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Antioxidant Activity and Cytotoxicity of Six Selected, Regional, Thai Vegetables

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Abstract: Six selected, regional, Thai ethanolic leaf extracts, *Barringtonia acutangula* (BaLE), *Cratoxylum formosum* (CfLE), *Limnophila aromatica* (LaLE), *Polygonum odoratum* (PoLE), *Syzygium gratum* (SgLE) *andSchinus terebinthifolius* (StLE) were measured for total phenolic content (TPC), total flavonoid content (TFC). Some major phytochemicals were qualitatively evaluated. Their antioxidant activity and cytotoxicity were determined. BaLE, SgLE andStLE contained high total phenolic compounds, but low flavonoids, with high antioxidant activity assayed by DPPH, LPO inhibition and FRAP. CfLE, LaLE andPoLE were rich in flavonoids, but low in antioxidant activities. TPC well correlated to DPPH scavenging activity and FRAP, but FTC correlated to lipid peroxidation inhibition. All extracts were not toxic to normal human lymphocytes. BaLE and StLE were highly toxic to Jurkat cells and they completely induced plasma membrane lysis of human red blood cells. SgLE was particularly high toxic to MCF7 cells. Only BaLE inhibited HepG2 cell growth. This information illustrated that these six selected, regional Thai vegetables possessed potent antioxidants and cytotoxic activity against cell lines, which will be beneficial for health, cancer prevention and food preservation.

Key words: Barringtonia Acutangula · Cratoxylum Formosum · Limnophila Aromatica · Polygonum Odoratum · Syzygium Gratum · Schinus Terebinthifolius

INTRODUCTION

Oxidative stresses play harmful physiological responses which may lead to develop cell damages and various diseases such as diabetes, atherosclerosis, ischemic injury, inflammation andcarcinogenesis [1]. Phytochemicals from fruits and vegetables significantly reduce the risk of chronic disease development, probably due to their antioxidant properties [2]. Tropical plants have been increasingly received attention as sources of many effective antioxidants and anticancer agents. Many of them are normally consumed in dietary food and also claimed for treatments of a number of symptoms, including asthma, hypertensive, indigestion, laxative, peptic ulcer, diarrhea andbacterial infection [3]. Some regional Thai vegetables were screened. Six selected vegetables, phak jig *Barringtonia acutangula* L., Gaerin (Lecythidaceae); phak tiew *Cratoxylum formosum* (Jack) Dyer (Guttiferae); phak khayaeng *Limnophila aromatica* Merrill (Scrophulariaceae); phak paew *Polygonum odoratum* Lour (Polygonaceae); phak mek *Syzygium gratum* (Wight) S.N. Mitra (Myrtaceae); and matumkhaeg *Schinus terebinthifolius* Raddi (Anacardiaceae), are of our interest for more detailed investigation on their cytotoxic properties. These plants are widely grown and their young leaves have been traditionally consumed as vegetables in daily diet in the Northeast of Thailand. Their leaf extracts were reported to possess antioxidant [4- 6], antimicrobial [7- 9], antifungal [10] andantimutagenic activities [11]. The leaf extracts of *C. formosum*, *L. aromatica* and*S. gratum* possess antioxidant and vascular protective activities *in vitro* and *in vivo* [12]. The methanolic leaf extracts of *L. aromatica* and *P. odoratum* expressed potent antitumor-promoting activity induced by Epstein-Barr virus, *in vitro* [13]. Moreover, *P. odoratum* leaf extract strongly inhibited MCF7 breast cancer cell proliferation [14]. The polyphenols from *S. terebinthifolius* leaves induced cell growth arrest and apoptosis in the androgen-insensitive DU145 prostate carcinoma cells [15]. Presently, little information on the cytotoxic efficacy of the above mentioned Thai vegetables is available. Therefore, this study aims to investigate phytochemicals, antioxidant activities andcytotoxic effects of leaf extracts *of*

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B. acutangula (BaLE), *C. formosum* (CfLE), *L. aromatica* (LaLE), *P. odoratum* (PoLE), *S. gratum* (SgLE) and *S. terebinthifolius* (StLE) on normal human erythrocytes and lymphocytes andsome human cancer cell lines.

MATERIALS AND METHODS

Chemicals: Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, penicillin-streptomycin andfetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) from Invitrogen (Carlsbad, CA, USA). Catechin, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), bismuth (III) subnitrate, ρ-anisaldehyde, vanillin, 3,5-dinitrobenzoic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), malonaldehyde (MDA) andHISTOPAQUE®-1077 were from Sigma-Aldrich (St. Louis, MO, USA). Butylated hydroxytoluene (BHT) and gallic acid was obtained from Fluka (Germany). 2-4-6-Tripyridyl-s-triazine (TPTZ) was obtained from Acros Organics (Geel, Belgium). Folin-Ciocalteu's reagent was purchased from Carlo Erba Reagents (Milano, Italy). Trichloroacetic acid (TCA) and bovine serum albumin (BSA) were purchased from BDH (Poole, UK). Dimethylsulfoxide (DMSO) and sodium bicarbonate were obtained by Amresco (Ohio, USA). The other chemicals were commercially available and analytical grade.

Plant Materials and Extraction: Fresh young leaves of *Barringtonia acutangula* (Ba), *Cratoxylum formosum* (Cf), *Limnophila aromatica* (La), *Polygonum odoratum* (Po), *Syzygium gratum* (Sg) and*Schinus terebinthifolius* (St) were purchased from local markets in Nakhon Ratchasima, Northeast of Thailand. The leaves were freeze-dried and grounded into powder. The powder of 60 g was macerated in 70% ethanol for 24 h. The extract was filtered, evaporated andlyophilized at -84°C. The extract powder was stored at -20ºC until analysis.

Thin Layer Chromatography (TLC): TLC was performed using silica gel 60 F254, 0.25 mm precoated plates (Merck, Darmstadt, Germany) and mobile phases of hexane:acetone (70:30); ethyl acetate: methanol:water (81:11:8) and chloroform:glacial acetic acid:methanol:water (64:32:12:8). The extracts were dissolved in 70% ethanol. The plates were visualized under UV at 254 and 366 nm and sprayed with specific reagents to detect some major phytochemicals [16], i.e., (a) Dragendorff reagent for an alkaloids group; (b) Potassium hydroxide (KOH) reagent for an anthraquinones (red), anthones (yellow, UV-366 nm) andcoumarins group (blue, UV-366 nm); (c) Kedde reagent for cardic glycosides; (d) Anisaldehyde reagent and (e) Vanillin-sulphuric reagent for essential oils, steroids, terpenoids andphenols. The extracts were also screened by specific tests; Salkowski test for terpenoids, froth test for saponins andgelatin-salt block test for tannins [17].

Total Phenolic Compounds (TPC) Measurement: TPC of the extracts was determined by Folin-Ciocalteu colorimetric method [18] with minor modification. Briefly, 0.1 ml of the extract, dissolved in DMSO, 1.5 ml of dH_2O and 0.1 ml Folin-Ciocalteu's reagent were mixed and incubated for 5 min. Then, 0.3 ml of 20% Na_2CO_3 was added, incubated for 45 min andwas measured the absorbance at 750 nm. The TPC was expressed as µg of gallic acid equivalents (GAE)/mg dried extract.

Total Flavonoids Content (TFC) Measurement: TFC was measured by the aluminium trichloride colorimetric assay [19]. An aliquot of 0.5 ml of the extract in 0.2% DMSO was mixed with 2 ml dH_2O and 0.15 ml of 5% $NaNO_2$. The mixture was incubated for 6 min and then 0.15 ml of 10% $AlCl_3$ was added. After allowed to stand for 6 min, 1 ml of 1 M NaOH was added and made up with dH_2O to 5 ml total volume and then measured the absorbance at 510 nm. Total flavonoid content was expressed as µg catechin equivalents (CAE)/mg of dried extract.

Free Radical Scavenging Activity: Free radical scavenging activity was determined by $DPPH^{\bullet}$ assay [20]. The extract of 0.1 ml at different concentrations was added to 3.9 ml of 40 mg/l methanolic $DPPH^{\bullet}$ solution, incubated for 45 min in the dark andmeasured at 515 nm. The percentage of free radical inhibition was calculated and expressed as median effective concentration (EC_{50}).

Microsome Preparation and Lipid Peroxidation: The microsomes were prepared from a rat liver (a Wistar rat from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand) at 4°C throughout. The liver was washed and motor-driven homogenized in phosphate buffer saline (PBS), pH 7.4, containing 50 mM phosphate and 0.15 M KCl, at 2000 rpm for 15 min. The homogenate was centrifuged at 9,000*g* for 10 min. The supernatant was collected and centrifuged at 100,000*g* for 60 min. The microsome pellets were collected, washed in PBS, recentrifuged at 100,000*g* for 60 min and then resuspended in PBS and stored at -80ºC. The protein content of microsome suspension was quantified by Lowry method.

The ferric-induced microsome lipid peroxidation inhibition was performed as described by Joubert *et al.* [21] with some modification. The extract was dissolved in DMSO and further diluted in PBS to obtain 0.2% DMSO in different concentrations. Fifty microlitters of extract solution, 200 µM $FeCl_2$, 100 µM sodium ascorbate and 1 mg/ml microsomes were mixed and incubated at 37ºC for 1 h. The chilled mixture of 0.5 ml of 20% TCA, 0.25 N HCl and0.01% BHT was added. Then, 0.5 ml of 0.8% TBA was added, incubated at 90ºC for 60 min, allowed to cool andcentrifuged at 4000*g* for 10 min. The supernatant was transferred onto a 96-well microplate and measured the absorption at 532 nm. The inhibition was calculated from the malonaldehyde (MDA) formed and expressed as EC_{50}.

Ferric Reducing Antioxidant Power (FRAP): FRAP assay was conducted according to Benzie and Strain [22]. FRAP reagent was freshly prepared by mixing 10 volumes of 1.0 mol/l acetate buffer, pH 3.6, 1 volume of 10 mmol/l TPTZ in 40 mmol/l hydrochloric acid and1 volume of 20 mmol/l ferric chloride. The extract of 50 µl was incubated with 1.5 ml of FRAP reagent for 40 min. The absorbance was measured at 595 nm. A standard curve of 100-1000 µmol $FeSO_4.7H_2O$ was prepared. The antioxidant power was expressed in µmol Fe^{2+}/mg dried sample.

Cell Culture: MCF7, human breast cancer cell line, was a gift from R.P. Shiu, Dubik and Shiu, 1992 and HepG2, human hepatocellular carcinoma cell line, was from American Type Culture Collection (ATCC). Jurkat, human acute lymphocytic leukemia cell line, was from Cell Lines Service (Eppelheim, Germany). MCF7 and HepG2 cells were cultured in high glucose DMEM medium supplemented with 100 IU/ml penicillin, 100 g/ml streptomycin and 10% fetal bovine serum (FBS). Jurkat cells and normal lymphocyte cells were cultured in RPMI 1640 medium supplemented with 1 mM sodium pyruvate, 100 IU/ml penicillin, 100 g/ml streptomycin and 10% FBS. The cells were incubated at 37ºC, 5% CO_2 and95% relative humidity.

Preparation for Normal Lymphocytes: Human blood collected from healthy donors (Blood Bank, Nakhon Ratchasima) was layered onto the HISTOPAQUE®-1077 and centrifuged at 400*g*, room temperature, for 30 min. The opaque interface containing mononuclear cells was carefully collected and washed, 3 times, with PBS, pH 7.4. The mononuclear cells were cultured in complete RPMI medium overnight to allow the monocytes and platelets to attach to the culture flask. The floating lymphocytes were collected for assay.

Cytotoxicity Assay: The cytotoxicity of the extracts on cancerous cell lines was investigated by Mosmann assay [23]. The cell lines and normal lymphocytes were grown in 96-well plates at 25,000 and 100,000 cells per well, respectively. Two hundred microlitters of the extracts, dissolved in 0.2% DMSO in medium, at different concentrations were added andincubated for 48 h. After removal of medium, 100 µl of 0.5 mg/ml MTT solution was added and incubated for 3 h. The formazan crystal formed was dissolved by adding 150 µl of DMSO. The absorbance was measured at 540 nm.

Hemolytic Assay: The extract induced cytotoxicity to cell membrane was observed by hemolysis assay according to El-Sayed *et al*. [24] with some modification. Red blood cells (RBCs) from healthy donors (Blood bank, Nakhon Ratchasima) was centrifuged at 900*g*, washed 3 times with PBS, pH 7.4 andthen resuspended in PBS (1:25, v/v). RBCs of 480 ìl were incubated with 1.32 ml of extract, diluted in PBS at various concentrations, at 37°C for 3 h and centrifuged at 900*g* for 10 min. The supernatant was carefully collected and measured the absorbance at 540 nm. The total hemolysis was obtained by incubating RBCs in 50 µl of 1% TritonX-100 for 3 h.

Statistical Analysis: All data were analyzed by analysis of variance (ANOVA), followed by Duncan's multiple range tests using the Statistical Package for Social Sciences (SPSS) version 17 for Windows. The correlations were analyzed using Pearson correlation test. The median inhibitory concentration, IC_{50}, the median effective concentration, EC_{50} andthe 95% confidence interval, CI 95%, were obtained by nonlinear regression using the GraphPad PRISM for Windows, v.5.

RESULTS AND DISCUSSIONS

Phytochemicals of the Leaf Extracts: The phytochemicals of these selected, regional Thai vegetable leaf extracts were partially separated and determined by TLC and semiquantitated by color intensity as shown in Table 1. Essential oils and tannins were found constituent in all extracts with different levels, but alkaloids, anthraquinones, quinones, coumarins andcardiac glycosides were not detected. Saponins were present in BaLE LaLE, SgLE and StLE while terpenoids were found only in LaLE and SgLE. However, LaLE contained low terpenoids, saponins and tannins. Terpenoids and saponins were not found in PoLE.

Table 1: Phytochemicals in the leaf ethanolic extracts of *Barringtonia acutangula* (BaLE), *Cratoxylum formosum* (CfLE), *Limnophila aromatica* (LaLE), *Polygonum odoratum* (PoLE), *Syzygium gratum* (SgLE) and*Schinus terebinthifolius* (StLE), detected by thin layer chromatography (TLC) and with specific tests

	Extract					
Phytochemicals	BaLE	CfLE	LaLE	PoLE	SgLE	StLE
Alkaloids[a]	-	-	-	-	-	-
Anthraquinones[a]	-	-	-	-	-	-
Quinones[a]	-	-	-	-	-	-
Coumarins[a]	-	-	-	-	-	-
Cardiac glycosides[a]	-	-	-	-	-	-
Essential oils[a]	+	++++	++	+++	+	+++
Terpenoids[b]	-	-	+	-	+++	-
Saponins[b]	+++	-	+	-	+	++++
Tannins[c]	+++	+	+	++	++++	+++++

Arbitrarily quantitative assigns of phytochemical contents: -, absent; +, very low; ++, low; +++, medium; ++++, high; +++++, very high

[a]detected by TLC; [b]detected by TLC and specific reagents; [c]detected with specific reagents

Table 2: Total phenolic compounds, flavonoid content, DPPH-scavenging activities, lipid peroxidation inhibition andferric reducing antioxidant power (FRAP) of the leaf ethanolic extracts of *Barringtonia acutangula* (BaLE), *Cratoxylum formosum* (CfLE), *Limnophila aromatica* (LaLE), *Polygonum odoratum* (PoLE), *Syzygium gratum* (SgLE) and*Schinus terebinthifolius* (StLE).

Extract	Phenolics (µg GAE/mg)	Flavonoids (µg CAE/mg)	DPPH EC_{50} (µg/ml)	LPO inhibition EC_{50} (µg/ml)	FRAP (µmol Fe^{2+}/mg)
BaLE	377.28±11.57[b]	15.47±4.11[e]	6.67±0.10[c]	3.92±0.01[a]	8.76±1.52[d]
CfLE	313.81±12.96[c]	251.36±1.83[a]	17.96±0.03[f]	23.13±1.34[e]	4.26±0.24[b]
LaLE	152.41±14.20[e]	112.35±0.50[c]	24.44±0.30[g]	17.58±1.04[d]	2.30±0.12[a]
PoLE	216.74±15.33[d]	117.30±0.68[b]	16.19±0.06[e]	11.44±2.61[c]	3.69±0.55[b]
SgLE	303.70±19.18[c]	37.24±0.86[d]	8.85±0.09[d]	3.54±0.21[a]	7.74±0.66[cd]
StLE	438.73±14.06[a]	39.80±1.18[d]	5.54±0.13[bc]	6.50±0.29[b]	8.09±0.44[d]
CA			3.73±0.07[a]	7.24±0.14[b]	11.98±1.52[e]
Trolox			6.66±0.02[c]	2.33±0.17[a]	6.65±2.91[c]
AA			4.49±0.13[ab]	NP	14.81±1.46[f]

Data represent mean±SD, n = 6. Numbers with different letters within the same column are significantly different ($P < 0.05$) analyzed by ANOVA and Duncan's multiple range test

CA, catechin; AA, ascorbic acid; LPO, lipid peroxidation; NP, not performed

Total phenolic content (TPC) and total flavonoid content (TFC) of the extracts are present in Table 2. TPC ranged from 152.41±14.20 µg GAE/mg in LaLE to 438.73±14.06 µg GAE/mg in StLE. TFC ranged from 15.47±4.11 µg CAE/mg in BaLE to 251.36±1.83 µg CAE/mg in CfLE. It was noticed that the CfLE contained high amounts of both TPC and TFC. BaLE, SgLE and StLE had considerably high TPC but low TFC.

BaLE possessed high TPC, saponins andtannins, but low essential oils (Table 1) and TFC, which were similar to the study of Narayan *et al.* [25]. CfLE contained high TPC, TFC and essential oils, low tannins andno terpenoids and saponins. The flavonoids in CfLE were reported as chlorogenic, dicaffeoylquinic and ferulic acids [26].

The aromatic plants, LaLE and PoLE, possessed moderate TPC and TFC but high essential oils. The flavonoids in *L. aromatica* were reported as nevadensin, nevadensin-7-o-β glycopyranoside, gardenin B and other flavones [27]. The major essential oils in *L. aromatica* were limonene, *trans*-isolimonene andα-humulene [28]. *P. odoratum* was reported to contain volatile compounds of (Z)-3-hexenal,

(Z)-3-hexenol, decanal, undecanal anddodecanal [29], which contributed to its aromatic flavor. The flavonoids of rutin, quercetin, catechin, isorhamnetin and kaempferal in PoLE were also reported [14].

StLE contained mainly high amount of essential oils, saponins andtannins, which was in agreement with other reports [10, 30, 31]. The phenolic compounds in StLE were identified as caffeic acid, syringic acid, coumaric acid, ellagic acid andgallic acid and terpenes [32].

However, there was no information available on secondary metabolites of SgLE. Hence, this study is the first report on terpenoids, tannins, essential oils and saponins found in *S. gratum*.

Antioxidant Activities of the Extracts: The antioxidative properties of the extracts evaluated by DPPH radical scavenging, microsome lipid peroxidation inhibition andFRAP assays are summarized in Table 2. According to the antioxidant activity, all extracts could obviously be divided into 2 groups. Group I with high antioxidant activity was BaLE, SgLE and StLE andgroup II with low antioxidant activity was CfLE, LaLE and PoLE. The radical scavenging activity of group I expressed as EC_{50} values was 6.67±0.10, 8.85±0.09 and5.54±0.13 µg/ml, respectively. They were similar to the standard compounds, catechin, trolox and ascorbic acid. The radical scavenging activity of group II was low with much higher EC_{50} values of 17.96±0.03, 24.44±0.30 and 16.19±0.06 µg/ml, respectively ($P < 0.05$).

The inhibition capacity of ferric-induced microsomal lipid peroxidation of extract group I was high with EC_{50} of 3.92±0.01, 3.54±0.21 and 6.50±0.29 µg/ml, respectively. These were similar to those of catechin and trolox. The inhibition capacity of the extract group II was less, with EC_{50} values of 23.13±1.34, 17.58±1.04 and 11.44±2.61 µg/ml, respectively ($P < 0.05$).

Ferric reducing antioxidant power (FRAP) indicates an electron donating of the extract. The FRAP of the extract group I was 8.76±1.52, 7.74±0.66 and 8.09±0.44 µmol Fe^{2+}/mg extract, respectively. These were similar to that of trolox and about 0.65 - 0.73 fold of catechin ($P < 0.05$). The FRAP of the extract group II was 4.26±0.24, 2.30±0.12 and 3.69±0.55 µmol Fe^{2+}/mg extract, respectively andsignificantly lower than those of group I and the standard compounds ($P < 0.05$).

The antioxidant properties of phytochemicals are very complex and vary due to the test systems. The extract group I, BaLE, SgLE and StLE, could act as the potent electron transfers (ET) evaluated by DPPH free radical scavenging and FRAP assays [33]. In addition, the *in vitro* inhibition of ferric-induced microsome lipid peroxidation indicated that these extracts also acted as the hydrogen atom transfer (HAT) in quenching peroxyl radicals. However, it was obviously seen that the extracts those possessed similar amount of phytochemical contents expressed similar antioxidant capacities. The high amount of tannin phenolics in BaLE, SgLE andStLE could importantly contribute to the high potency of antioxidant activity. There was a report that polyphenolic tannins from plants were more potent DPPH free radical scavenging than flavonoids [34], which supported the correlation between phytochemical constituents and antioxidant property of the extract group I. On the contrary, the extract group II, CfLE, LaLE andPoLE, containing very high flavonoids were less potent antioxidants.

Relationships Between Phytochemicals and Antioxidant Activities: The TPC, TFC and the antioxidant activities of the 6-vegetable leaf extracts were graphically correlated (Figure 1). The high TPC was well correlated with the EC_{50} of DPPH scavenging activity ($R = -0.856$) but the low TFC was poorly correlated with this antioxidant activity ($R = 0.673$) (Figure 1 A and B). In contrast, the TPC showed low correlations with the EC_{50} of lipid peroxidation inhibition ($R = -0.469$) and the TFC showed high correlation (Figure 1 C and D). The extracts containing polyphenol antioxidant could be more complex in microsomal lipid peroxidation system than in DPPH free radical scavenging according to the configuration of lipids in the microsomal membrane [21]. In addition, FRAP values were significantly correlated to the amount of TPC ($R = 0.855$), but were poorly correlated to TFC ($R = -0.678$) (Figure 1 E and F). This study was in agreement with other reports [35, 36, 37]. Therefore, it could conclude that the polyphenolic components in these vegetable leaf extracts were the main potent antioxidants in single electron transfer (ET) in DPPH free radical scavenging and FRAP, while small amount of flavonoid components contributed to hydrogen atom transfer (HAT) in *in vitro* microsome lipid peroxidation. The potent antioxidant property of these vegetable leaf extracts is beneficial for good health in consumption and value added to them. They can be also used in stabilizing or increasing the shelf life of lipid containing food products and in replacing the synthetic antioxidants, BHA (3-*tert*-butyl-4-hydroxyanisole) and BHT (butylated hydroxyl toluene) in food industry [38].

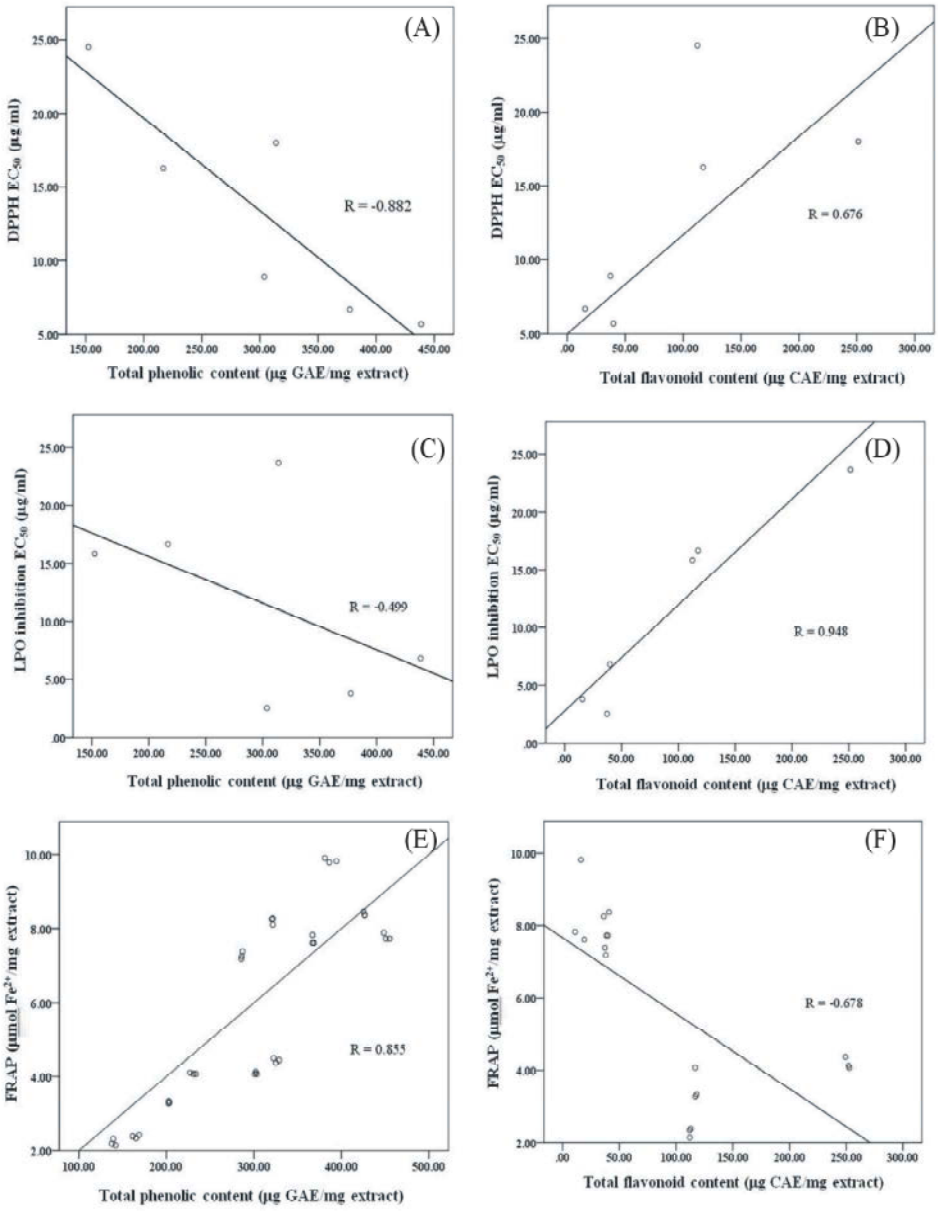

Fig. 1: Relationships between phytochemical contents and antioxidant activity (EC_{50}) of the extracts. TPC and DPPH, (A); TFC and DPPH, (B); TPC and lipid peroxidation (LPO) inhibition, (C); TFC and LPO inhibition, (D); TPC and FRAP (E); TFC and FRAP (F).

Cytotoxicity of the Extracts: The cytotoxic effect of the 6-vegetable leaf extracts on normal and cancer cells was evaluated by cell proliferation MTT assay. BaLE and StLE highly inhibited the proliferation of Jurkat cells at 48 h with IC_{50} values of 66.90 and 75.36 µg/ml, respectively (Table 3). SgLE and AA highly inhibited the proliferation of MCF7 cells with IC_{50} values of 66.71 and 78.60 µg/ml, respectively. CfLE expressed cytotoxicity only to MCF7 cells with IC_{50} of 176.50 µg/ml. LaLE, PoLE and SgLE were moderately reduced proliferation of Jurkat cells with IC_{50} 128.60, 146.80 and 141.30 µg/ml, respectively. It was reported that prolong incubation of PoLE and LaLE up to 72 h could induce highly potent cytotoxcity to MCF7 cells [14]. It is noticed that most extracts and all standard controls were low cytotoxic to HepG2 cells. Only BaLE inhibited the proliferation of n-lymphocytes and HepG2 cells with moderate IC_{50} values of 158.80 and 112.70 µg/ml, respectively.

Table 3: Cytotoxic effects of leaf extracts of *Barringtonia acutangula* (BaLE), *Cratoxylum formosum* (CfLE), *Limnophila aromatica* (LaLE), *Polygonum odoratum* (PoLE), *Syzygium gratum* (SgLE) and*Schinus terebinthifolius* (StLE) on n-lymphocytes and3-cancer cell lines, Jurkat, MCF7 and HepG2.

		Cell lines, IC_{50} (µg/ml)		
Extract	n-lymphocyte	Jurkat	MCF7	HepG2
BaLE	158.80 (150.70 - 166.70)	66.90 (61.90 - 72.30)	124.80 (113.0 - 137.80)	112.70 (103.00 - 123.30)
CfLE	358.90 (336.50 - 386.40)	> 400	176.50 (154.20 - 202.00)	> 400
LaLE	> 400	128.60 (109.30 - 151.30)	291.70 (270.70 - 315.70)	349.90 (316.20 - 393.60)
PoLE	332.90 (318.00 - 348.80)	146.80 (103.40 - 208.40)	205.20 (163.70 - 257.10)	> 400
SgLE	202.50 (175.10 - 234.10)	141.30 (117.50 - 170.00)	66.71 (47.37 - 93.93)	> 400
StLE	260.60 (252.00 - 269.30)	75.36 (40.95 - 104.30)	238.20 (223.10 - 254.60)	231.20 (146.30 - 365.50)
CA	>400	> 400	> 400	> 400
Trolox	>400	> 400	> 400	> 400
AA	> 400	> 400	78.60 (74.90 - 82.60)	> 400

Data represent as mean±SD, n = 6. IC_{50}, mean inhibition concentration at 48 h obtained by non-linear regression. CA, catechin; AA, ascorbic acid;

Table 4: Hemolytic activity of *Barringtonia acutangula* (BaLE), *Cratoxylum formosum* (CfLE), *Limnophila aromatica* (LaLE), *Polygonum odoratum* (PoLE), *Syzygium gratum* (SgLE) and*Schinus terebinthifolius* (StLE) leaf extracts on human erythrocytes.

	Percentage of hemolysis (%)				
Sample	200 µg/ml	400 µg/ml	600 µg/ml	1200 µg/ml	EC_{50} (µg/ml)
BaLE	100	NP	NP	NP	6.69 (6.66-6.72)
CfLE	0.23±0.15	2.34±0.17	6.63±0.78	33.28±2.26	> 1200
LaLE	0.90±0.11	1.50±0.41	6.08±0.99	22.78±5.93	> 1200
PoLE	0.11±0.04	0.17±0.06	3.06±0.70	9.64±0.50	> 1200
SgLE	3.20±0.85	5.20±1.21	7.38±1.08	15.64±4.62	> 1200
StLE	70.72±2.76	79.50±0.78	100	NP	134.60 (125.30-144.60)
CA	0.17±0.15	0.13±0.06	0.35±0.15	0.63±0.46	> 1200
Trolox	0.04±0.01	0.08±0.01	0.63±0.47	4.22±3.79	> 1200
AA	0.09±0.01	0.23±0.10	0.58±0.32	63.61±1.45	960.70 (951.50-970.10)

Data represent as mean±SD, n = 6. EC_{50}, mean effective concentration obtained by non-linear regression. CA, catechin; AA, ascorbic acid; NP, not performed

The phytochemical composition of plants might influence their cytotoxic properties. Tannins and saponins in *B. acutangula* and *S. terebinthifolius* could be the major components that inhibited the growth of Jurkat cells. Terpenoids, saponins and tannins in *S. gratum* could inhibit the growth of MCF7 cells. There were reports that stigmasterol, β-sitosterol [39] and gallic acid [40] in *B. acutangula* inhibited cancer cell proliferation and induce apoptosis; and barringtonic acid and acutangulic acid produced inflammatory effect [41, 42]. *C. formosum* extract selectively inhibited the MCF7 cell proliferation, but not other cell types. This plant extract was also found to selectively and moderately toxic to HepG2 cells [43]. LaLE and PoLE were potent against the proliferation of Jurkat and MCF7 cell lines, which were in agreement with the *in vitro* antitumor promotion [13, 14]. Their high flavonoids content was demonstrated strong cancer inhibitory effects [44]. SgLE possessed high antioxidant capacity and inhibitory effect on MCF7 and Jurkat cells, but less toxic to n-lymphocytes and erythrocytes. Tannins and terpenoids, the main compound in SgLE, could be responsible for this cytotoxicity. Tannin and terpenoids were chemopreventive agents against breast carcinogenesis [45]. However, there is no evidence reported on any compounds isolated from this plant. Nevertheless, some plants in the genus Syzygium compose of phenolic compound such as ferulic acid and catechin which were responsible for their antioxidant activity [46]. More studies of SgLE on its phytochemical compounds and effects on cancerous cells are needed. In our work, StLE exhibited strong antioxidant activity and cytotoxicity to Jurkat cells and moderate effects on n-lymphocytes, MCF7 and HepG2 cells. There was also a report that the ethyl acetate fraction of aqueous extract of *S. terebinthifolius,* rich in polyphenol, strongly inhibited the proliferation of DU145 human prostatic carcinoma, oestrogenosensitive breast cancer MDA-MB435 and androgen-sensitive LNCaP prostate cells [15].

Hemolytic Effect on Red Blood Cells: The hemolytic activity indicates the cytotoxicity of extracts on plasma membrane. There were two extracts caused hemolytic effect on human RBCs. BaLE was highly toxic with EC_{50} of 6.69 µg/mL and StLE was poor toxic with EC_{50} of 134.60 µg/ml (Table 4). Complete RBC lysis induced by 200 ìg/ml of BaLE and by 600 ìg/ml of StLE. The strong lytic effect of BaLE and StLE could be the major contribution in cytotoxicity to Jurkat leukemia cells (Table 3). At highest concentration tested, 1200 ìg/ml, PoLE produced least lysis of 9.64±0.50% followed by SgLE lysis of 15.64±4.62%. Similarly, catechin and trolox were also low lytic effects. The other extracts expressed low lytic effects with EC_{50} over 1200 µg/ml.

In conclusion, this study provides the information of the leaf ethanolic extracts of *Barringtonia acutangula*, *Cratoxylum formosum*, *Limnophila aromatica*, *Polygonum odoratum*, *Syzygium gratum* and*Schinus terebinthifolius*, commonly grown in the Northeast of Thailand. It is demonstrated that the less flavonoids containing extracts, BaLE, SgLE and StLE, the more potent antioxidants. CfLE, LaLE andPoLE, contained high flavonoids, but less potent antioxidants. All extracts were not toxic to normal human red blood cells. BaLE and StLE were most likely to be cytotoxic to Jurkat cells, while SgLE prominently inhibit the proliferation of MCF7 cells. The others were moderately cytotoxic to Jurkat, MCF7 and HepG2 cells. This information will be useful for further studies of these vegetables for various applications, such as for cancer prevention and food preservation.

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