Original Article

ฤทธิ์ต้านอนุมูลอิสระและปริมาณสารประกอบฟีนอลิกรวมของตำรับยาแผนโบราณบางตำรับ

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บทคัดย่อ

บทนำ: สารต้านอนุมูลอิสระสามารถลดปริมาณอนุมูลอิสระและโมเลกุลที่ประกอบด้วยอะตอมของออกซิเจนที่พร้อมเข้าทำปฏิกิริยาในร่างกายได้ ยาสมุนไพรและยาแผนโบราณมีฤทธิ์ต้านอนุมูลอิสระที่ดี ตำรับยาแผนโบราณประกอบด้วยสมุนไพรหลายชนิดและปริมาณที่แตกต่างกัน ซึ่งสามารถรักษาได้หลายโรค ได้แก่ระบบทางเดินอาหาร ระบบหัวใจและหลอดเลือด ระบบทางเดินหายใจ ระบบไหลเวียนโลหิต ระบบกล้ามเนื้อและกระดูก และบำรุงร่างกาย ฯลฯ มีตำรับยามากมายที่ใช้ในประเทศไทยมาอย่างยาวนาน ได้แก่ ยาหอม ยากษัยเส้น ยาเขียวหอม และยาลม วัตถุประสงค์ของการวิจัยนี้คือเพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระและปริมาณสารประกอบฟีนอลิกรวมของตำรับยาแผนโบราณ 7 ตำรับ **วิธีดำเนินการวิจัย:** การทดสอบฤทธิ์ต้านอนุมูลอิสระโดยวิธี ABTS assay และหาปริมาณสารประกอบฟีนอลิกรวมด้วยวิธี Folin-ciocalteu **ผลการวิจัย:** จากผลการทดลองพบว่าตำรับยา A แสดงสมบัติต้านอนุมูลอิสระสูงที่สุด (IC_{50} 1.60 มิลลิกรัมต่อมิลลิลิตร) ขณะที่ตำรับยา F มีฤทธิ์ต้านอนุมูลอิสระน้อยที่สุด (IC_{50}17.11มิลลิกรัมต่อมิลลิลิตร) ร้อยละของการยับยั้งการดูดกลืนแสงของ $ABTS^{+}$เพิ่มขึ้นตามความเข้มข้นของตำรับยาแผนโบราณ ตำรับยา A มีปริมาณสารประกอบฟีนอลิกรวมสูงสุด (897.78 มิลลิกรัมกรดแกลลิคต่อกรัมน้ำหนักแห้ง) ขณะที่ตำรับยา G มีปริมาณสารประกอบฟีนอลิกรวมต่ำสุด (158.15 มิลลิกรัมกรดแกลลิคต่อกรัมน้ำหนักแห้ง) **สรุปผลการวิจัย:** ตำรับยาแผนโบราณทั้ง 7 ชนิดแสดงสมบัติต้านอนุมูลอิสระและมีสารประกอบฟีนอลิกเป็นองค์ประกอบตำรับยา A ประกอบด้วย จันทน์แดง โกศหัวบัว ดอกมะลิ ดอกพิกุล ดอกบุนนาค ดอกสารภี และเกสรบัวหลวง เป็นตำรับที่มีฤทธิ์ต้านอนุมูลอิสระและมีสารประกอบฟีนอลิกรวมสูงกว่ายาแผนโบราณตำรับอื่น ดังนั้นองค์ประกอบในตำรับยา A จึงน่าสนใจที่จะศึกษาฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ทางชีวภาพอื่นๆ ต่อไป

คำสำคัญ: ฤทธิ์การต้านอนุมูลอิสระ สารประกอบฟีนอลิกรวม ยาแผนโบราณ

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Antioxidant Activity and Total Phenolic Contents of Some Thai Traditional Formulation

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Abstract

Introduction: Antioxidants decrease the amount of free radicals and reactive oxygen species *in vivo***.** The traditional herbal medicines have been reported fortheir good antioxidant activities**.** Thai traditional herbal formulations consist of various herbs with different amounts which could treat many symptomsof gastrointestinal, cardiovascular, respiratory, central nervous systems, musculoskeletal and joint diseases, etc. Many preparations have long been used in Thailand such as Ya-Hom, Ya-Kasaisen, Ya-KeawHomand Ya-Lom. The objectives of this study were to investigate the antioxidant activity and total phenolic contents of seven types of Thai traditional herbal formulations. **Methods**: Antioxidant activity of seven Thai traditional for mulations was investigated using ABTS radical scavenging assay, and the amount of total phenolic contents was examined using Folin-ciocalteu method. **Results**: Ya-A formulation showed the highest antioxidant activity (IC_{50} 1.60 mg/mL), while Ya-F formulation showed the lowest antioxidant activity (IC_{50}17.11 mg/mL). The percentage of inhibition of the absorbance of the $ABTS^{+}$was increased with the increased Thai traditional concentration. Ya-A formulation showed the highest total phenolic contents (891.78 mg GAE/g dry weight)**.** Whereas, the total phenolic contents of Ya-G formulation had the lowest values (158.15 mg GAE/g dry weight). **Conclusion**: All seven types of Thai traditional herbal formulations had the antioxidant activity and contained phenolic compounds. Ya-A formulation comprising *Dracaena loureiri* Gagnep.,*Ligusticum striatum* DC.,*Jasminum sambac* L., *Mimusopselengi* L., *Mesuaferrea* L., *Mammeasiamensis* T. and *Nelumbonucifera* Gaertn. Exhibited higher antioxidant activity and total phenolic contents than other test Thai traditional herbal formulations. Therefore, the component in Ya-A was interesting for further investigating antioxidant and other biological activities

Keywords: antioxidant activity, total phenolic content, Thai traditional herbal formulations

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Introduction

Thai traditional herbal drugs consist of various herbs with different amounts. They could be divided based on therapeutic uses such as for gastrointestinal system, cardiovascular system, respiratory system, central nervous system, musculoskeletal and joint diseases and nutrition (Suwangasawong, 2016). Many researchers reported that the herbal medicine and traditional drugs had the good antioxidant activity (Luanchoy *et al*., 2014; Manok and Limcharoen, 2015). Ya-hom preparations have been reported for their high amounts of total phenolic compounds and high FRAP values which might exhibit a potential benefit as a natural antioxidant (Channarong*et al*., 2012). Several herbals in Ya-Homintajak have antioxidant activity and phenolic compounds (Tuekaew *et al*., 2014).

Antioxidants are great to prevent the formation and oppose the actions of reactive oxygen and nitrogen species, which are generated *in vivo* and cause damage to DNA, lipids, proteins, and other biomolecules. (Halliwell, 1996). Antioxidants play important role in neutralizing free radicals (Rice-Evans *et al.*, 1997). Antioxidants are divided into several groups(by inhibiting formation of free radicals or by interrupting propagation of the free radical)based on the mechanisms of action: scavenging species that initiate peroxidation and chelating metal ions that they are unable to generate reactive species or decompose lipid peroxides, quenching radical oxygen preventing formation of peroxides, breaking the auto oxidative chain reaction, and reducing localized oxygen concentrations (Nawar, 1996).

Various methods have been evaluated to measure the antioxidant activity including oxygen radical absorbance capacity (ORAC), Total antioxidant scavenging capacity (TOSC), Trolox equivalent antioxidant capacity (TEAC), DPPH radical scavenging activity assay, ferric reducing antioxidant power (FRAP) assay and ABTS assay (Re *et al., 1999*; Regoli, 2000; Su *et al.,* 2007). DPPH method was sensitive to acidic environment where as ABTS method could be used at different pH. The samples with ABTS method reacted rapidly in the aqueous buffer solution reaching a steady state within 30 min but the sample with DPPH method reacted very slowly reaching a steady state after 8 h (Shalaby and Shanab, 2013).

The objectives of this study were to compare the antioxidant activity and total phenolic contents of seven types of Thai traditional herbal formulations.

Materials and Methods

Materials

ABTS (2,2'-azinobis (3-thylbenzothia-zoline-6-sulfonic acid)) was obtained from Merck (Germany). Folin-Ciocalteu reagent was purchased from Fluka. All other reagents were of analytical reagent grade. Seven types of Thai traditional herbal formulations (Ya-A, Ya-B, Ya-C, Ya-D, Ya-E, Ya-F, Ya-G) were purchased from drugstores from Prachuap Khiri Khan, Thailand.

Preparation of Thai traditional herbal drug extracts

Seven Thai traditional herbal formulation were extracted individually using methanol. One-hundred milligrams of each sample was soaked in 10 mL methanolfor 24 hours.The samples were used for ABTS assay and total phenolic content. Herbal ingredients in each Thai traditional herbal formulation is shown in Table 1.

Determination of ABTS radical scavenging assay

The ABTS assay was studied on the ability of the antioxidants to scavenge the long life radical cation $ABTS^+$(Re et al., 1999). The scavenging produces a decrease in the absorbance at 734 nm. ABTS was dissolved in phosphate buffer (pH 7) and activated to $ABTS^+$ radical by addition of 2.45 mM potassium persulfate with stirring. The mixture was to stand in the dark at room temperature. The $ABTS^+$ solution was diluted with ethanol and time of activation 30 mins. The diluted ABTS solution was mixed with sample extracts at various concentrations (500-10,000µg/mL). Then the sample was measured the absorbance at 734 nm.

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample})/ A_{sample}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the sample.The effective concentration at 50%inhibition of $ABTS^+$(IC_{50}) was calculated by plotting percentage inhibition against different concentrations of Thai traditional herbal formulation (Rubalya and Neelamegam, 2012). The antioxidant capacity of samples was expressed as IC_{50}.

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu method. An aliquot of the extract was mixed with 5.0 mL of 0.2 M Folin-Ciocalteau reagent for 5 min then 4.0 ml of sodium carbonate (7.5 %w/v) was added in the mixture. The absorbance of the mixture was measured at 765 nm after 30 min in the dark at room temperature. Total phenolic content of each sample was expressed as gallic acid equivalent in mg/g dry powder based on the calibration curve conducted from the standard gallic acid solutions (10-60 mg%) and reacted in the same manner as the tested samples.

Statistical analysis

The data of antioxidant activity and total phenolic contents were the average of triplicate. The data were recorded as mean±SD and analyzed by One-way ANOVA (SPSS version 11). Significant differences between means were determined by Duncan's multiple range test

Table 1 Herbal ingredients in seven types of Thai traditional formulations

Formulation	Herbal ingredients
Ya-A	*Dracaena loureiri* Gagnep., *Ligusticum striatum* DC., *Jasminum sambac* L., *Mimusopselengi* L., *Mesuaferrea* L., *Mammeasiamensis* T., *Nelumbonucifera* Gaertn.
Ya-B	*Angiopterisevecta* (G.Forst) Hoffm.,*Globbamalaccensis* Ridl., *Rauvolfiaserpentina* (L.) Benth.,*Dryopterissyrmatica* (O.kze),*Graptophyllumpictum*(L.) Griff.
Ya-C	*Citrus aurantifolia* (Christm.) Swingle., *Zingiber zerumbet* (L.) Smith., *Alpiniagalanga* (L.) Willd., *Zingiber montanum* (J.Koenig) Link ex A.Dietr., *Zingiberofficinale* Roscoe.
Ya-D	*Angelica dahurica* Benth., *Cuminum cyminum* L., *Myristica fragrans* Houtt., *Glycyrrhizaglabra* Linn., *Zingiberofficinale* Roscoe., *Zingiber zerumbet* (L.) Roscoe ex Sm., *Zingibercassumunar* Roxb., *Ocimumtenuiflorum* L.
Ya-E	*Rheum palmatum* L.,*Senna alexandrina* Mill., *Ferula assafoetida* L., *Aloe barbadensis* Mill., *Garcinia hanburyi* Hook.f.
Ya-F	*Piper nigrum* Linn., *Piper retrofractum* Vahl., *Acoruscalamus* L., *Zingiber mekongense* Gagnep., *Citrus hystrix* DC.
Ya-G	*Piper nigrum* Linn., *Alpiniagalanga* (L.) Willd., *Zingiber montanum* (Koenig) Link ex Dietr., *Zingiber zerumbet* (L.) Roscoe ex Sm., *Zingiber officinale* Roscoe., *Curcuma xanthorrhiza* Roxb.

Results

The antioxidant activity of seven Thai traditional formula extracts was assessed by ABTS assays.The effects of the duration of interaction of Thai traditional herbal formulations on the suppression of the absorbance of the $ABTS^+$radical cation at 734 nm are shown in Figure 1. The resulted indicated that all of Thai traditional herbal formulations showed the free radical scavenging ability. The reaction with $ABTS^+$ of only Ya-F formulation was already completed at 1 min, while the others Thai traditional herbal formulation could be further reacted upto 6 mins. However, the reaction with $ABTS^+$of all Thai traditional formulations was increased as the Thai traditional herbal drug concentration was increased.

The percentage of inhibition versus concentration of Thai traditional herbal formulation at 1 and 6 mins was plotted as shown in Figure 2.

The percentage of inhibition of the absorbance of the $ABTS^+$was increased withthe increased Thai traditional herbal drug concentration. Ya-A showed the steepest curves of percentage of inhibition versus concentration, indicating that the anti-radical activity increased rapidly with concentration. Therefore, the antioxidant activity of Ya-A was higher than the others Thai traditional herbal formulation.

The concentration of Thai traditional herbal formula required to scavenge 50%ABTS free radicals (IC_{50}) is shown in Table 2. Low IC_{50} values indicated high radical scavenging

activity. IC_{50} of Ya-A (1.60 mg/mL) was lower than that of the others. Whereas the IC_{50} of Ya-F was 17.11 mg/mL that was higher than that of the others. There fore Ya-A showed the highest radical scavenging activity and Ya-F showed the lowest radical scavenging activity.

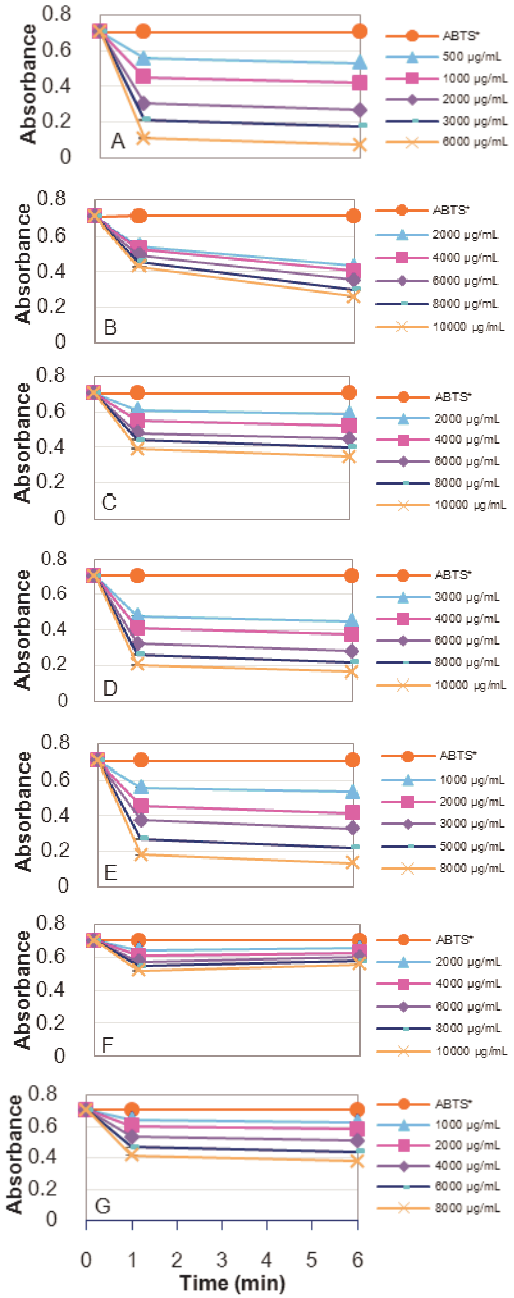

Figure 1 Effect of time on the suppression of the absorbance of the $ABTS^{+}$; (A)Ya-A, (B)Ya-B, (C)Ya-C, (D)Ya-D, (E)Ya-E, (F) Ya-F and (G)Ya-G at different Thai traditional herbal drug concentrations (500-1000µg/mL).

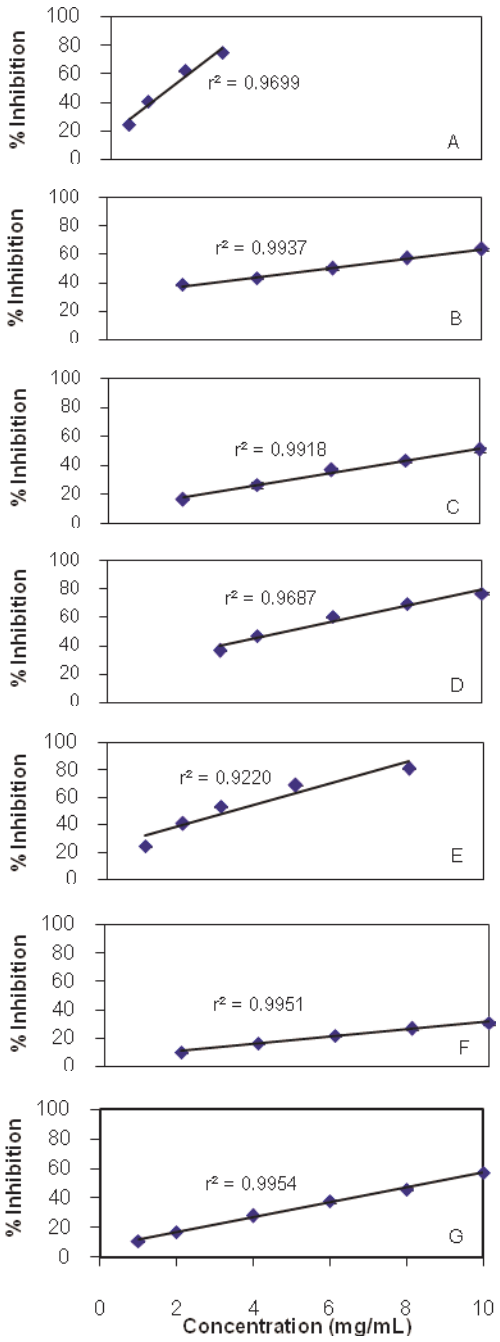

Figure 2 Effect of traditional herbal drug concentrations on the inhibition of the $ATBS^{+}$. (A)Ya-A, (B)Ya-B, (C)Ya-C, (D)YA-D, (E)Ya-E, (F)Ya-F and (G)Ya-G.

Table 2 IC_{50} of ABTS free radical and total phenolic content of Thai traditional herbal formulations.

Formulation	IC_{50} (mg/mL)	Total phenolic content (mg GAE/g dry weight)
Ya-A	1.60±0.04[a]	891.78±0.34[a]
Ya-B	5.84±0.01[b]	400.48±0.25[c]
Ya-C	9.55±0.05[c]	338.26±0.19[c]
Ya-D	4.78±0.01[b]	651.16±0.26[b]
Ya-E	3.33±0.02[b]	626.93±0.32[b]
Ya-F	17.11±0.07[d]	332.26±0.21[c]
Ya-G	8.57±0.02[c]	158.15±0.33[d]

Mean value ± SD (n=3)

[a-d]means with different letters within the same column were significantly different ($p<0.05$)

Total phenolic content of seven Thai traditional herbal formulations is shown in Table 2. Ya-A had higher total phenolic contents than other formula. Total phenolic contents of Ya-A was 891.78 mg GAE/g dry weight, while Ya-G showed the lowest total phenolic contents (158.15mg GAE/g dry weight). The results indicated that Ya-A Thai herbal tradition formulation had the high antioxidant activity and high total phenolic contents. Many studies supported that there was a high correlation between the antioxidant activity of some plants and their phenolic contents (Yan and Asmah, 2010).

Discussions and Conclusion

All seven Thai traditional herbal formula had the antioxidant activity and phenolic compounds. The antioxidant activity of all Thai traditional herbal formula was increased with concentration dependence. The antioxidant activity and total phenolic contents of Ya-A were higher than those of the other test Thai traditional herbal formulations. For on-going research the further antioxidant and other biological activities of the components in Ya-A including *Dracaena loureiri* Gagnep. ,*Ligusticum striatum* DC., *JasminumSambac* L., *Mimusopselengi* L., *Mesuaferrea* L., *Mammeasiamensis* T. and *Nelumbonucifera* Gaertn. should be investigated.

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