH	0.225±0.001	0.098±0.002	0	0.111±0.005	0.322±0.005	0.106±0.001
	95% EtOH	9.059±0.007	0.062±0.000	0.029 ± 0.001	0	0.471±0.004	0.135±0.010

All values are expressed as mean±SD (n = 2).

in DPPH scavenging and FRAP reducing properties and Food 8 with high in both DPPH scavenging and FRAP reducing properties. An ethanol extract of red curry paste showed anti-inflammatory activities on same macrophage cell line at 0.065-0.260 mg/ml by Tuntipopipat (2011) and at 0.97 mg/ml in Stirred-fried chicken green curry paste (Charoenkiatkul *et al.*, 2011).

For the case of 50% ethanol extracts, only Food 1 showed NO inhibitory effect whereas other food extracts NO inhibition were not effective in NO inhibition. It could be possible that only Food 1 at 50% ethanol extract was the only sample that contained a higher amount of gallic acid (Table 4). It could be concluded from this investigation that among water extract from defatted samples, Southern Thai foods including Food 3, Food 1, Food 2, Food 6 and Food 4 exhibited anti-inflammatory effects without cytotoxicity effect. Some of such properties were found to be related to free radicals scavenging and reducing capacity, such as gallic acid. More research is warrant to further study understanding.

Total phenolic contents (TPC) by Folin-Ciocalteu method

The total phenolic contents (TPC) for these samples and foods are presented in Table 3. The data are expressed in mg GAE per g dry crude extract and per 100 g dry food. Total phenolic contents in food extracts varied widely ranging from 22.59 to 971.7 mg GAE/ 100 g dry food. While there was no particular trends, most of non-defatted samples contained higher amounts of phenolics than their

defatted counterparts because of the great amount of fat-soluble phenolics in several of the samples. It was noted that typical Southern-Thai soups (namely Food 5, Food 2 and Food 7) contained higher phenolic compounds (855.8, 753.2 and 706.7 mg GAE / 100 g dry food, respectively) primarily from vegetables and herbs used. Phenolic compounds recovered varied greatly among different solvents, defatting treatments and ingredient mixtures unique Southern Thai foods. These values are difficult to interpret due to the complexity and interference of solvents and reaction involved (Chandrasekara and Shahidi, 2010). Recommended defatting food fractions in order to remove lipids and soluble components which could interfere in the analysis of phenolic antioxidants and also to remove lipids that are prone to oxidation and deterioration resulting in poor specificity of the phenolics analysis (Kaefer and Milner, 2008). In this case, an interference of Folin oxidation of phenolics on lipid components (i.e., it oxidized lipids components as well) could also occur and might have distorted some of the data resulting in an overestimation of total phenolics in the non-defatted samples.

The Folin-Ciocalteu method suffers from a number of interfering substances both phenolic and non-phenolic substances as described by Prior and colleges (2005). From Table 2, some foods studied contained interfering components, such as ascorbic acid from lime in Food 8 and proteins (amines) from fermented fish in Food 7. Therefore, the data on TPC need to be further characterized should the foods are to be further investigated in depth.

Identification of phenolic compounds by HPLC

Common phenolic compounds, namely gallic acid, protocatechuic acid, chlorogenic acid, ρ -coumaric acid and ferulic acid, were analyzed by HPLC. The contents of each extracts (Table 4) for all 8 foods extracted with water, 50% ethanol and 95% ethanol. Phenolic compounds were present in significant amounts. Mostly all samples contained gallic acid as the major phenolic component (e.g. data from 95% ethanol extract, Table 4) which had a tendency to increase with reducing solvent polarity.

The phenolic compounds found in 24 food extracts (8 foods \times 3 solvents) noticeably varied with the types of foods and the extracting solvents. For foods that showed relatively lower scavenging properties (higher IC_{50}) such as Food 1, Food 3 and Food 7 were relatively lower in phenolic compounds (relatively lower in hydroxycinnamic acids). Observed data showed that extracts with high phenolic contents might not be accompanied with high DPPH scavenging property, for example Food 5 and Food 7 (Table 3). On the other hand extracts with high scavenging properties might be low in TPC suggesting other non-phenolics involved. It has been reported that radical scavenging activities performance of 5 phenolic acids are in the following order: gallic > protocatechuic > ferulic > ρ -coumaric > chlorogenic (Karamac *et al.*, 2005).

Various foods in the study are complex mixtures undergone different preparation methods. Nevertheless, some strong anti-inflammation was found in products with relatively high gallic acid such as Food 6, Food 3, and Food 7. These data may be useful for future more in-deeply research of each food.

Conclusion

This study is a preliminary study of eight Southern Thai foods that are rich in indigenous vegetables, herbs and spices. Scavenging, reducing properties and NO inhibition were found to be significant. Phenolic compounds found included gallic acid, protocatechuic acid, chlorogenic acid, ρ -coumaric acid and ferulic acid. Anti-inflammatory effects were significant in water extracts of foods which showed some correspondingly high in key phenolics such as gallic acid. In addition, water extracts of Food 3, Food 4 and Food 6, were much more effective as seen from IC_{50} by anti-inflammation by 7-26 times the magnitude (compared with 95% ethanol extracts). More investigation is warranted to further elucidated the health implication of Southern Thai foods.

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