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Thai Indigenous Plants: Focusing on Total Phenolic Content, Antioxidant Activity and Their Correlation on Medicinal Effects

Varipat Areekul[1] and Naraporn Phomkaivon[2*]

[1]Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, 10520 and [2]Department of Food Chemistry and Physics, Institute of Food Research and Product Development, Kasetsart University, 10900

Abstract

Total phenolic content and antioxidant activity have involved in medicinal effects of plants. The 25 species of Thai indigenous plants were classified into three groups depending on their medicinal effects; (1) anti-inflammatory (2) analgesic and (3) diuretic groups. Both of fresh and dried plant extracts were determined total phenolic content (TPC) and antioxidant activity by four different methods; DPPH-free radical scavenging activity (DPPH), Trolox equivalent antioxidant capacity (TEAC), Ferric-reducing antioxidant power (FRAP) and thiobarbituric acid reactive substances (TBARS) assays. Then, the correlation between each parameter was examined. The results showed that TPC and antioxidant activities of plant extracts were highly variable among different plant species and plant parts. The analgesic plants showed the highest change of TPC and antioxidant activities after drying. A positive linear correlation was found between TPC, DPPH, TEAC and FRAP but close relationship of these parameters with medicinal effects was occurred in the diuretic plants group (r =0.808-0.970). From the results, five plants, *Saraca indica* L., *Litsea glutinosa* (Lour.) C.R. Rob, *Glochidion sphaerogymum* (Müll. Arg.) Kurz, *Schima wallichii* (D.C.) Korth. and *Camellia sinensis* (L.) Kuntze var. *assamica* (J. Masters) Kitam. could be potential rich sources of phenolic compound, antioxidant and new pharmaceutical from indigenous plants.

Keywords: Thai indigenous plants, total phenolic content, antioxidant activity, medicinal effects

1. Introduction

Thai indigenous plants are used widely throughout several regions of Thailand, especially in the north. The indigenous plants are common food plants and also traditional medicine of Thai people for long time [1]. Thus, the medicinal effect of these plants is well known while theirscientific information likea chemical composition and/oran active constituent isvery limited. The various pharmacological effects are directly linked to the different types of phytochemicals. A large number of the secondary metabolites in plants have been studied but the phenolic compounds show a strong ability to decrease the risk of various disease and health protection [2]. In addition, the antioxidant activityof phenolic compound is related toreduce or inhibit oxidative stress in biological substancesand also shows high potential of anti-inflammatory, anti-mutagenicity and anti-carcinogen, etc. [3-4]. Increased interest in the disease prevention, many plants are a new source of natural pharmaceuticals and functional products. Similarly, the total phenolic content

*Corresponding author: E-mail: ifrnpph@ku.ac.th

and antioxidant activityassays havebeen developed and become the primary indexto screening the ability of fruits, vegetables, food plants and herbs [5]. Recently, the total phenolic content by Folin-Ciocalteau assays and the antioxidant activity by DPPH-free radical scavenging activity (DPPH), Trolox equivalent antioxidant capacity (TEAC), Ferric-reducing antioxidant power (FRAP) and thiobarbituric acid reactive substances (TBARS) assays are common methods and very popular in plant antioxidant studies. These methods are simple, highly sensitive, good reproductively and using simple equipment [5]. They show the high correlation between total phenolic content and antioxidant activity [6]. However, the total phenolic content and antioxidant activity depending on species, part of plant, sample preparation, drying methods, solvent and extraction [7].The change of phenolic content affect the antioxidant activity and also the pharmacology.

This study was focusing on the total phenolic content and antioxidant activities of 25 species of Thai indigenous plants thatwere classified into three groups depending on medicinal effects; (1) anti-inflammatory (2) analgesic and (3) diuretic groups. In addition, the correlation between medicinal effects and their activity were examined.

2. Materials and Methods

2.1 Plant materials

All of selected Thai indigenous plants were listed in Table 1. Each plant was collected the edible part, cleaned, air-dried and separated in two parts for fresh and dried plant extraction.

Table 1 Scientific Names, Plant Parts, Method of Food Preparation and Active Constituents of 25 Selected Thai Indigenous Plants

Scientific name	Part used	Method of food preparation	Location*	Active constituents [a]
(1) anti-inflammatory				
Azadirachta indica A. Juss. var.*siamensis* Valeton	Flower	Fresh, boiling	Sa	Coumarins, Flavonoids, Steroids,
Buddleja asiatica Lour	Leaves	Fresh	A	Essential oils
Commelina diffusa Burm.f.	Stem,leaves	Fresh, boiling	A	-
Emilia sonchifolia (L.) DC	Stem,leaves	Fresh, boiling	A	Alkaloids, Flavonoids, Steroids
Justicia adhatoda L.	Leaves	Fresh, boiling	A	Alkaloids, Phenolic acid
Limnophila aromatic Merr.	Stem,leaves	Fresh	Sa	Phenolic acid, Terpenes
Litsea glutinosa (Lour.) C.B. Rob.	Leaves	Traditional medicine	A	Alkaloids, Saponin, Terpenes
Neptunia oleracea Lour.	Stem,leaves	Boiling	Su	Polyphenols, Vitamin C, Vitamin E
Momordica charantia L.	Stem,leaves	Fresh	Sa	Flavonoids, Steroids
Saraca indica L.	Leaves	Fresh	A	-
(2) analgesic				
Acmella oleraceae (L.) R.K. Jansen	Leaves	Boiling	A	-
Bidens bipinnata L.	Stem,leaves	Fresh, boiling	A	-
Bidens pilosa Lour	Stem,leaves	Boiling	A	Flavonoids, Phenolic acid, Steroids

Table 1 Scientific Names, Plant Parts, Method of Food Preparation and Active Constituents of 25 Selected Thai Indigenous Plants (Con.)

Scientific name	Part used	Method of food preparation	Location*	Active constituents [a]
Conyza sumatrensis (Retz.) Walker	Stem,leaves	Fresh, boiling	A	Essential oils, Flavonoids
Glochidion sphaerogymum (Müll. Arg.) Kurz	Leaves	Fresh, boiling	A	-
Mosia dianthera (Buch-Ham. Ex Roxb.) Maxim.	Stem,leaves	Boiling	A	-
Schima wallichii (D.C.) Korth.	Leaves	Boiling	A	Saponin, Tanin
Tiliacora triandra (Colebr.) Diels	Leaves	Boiling	Sa	Alkaloids, Polyphenols
(3) diuretic				
Basella alba L.	Leaves	Fresh, boiling	Sa	Flavonoids, Vitamin C, Vitamin E
Basella rubra L.	Leaves	Fresh, boiling	Sa	Flavonoids, Vitamin C, Vitamin E
Camellia sinensis (L.) Kuntze var. *assamica* (J. Masters) Kitam.	Leaves	Beverage	A	Polyphenols
Limnophilia geoffrayi Bonati	Leaves	Fresh, boiling	Sa	Essential oils
Persicaria odorata (Lour) Soja'k	Stem,leaves	Fresh, boiling	Sa	Essential oils, Polyphenols, Vitamin C, Vitamin E
Senna siamea(Lam.) Irwin & Barneby	Flower	Fresh, boiling	A	Polyphenols, Vitamin C, Vitamin E
Smilax macrophylla Roxb.	Leaves	Fresh, boiling	A	-

* = the locations of plants collected consist of A= the Royal Angkhang Agricultural Station Project of the Royal Project Foundation, Chiang Mai, Sa= the local markets at Sakon Nakhon, Su= the local markets at Surat Thani

[a] = the actives constituents of selected plants were listed following Areekul [1].

2.2 Extraction

For the fresh plant extraction, the plant was cut into small pieces and chopped with food processer (Moulinex, France). The extraction was done according to the method of Phomkaivon and Areekul [8]. Briefly, 10g of sample was blended with 80% ethanol 100 ml (0.1%,w/v) for 1 min after that the extract was placed in water bath at 70°C for 15 min. The extract was cooled at room temperature for 15 min and filtered through Whatman No. 4 filter paper.

In the case of dried plant extraction, the plants were dried in a hot air oven at 50°C for 18 hours and grounded to powder approximately 60 mesh. The dried powdered was extracted following the method as described previously.

Both of fresh and dried plant extracts were stored at -20°C until analysis.

2.3 The total phenolic content (TPC)

Total phenolic content was determined using Folin-Ciocalteau method [9]. A 0.25 mlof the plant extract and 4.75 ml of distilled water were mixed. Then 0.25 ml of Folin-Ciocalteau reagent was added, vortexed and placed for 5 min. After 5 min, 1 ml of 10% sodium carbonate solution was added to stop reaction. The mixtures were incubated in the dark for 10 min. The 760 nm absorbance was measured using gallic acid as standard. The results were expressed as milligrams of gallic acid equivalence (GAE)/g dry basis.

2.4 DPPH-free radical scavenging activity (DPPH) assay

The DPPH assay was measured with a slightly modification from Brand-Williams *et al.* [10]. Briefly, 1 ml of a series of extract concentrations was mixed with 3 ml of 0.2 mM DPPH. After 30 min of incubation in the dark, the absorbance of mixtures were measured at 517 nm. The % inhibition was calculated according to this equation:

$$\%\text{inhibition} = (1\text{-}A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the assay without sample and A_{sample} is the absorbance in the presence of the plant extracts.

The results were expressed as EC_{50} (the concentration of test compounds demonstrating 50% radical scavenging activity).

2.5 Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was evaluated based on the method described by Zhou and Yu [11]. The stock solution of ABTS radical cation ($ABTS^{\cdot+}$) was prepared by reacting 5 mM ABTS solution with excessed Manganese dioxide (MnO_2). The mixture was stirred with magnetic stirrer for 30 min in the dark. Then, the solution was filtered through Whatman No. 4 filter paperand passed again through syringe filter (0.2 micron) before analysis.

For this study, the $ABTS^{\cdot+}$ solution was diluted with distilled water to obtain an absorbance of 0.700 ± 0.002 at 734 nm. The plant extract (0.3 ml) was mixed with 3 ml of diluted $ABTS^{\cdot+}$ solution and allowed to react in the dark for 6 min. The absorbance was recorded at 734 nm and the antioxidant activity was calculated as Trolox equivalence against the Trolox standard curve and expressed as milligrams of Trolox equivalence (TE)/g dry basis.

2.6 Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was tested by the method of Benzie and Strain [12]. Briefly, the FRAP reagent was freshly prepared by mixing sodium acetate buffer (300 mM at pH 3.6), TPTZ solution (10 mM in 40 mM HCl) and Ferric chloride (20 mM in distilled water) at a ratio of 10:1:1 (v/v/v). A 0.1 ml of the plant extract and 1 ml of the FRAP reagent were mixed and incubated in the dark for 8 min. The absorbance at 593 nm was measured using FRAP reagent as a blank. The FRAP values expressed as milligrams of Trolox equivalence (TE)/g dry basis.

2.7 Thiobarbituric acid reactive substances (TBARS) assays

The TBARS assays was obtained following McDonald and Hutin [13] with slight modifications. Firstly, the linoleic acid emulsion 1% with Tween 40% was prepared. The mixture of plant extract (0.2 ml) and linoleic acid emulsion 1% (0.8 ml) was incubated in water bath at 50°C for 18 h in the dark. This reacted solution was mixed with 2 ml of TCA-TBA-HCl solution, boiled immediately for 15 min and cooled in ice for 5 min. The absorbance was measured at 532 nm and antioxidant activity (AOA) was calculated by equation:

$$\%\text{AOA} = (1\text{-}A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the assay without sample and A_{sample} is the absorbance in the presence of the plant extracts.

Then, the TBARS values were expressed as milligrams of Trolox equivalence (TE)/g dry basis.

2.8 Statistical analysis

All results were obtained in triplicate and data were expressed as mean±standard deviation. Analysis of variance was performed using ANOVA procedures. In addition, correlations among the methods were established by regression analysis.

3. Results and Discussion

3.1 Total phenolic content and antioxidant activities

The results of total phenolic content and antioxidant activities of 25 selected Thai indigenous plants in both fresh and dried extracts were shown separately following by the different medicinal effects in threegroups that are anti-inflammatory (n=10), analgesic (n=8) and diuretic (n=7).

3.1.1 Anti-inflammatory

For the anti-inflammatory plants (10 species), TPC, DPPH, TEAC, FRAP and TBARS values are presented in Table 2. TPC of fresh plant extracts varied between 7.02 and 117.07 mg GAE/g db, while *S. indica* and *L. glutinosa* had the highest TPC (>100 mg GAE/g db). It was observed that the range of TPC values of dried plant extracts was lower than the fresh ones (from 2.08 to 41.73 mg GAE/g db).

The determination of antioxidant activity has various assays based on different mechanismsof reactionssuch as electron transfer, hydrogen atom transfer or both. The DPPH free radical scavengingis the most popularassay for natural antioxidants. In this study, the EC_{50} values, the number of plants (mg db) to decrease 50% of initial DPPH concentration, represented the value of DPPH assay. It was cleared that the high antioxidant activity of plants showed the low EC_{50}, whereas the other assays showed the high antioxidant activity with the high values.

The different species of the anti-inflammatory plants had an important role on the variation of EC_{50}. The fresh plant extracts showed the high antioxidant activity with low EC_{50} values (0.13-12.81 mg db) but the low antioxidant activity was found in the dried (EC_{50} = 0.42-17.62 mg db). Both of fresh and dried*S. Indica* appeared to have the highest antioxidant activity measuring by this assays. While the results of TEAC and FRAP assays showed similar trend to the DPPH assays and the fresh plant extracts had the higher values than dried. Moreover, the highest TPC plants showed the greatest antioxidantactivities. *S. indica* had the highest reducing power both fresh and dried plants (147.88 and 56.21 mg TE/g db, respectively) and *L. glutinosa* exhibited the highest TEAC values as 249.65 and 80.91 mg TE/g db in fresh and dried plants, respectively.

In contrast, TBARS values were extremely variable comparing with other three assays. The mechanism of TBARS assays was based on hydrogen atom transfer for inhibiting chain reaction while the donation of electron to free radical or metal ion was the main mechanism of DPPH, TEAC and FRAP assays. Thus, the fresh leaves of *B. asiatica* had the highest TBARS values (40.49 mg TE/g db) followed by *L. aromatic*and *A. indica* (10.97 and 10.54 mg TE/g db), respectively. The TBARS values of dried plants extracts were very low ranging between 1.61 to 8.13 mg TE/g db and these results indicated the reduction of antioxidant activities causing by drying process.

However, the leaves of *L. glutinosa*had a great TPC and antioxidant activity like the bark parts [14]. The *M. charantia* extracts had TPC and EC_{50} lower than the previous reports that could depend on the different parts used and extraction methods [15-16]. While the *A. indica* showed the fair TPC and antioxidants comparing with Maisuthisakul *et al.* [17].

Table 2 Total Phenolic Content and Antioxidant Activities of Anti-Inflammatory Plants

Scientific name	Part used		TPC	DPPH	ABTS	FRAP	TBARS
A. indica A. Juss.var. *siamensis* Valeton	Flower	F	69.33±1.72	1.71±0.08	37.45±1.76	27.20±0.37	10.54±1.09
		D	5.24±0.40	15.67±1.04	3.50±0.09	2.64±0.09	1.61±0.14
B. asiatica Lour	Leaves	F	50.74±3.22	1.03±0.07	71.00±2.45	60.42±5.84	40.49±3.46
		D	15.81±0.71	1.46±0.05	26.77±0.69	32.05±4.39	8.13±0.15
C. diffusa Burm.f.	Stem, leaves	F	19.46±0.67	3.03±0.12	27.08±1.06	14.94±0.54	5.90±0.20
		D	2.08±0.04	2.17±0.00	36.35±2.42	18.45±1.74	4.12±0.09
E. sonchifolia (L.) DC	Stem, leaves	F	7.02±0.56	8.34±0.16	2.45±0.39	8.96±0.38	7.48±0.19
		D	26.56±2.04	4.44±0.01	11.58±0.66	37.04±2.12	7.29±0.39
J. adhatoda L.	Leaves	F	46.05±3.29	1.38±0.01	28.71±0.70	21.32±1.02	7.52±0.48
		D	9.90±0.31	13.3±1.2	6.15±0.30	5.34±0.43	3.09±0.38
L. aromatic Merr.	Stem, leaves	F	48.66±1.0	0.62±0.02	22.49±0.56	54.39±2.70	10.97±0.70
		D	41.73±1.3	0.68±0.00	36.22±4.35	55.24±2.18	4.44±0.42
L. glutinosa Lour.) C.B. Rob.	Leaves	F	106.30±8.35	0.31±0.01	249.65±17.62	104.49±5.82	6.14±0.32
		D	29.96±2.53	0.73±0.00	80.91±4.99	31.83±5.62	3.63±0.49
N. oleracea Lour.	Stem, leaves	F	45.91±1.90	0.77±0.08	89.19±3.98	42.87±1.48	7.58±0.44
		D	22.38±2.13	0.72±0.05	21.70±3.49	28.81±3.60	5.45±0.22
M. charantia L.	Stem, leaves	F	6.88±0.22	12.81±0.82	5.09±0.45	4.57±0.26	2.41±0.24
		D	2.94±0.23	17.62±0.03	1.67±0.06	3.38±0.53	3.60±0.10
S. indica L.	Leaves	F	117.07±6.93	0.13±0.00	78.63±4.13	147.88±14.52	9.46±0.49
		D	19.9.0±0.48	0.42±0.02	30.51±2.45	56.21±12.10	1.78±0.19
range		F	7.02-117.07	0.13-12.81	2.45-249.65	4.57-147.88	2.41-40.49
		D	2.08-41.73	0.42-17.62	1.67-80.91	2.64-56.2	1.61-8.13

F=fresh plant extract, D= dried plant extract

TPC = total phenolic content (mg GAE/ g db), DPPH=DPPH free radical scavenging activity (EC_{50}, mg db),

TEAC = Trolox equivalent antioxidant capacity (mg TE/g db), FRAP= Ferric-reducing antioxidant power (mg TE/g db), TBARS = Thiobarbituric acid reactive substances (mg TE/g db)

3.1.2 Analgesic

The selected analgesic plants 8 species were determined TPC and antioxidant activities (Table 3). In this study, the analgesic group had the highest TPC and antioxidant activities comparing with the anti-inflammatory and diuretic groups. As it can be seen from the table, all tested parameters were investigated the high values in fresh plant extracts while for dried plants, the low values were found. From the results, *S. wallichii* and *G. sphaerogymum* presented the high values of TPC, DPPH, TEAC and FRAP. In addition, their fresh and dried extracts also showed the greatest values in these assays. The high antioxidant activity was obviously detected in the DPPH assays, these fresh plants extracts showed the EC_{50} values lower than 0.1 mg db while the dried ones showed a good DPPH radical scavenging too. The high amount phenolic compounds in these plants might contribute to the high antioxidant activities in this experiment. By the way, the lowest values of four assays mainly found in *T. triandra* followed by *C. sumatrensis* and *B. pilosa*, respectively.

Based on the TBARS assays, it had different trend from the other assays like found in the anti-inflammatory plants. From Table 3., *B. bipinnata* and *M.dianthera* showed the highest antioxidant activities in fresh and dried plant extracts respectively. According to the results,focusing on two of high antioxidants activities plants,*S. wallichii* exhibited the highTBARS values but *G. sphaerogymum* did not show high antioxidant activity. It indicated that the antioxidant mechanisms might be differed on types of phenolic compounds in plants. The high TPC and antioxidant activities of *S. wallichii* were supported with the high level of TPC, EC_{50}, TACE and FRAP values that reported by Phomkaivon and Areekul [8] and Das *et al*. [18] reported that the ethyl acetate extracts had the ability to scavenge reactive nitrogen species. Moreover, *B. pilosa* had the moderate effect on DPPH free radical scavenging and lipid oxidation [19-20].

Generally, the drying process and the variation of plants species affect directly to decrease in TPC and antioxidant activities. Where as many analgesic plants including *A. oleraceae*, *B. bipinnata*, *B. pilosa*, *C. sumatrensis* and *M. dianthera*increased their tested values. Increasing of tested values might connect to a heat stability of antioxidant compounds founded in each plant species.

Table 3 Total Phenolic Content and Antioxidant Activities of Analgesic Plants

Scientific name	Part used		TPC	DPPH	ABTS	FRAP	TBARS
A. oleraceae (L.) R.K. Jansen	Leaves	F	9.24±0.51	6.26±0.30	2.84±0.18	8.92±0.28	6.41±0.38
		D	17.97±1.05	8.24±0.30	7.66±0.26	21.59±1.80	3.51±0.20
B. bipinnata L.	Stem, leaves	F	27.21±1.0	0.75±0.06	22.60±1.18	26.11±3.07	40.25±2.51
		D	48.38±2.01	1.07±0.10	39.01±3.08	34.63±1.50	16.33±1.27
B. pilosa Lour	Stem, leaves	F	9.82±0.61	4.56±0.08	14.77±0.81	13.22±0.68	11.37±0.39
		D	11.39±0.35	3.34±0.11	16.29±0.73	15.19±0.97	4.20±0.18
C. sumatrensis (Retz.) Walker	Stem, leaves	F	15.11±0.36	2.17±0.18	17.47±0.41	16.38±1.47	3.07±0.09
		D	8.36±0.56	3.99±0.27	11.0/±0.78	10.60±0.45	4.38±0.20
G. sphaerogymum (Müll. Arg.) Kurz	Leaves	F	59.36±3.94	0.06±0.00	742.36±94.27	351.97±18.39	4.21±0.08
		D	153.15±7.79	0.79±0.02	499.37±65.80	253.93±18.40	4.25±1.07
M. dianthera (Buch-Ham. Ex Roxb.) Maxim.	Stem, leaves	F	12.38±0.84	3.43±0.16	18.41±0.93	13.53±0.85	12.01±0.12
		D	86.50±7.49	0.74±0.02	44.97±3.91	46.05±2.50	33.19±2.06
S. wallichii (D.C.) Korth.	Leaves	F	213.84±10.52	0.08±0.00	525.61±48.48	275.47±13.60	18.81±1.13
		D	105.42±7.96	0.13±0.00	306.34±27.77	222.23±18.85	17.69±0.67
T.triandra (Colebr.) Diels	Leaves	F	8.48±0.49	8.20±0.64	2.39±0.09	6.96±0.11	3.25±0.32
		D	10.13±1.07	4.43±0.18	1.22±0.07	2.98±0.14	9.54±0.59
range		F	8.48-213.84	0.06-8.20	2.39-742.36	6.96-351.97	3.07-40.25
		D	8.36-153.15	0.74-8.24	1.22-499.37	2.98-253.93	3.51-33.19

F=fresh plant extract, D= driedplant extract

TPC = total phenolic content (mg GAE/ g db), DPPH=DPPH free radical scavenging activity (EC_{50}, mg db),

TEAC = Trolox equivalent antioxidant capacity (mg TE/g db), FRAP= Ferric-reducing antioxidant power (mg TE/g db), TBARS = Thiobarbituric acid reactive substances(mg TE/g db)

3.1.3 Diuretic

Among all the diuretic plants species, the results of TPC and four antioxidant activities show in Table 4. It is important to note in this study, the diuretic plants showed the lowest range of values in all parameters. Moreover, high TPC, DPPH and FRAP values were detected in the fresh and dried leaves of *C. sinensis*. For other plants in this group, *S. macrophylla* had the highest TEAC values in fresh extract andshowed the highest DPPH values on dried one. While the two species of *Basella* showed the highest antioxidant activitieson TBARS assays only. The stem and leaves of *P. odorata* also showed the high TPC and antioxidant activity measuring by different mechanisms in both fresh and dried extracts.

In addition, *C. sinensis*had the highest TPC and antioxidant activities.Similar to other plants in genus *Camellia,* a source of numerous catechins cause the strong antioxidant and pharmacological activity [21-23]. The phenolic compounds of *S. siamea* are protocatechuic acid, ferulic acid, sinapic acid, rutin and quercetin [24].

Table 4 Total Phenolic Content and Antioxidant Activities of Diuretic Plants

Scientific name	Part used		TPC	DPPH	ABTS	FRAP	TBARS
B. alba L.	Leaves	F	13.87±1.17	10.82±0.06	4.32±0.16	5.66±0.42	6.58±0.66
		D	7.11±0.38	25.33±1.10	1.38±0.04	3.85±0.22	2.23±0.03
B. rubra L.	Leaves	F	18.91±0.44	4.35±0.11	6.19±0.10	8.26±0.50	7.56±1.25
		D	13.78±0.51	23.73±0.14	2.96±0.09	5.39±0.31	1.87±0.15
C. sinensis (L.) Kuntze var. *assamica* (J. Masters) Kitam.	Leaves	F	140.64±3.34	0.17±0.00	85.73±10.22	176.74±13.31	5.72±0.32
		D	66.24±2.54	0.41±0.00	49.31±2.1.97	100.98±5.87	2.03±0.15
L. geoffrayi Bonati	Leaves	F	8.57±0.54	5.15±0.40	3.94±0.14	5.16±0.66	2.56±0.37
		D	3.50±0.28	16.83±0.38	2.65±0.08	2.40±0.04	1.53±0.22
P. odorata (Lour) Soja'k	Stem, leaves	F	85.31±4.52	0.28±0.02	39.54±1.95	83.91±4.51	2.72±0.24
		D	28.60±1.54	0.72±0.00	17.46±2.07	38.49±5.09	7.23±0.33
S. siamea (Lam.) Irwin &Barneby	Flower	F	31.04±1.46	1.75±0.04	27.84±0.63	28.58±1.70	0.75±0.20
		D	11.93±0.71	3.23±0.07	6.47±0.42	15.27±3.41	1.63±0.12
S. macrophylla Roxb.	Leaves	F	106.39±7.47	0.25±0.01	131.18±7.79	96.17±7.94	3.28±0.19
		D	26.86±1.47	0.29±0.01	45.91±3.32	33.53±1.95	1.13±0.03
range		F	8.57-140.64	0.17-10.82	3.94-131.18	5.16-176.74	0.75-7.56
		D	3.50-66.24	0.29-25.3	1.38-49.31	2.40-100.98	1.13-7.23

F=fresh plant extract, D= driedplant extract
TPC = total phenolic content (mg GAE/ g db), DPPH=DPPH free radical scavenging activity (EC_{50}, mg db),
TEAC = Trolox equivalent antioxidant capacity (mg TE/g db), FRAP= Ferric-reducing antioxidant power (mg TE/g db), TBARS = Thiobarbituric acid reactive substances (mg TE/g db)

TPC and antioxidant activities of selected indigenous plants with various medicinal effects in this study showed that the differences of plants species, part used are major roles on TPC and antioxidant activities including the changes of values after drying process. The leaves were the effective part of selected plants with high values, but the phytochemicals in leavelost easily during

drying process. While the types and quantities of phytochemicals affected on antioxidation mechanism and heat sensitivity.

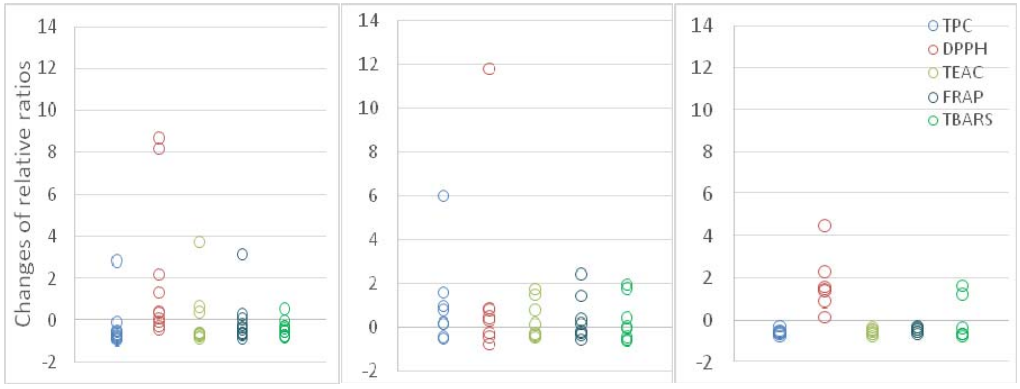
3.2 Effect of drying process on TPC and antioxidant activities

After drying process at 50°C for 18 h, the changes of TPC and antioxidant activities in dried plants were observed. The results expressed as positive (+) and negative (-) relative ratio between fresh and dried plants that were referred to the increasing and decreasing of their values in each assay except the DPPH assay. Figure 1 present the changes of relative ratios based on medicinal effect. Form the results of three medicinal plant groups, the changes of relative ratios showed very differences among the groups. The range of anti-inflammatory plants ratios were -0.92 to +8.65 (Figure 1-A). This groups found the increasing and decreasing ratios on TPC, TEAC, FRAP and TBARS assays, whereas the decreasing of EC_{50} values (the minus ratios) showed the high DPPH scavenging activity.

As shownin Figure 1-B, the analgesic plants had similar change in ratios as the anti-inflammatory plants, but this group showed the highest variation of ratios (-0.79 to +11.77). The dried *M. dianthera* showed the increasing values in all tested parameters while the other dried plants had the high values found at least 2 assays except *C. sumatrensis* and *S. wallichii*. In addition, the dried *S. wallichii* exhibited the low values in all assays compared to the fresh plant.However, all diuretic plants showed a decrease in TPC and antioxidants activities (-0.77 to +4.45) except *S. siamea* and *S. macrophylla* in TBARS assays (Figure 1-C). The low TPC and antioxidants activities in this group might be related to the low content of phytochemicals that showed the lowest range in previous discussion. It was noted that these plants was the most sensitivity to heat processing in this study.

After drying process, the degradation of phenolic acids inplants from bound form to free form cause the higher TPC. While the heating also destroy a cell wall structure that make it easy to extract phytochemicals [25-26]. In contrast, the loss of phytochemicals in plants occurred in the species which is very sensitive to heat. The drying condition such as temperature, time and oxygen and enzyme activity also increase the rate of phytochemicals loss [27-29]. The changes of TPC affected directly to antioxidant activities [30]. The drying process became an important factor to altered antioxidant activity and phytochemicals on these plants.

Thus, the changes of TPC and antioxidants activities of dried plants indicated that the preparations of food or tradition medicine by heat processing directly affects to the phytochemicals in plants leading to the decrease and increase in TPC and antioxidant activities. Furthermore, the changes occurred in diversity due to plant species, types and quantities of phytochemicals.



(A) (B) (C)

Figure 1 Changes of relative ratios of selected Thai indigenous plants classified in (A) the anti-inflammatory plants, (B) the analgesic plants and (C) the diuretic plants

3.3 Correlationof TPC and antioxidant activities on medicinal effects

The relationship between 5 studied parameters (TPC, DPPH, TEAC, FRAP and TBARS) are expressed as a correlation coefficient (r) and show in Table 5. The results showed a strongcorrelation between TPC, DPPH, TEAC and FRAP assays, whereas TBARS assay had no significantly correlated with the other assays (data not shown).

For the anti-inflammatory plants, TPC had moderate correlation with DPPH, TEAC and FRAP (r =0.724-0.882, p<0.01) while DPPH and FRAP assays showed the highest correlation (r =0.932, p<0.01). Based on these correlations, the anti-inflammatory plants might be the other groups of antioxidants over the phenolic compounds. However, several reports supported the great anti-inflammatory effect of phenolic compounds and flavonoids which inhibit or destroy the intermediate in pro-inflammatory process such as cytokines, COX-2, leukote adhesion and nitric oxide, etc [3, 31]. Additionally, *S. indica* and *L. glutinosa*, the high TPC and antioxidant activities, could be determined further in topic of active constituents and the anti-inflammatory effect both *in vitro* and *in vivo*.

From Table 5-B, the analgesic plants had weakly correlated between TPC and antioxidant activities comparing with other plants but they showed the strong correlation on DPPH, TEAC and FRAP assays (0.878-0.989, p<0.01). For analgesic effect, the free radical scavenging ability was important to protect oxidative stress in analgesic mechanisms that lead to inflammatory in body. The phenolic compounds and flavonoids such as quercitin, myricetin and flavone could reduce pain and inhibit inflammatory process [31-32], but the other phytochemicals like steroids and alkaloids have the strong analgesic effect.

Interestingly, the diuretic plants showed a close correlation between TPC and all antioxidants activities ranging from 0.873 to 0.970 (Table 5-C).The phenolic compounds play the major role as antioxidants in this group and they alsoplay role in exerting diuretic effect similar to flavonoids, saponin and some lipid acid in plants [33].In addition, plants was extracted with the high polar solvent that showed the higher diuretic effect than using non-polar solvent [34-35].While the DPPH assays strongly correlated to FRAP and TEAC, the great correlation was confirmed that antioxidants inhibit intermediates production in prostaglandin pathways due to inflammatory, pain and diuretic in body systems.

According to this study, the medicinal effects of Thai indigenous plants can be screening by using total phenolic content and the antioxidant activity assays. The diuretic plants showed a strong correlation, but the anti-inflammatory and analgesic plant needed more *in vivo* specific test. However, TPC and antioxidant activities could be the index to selected plants for further study.

Table 5 The Correlations Between Tpc and Antioxidant Activities of Aelected Thai Indigenous plants Based on Medicinal Effects: (a) Anti-Inflammatory, (b) Analgesic and (c) Diuretic

(A) anti-inflammatory

	TPC	DPPH ($1/EC_{50}$)	TEAC
DPPH	0.795**		
TEAC	0.724**	.497*	
FRAP	0.882*	.932**	.656**

(B) analgesic

	TPC	DPPH ($1/EC_{50}$)	TEAC
DPPH	0.613*		
TEAC	0.730**	0.878**	
FRAP	0.775**	0.881**	0.989**

(C) diuretic

	TPC	DPPH ($1/EC_{50}$)	TEAC
DPPH	0.926**		
TEAC	0.873**	0.856**	
FRAP	0.970**	0.921**	0.808**

* = $p<0.05$, **= $p<0.01$

TPC = total phenolic content, DPPH=DPPH free radical scavenging activity,
TEAC = Trolox equivalent antioxidant capacity, FRAP= Ferric-reducing antioxidant power,
TBARS = Thiobarbituric acid reactive substances

4. Conclusions

The selected indigenous plants which different medicinal effects had the different amount of total phenolic contents and antioxidant activities depending on their species and part used. Drying process affected directly on the decrease in TPC and antioxidant activity values except some species such as *M. dianthera*, *A. oleraceae*, *B. bipinnata*,*B. pilosa* and *E. sonchifolia*. These changes was the effect of type, quantity and heat sensitivity of phytochemicals occurring in each species. Correlation between TPC and antioxidant activities on medicinal effects varied on the groups of plants while TPC showed a strong correlation with DPPH, TEAC and FRAP assays. In addition, the correlation of TPC and antioxidant activities could be the great index for diuretic effect. For anti-inflammatory and analgesic plants, the study on clinical efficacyshould be further investigated. These results indicated the new sources of phenolic compounds and antioxidants from Thai indigenous plants consisting of*S. indica*, *L. glutinosa*, *G. sphaerogymum*, *S. wallichii* and *C. sinensis*. Moreover, thepharmacology of these plantsshould be examinedand could be applied to new pharmaceutical or functional supplement in future.

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