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Original Article

Antioxidant activity and DNA fingerprint of four varieties of lotus stamens (*Nelumbo nucifera* Gaertn.)

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Abstract

DNA fingerprints of stamens of four varieties of *Nelumbo nucifera* (Gaertn) were established and their antioxidant activity was studied. PCR amplification was used for identification of Lotus DNA by using OPS3, OPS11, OPS13 and OPE3 random decamer-primers. The result showed variety-specific markers of Pathum, Sattabongkot, Boontharik and Sattabuut varieties. The antioxidant activities of solutions in methanol and mixed-solvent of the four varieties were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) model. The antioxidant activities (IC_{50}) of methanol solution of the four varieties were 68.30 6.30, 62.22 4.00, 31.60 3.40 and 40.90 1.50 $g.mL^{-1}$, respectively, whereas those of the mixed-solvent solution were 2.21 0.06, 2.23 0.05, 1.29 0.02 and 1.83 0.07 $mg.mL^{-1}$. The result showed that IC_{50} of both solutions of Sattabongkot were significantly low ($p<0.05$, at the confidence level of 95%), indicating a higher activity than the others.

Keywords: DNA fingerprint, *Nelumbo nucifera*, DPPH

1. Introduction

Three commonly used and well known recipes of Thai herbal medicine are "The Total 5 Types Stamens", "The Total 7 Types Stamens" and "The Total 9 Types Stamens". Lotus stamen is one of the type in all the recipes. The indications of all recipe's are antipyretic, nerve and heart tonic and refreshing (Picheansoonthon, 1999; Boonyapraphatsara, 1980). Main chemical compositions of lotus stamens are flavonoids, alkaloids and carotenoids (Chinese-Thai Expert Committee on Technology Transfer'of Herbal Medicines, 2004). None of the carotenoids detected was betacarotene (Aromdee and Phonkot, 2005). The indications and pharmacological activity of lotus stamen has not been proved. However, it has been long known that flavonoids are good as chelating agents with transitional metals, and have been proved to be cardioprotective and also easily oxidized due to the phenolic constituent (Heim *et al.*, 2002). Carotenoids also act as scavengers for alkyl radicals by stabilization with resonance formed, and stop the initiation of lipid peroxidation which causes of many diseases (Belitz and Grosch, 1999). The antioxidant activity of lotus stamen of methanol and ethyl acetate extracts in scavenging authentic peroxynitriles (ONOO-) system, 1,1-diphenyl-2-picrylhydrazyl (DPPH) system and total reactive oxygen species (ROS) system were reported (Jung *et al.*, 2003). In Thailand, there are at least four varieties of commonly available Royal Lotus that is used in Thai herbal medicine, Pathum, Boontharik,

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Sattabongkot and Sattabuut (Picheansoonthon, 1999). The purpose of this study is to compare the antioxidant activity, using DPPH as a representative model, of the four varieties. Once stamens were picked out from flowers, it is difficult to distinguish or identify the origin. Thus DNA fingerprinting of these lotus stamens was carried out to establish their identity.

2. Material and Methods

2.1 Plant specimens

Four varieties of lotus stamens (*Nelumbo nucifrea* Gaertn.) were collected from the central and northeast of Thailand. Stamens were picked out and air dried for 7 days and turned over occasionally.

2.2 Equipment and Chemicals

UV-VIS Spectrophotometer (Shimadzu UV-1240 mini, Japan), Electrophoresis instrument, DNA amplifying instrument (Palm Cycle, Corbett research, Australia) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Fluka, techn.; ≥ 85% (CHN)), solvents and chemicals were analar or molecular grades. The 100 bp DNA Ladder and chemical for PCR mixture were from Fermentas and primers from Operon.

2.3 Assay for DPPH radical scavenging activity

1) Preparation of sample solutions

Methanol extracts: weighed 0.5 g of sample into a 10 mL volumetric flask, added methanol to the volume and mixed. Each sample mixture was allowed to stand in the dark for 24 hrs, then filtered and the solution kept in the dark.

Mixed-solvent extracts: weighed 2.5 g of sample into a 50 mL volumetric flask, added a mixture of solvents (hexane : acetone : methanol : toluene = 10 : 7 : 6 : 7) to the volume and mixed. Allowed each sample mixture to stand in the dark for 24 hrs, then filtered and kept the solution in the dark.

2) Determination of the DPPH radical scavenging activity (Pourmorad et al., 2006)

Varying amounts of the solutions were mixed with 200 L of 1mM of DPPH, and methanol was then added to make a final volume of 3 mL. The mixture was allowed to stand for 15 minutes. The absorbances of the resulting solutions were measured at 517 nm. The IC_{50} was calculated from the concentration at which 50% of 1mM DPPH was scavenged.

2.4 DNA fingerprint

1) DNA extraction

DNA extraction method was adapted by Wangsomnuk *et al.* from the method described by Tai and Tanksley, 1990; Singh and Ahuja, 1999. The quantity and quality of the isolated DNA was determined by electrophoresis using 1% agarose gel, and stained with ethidium bromide and observed under the UV light at 366 nm.

2) DNA amplification

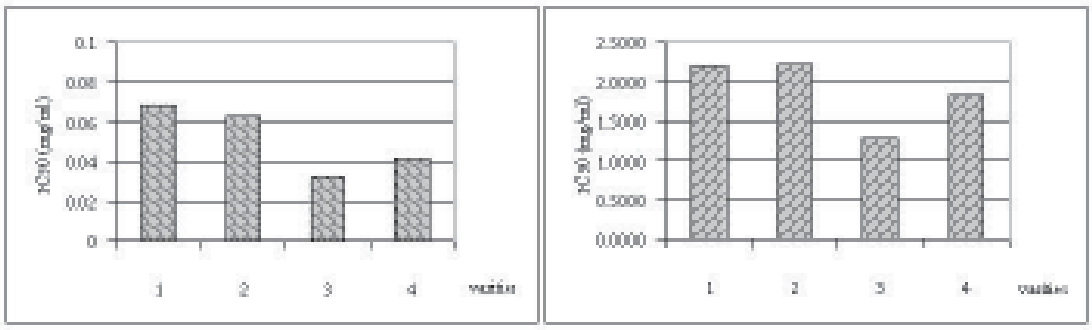
Approximately 40 ng of genomic DNA was used as a template for PCR with rRNA specific primers (5S-P1 + 5S-P2) and thirteen random decamer-primers (OPA10, OPA15, OPA20, OPE2, OPE3, OPE5, OPE9, OPE10, OPS2, OPS3, OPS4, OPS11, OPS13). The reaction was in a final volume of 10 L containing 5.32 L distilled water, 1 L 10x PCR buffer, 0.6 L 25 mM $MgCl_2$, 1 L 2 mM dNTP, 0.08 L 0.625 U Taq DNA polymerase, and 1 L of each primer (10pM). RAPD-PCR amplification was carried out using a thermal cycle programmed (Corbett Research) of 3 min at 95°C for initial DNA denaturation, followed by 45 cycles of denaturation at 94°C for 30s; annealing at 40°C for 30s; extension at 72°C for 1.5s. The final cycle was followed by a 5 min at 70°C. The PCR amplification products were resolved in 1.2% agarose gels in 0.5x TBE buffer at 100 V for 90 min and stained with ethidium bromide. The PCR profiles were visualized under UV light at 366 nm, photographed and stored for further analysis.

2.5 Statistical analysis

Program SPSS was used to analyze DPPH data with descriptive statistics and t-test.

3. Results and Discussion

The value IC_{50} of DPPH scavenging of lotus stamen solutions in methanol of Pathum, Boontharik, Sattabongkot and Sattabuut were 68.30 6.30, 62.22 4.00, 31.60 3.40 and 40.90 1.50 $g.mL^{-1}$ respectively, and those of mixed-solvent solutions were 2.21 0.06, 2.23 0.05, 1.29 0.02 and 1.83 0.07 $mg.mL^{-1}$, respectively. Graphs illustrating the activity are shown in Figures 1(A) and 1(B). The IC_{50} of DPPH scavenging of Sattabongkot solutions in both solvents was significantly lower than that of others ($p<0.05$, at the confidence level of 95%), whereas the methanol solutions were stronger than the mixed solvents about 40 folds. The methanol solution contained flavonoids due to their similar polarity (Phonkote and Aromdee, 2006; Harborne, 1998). The mixed-solvent solution contained carotenoids (Phonkote, and Aromdee, 2006; Horwitz, 2000). DPPH method is useful as a simple screening test to see the difference of lotus stamens samples.



(A) Methanol extract **(B) Mixed solvent extract**

Figure 1. Antioxidant activity (IC_{50}) of 4 varieties Lotus Stamens Pathum (1), Boontharik (2), Sattabongkot (3) and Sattabuut (4)

A yield of good quality and quantity of genomic DNA of four varieties lotus stamens was obtained using this method and enough to conduct PCR reactions. Using rRNA specific primers, 5S-P1 and 5S-P2 could give approximately 470 bp-PCR products from all of the lotus studies. These could be further analyzed for their sequences, and compared with available data in the public database. Four primers, S3, S11, S13 and E3, were chosen from thirteen random decamer primers for RAPD-PCR amplification from Lotus DNA. The results showed that the amplified DNA fragments of four varieties lotus stamens were different in size (Figure 2) ranging from 300 to 2,300 bp. Consideration of S3 and S13 primers amplification products, 450, 370 and 850 bp were variety specific to Pathum, Boontharik and Sattabuut, respectively. There were also monomorphic bands obtained when using S3, S11 and E3 primers on four lotus varieties. E3 primer exhibited monomorphic of 4 varieties at PCR product band size 300 bp. Furthermore a polymorphic 450 bp fragment was only present while using DNA of Sattabongkot and Sattabuut as templates.

4. Conclusion

In this study identity and antioxidant activity of lotus stamen specimens was determined. This preliminary study indicated that stamens from Sattabongkot gave higher antioxidant activity than the others. The PCR amplification used for identification of these four varieties of lotus stamens can be used to establish the DNA fingerprint. Overall, this study should be applicable to the quality of lotus stamen specimens four varieties of lotus stamens by antioxidant activity and genetic identification.

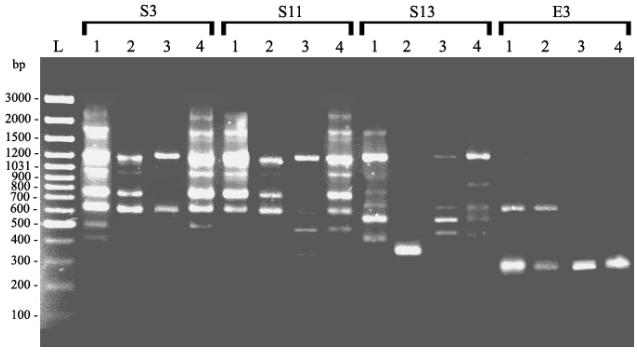


Figure 2. PCR product, using primer S3, S11, S13 and E3 L: 100 bp DNA Ladder Plus (Fermentas), 1-4: DNA of 4 varieties Lotus Stamens

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