ฤทธิ์ต้านอนุมูลอิสระและปริมาณฟีนอลิกรวมจากส่วนต่าง ๆ ของบัวหลวง 2 พันธุ์

Antioxidant Activity and Total Phenolic Contents of Various Parts from Two Cultivars *of Nelumbo nucifera* Gaertn.

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บทคัดย่อ

อนุมูลอิสระจากกระบวนการทำงานของร่างกายหรือจากสิ่งแวดล้อมเกี่ยวข้องกับกระบวนการเสื่อมของร่างกายและการเกิดโรคเรื้อรัง สารต้านอนุมูลอิสระจากธรรมชาติจึงมีบทบาทสำคัญในการป้องกันและรักษาโรค บัวหลวง (*Nelumbo nucifera* Gaertn.) เป็นสมุนไพรที่ถูกนำมาใช้ประโยชน์ได้ทั้งทางยาและอาหาร บัวหลวงที่ปลูกในเมืองไทยมีหลายพันธุ์ การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบฤทธิ์ต้านอนุมูลอิสระของสารสกัดเมทานอลจากส่วนใบ เกสรตัวผู้ กลีบดอกชั้นนอก กลีบดอกชั้นใน ก้านใบ และฝัก ของบัวหลวง 2 พันธุ์ คือ บัวหลวงสีชมพูดอกซ้อน (สัตตบงกช) และบัวหลวงสีขาวดอกซ้อน (สัตตบุษย์) ผลการศึกษาพบว่าฤทธิ์ต้านอนุมูลอิสระโดยสมมูลกับสารมาตรฐานโทรลอกซ์ของตัวอย่างพืชที่ทำการทดสอบด้วยวิธี ABTS และ DPPH อยู่ในช่วง 14.9±3.54 ถึง 227.63±6.93 มก.โทรลอกซ์/ก.พืชแห้งและ 19.66±2.05 ถึง 285.05±22.51 มก.โทรลอกซ์/ก.พืชแห้ง ตามลำดับ ปริมาณฟีนอลิกรวม โดยสมมูลกับกรดแกลลิกอยู่ในช่วง 8.80±1.17 ถึง 109.90±4.37 มก. กรดแกลลิก/ก.พืชแห้ง โดยฝักของบัวหลวงสัตตบุตย์มีฤทธิ์ต้านอนุมูลอิสระและปริมาณฟีนอลิกรวมสูงที่สุด ผลการศึกษานี้เป็นแนวทางในการพัฒนาสารต้านอนุมูลอิสระจากธรรมชาติและการใช้ประโยชน์จากบัวหลวงทั้ง 2 พันธุ์

คำสำคัญ: บัวหลวง *Nelumbo nucifera* Gaertn. ปริมาณฟีนอลิกรวม ฤทธิ์ต้านอนุมูลอิสระ

Abstract

Free radicals generated from human body metabolisms or from environments are involved in development of degenerative diseases. Natural antioxidants could play an important role in prevention and cure of chronic diseases caused by free radicals. Sacred lotus or lotus (*Nelumbo nucifera* Gaertn.) is a widely used medicinal plant. Parts of lotus were used in Thai traditional medicine as cardio tonic and culinary. Different cultivars of lotuses are cultivated in Thailand. This study aimed to evaluate antioxidant activity and determine total phenolic contents from methanolic extract of leaf, stamen, outer petal, inner petal, stalk and seed pod of two cultivars of lotuses including white short flower (Sattabut) and pink short flower (Sattabongkoj) lotuses. The antioxidant activity by ABTS and DPPH methods ranged from 14.9±3.54 to 227.63±6.93 mgTEAC/gDW and 19.66±2.05 to 285.05±22.51 mgTEAC/gDW, respectively. Total phenolic contents were ranged from 8.80±1.17 to 109.90±4.37 mgGAE/gDW. The seed pod of the white short flower lotus (Sattabut) exhibited the highest antioxidant activity and total phenolic contents. This study provides basic information for development of natural antioxidants from the two lotus cultivars.

Keywords: lotus, *Nelumbo nucifera* Gaertn., antioxidant activity, total phenolic contents

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Introduction

Reactive oxygen species (ROS) or free radicals are generated as by-products of metabolisms in the human body. An excess of free radicals caused by losing balance in the oxidative defense system and environmental factors can damage biomolecules (lipids, proteins, DNA and etc.) and lead to many degenerative chronic diseases such as cancer and Alzheimer's disease. Current research has proved that antioxidants are able to decline the oxidative damage thus benefit human health[1]. The major natural antioxidants from plant food and medicinal plants are the phenolic compounds[2, 3].

Sacred lotus (*Nelumbo nucifera* Geartn.) a perennial aquatic plant, belongs to the family Nelumbonaceae It originated from East Asia and widely distributed throughout Asia including Thailand. Sacred lotus is one of the most useful plants. Almost all parts of the plants are used in different ways such as in religious ceremony and culinary[4]. In traditional medicine, parts of of *N. nucifera* have been used to treat many diseases and disorders. For example pollen was used as cardio tonic; seed was used as anti-cough agent, embryo of lotus was used to reduce high fever and increase blood circulation, seed and fruit were used in tissue inflammation[5]. Phytochemical studies reported alkaloids (nuciferine, neferine, nornuciferine, etc.) and flavonoids (quercetin, kaemferol, catechin etc.) as the main constituents[5]. Pharmacological studies of lotus reported antimicrobial activity of the essential oil from pollens[6], anti-inflammatory polysaccharides from leaf[7], hypoglycemic activity of rhizome[8] and leaf extracts[9], antipyretic activity of stalk extract[10], hepatoprotective effect of seed extract[11] and antioxidant activity of various parts of lotus e.g. seed, leaf, flower, etc[11-13].

The ABTS and DPPH scavenging assays are the well established methods for evaluation of *in-vitro* antioxidant activity of food, beverage and plant extracts[14]. In the ABTS method, ABTS is oxidized with potassium persulfate to give cation radical, $ABTS^{\cdot+}$, while in the DPPH method, a stable radical of $DPPH^{\cdot}$ is used. The antioxidant capacity of the test compound is estimated by hydrogen donating or single electron transfer property therefore scavenged the free radicals and resulted in inhibition of oxidation reaction[14,15].

N. nucifera was divided into cultivars by the shape and color of their flowers. White short flower (Sattabut) and pink short flower (Sattabongkoj) lotus (Figure 1) are cultivated widely as ornamental plants and they are of preference to be used in ceremony[4]. In terms of searching for sources of natural antioxidants, herein this work the antioxidant potential of various parts of two cultivars of *N. nucifera,* which are white short flower lotus (Suttabut) and pink short flower lotus (Sattabongkoj) were investigated by $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ scavenging assays. Total phenolic contents in parts of plants were determined in order to gather the information about antioxidant property of plant parts of the two cultivars of *N. nucifera*.

Materials and methods

Plant material

Nelumbo nucifera Gaertn. (Sattabut) and (Sattabongkoj) were collected from the horticulture field in Warinchamrab, Ubon Ratchathani Province, Thailand. The voucher specimens of the plants were deposited at the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University. The plants were cleaned and separated in to 6 parts, which were leaf, stamen, outer petal, inner petal, stalk and seed pod. The plant materials were oven dried at 50°C for 36-48 hr and then pulverized by blender. Powdered materials were kept in close container until used.

Extraction

Plant materials (400 mg) were soaked with 20 mL MeOH for 30 min. The mixtures were then placed on an orbital shaker with 200 rpm for 1 h. The extracts were centrifuged at 3000 rpm for 15 min. The methanol extracts were collected and subjected directly to determination of total phenolic contents and evaluation of antioxidant activity. Meanwhile, 2 mL of the methanol extracts were taken to dryness. Yields of extraction were calculated.

2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) free radical scavenging assay

The antioxidant activity was performed as described in Thaipong *et al* (2006) with modification[16].

The 0.15 mL of the extract (equal to 50 mg dry material/ mL) was transferred to a test tube. The 2.85 ml of ABTS assay solution was added, gently mixed and let stand in the dark for 7 min. The absorbance of the mixture was measure at 734 nm. The percentage of radical scavenging activity was calculated from the following equation: Radical scavenging activity (%) = $[(Abs_{control}-Abs_{sample})/Abs_{control}]\times100$. Trolox (0.025-0.6 mM) was used to create a calibration curve. The antioxidant capacities were calculated and expressed in mg equivalent to trolox antioxidant capacity per g dry material (mgTEAC/g DW).

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay

The antioxidant activity was investigated by DPPH radical scavenging assay based on the procedure of Kaewseejan *et al* (2015)[17]. A 0.2 mL of the extract (equal to 50 mg dry material/mL) was mixed with 1.8 mL of 0.1 mM DPPH. The mixture was kept in the darkness at room temperature for 30 min. After that the absorbance was immediately determined at 517 nm. Trolox (0.025-0.8mM) was used to create a calibration curve. The antioxidant capacities were calculated as described in ABTS method and expressed in mg TEAC/g DW.

Determination of total phenolic contents

Total phenolic contents were determined by Follin-Ciocalteu (FC) method as described in Ikram *et al* (2009) with modification[18]. In brief, a 0.75 mL of FC reagent (1/10 in distilled water) was placed in a test tube. A total 0.1 mL of the extract was added, mixed and kept in room temperature for 5 min. After that 0.75 mL of 6% (w/v) Na_2CO_3 was added, mixed and let stand in room temperature for 90 min, the absorbance at 725 nm was read. Gallic acid (0.05- 0.15 mg/ml) was used to create a calibration curve. Total phenolic contents were expressed in mg equivalent to gallic acid per g dry material (mgGAE/g DW).

Statistical analysis

The results were expressed as means±standard deviation (SD) of the three replications. The variance analysis (ANOVA) was performed to determine any significant differences ($p < 0.05$) between mean values using SPSS statistical software (SPSS 13.0 for windows). The correlation analysis of chemical constituents and antioxidant activities was carried out using the Pearson test.

Results

Extraction

The dry plant materials (400 mg) were extracted with 20 mL of MeOH. Two milliliter of the methanolic extract was taken to calculate for extraction yield. The extractable matters from different morphological parts of plants were varied as shown in (Table 1). The yield percentages of extractions from over all samples ranged from 9.58±0.80 to 30.25±1.80 % (w/w) in *N. nucifera* white short flower (Sattabut). The extraction yields of plant parts were descending as follow stamen> seed pod>leaf>inner petal>outer petal>stalk. In *N. nucifera* pink short flower (Sattabongkoj) the extraction yield ranged from 15.57±1.29 to 31.42±0.80 % (w/w) and the extraction yields of plant parts were in descending from stamen>outer petal>inner petal>leaf >seed pod>stalk. In both cultivars, stamen gave the highest extraction yield, whereas stalk gave the lowest extraction yield.

Antioxidant activity

The MeOH extracts from six parts of from *N. nucifera* white short flower (Sattabut) and pink short flower (Sattabongkoj) were evaluated for their antioxidant activity by ABTS and DPPH radical scavenging assays. The antioxidant activities were expressed in mg equivalent to trolox antioxidant capacity per gram dry plant material (mgTEAC/gDW). As shown in (Table 1), all the test extracts showed significantly different in antioxidant activity. In the evaluation of antioxidant by ABTS method, the antioxidant activity ranged from 16.92±1.58 to 227.63±6.93 mgTEAC/gDW in *N. nucifera* white short flower (Sattabut). The effectiveness of the extracts in scavenging of ABTS radical was in order: seed pod>stamen>inner petal>leaf> outer petal>stalk. In *N. nucifera* pink short flower (Sattabongkoj), the antioxidant capacity ranged from 14.90±3.54 to182.42±0.33 mgTEAC/gDW. The effectiveness of the extracts in scavenging of ABTS radical was in order: seed pod>stamen>leaf > inner petal >outer petal>stalk. By DPPH radical scavenging assay, the

antioxidant capacity of *N. nucifera* white short flower (Sattabut) ranged from 19.66±2.05 to 285.05±22.51 mgTEAC/gDW. The effectiveness of the extracts in scavenging of DPPH radical was in order: seed pod> stamen>leaf>inner petal>outer petal>stalk. The antioxidant capacity of *N. nucifera* pink short flower (Sattabongkoj) ranged from 19.84±0.48 to 224.70±11.55 mgTEAC/gDW. The effectiveness of the extracts in scavenging of the DPPH radical was in order: seed pod>stamen>outer petal>inner petal>leaf>stalk. The results revealed that in both lotus cultivars, seed pod extract exhibited strongest antioxidant activity followed by stamen extract. The extract that showed lowest antioxidant activity was from the stalk in both lotus cultivars. Comparing among all test samples seed pod of *N. nucifera* white short flower (Sattabut) was identified as the best source of antioxidants.

Total phenolic contents (TPC)

In this study, total phenolic contents (TPC) of various parts of the two lotus cultivars were determined by Follin-ciocalteu methods. The TPCs were expressed in mgGAE/gDW. The TPCs were varied in different parts of both lotus cultivars as shown is (Table 1). The TPCs of *N. nucifera* white short flower (Sattabut) ranged from 8.80±1.17 to 109.90±4.37 mgGAE/gDW. The TPC values of plant parts were descending in order: seed pod>stamen> leaf>inner petal>outer petal>stalk. In *N. nucifera* pink short flower (Sattabongkoj) the TPCs ranged from 9.20±0.88 to 84.90±3.51 mgGAE/gDW. The TPC values in different plant parts were descending in order: stamen>seed pod>leaf>outer petal>leaf>inner petal>stalk. Among all test samples, the highest TPC was detected in seed pod of *N. nucifera* white short flower (Sattabut).

The correlation analysis

The Pearson's correlation coefficient (*r*) between the antioxidant activity evaluated by ABTS and DPPH radical scavenging assays was 0.937. The Pearson's correlation coefficient (*r*) between total phenolic contents and antioxidant activity by ABTS assay were 0.950. The DPPH antioxidant activity was correlated to total phenolic contents with the coefficient (*r*) of 0.891.

Figure 1 *Nelumbo nucifera* white short flower (Sattabut) (A-B) and pink short flower (Sattabongkoj) (C-D)

Table 1 Extraction yield, total phenolic contents and antioxidant activity of *Nelumbo nucifera* "Sattabut" and *Nelumbo nucifera* "Sattabongkoj"

Name of cultivars	Parts used	Extraction yield (% w/w)	TPC (mgGAE/ g DW)	ABTS (mgTEAC/g DW)	DPPH (mgTEAC/g DW)
Sattabut	Leaf	25.33 ±2.04[a]	73.20 ± 6.10[a]	117.74 ±13.37[a]	156.98 ±15.79[a]
	Stamen	30.25 ±1.80[b]	88.20 ± 6.49[b]	153.14 ±4.30[b]	203.99 ±8.42[b]
	Outer petal	22.92 ±0.29[ac]	50.40 ± 0.51[c]	85.53 ±8.41[c]	105.33 ±2.92[c]
	Inner petal	24.58 ±0.88[a]	72.80 ± 2.52[a]	126.05 ±5.11[a]	133.83 ±3.68[d]
	stalk	9.58±0.80[d]	8.80 ± 1.17[d]	16.92 ±1.58[d]	19.66 ±2.05[e]
	Seedpod	27.42 ±0.63[e]	109.90 ± 4.37[e]	227.63 ±6.93[e]	285.05 ±22.51[f]
bold	Leaf	21.58 ±1.44[c]	61.90 ± 2.29[f]	93.47 ±2.29[cg]	69.96 ±2.98[g]
	Stamen	31.42±0.80[b]	84.90 ± 3.51[b]	156.33 ±2.83[f]	180.13 ±3.68[b]
	Outer petal	24.67 ±0.80[a]	61.40 ± 2.02[f]	108.48 ±0.65[g]	70.56 ±2.98[g]
	Inner petal	22.33 ± 0.38[c]	57.00 ± 3.41[f]	102.25 ±5.72[g]	69.25 ±9.45[g]
	stalk	15.57±1.29[f]	9.20 ± 0.88[d]	14.90 ±3.54[d]	19.84 ±0.48[e]
	Seedpod	19.48 ±1.17[g]	77.00 ± 6.22[a]	182.42 ±0.33[h]	224.7 ±11.55[h]

All values are means±SD obtained by triplicate analysis.

Values bearing the different letters in the same column are significantly different ($P<0.05$)

Discussion and Conclusion

An excess of free radicals leads to deterioration of cellular biomolecules, which could develop to aging and degenerative chronic diseases. Consuming of natural antioxidants may prevent and attenuate the progress of ailments[1]. Polyphenolic compounds are the major natural antioxidants detected in vegetables and medicinal plants[2,3]. In previous studies antioxidant activity from various parts of *N. nucifera* was reported[12,13, 19-22]. However the comparative study of antioxidant capacity from various parts of different cultivars of *N. nucifera* was not illustrated. Therefore the evaluation of antioxidant activity and determination of total phenolic contents of the two lotus cultivars, including, white short flower (Sattabut) and pink short flower (Sattabongkoj) was conducted.

In this current study we found that methanolic extracts from parts of *N. nucifera* which were, leaf, stamen, outer petal, inner petal, seed pod and stalk were able to scavenge free radicals. The antioxidant capacity tested by ABTS and DPPH methods were highly correlated (r=0.937). This indicated that the antioxidant capacities of the plant parts tested by DPPH and ABTS methods were in agreement. These results were in agreement with previous reports[12,13, 19-22]. The extractable yields displayed the potential of different plant tissues as sources of natural antioxidants. The stamen of both cultivars offered the highest extraction yields which were 30.25 ±1.80% in white short flower lotus (Sattabut) and 31.42±0.80% in pink short flower lotus (Sattabongkoj) when methanol was used as a solvent. Wu *et al* (2011) reported the highest extraction yield of lotus from leaf (24.54±1.72%) when acetone was used as a solvent, while stamen yielded 10.20% of extract[13]. The higher polarity of methanol may affect the extraction yield as well as the total phenolic contents. Choe *et al* (2010) reported the antioxidant activity of lotus leaf extracted by different solvents. The yield of extract using MeOH (9.67%) was higher than EtOH (6.95%). At the concentration of 5 mg/ ml, the methanolic extract (92.8±0.15%) was able to inhibit DPPH radical better than ethanolic extract (59.2±0.54%). The total phenolic contents of methanolic extract (15.2±0.1 mgGAE/g extract) was also higher than that of ethanolic extract (9.8±0.4 mgGAE/g extract)[20]. This suggested that MeOH is a suitable solvent for extraction of antioxidant compounds.

Antioxidant activity of both *N. nucifera* was significantly different among various morphological parts. Considering the effectiveness of plant parts in radical

scavenging both cultivars showed the similar trend of antioxidant activity in parts of plants. In both ABTS and DPPH antioxidant assays, seed pod showed the highest antioxidant activity follow by stamen, while the stalk exhibited the lowest antioxidant activity. The antioxidant activity of the seed pod was about 10 times higher than that of the stalk. These finding was in agreement with Wu *et al*. (2011), therein antioxidant activity of acetone extracts from 10 parts *N. nucifera* was studied. Considering only the same plant parts with our study, they reported that seed pod was the most active part (IC_{50}= 9.39 µg by DPPH method and 132.50 µg by ABTS method)[13]. The dimeric procyanidins was antioxidative content in seed pod[23]. In addition, Wu *et al.* (2011) reported the antioxidant activity of other parts of *N. nucifera* that were stamen (IC_{50} = 22.87µg by DPPH method and 390.82 µg by ABTS method), leaf (IC_{50} = 21.15 µg by DPPH method and 285.17 by ABTS method), flower (IC_{50} = 21.96 µg by DPPH method and 16.30 µg by ABTS method) and stalk (IC_{50} = 144.30 µg by DPPH method and 1506.64 µg by ABTS method). The leaf part of *N. nucifera*, which is the most available part of plants showed good antioxidant activity[13]. In our study, leaf and petal were ranked together after stamen and seed pod in antioxidant capacity. The stalk showed the weakest antioxidant activity in all test materials corresponding to the previous study[13]. Lin *et al* (2009) found that the methanolic extract of lotus leaf showed DPPH scavenging activity at EC_{50} of 12.3 ±0.7 µg/ml and TPC 118.0+1.7 mgGAE/g extract. The phenolic compounds of flavonoid structures such as quercetin, catechin and their glycosides are responsible for antioxidant activity of the leaf extract[19]. In addition, here in this study we measured antioxidant activity of outer petal and inner petal separately. The results displayed that the antioxidant activity of the two parts were not significantly different in pink short flower lotus (Sattabongkoj), while in white short flower lotus (Sattabut) the inner petal showed significantly higher antioxidant activity than the outer petal. In over all the petals of white short flower lotus (Sattabut) exhibited stronger antioxidant activity than that of pink short flower lotus (Sattabongkoj). This finding is consistent with previous study. Venkatesh and Dorai (2011) described that the white flower lotus was more potent than the pink flower lotus[22]. Comparison between two cultivars, almost all parts of *N. nucifera* white short flower lotus (Sattabut) possess higher antioxidant activity and contain higher level of total phenolic contents than pink short flower lotus (Sattabongkoj). To our knowledge this study is the first study that provided a comparative activity between plant parts from different lotus cultivars.

The correlation between the polyphenol contents and antioxidant activity has been demonstrated in many plant species. Total phenolic contents and ABTS and DPPH radical scavenging activity are correlated, indicating that the polyphenol compounds are attributed to antioxidant activity. The seed pod of white short flower lotus (Sattabut) contained the highest total phenolic contents and showed the highest antioxidant activities determined by both methods hence the phenolic compounds were the main component in these plant parts and contributed to antioxidant capacity. The correlations between the total phenolic contents and DPPH and ABTS radical scavenging activity were supported by the high Pearson's coefficients (r).

In conclusion this study has demonstrated the antioxidant activity of various parts of two different cultivars of *N. nucifera*, namely white short flower lotus (Sattabut) and pink short flower lotus (Sattabongkoj). Comparing among parts of plants, seed pods showed the highest antioxidant activity and total phenolic contents, while stalks showed lowest antioxidant activity and total phenolic contents in both cultivars. The seed pod of *N. nucifera* white short flower lotus (Sattabut) was identified as the best source for antioxidants among the test materials.

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