บทความวิจัย

การประเมินฤทธิ์การต้านอนุมูลอิสระของพืชสมุนไพรไทยสิบเอ็ดชนิด

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บทคัดย่อ

สารสกัดจากพืชสมุนไพรไทยหลายชนิดมีฤทธิ์ทางชีวภาพ เช่น ฤทธิ์การต้านอนุมูลอิสระ งานวิจัยนี้ได้ทำการศึกษาฤทธิ์การต้านอนุมูลอิสระของใบสดพืชสมุนไพร 11 ชนิด ได้แก่ หูกวาง *(Terminalia catappa)* กำจาย *(Caesalpinia decapetala)* หว้า *(Syzygium cumini)* ดันหมี *(Gonocaryum lobbianum)* พิกุล *(Mimusops elengi)* เทียนกิ่ง *(Lawsonia inermis)* พุดจีบ *(Tabernaemontana divaricata)* น้อยโหน่ง *(Anona recticulata)* คงคาเดือด *(Arfeuillea arborescens)* ตีนเป็ดฝรั่ง *(Crescentia alata)* และ สำมะงา *(Clerodendrum inerme)* นำมาทำการสกัดด้วย 80% เมทานอลในน้ำโดยปริมาตร แล้วนำสารสกัดมาทดสอบฤทธิ์การต้านอนุมูลอิสระโดยวิธี DPPH (2,2-diphenyl-1-picryhydrazyl) radical scavenging พบว่ามีค่า EC_{50} เท่ากับ 38.31, 39.91, 58.18, 126.00, 146.48, 267.00, 417.11, 664.56, 2010.24, 7313.61 และ 7819.00 μg/ml ตามลำดับ ส่วนการหาปริมาณหมู่ฟีนอลโดยใช้ Folin-Ciocalteu reagent ได้ค่า GAE (gallic acid equivalent) เท่ากับ 3.75, 5.23, 2.39, 1.79, 2.78, 1.27, 0.76, 2.65, 0.41, 0.16 และ 846.00 mg, gallic acid/100 mg, wet samples ตามลำดับ ผลการศึกษานี้ยังได้แสดงให้เห็นความสัมพันธ์ระหว่างค่าฤทธิ์การต้านอนุมูลอิสระและปริมาณหมู่ฟีนอลของพืช 6 ชนิด ได้แก่ หูกวาง กำจาย หว้า ดันหมี พิกุล และเทียนกิ่ง พบว่าพืชเหล่านี้มีปริมาณสารฟีนอลสูงจะมีแนวโน้มที่จะมีฤทธิ์การต้านอนุมูลอิสระสูงตามไปด้วย (r = -0.750, p < 0.05) ซึ่งน่าจะเป็นแหล่งให้สารต้านอนุมูลอิสระแหล่งใหม่ที่สำคัญอีกแหล่งหนึ่งและน่าจะนำมาศึกษาหาสารสำคัญและฤทธิ์ทางชีวภาพอื่นต่อไป

คำสำคัญ: สมุนไพรไทย ฤทธิ์การต้านอนุมูลอิสระ สารหมู่ฟีนอล

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Evaluation of Antioxidant Activity of Eleven Thai Medicinal Herbs

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ABSTRACT

Many Thai medicinal herbs have been reported to exhibit biological activity such as antioxidant activity. This work aims to evaluate antioxidant activity of eleven fresh leaves of the following herbs: *Terminalia catappa, Caesalpinia decapetala, Syzygium cumini, Gonocaryum lobbianum, Mimusops elengi, Lawsonia inermis, Tabernaemontana divaricata, Anona recticulata, Arfeuillea arborescens, Crescentia alata* and *Clerodendrum inerme* which were extracted by 80% aqueous methanol. The extracts were tested for antioxidant activity by using DPPH radical scavenging method. The results shown in term of EC_{50} were 38.31, 39.91, 58.18, 126.00, 146.48, 267.00, 417.11, 664.56, 2010.24, 7313.61 and 7819.00 μg/ml, respectively. The phenolic compounds were determined by using Folin-Ciocalteu reagent colorimetric method at 765 nm. The values in term of GAE (gallic acid equivalent) were 3.75, 5.23, 2.39, 1.79, 2.78, 1.27, 0.76, 2.65, 0.41, 0.16 and 846.00 mg, gallic acid/100 mg, wet samples, respectively. The study showed that the six selected herbs, *Terminalia catappa, Caesalpinia decapetala, Syzygium cumini, Gonocaryum lobbianum, Mimusops elengi* and *Lawsonia inermis,* possessed phenolic content which was associated with a higher antioxidant activity (r = -0.750, $p < 0.05$). These potential herbs should be regarded as new sources containing antioxidant compounds and there will be further studied of their active substances and other biological activities.

Keywords: Thai herbs, antioxidant activity, phenolic compound

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Introduction

Up to present, a number of Thai medicinal herbs have long been used for primary health care. For instance, dried flower of *Mimusops elengi* is cardiotonic and is used for treatment of sore throat and muscular pain [1], roots of *Caesalpinia decapetala* is used as emmenagogue [2], roots or stems of *Gonocaryum lobbianum* are used for jaundice treatment [3], leaves of *Tabernaemontana divaricata* are cough relieved [4]. All leaves of *Anona recticulata, Arfeuillea arborescens, Lawsonia inermis* and *Crescentia alata* are shown as contusion [5], pain relieved [6], diuretic action and haemostatic effect [5], respectively. Moreover, a pruritic rash activity is found in leaves of *Terminalia catappa* [7] and *Clerodendrum inerme* [2], as well as, leaves of *Syzygium cumini* displayed dysentery function [7].

In addition, the antioxidant activity of the seed of *Syzygium cumini* has been reported and found to have high total phenolic contents with high antioxidant activity in DPPH free radical scavenging method [8, 9]. The leaves extracts of *Lawsonia inermis* are active with ABTS free radical scavenging method, and *p*-coumaric acid, 2-methoxy-3-methyl-1,4-naphthoquinone and apinin were isolated [10]. Its fruits were found to have high phenolic compounds [11]. The leaves of *Tabernaemontana divaricata* showed hydroxyl radical scavenging activity [12]. Natural products in *Anona recticulata* were reported as potential anticarcinogenic agents [13] and as an effective free radical scavenger [14].

Recently, an investigation of *Terminalia catappa* exhibited antioxidant effect in $FeCl_2$-ascorbic acid induced lipid peroxidation in rat liver homogenate [15]. The extract contains tannins which possess superoxide radical scavenging activity [16]. An extensive studied of the plant species by Kinohshita *et al.* [17] led to the isolation of chebulagic acid and corilagin as the antioxidants. Both compounds showed strong scavenging actions for superoxide free radical scavenging ($O_2^{\cdot -}$) and peroxyl radicals. They also exhibited reactive oxygen species production from leukocytes stimulated by phorbol-12-myristate acetate. In addition, an inhibition effects of *Terminalia catappa* leaves extract on the invasion and mobility of highly metastatic A549 and Lewis lung carcinoma (LLC) cells were also studied [18]. However, the antioxidant activity of *Caesalpinia decapetala, Gonocaryum lobbianum, Mimusops elengi, Arfeuillea arborescens, Crescentia alata* and *Clerodendrum inerme* has not been reported.

The purpose of this study was to evaluate eleven selected Thai medicinal herbs as mentioned above, which could be used as new potential sources of natural antioxidants. The active compounds play an important role, mainly contains phenolic compounds, vitamin A, C and β-carotenes [19, 20]. Methodologies for determination of radical scavenging activities *in*

vitro have been reported such as ABTS, DMPD and DPPH assay [21]. The DPPH assay is based on the measurement of radical scavenging ability of antioxidants (hydrogen donors) towards the stable free radical DPPH$^{\cdot}$ which is reduced to the corresponding hydrazine [22] and monitoring decoloration solution decreased at 515 - 528 nm. The phenolic content determined by using Folin-Ciocaluteu reagent and the absorbance was recorded at 765 nm. The study also determined a possible relationship between phenolic compound and antioxidant activity using DPPH radical scavenging method.

Materials and methods

Eleven plants were obtained from Siri Ruckhachati Medicinal Plant Garden of Mahidol University (Salaya) in August-October 2009. DPPH radical (2, 2-Diphenyl-1-picryhydrazyl) was purchased from Sigma Aldrich. Folin-Ciocalteu reagent and gallic acid were purchased from Fluka. Sodium carbonate and ascorbic acid were obtained from Reidel-dettaën and Polskie Odczynnki Chemiczne S.A. Methanol (a commercial grade) was distillated and collected at 66-67°C before use.

Statistical analysis

Statistical analysis for each sample was performed. Correlation analyses of antioxidant activities (EC_{50}) and phenolic compound values (GAE) were carried out using correlation program in SPSS for Windows (ver 10.0.1).

Preparation of crude extracts

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

Determination of antioxidant activity by DPPH free radical scavenging method

A suitable concentration of samples was prepared using 80% aqueous methanol as a solvent. A portion of sample mixed with 200 μM DPPH radical solution in 80% aqueous methanol at a ratio of 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. The EC_{50} was determined by a concentration of the sample that was required to give 50% decrease of the absorbance from that of 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 [23, 24], distillated water and 7.5% (w/v) Na_2CO_3 at 0.40, 3.60 and 4.00 ml, respectively. The mixture was 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 using gallic acid as a standard compound. The phenolic compounds were expressed as mg gallic acid equivalent (GAE)/100 mg, wet sample.

Results and Discussion

It has been reported that the aqueous methanol fresh leaves extract of *Caesalpinia coriaria* showed strong antioxidant activity (15.05 μg/ml) with DPPH free radical scavenging method. In comparison with ascorbic acid, its activity was lower than 8 times [25]. This work aims to continue to evaluate new sources of plants containing antioxidant components. The experiment showed that all the selected herbal plants contain antioxidant components. The values were reported in term of EC_{50} of fresh leaves extracts of *Terminalia catappa* (1), *Caesalpinia decapetala* (2), *Syzygium cumini* (3), *Gonocaryum lobbianum* (4), *Mimusops elengi* (5), *Lawsonia inermis* (6), *Tabernaemontana divaricata* (7), *Tabernaemontana divaricata* (8) *Arfeuillea arborescens* (9), *Crescentia alata* (10), and *Clerodendrum inerme* (11) were 38.31, 39.91, 58.18, 126.00, 146.48, 267.00, 417.11, 664.56, 2010.24, 7313.61 and 7819.00 μg/ml, respectively. The phenolic contents in term of GAE of samples 1-11, were 3.75, 5.23, 2.39, 1.79, 2.78, 1.27, 0.76, 2.65, 0.41, 0.16 and 846.00 mg, gallic acid/100 mg, wet samples, respectively (Table 1).

Table 1 The values, EC_{50} and GAE, of the extracts from selected herbs

Entry	EC_{50} [a]	GAE [b]
1. *Terminalia catappa*	38.31	3.75
2. *Caesalpinia decapetala*	39.91	5.23
3. *Syzygium cumini*	58.18	2.39
4. *Gonocaryum lobbianum*	126.00	1.79
5. *Mimusops elengi*	146.48	2.78
6. *Lawsonia inermis*	267.00	1.27
7. *Tabernaemontana divaricata*	417.11	0.76
8. *Anona recticulata*	664.56	2.65
9. *Arfeuillea arborescens*	2,010.24	0.41
10. *Crescentia alata*	7,313.61	0.16
11. *Clerodendrum inerme*	7,819.00	846.00
12. Ascorbic acid	1.96	

Note: [a]in term of μg/ml
[b]in term mg, gallic acid/100 mg, wet sample

Basically, it was believed that aqueous methanol can dissolved both aqueous and organic phases of natural antioxidant compounds, resulting from the antioxidant activity which could be the total antioxidant activity in herbal plants. The strongest antioxidant activity group is the samples 1-6, the moderate and lowest exhibition groups are 7-9 and 10-11, respectively. The relationship between EC_{50} (y) and GAE (x) of the group 1-6 is more linearly correlated (Fig. 1, r = -0.750, $p < 0.05$) than sample group 7-9 (Fig. 2, r = 0.501, $p < 0.05$). The coefficient of determination (r^2 = 0.563) of 1-6 is not high, and it reflected that the contribution of phenolic content capacity (PAC) was 56%. The other 44% could have come from tannins, volatile oils, vitamins and β-carotenes. It suggests that samples 1-6 contained a phenolic content which associated with antioxidant activity in moderation, but the other groups are less associable. However, the present study showed that the strongest group 1-6 exhibited strong free radical scavengers by comparison with ascorbic acid as a reference compound (1.96 μg/ml). In addition to complexity and diversity of natural mixtures of phenolic and other active compounds in herbal plant extracts, it is difficult to characterize and assess each compound or compare it to any antioxidant activities. Generally, each herbal plant contained same or different active compounds, and each of these compounds showed different amounts of antioxidant activity.

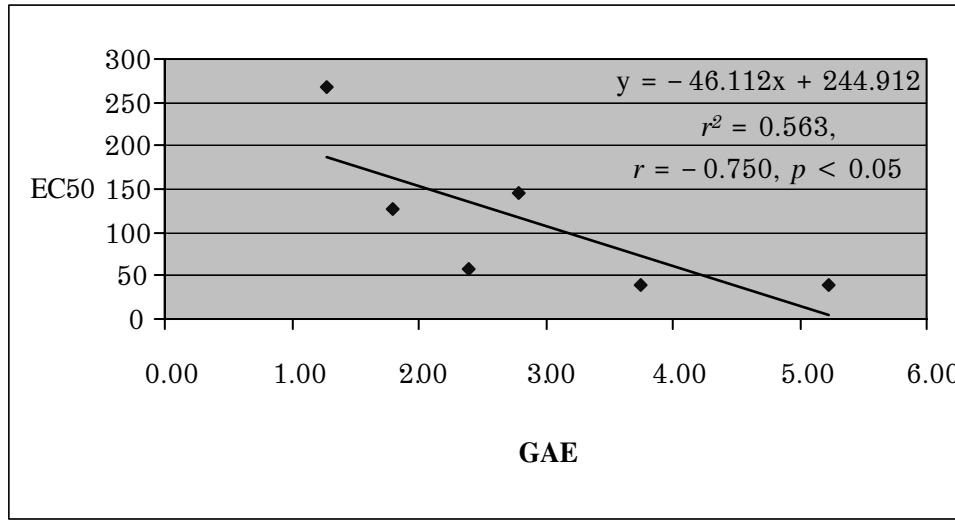

Fig. 1 The relationship between EC_{50} (µg/ml) and GAE (mg, gallic acid/100 mg, wet sample) of samples 1-6

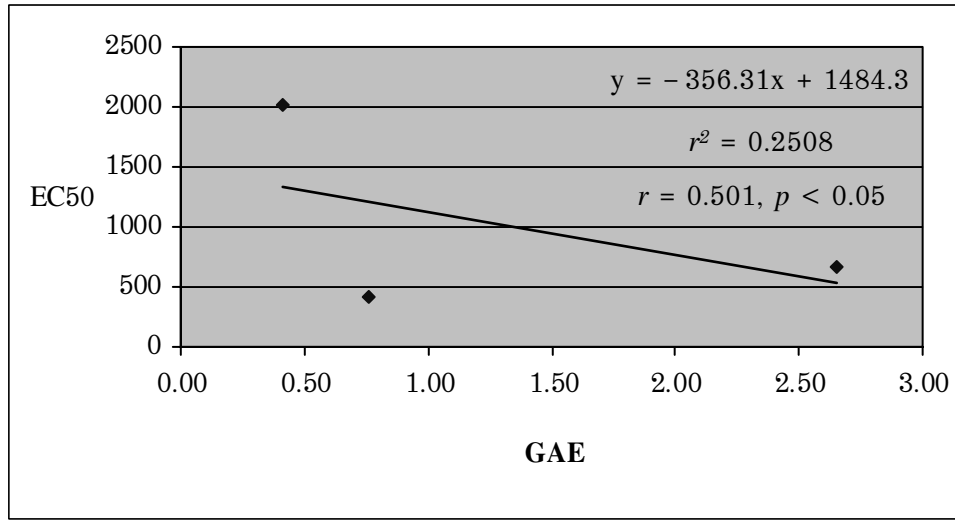

Fig. 2 The relationship between EC_{50} (µg/ml) and GAE (mg, gallic acid/100 mg, wet sample) of samples 7-9

Accordingly, it is necessary to isolate the pure compounds from natural complex mixtures and their structures would be characterized and assessed whether it is active with any antioxidant activity test. Finally, the finding of our study suggests that groups 1-6 may be regarded as new sources containing antioxidant compounds and it would be further studied of bioactive compounds and other biological activity aspects.

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