Antioxidant activity of *Nymphaea odorata* and *Nelumbo nucifera* from Nymphaeales order

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Summary

The antioxidant activity of *Nymphaea odorata* and *Nelumbo nucifera* collected from AIMST University pond were determined. The free radical scavenging activity of the different parts (flowers, leaves and stems) of *N. odorata* and *N. nucifera* were assessed with the aid of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. In general, *N. odorata* showed high free radical scavenging activity compared with *N. nucifera* especially in the case of *N. odorata* leaves with 34.71% scavenging activity at 1.0 mg/ml concentration tested. In particular, both plant leaves showed a higher free radical scavenging activity followed by flower and stem. The reference antioxidant BHT and vitamin E showed relatively higher antioxidant activity compared with the both plants tested in this study. This finding indicated that the methanolic extract of *Nymphaea odorata* and *Nelumbo nucifera* was a starting material for the isolation of compound(s) with effective activities as radical's scavengers.

Key words: Antioxidant activity, *Nymphaea odorata, Nelumbo nucifera,* DPPH radical scavenging

Introduction

Lipids containing polyunsaturated fatty acids are readily oxidized by molecular oxygen, and such oxidation proceeds by a free radical chain mechanism[1]. Lipid peroxidation can lead to aging, coronary heart disease, stroke, diabetes mellitus, rheumatic disease, liver disorders, multiple sclerosis, Parkinson's disease, autoimmune disease, Alzheimer's and carcinogenesis[2,3]. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases[4]. The adverse effect showed by the common antioxidant has forced scientists into looking for new antioxidant substances from various sources like medicinal plants[5]. In this respect, in the present study, 2 medicinal and ornamental plants *Nymphaea odorata* and *Nelumbo nucifera* different parts i.e. flowers, leaves and stems extracts were screened to determine their free radical scavenging and antioxidant activities.

Material and methods

Plant Collection and Extraction

Samples of two different plants species of Nymphaeales order from namely *Nymphaea odorata* and *Nelumbo nucifera* were collected from AIMST University, Kedah, Malaysia, in June of 2007. Plants were identified by a botanist of Department of Biotechnology, AIMST University, Kedah, Malaysia. Flowers, leaves and stems of selected plants were dried (room temperature) and powdered with a mortar.

Preparation of Plant Extracts

Some 100g of dried and powdered plant material were extracted at room temperature with 500 mL of methanol under constant shaking for 24 h. After filtration, the methanolic (MeOH) solutions were evaporated to dryness in a rotary evaporator for the antioxidant assays.

2,2-Diphenyl-1-Picrylhydrazyl Radical (DPPH) Scavenging Assay

Quantitative measurement of radical scavenging properties was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (80% (v/v) MeOH as a blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol. The commercial antioxidant butylated hydroxytoluene (BHT, Sigma) was used for comparison or as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements were taken at least in triplicate. DPPH radical's concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = A_o - A_1 / A_o \text{ X100}$$

Where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample (crude extract of the selected plants)[6]. The actual decrease in absorption induced by the test compounds was compared with the positive controls.

Statistical Analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard error or standard deviation (SD). The differences between the extracts were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using the SPSS version 12 programmer.

Results and discussion

To find new natural sources of active compounds, we studied the antioxidant potential of different parts of *Nymphaea odorata* and *Nelumbo nucifera*. Table 1 presents extraction yields (expressed as w/w percentages), obtained for all the extracts of flowers, leaves and stems of *Nymphaea odorata* and *Nelumbo nucifera*. Despite the low values obtained for the extraction yields, the antioxidant contents found were very good, indicating that the methanol extraction was efficient in this study.

Table 1. Extraction yields of 100g *Nymphaea odorata* and *Nelumbo nucifera*

Extraction yield		**leaves**	**flowers**	**stem**
Nymphaea odorata	(%)	9.53	5.93	3.11
Nelumbo nucifera	(%)	8.25	5.18	3.17

The antioxidant properties of the methanol extracts of flowers, leaves and stems of *Nymphaea odorata* and *Nelumbo nucifera* were examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Figure 1 and Figure 2 as comparable with known antioxidant BHT and vitamin E. The radical-scavenging activity (RSA) values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of $DPPH^{\bullet}$ solution in the absence of extract at 517 nm. From the analysis of Figure 1 and Figure 2, we can conclude that the scavenging effects of *Nymphaea odorata* better than the *Nelumbo nucifera* extracts on DPPH radicals, especially in the case of *Nymphaea odorata* (34.71% at 1.0 mg/ml for the leaves and 19.09 % for the flower, at the same concentration). However, *Nymphaea odorata* stem revealed a low value of antioxidant activity compared with other parts i.e. 4.24% at 1.0 mg/ml of extract tested. The free radical scavenging activity for various parts of *Nelumbo nucifera* were 12.40%, 17.73% and 33.70% for the stem, flower and leaves respectively.

As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant BHT and vitamin E, the reference antioxidant for the *Nymphaea odorata* and *Nelumbo nucifera* (Figure 1 and Figure 2). In other words, BHT showed the highest antioxidant activity follow by vitamin E, stem, Flower and leaves for both plant extract tested.

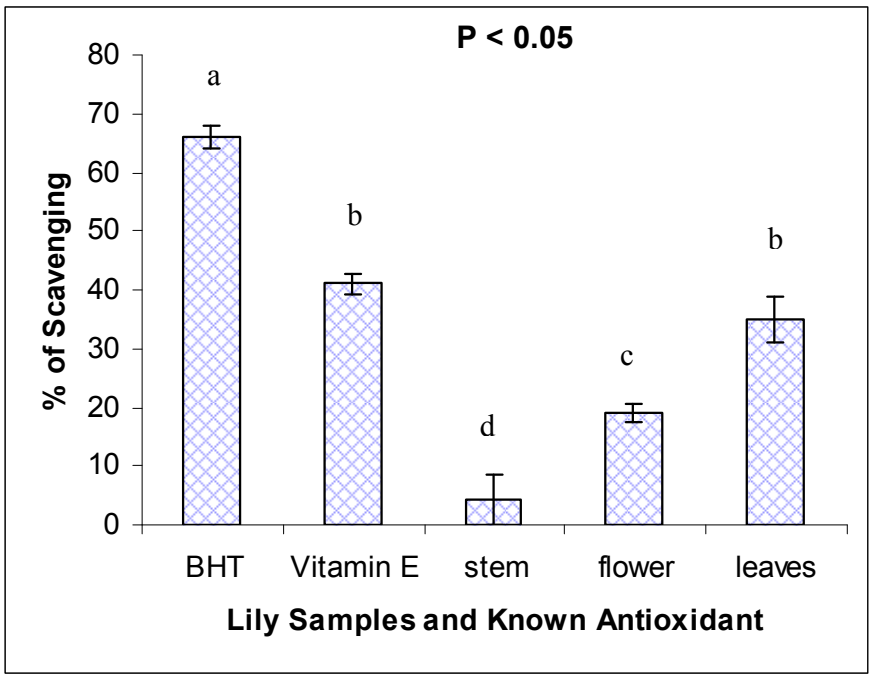

Figure 1. Scavenging effect (%) of crude extract of *Nymphaea odorata* and known antioxidant at 1.0 mg/ml

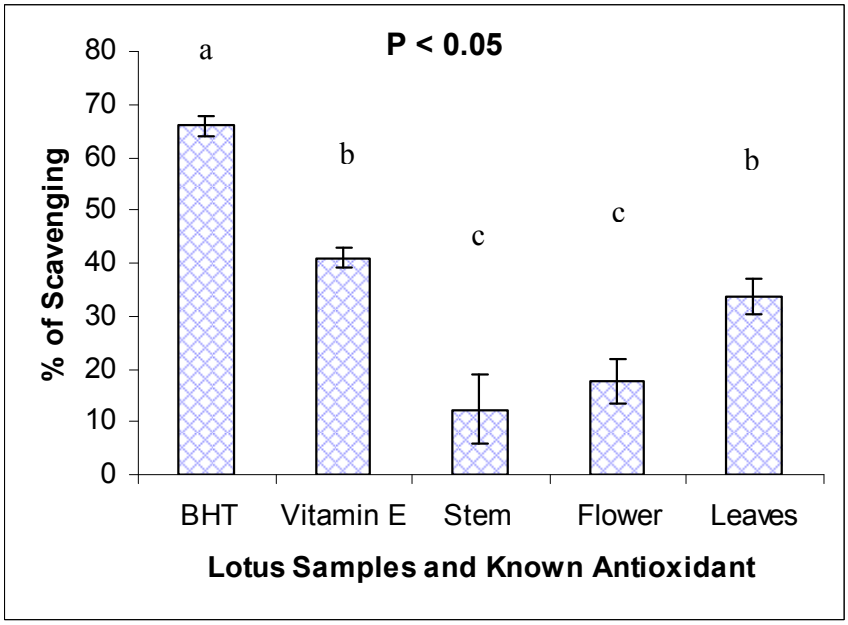

Figure 2. Scavenging effect (%) of crude extract of *Nelumbo nucifera* and known antioxidant at 1.0 mg/ml

In conclusion, the screening of antioxidant activity performed on various parts of *Nymphaea odorata* and *Nelumbo nucifera* which was traditionally used as herbs shows that they are endowed with potentially exploitable free radical scavenging activity. Hence, *Nymphaea odorata* and *Nelumbo nucifera* could be used as an easy accessible source of natural antioxidants, as a food supplement, or in the pharmaceutical and medical industries. Further work should be performed to isolate and identify the antioxidant components.

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