Antioxidant Activity of Fourteen Herbal Plants In Thailand

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Abstract

Herbal plants are responsible as the good sources of bioactive substances, which are potential to be further alternative remedies for mankind and animals. This work studies the antioxidant activity of fourteen fresh leaf extracts; *Ardisia eliptica* **(1),** *Bauhinia strychnifolia* **(2)**, *Carissa carandas* **(3)**, *Couroupita guianensis* **(4)**, *Cynometra ramiflora* **(5)**, *Erycibe cochinchinensis* **(6)**, *Hopea ferrea* **(7)**, *Jatropha curcas* **(8)**, *Maclura cochinchinensis* **(9)**, *Mammea siamensis* **(10)**, *Olax psittacorum* **(11)**, *Salacia chinensis* **(12)**, *Scaevola taccada* **(13)** and *Madhuca grandiflora* **(14)** using by DPPH (1,1-diphenyl-1-pricylhydrazyl) radical scavenging assay at 515 nm. The phenolic content was determined by Folin-Ciocalteu reagent at 765 nm. The results showed that the most active group is contained by *M. grandiflora* **(14)**, *B. strychnifolia* **(2)**, *A. eliptica* **(1)** and *C. guianensis* **(4)** in addition to the EC_{50} values are 41.64, 54.16, 70.06 and 99.81 μg/ml, respectively. The corresponding phenolic content (GAE) is 4.70, 4.43, 1.60 and 2.43 mg gallic acid/100 mg sample. *M. grandiflora* **(14)** showed the strongest antioxidant activity which is lower than that ascorbic acid of the 21-fold. In addition, the relationship of phenolic content (GAE) and antioxidant activity (EC_{50}) of six plants; *C. guianensis* **(14)**, *C. ramiflora* **(5)**, *C. carandas* **(3)**, *J. curcas* **(8)**, *M. siamensis* **(10)** and *S. chinensis* **(12)** showed a strong correlation (r = -0.918, $p < 0.01$, r^2 = 0.84). It indicates that the antioxidant activity is a highly contributed to phenolic content, and the six samples possessed a higher content of phenolic compounds is associated with a higher antioxidant activity. These potential herbs should be further studied for their active substances and other biological activities.

Keywords: herbal plant and antioxidant activity

Introduction

For hundreds of years, medicinal plants have been used in the basis of traditional medicinal treatment, and it is a repository of new remedies for mankind in many parts of the world. In Southeast Asian countries such as Thailand has been prolonged history of using medicinal plants as an alternatively medical

treatment. In this regard, leaves of *Ardisia eliptica* **(1)** have been healed liver diseases, treatment of infectious diarrhea, leprosy and venereal diseases (SRNP). Its bioactive compounds can scavenge DPPH radical. (Wetwitayaklung et al., 2012; Siti-Azima et al., 2013). In addition, the stem and root of *Bauhinia strychnifolia* **(2)** have been used to treat cancer, fever, alcoholic toxication and allergy (Wutthithammavet et al., 1997). Recently, supporting data have been reported the use of stem for breast and colon cancer treatment by boiling with water (Yuenyongsawad et al., 2013). Additionally, the vine part extract (but not leaves) shows good inhibitory activity against several of human transformed cancer cells. (Kaewpiboon et al., 2012; (Yuenyongsawad et al., 2013). In addition, Wood of *Carissacarandas* **(3)** is administered for tonic sliming (SRNP) in addition to its fruits contain vitamin C and flavonoid compounds. (Kubola et al. 2011). Use infusions or teas, people from Amazonian region and other states of North of Brazil, obtained from the leaves and flowers of *Couroupita guianensis* **(4)** to treat hypertension, tumors, pain and inflammatory processes (Revilla et al., 2002). Premanathan et al. (2002) had discovered isatin from flowers, which was cytotoxic to promylocytic leukemia (HL60) cells. *Cynometra ramiflora* **(5)** showed an inhibiting proliferation of MCF-7 human breast cancer cells (Subarnas et al., 2002). Interestingly, *Erycibe cochinchinensis* **(6)** was newly recorded as a medicinal plant in Thailand (Chuakul 2002). *Hopea ferrea* **(7)** is used for any disorder or disease causing cachexia and anti-tooth decay (SRNP). However, the claimed supports of **6** and **7** about antioxidant activity are no data reported. More researches of *Jatropha curcas* **(8)** indicates the potential as an antioxidant source (Igbinosa et al., 2011; Diwani et al., 2009; Oskoueian et al., 2011). Antidiarrheal and plasma activity improvement has been used by *Maclura cochinchinensis* **(9)** (SRNP), and it has been reported to possess antioxidant compounds as well (Nikolova et al., 2011). *Mammea siamensis* **(10)** is used for cardiotonic (SRNP) as well as isolated β-sitosterol, stigmasterol and friedelin exhibited antioxidant activity (Subhadhirasakul et al., 2005). *Olax psittacorum* **(11)** serves as decoction; treatment of kidney dysfunction (SRNP), and it is a capable of bleaching DPPH solution (Sahu et al. 2011). *Salacia chinensis* **(12)** has been used to cure laxative, decoction for muscular pain (SRNP) in addition to many biological works were studied, including DPPH radical scavenging assay (Salunkhe et al., 2009; Yoshikawa et al., 2003). Beriberi and dysentery root could be cured by *Scaevola taccada* **(13)** (SRNP), and the antioxidant activity report was found (Rahmawati et al., 2014) but *Madhuca grandiflora* **(14)** are no data supports of the antioxidant aspect.

Purposes

At present, many herbal plants in the preceding paragraph have been reported the antioxidant activity in which many bioactive compounds play an important role in an antioxidant activity, especially the polyphenol compounds. In this regard, this work studies

the relationship between antioxidant activity (EC_{50}) and phenolic content (GAE) of these fourteen plants. We evaluate antioxidant activity of the plant extracts which determined by DPPH radical scavenging assay. This method is based on the measurement of radical scavenging ability of antioxidants (hydrogen donors) towards the stable DPPH free radical which is reduced to the corresponding hydrazine (Brand-Williams 1995) and monitoring decoloration solution decreased at 515-528 nm, while the phenolic content was performed by Foin-Ciocalteu reagent at 765 nm.

Benefit of Research

A new source of antioxidant activity in the studied plants will be discovered.

Research Process

Materials and methods

Fresh leaves of fourteen plants; *A. eliptica* **(1)**, *B. strychnifolia* **(2)**, *C. carandas* **(3)**, *C. guianensis* **(4)**, *C. ramiflora* **(5)**, *E. cochinchinensis* **(6)**, *H. ferrea* **(7)**, *J. curcas* **(8)**, *M. cochinchinensis* **(9)**, *M. siamensis* **(10)**, *O. psittacorum* **(11)**, *S. chinensis* **(12)**, *S. taccada* **(13)** and *M. grandiflora* **(14)** were obtained from SRNP, Faculty of Pharmacy, Mahidol University (Salaya) in June 2012. DPPH radical (2, 2-Diphenyl-1-picryhydrazyl) was purchased from Sigma Aldrich. Folin – Ciocalteu reagent and gallic acid purchased from Fluka. Sodium carbonate and ascorbic acid purchased from Reidel – dettaën and Polskie Odczynnki Chemiczne S.A. Methanol (a commercial grade) was distillated and collected in 66-67 ^{o}C before use.

Statistical analysis

Each sample was used for statistical analysis. Correlation analyses of antioxidant activities (EC_{50}) and phenolic compound values (GAE) were carried out using correlation and regression function in IBM® SPSS® Statistics for Windows Version 20.

Determination of the moisture content

The fresh leaves were dried in 90-100 ^{o}C for 6 h and kept in a desiccator until at room temperature. To calculate a percentage of moisture based on a wet weight sample and apply the preceding method with samples, respectively.

Preparation of crude extracts

All the fresh leaves of the selected medicinal herbs were cut into pieces, crushing and the exactly 1 g of samples was stirred with 20 ml of 80 % aqueous methanol for 30 minutes at room temperature. The mixture was filtered and diluted to the volume with the same solvent in a 25 ml volumetric flask and used as working solution of extracts at an appropriate concentration.

Determination of antioxidant activity by DPPH assay

A suitable concentration of samples by 80% aqueous methanol as a solvent was prepared. A portion of sample mixed with 200 μM, DPPH radical solution in 80% aqueous methanol at a ratio 1:1. After the mixture was left at room temperature for 30 min. Its absorbance was read at 515 nm using a spectrophotometer (Spectronic 20+). All

samples were run in triplicates and their antioxidant activities were averaged using ascorbic acid as a reference compound. The free radical scavenging activity of the samples was expressed as percent inhibition of DPPH decoloration was estimated. The EC_{50} was determined by a concentration of the sample and its required to give 50% decrease of the absorbance from that the blank solution (aqueous methanol and DPPH).

Determination of phenolic compounds

A 2 ml suitable concentration of each sample was mixed with Folin – Ciocalteu reagent (Javanmardi et al., 2003; Georgé et al., 2005) distillate water and 7.5% (w/v) Na_2CO_3 equaled 0.40, 3.60 and 4.00 ml, respectively. The mixture left at room temperature for 1 h. After filtering using a microfibre filter glass (Whatmann Cat. No. 1822 090), its absorbance was measured at 765 nm, and the gallic acid used as a standard compound. The phenolic compounds were expressed as mg gallic acid equivalent (GAE)/100 mg wet sample.

Data Analysis

The leaf extracts of fourteen plants exhibited promising antioxidant activity, in which containing various kinds of compounds to scavenge DPPH radical. Table 1 shows that the most antioxidant activity group contained *M. grandiflora* **(14)**, *B. strychnifolia* **(2)**, *A. eliptica* **(1)** and *C. guianensis* **(4)**. That those EC_{50} are 41.64, 54.16, 70.06 and 99.81 μg/ml. The corresponding GAE is 4.70, 4.43, 1.60 and 1.28 mg gallic acid/100 mg sample, respectively. The extracts are richer in phenolic content; they showed the stronger antioxidant activity. Consequently, the phenolic content could be responsible to the antioxidant activity, and it plays an important role in the group. However, the other active component being from stem of *B. strychnifolia* **(2)** is responsible for anticancer such as 3, 5, 7, 3', 5'-pentahydroxy-flava-nonol-3-*O*-α-L-rhamnopyranoside and 3, 5, 7-trihydroxy-chromone-3-*O*-α-L-rhamnopyra-noside. In addition, the former is a higher active 10-fold than that of the Camptothecin (anticancer drug) (Yuenyongsawad et al., 2013). Isatin from flowers of **4** showed antioxidant activity in terms of lipid peroxidation and cytotoxicity against HL60 cells. Moreover, flavonoids (flavone and flavonol) and anthocyanins in **1** play a major role as compared to phenols (Siti-Azima et al., 2013).

The lower active group filled with *H. ferrea* (**7**), *J. curcas* **(8)**, *C. carandas* **(3)**, *C. ramiflora* **(5)**, *M. siamensis* **(10)** and *S. chinensis* **(12)**. EC_{50} and GAE are in the range of 181.51-534.71 μg/ml and 0.59-3.47 mg gallic acid/100 mg sample, respectively. The highest of antioxidant potential and phenolic content was found in **7**, while **12** deserved the lowest values compared to the studied group (Table 1). Thereby, the antioxidant activity of this group mainly depends on the amount of phenolic content as well. In according to recent works of **8** were studied the antioxidant potential in seeds, kernel

meal, root, stem and leaves (Igbinosa et al., 2011; Nithiyanantham 2013; Oskoueian 2011) as well as the reporting of root and stem of **7** was contained many bioactive compounds: gallic acid, benzoic acid, tannins, coumaric acid and ellagic acid. However, its spectroscopic data are not available for its leaf extracts (Diwani et al., **2009**). Essential oils of **10** leaf extracts bleached efficiently the DPPH radical solution in comparison to kaemferol, trolox and quercetin. The activity is lower than that one of the 29-, 31- and 55-fold, respectively (Leelapornpisid et al., 2008). Additionally, a mixture of β-sitosterol and stigmasterol from **10**, at the equaled ratio, exhibited EC_{50} > 200 μg/ml with DPPH radical scavenging assay (Subhadhirasakul et al., 2005). Magiferin and its derivatives in *Salacia* species; *S. oblonga, S. recticulata* and *S. chinensis* **(12)** exhibited the anti-oxidative stress which causing of diabetic diseases (Sellamuthu et al., 2013; Dar et al., 2005). Earlier year's reporting, **3** contained vitamin C, and flavonoids; apigenin, rutin, luteolin, myricertin and quercetin (Kubola et al., 2011) which are responsible for decolorized DPPH solution. Due to the leaves of **5** are contained with betulinic acid, β-sitosterol-3-*O*-β-D-glucopyranoside, oleanolic acid, ursolic acid and 4-hydroxybenzoic acid (Begum et al., 2013), resulted in it could be considered an efficient antioxidant source. However, **7** has been newly reported this biological manner.

In addition, the lowest activity group includes *E. cochinchinensis* **(6)**, followed by *M. cochinchinensis* **(9)**, *O. psittacorum* **(11)** and *S. taccada* **(13)**. In accord to

Table 1 The values, EC_{50}, GAE, and % moisture, of the extracts from fourteen selected plants

Sample	EC_{50}[a]	GAE[b]	%Moisture
1. *A. eliptica*	70.06	1.60	29.30
2. *B. strychnifolia*	54.16	4.43	55.75
3. *C. carandas*	228.30	1.28	61.63
4. *C. guianensis*	99.81	2.43	67.63
5. *C. ramiflora*	250.00	1.34	60.00
6. *E. cochinchinensis*	1,418.01	0.30	57.10
7. *H. ferrea*	181.51	3.47	59.63
8. *J. curcas*	249.80	1.16	77.92
9. *M. cochinchinensis*	2,214.20	1.41	61.00
10. *M. siamensis*	337.96	0.79	59.84
11. *O. psittacorum*	4,397.74	0.28	67.11
12. *S. chinensis*	534.71	0.59	46.65
13. *S. taccada*	15,526.00	0.14	83.00
14. *M. grandiflora*	41.64	4.70	44.15
15. Ascorbic acid	1.96	-	-

[a] in term of μg/ml

[b] in term mg gallic acid/100 mg sample

leaf extracts of **9** showed antioxidant potential (Nikolova et al., 2011). **11** dried leaves extracted by various kinds of organic solvents, *e.g.* methanol, ethanol and hexane showed a good of potent antioxidant sources. (Sahu et al. 2011). Recent research reported the positive result to scavenging DPPH radical solution by leaf extracts of **13** (Rahmawati et al., 2014). Apart from above, **6** has been just newly reported as the antioxidant activity. A carefully considered in the relationship of antioxidant potential and phenolic content; it

gives similarly the conclusion to the previous group.

The relationship between EC_{50}(y) and GAE (x) of fourteen plants was found a moderate correlation (r = -0.421, p > 0.05, r^2 = 0.177) in according to the low contribution of phenolic content to antioxidant activity is 18%. In this manner, seven plants; **2**, **3**, **4**, **5**, **8**, **10** and **12** were chosen. The relationship of two parameters was shown a stronger correlation (r = -0.816, p < 0.05, r^2 =

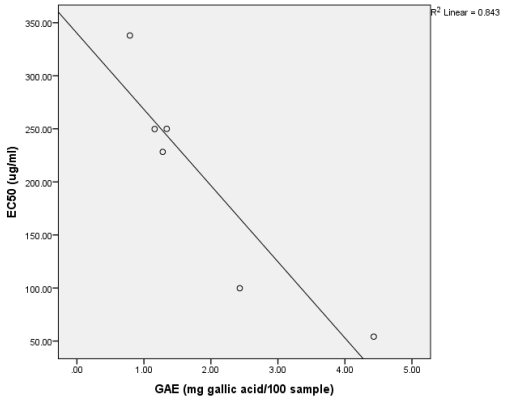

Figure 1 A plotting of EC_{50} vs. GAE of chosen six plants (**2**, **3**, **4**, **5**, **8** and **10**)

0.665) in addition to the contribution of phenolic content to antioxidant activity was increased to 67%. Furthermore, if a **12** is neglected from the group. Certain data were analyses; **2**, **3**, **4**, **5**, **8** and **10**, resulted in the phenolic content was shown the strongest correlation with the antioxidant activity (r = -0.918, p < 0.01, r^2 = 0.843, Figure 1). Phenolic content contribution was increased to the highest at 84% and in among others the 16% activity may also come from other natural antioxidant compounds such as volatile oils, vitamins or beta-carotene. It suggests that this group possessed a higher content of phenolic compounds, which was associated with a higher antioxidant activity.

However, based on the strongest antioxidant activities of **14**, **2**, **1** and **4**. This group could be considered the strong radical scavengers and recognized as good sources of natural antioxidant. Interestingly, using ascorbic acid as reference compound that the EC_{50} is 1.96 μg/ml (Table 1) (Premkaisorn 2008). The activities of four extracts are nearly lower than that ascorbic acid of the 21-, 28-, 41- and 60-fold, respectively. Moreover, **14** being that the strongest antioxidant activity has never been reported on this aspect. Finally, due to the complexity and diversity of antioxidants, the isolation and identification of the active components are needed to elucidate its mechanism as well as other biological activities, including antioxidant activity is ongoing.

Conclusion

The DPPH radical scavenging assay was chosen as a preliminary tool, to evaluate for new sources of antiradical scavengers. The result showed that leaf extracts with exhibiting the highest antioxidant activity are *A. eliptica* **(1)**, *B. strychnifolia* **(2)**, *C. guianensis* **(4)** and *M. grandiflora* **(14)**. Thereafter, these herbs could enrich of antiradical scavengers in which to be considerable for further studies.

Recommendation

A. eliptica **(1),** *B. strychnifolia* **(2)**, *C. guianensis* **(4)** and *M. grandiflora* **(14)** should be isolate the bioactive compounds as well as to elucidate its mechanism and other biological activities, including the other antioxidant activity is ongoing.

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