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Evaluation of total phenolic content and antioxidant activity of *Nelumbo nucifera* seed from north of Iran

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Abstract

In this study antioxidant properties and total phenolic content of the extracts of *Nelumbo nucifera* seeds were examined. The extracts were prepared using the solvents water, methanol, water : methanol (1:1), ethyl acetate, acetone and chloroform. Two experimental methods including DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging activity and total antioxidant capacity were used for characterization of antioxidant activity of the extracts. The six extracts showed varying degrees of efficacy in each assay in a dose-dependent manner. The acetone extract with the highest amount of total phenolic content (20.12 ± 0.02 g GAE/100 g dried extract), was the most potent antioxidant in both assays used. The LSA exhibited strong free radical scavenging activity as evidenced by the low EC_{50} values in DPPH assay (4.28 µg/ml) and in total antioxidant assay by maximum activity (25% of ascorbic acid). On the basis of the results, the acetone extract of *Nelumbo nucifera* seeds were found to serve as a potential source of natural antioxidants.

Keywords: Total phenolic content; DPPH˙ scavenging activity; Antioxidant activity

1. Introduction

Nelumbo nucifera Gaertn. (Lotus), of the family Nymphaeaceae, is a perennial, rhizomatous, and aquatic plant distributed throughout Asia and Egypt. All parts of *N. nucifera*, namely the leaves, flowers, embryos and rhizomes, have been used as foodstuffs and as traditional medicines in china and India [1]. Moreover, many biological and pharmacological studies of each part of the plant have been performed [2].

There is great interest in the use of naturally occurring antioxidants for treatment or prophylaxis of various oxidative stress-related diseases [3]. *Nelumbo nucifera* seeds are commonly used as folk remedy in the treatment of tissue inflammation, cancer, antiemetic, given to children as diuretic and refrigerant. It is also used as a cooling medicine for skin diseases, leprosy and considered as antidote to poison [4]. The hydro alcoholic extract of *Nelumbo nucifera* seeds was reported to possess hepatoprotective and free radical scavenging activity [5,6], antifertility activity[7] and also suppress cell cycle progression, cytokine gene expression, and cell proliferation in human peripheral blood mononuclear cells [8].

Phenolic compounds from vegetables, fruits, grains, beverages and medicinal plants have attracted a great deal of attention because of their significant antioxidative activities [9]. Antioxidant activity of various parts of *Nelumbo nucifera* is well established, e. g. leaf, stamens and rhizomes [6]. A literature survey showed that the methanolic extract of *N. nucifera (Lotus)* seed and its various fractions (dichloromethane, ethyl acetate, *n*-butanol and water) have been found to be rich in phenolic compounds [10]. Several phytochemical constituents, including caffeic acid, chlorogenic

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acid, *p*-hydroxybenzoic acid, gallic acid and a large amount of phenolic compounds, as found in boiling water extract of lotus seeds, were contributed to the antioxidant activity of this extract and its effect on DNA damage in human lymphocytes [11]. Antioxidant activity of hydro alcoholic extract of *N. nucifera* seeds was studied using *in vivo* and *in vitro* models. The hydro alcoholic extract exhibited strong free radical scavenging activity as evidenced by the low EC_{50} value in DPPH method (6.12±0.41 µg/ml) [6]. A methanol extract of the stamens of *N. nucifera* was evaluated for its potential to scavenge DPPH radicals. The methanolic extract was fractionated with dichloromethane, ethyl acetate and *n*-butanol. The ethyl acetate exhibited strong antioxidant activity in DPPH, total ROS and $ONOO^-$ scavenging/inhibitory tests.[10]

To our knowledge, there are no reports that detail the antioxidant activity of the various extracts from the *N. nucifera* seeds. Therefore, the aim of the present study was to evaluate the antioxidant properties of the various solvent extracts (water, methanol, aqueous methanol, ethyl acetate, acetone and chloroform) of lotus seeds using DPPH radical scavenging activity and total antioxidant capacity methods.

2. Experimental

2.1. Chemicals and methods

The extraction of antioxidant compounds and total phenolics from dried and finely powdered seeds were carried out using six various solvents to compare the effect of extraction solvents on antioxidant activity, content of total phenolic compounds and radical scavenging activity. These solvents included water, methanol, methanol/water (50:50, v/v), acetone, ethyl acetate and chloroform. 1 g of plant sample was extracted with 3 × 20 ml of solvent on shaker at room temperature, which the solvent became colorless. Each extract was centrifuged and filtered through Whatman No. 1 filter paper. The filtrate was evaporated to dryness in vacuo at 40 °C in a rotavapor. The dried sample of each extract was weighed to determine the yield of soluble constituents and stored at 4 °C until use.

2.2. Determination of total phenolic content

The total phenolic contents of the extracts were determined by the Folin-Ciocalteu assay; a modified method was proposed by Salehi [12]. In the test tube appropriate volume of extract solutions in 1.6 ml water (10-100 microgram of dried extract) was mixed with 100 µl Folin-Ciocalteu reagent. After 3 min. 300 µl of the sodium carbonate solution (7% w/v) was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. All the experiments were conducted using gallic acid as a calibration standard and the results were recorded as mg of gallic acid equivalent per g of dried extracts (GAE, g/100 g of each extract).

2.3. DPPH radical scavenging capacity

The antioxidant activity of the plant extracts was determined according to the Blois method [13] with a slight modification. Free radical scavenging capacity was evaluated by measuring the scavenging activity of LS extracts on the 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical. Briefly, 1 ml of a 0.1 mM solution of DPPH radical in methanol was mixed with 2 ml of extract solution in 50% methanol. The absorbance of the resulting solutions and the blank (with same chemicals, except for the sample) were recorded after 15 min at room temperature against acid ascorbic and *tert*-butylated hydroxy anisole (BHA) as positive controls. For each sample, three replicates were recorded. This activity is given by %DPPH radical scavenging calculated according to the following equation:

%DPPH radical scavenging= [(control absorbance- extract absorbance/control absorbance)] ×100.

2.4. Antioxidant activity

The antioxidative capacity of all extracts was determined by the reduction of Mo(VI) to Mo(V) and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH. An aliquot of 0.3 ml of sample solution (containing 10–300 µg of dried extract in corresponding solvent) was combined in a glass tube with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90 min. After cooling the samples to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples [14].

2.5. Statistical analysis

Data were recorded as means ± standard deviation of triplicate measurements. Analysis was performed using GraphPad Prism version 2.00 for windows.

3. Results and discussion

3.1. Total phenolic content

The total phenolic content in the six extracts of *Nelumbo nucifera* seeds were determined by the Folin-Ciocalteu assay. The yield and total phenolic contents for different extracts from *Nelumbo nucifera* seeds are shown in Table 1. The amount of extractable components expressed as percentage by weight of dried seeds ranged from 2.15% (ethyl

acetate extraction) to 16.95% (aqueous methanol extraction). The amount of total phenolic content (in gallic acid equivalents) expressed as percentage by weight of dried extract ranged from 0.56% in chloroform extract to 20.12% in acetone extract. Acetone was found to be the most effective solvent in extraction of phenolic compounds from *Nelumbo nucifera* seeds.

Table 1. Extract yield, total phenolic content and comparison of DPPH radical scavenging capacity of the *Nelumbo nucifera* seed extracts, BHA and ascorbic acid.

Extracts	Yield†	Total phenolics‡	EC_{50}(µg/ml)±
LSA	3.55 ± 0.08	20.12 ± 0.02	4.28
LSM	15.65± 0.12	2.05 ± 0.03	22.66
LSWM	16.95± 0.19	1.06 ± 0.05	42.24
LSW	10.30± 0.25	2.12 ± 0.01	27.00
LSEA	2.15± 0.02	3.06 ± 0.01	na
LSCh	2.90± 0.01	0.56 ± 0.08	na
BHA	-	-	0.27
AA	-	-	0.85

†Grams of extract per 100 g of dried seeds; ‡grams of gallic acid per 100 g (dry weight) of extract.
±The effective concentration of the extract at which DPPH radicals were scavenged by 50%. na, not active. LS, Lotus Seeds; W, Water; M, Methanol; A, Acetone; EA, Ethyl acetate; Ch, Chloroform, AA, Ascorbic acid and BHA, *tert*-butylated hydroxy anisole.

3.1. Scavenging effect on DPPH radical

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others. The scavenging activity of extracts against DPPH· was determined according to the Blois method. The EC50 value, which represented the amount of extract required to scavenge 50% of DPPH radicals present in the reaction mixture, was determined by nonlinear regression analysis from the obtained %DPPH radical scavenging values and the results are shown in Table 1. The acetone extract that contained the highest amount of total phenolics, was found to be the most active radical scavenger followed by methanol and water extracts. However, the acetone extract was not as effective as the positive controls, BHA and ascorbic acid (Table 1). The ethyl acetate and chloroform extracts have no activity demonstrated in test.

The relationship between the concentration of lotus seeds extracts and the scavenging activity for DPPH free radicals is shown in Fig 1. It can be seen that the acetone extract is the most active radical scavenger.

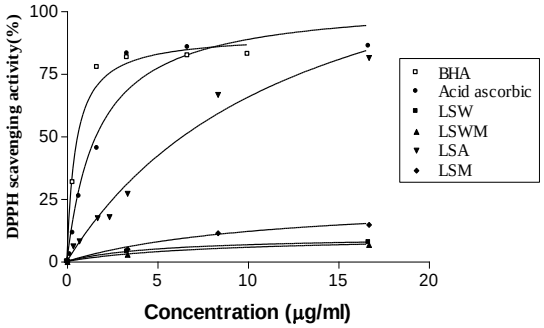

Fig. 1. DPPH radical scavenging activities of acetone, methanol, aqueous methanol and water extracts of Nelumbo nucifera seeds. BHA and ascorbic acid were used as positive controls. Percentage radical scavenging capacity relative to control. For abbreviation refer to table 1.

3.1. Total antioxidant activity

In the phosphomolybdenum assay, which is a quantitative method to evaluate total antioxidant capacity, the extracts exhibited some degree of activity in a dose-dependent manner; however, the activities were inferior to that of ascorbic acid. The results are shown in Fig. 2 simply in bar graph demonstration. In this test acetone extract was more effective and methanol and water extracts were found similar in their action, ethyl acetate and chloroform again being the lowest in activity. The extracts demonstrated electron-donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products [15].

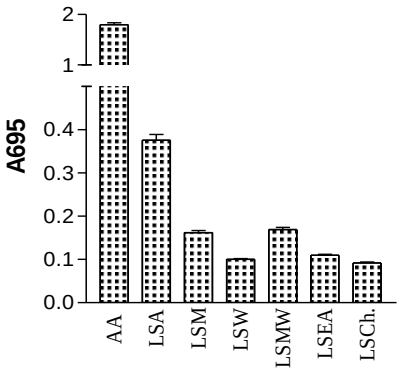

Fig. 2. Total antioxidant activities of six extracts of *Nelumbo nucifera* seeds. Acid ascorbic was used as positive control. Each bar represents mean ± standard deviation from triplicate samples. For abbreviation refer to table 1.

4. Conclusion

In conclusion, the various solvent extracts from *Nelumbo nucifera* seeds showed varying degrees of antioxidant activity in different test methods in a concentration-dependent manner. The acetone extract contained the highest amount of phenolic compounds and also has the strongest antioxidant capacity. Therefore acetone proved to be the most efficient solvent for extraction of antioxidants

(phenolics, alkaloids and saponins) from *Nelumbo nucifera* seeds grown in north of Iran.

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اندازه گیری محتوای فنلی تام و فعالیت آنتی اکسیدانی دانه لاله مردابی شمال ایران

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چکیده:

در مطالعه حاضر خواص آنتی اکسیدانی و محتوای فنلی تام عصاره های دانه لاله مردابی اندازه گیری شد. این عصاره ها در حلال های آب، متانول، آب:متانول(۱:۱)، اتیل استات، استن و کلروفرم تهیه شدند. برای تعیین خواص آنتی اکسیدانی عصاره ها از دو روش شامل فعالیت به دام اندازی رادیکال DPPH و توان آنتی اکسیدانی تام استفاده گردید. همه عصاره ها در هر تست اندازه تاثیر متفاوت و وابسته به غلظت را نشان دادند. عصاره استنی با بالاترین مقدار محتوای فنلی (۲۰/۱۲ ±۰/۰۲ g GAE در ۱۰۰ گرم عصاره خشک) در هردو آزمایش خاصیت آنتی اکسیدانی بالایی از خود نشان داد. LSA با پایین ترین مقدار EC50 (۴/۲۸ میکروگرم در میلی لیتر) فعالیت به دام اندازی رادیکال آزاد بالا و در آزمایش اندازه‌گیری فعالیت آنتی اکسیدانی کل حداکثر فعالیت (۲۵٪ فعالیت اسید اسکوربیک) را نشان داد. براساس این نتایج عصاره استنی دانه لاله مردابی می‌تواند به عنوان یک منبع غنی از آنتی اکسیدان های طبیعی در نظر گرفته شود.

کلمات کلیدی: محتوای فنلی تام، فعالیت به دام اندازی رادیکال DPPH ، فعالیت آنتی اکسیدانی

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