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Biochemical analysis of Lotus (*Nelumbo nucifera*) stem

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Abstract
In present study, lotus stem powder was analysed for total phenolic content, total flavonoid content, Ferric Reducing Antioxidant Power and radical scavenging properties.
Results revealed that the antioxidant value in terms of FRAP and DPPH was higher of lotus stem powder, 136.32 and 51.91mgTE/100g. Maximum total phenolic content (31.98 mg GAE/100g) and total flavonoid content (72.13 mg RE/100g) was observed in lotus stem powder.
The phenol and Phenolic (catechol and catechin) compounds, present in the lotus stem protects the body cells from oxidative damage.

Keywords: Lotus stem, antioxidants

1. Introduction
Lotus stem (*Nelumbo nucifera*), belongs to *Nelumbonaceae*, family and is a aquatic herb cultivated for its edible rhizomes, seeds and leaves, harvested in autumn or winter and dried for later use. It is an underwater vegetable whose consumption is confined indigenously to the South-East Asia. In India lotus plant is grown in all lakes and other water bodies, at high elevation territories of Kashmir, Himalayas, north India and in the lower heights of Kanya Kumari and Southern India. Lotus stem exterior is covered with a peel which is reddish brown in color. Its buds, flowers, fruits, leaves, stalks, rhizomes and roots are edible and have been used as herbal medicines for treatment of cancer, diarrhea, heart problems, hypertension and insomnia [1]. Secondary metabolites like alkaloids, flavonoids, steroids, triterpenoids, glycoside sand polyphenols present in lotus stem have stimulating action on the body. Polyphenols present in lotus stem possess antioxidant properties. Lotus stem inhibits the bone resorption of differentiated osteoclast cells and prevents bone metabolic disorders such as osteoporosis [2].
Uncooked lotus stem comes under “cold” food which improves the appetite, promotes fluids and relieves blood stagnation, thirstiness and hangovers. Cooked lotus stem is “warm” and help nourish the stomach and spleen reinforces the heart and blood, smooth nerves and relieves diarrhea [3]. The presence phenol and Phenolic (catechol, epicatechin, gallocatechin and catechin) in the lotus stem may be responsible for its antioxidant property (Yang *et al*. 2016) [16].

2. Materials and Method
2.1 Procurement of material
Lotus stem was procured in a single lot from local market of Hisar city. Atomic absorption spectrophotometer (AAS) (model UV-1800 Shimadzu UV Spectrophotometer) was used for analysis.

2.2 Preparation of sample
Extraction of samples for antioxidant activity
Finely ground samples were extracted with 80% methanol. Five gram of weighted sample was taken conical flask. Fifteen ml of 80% methanol were added and acidified to pH 2.0 with 6N HCl by shaking at room temperature for 30 minutes. Supernatant was decanted and re-extracted the residue for complete removal of phenolic and antioxidant compounds. This procedure was repeated for two times. Three supernatants were pooled and centrifuged at 6000 rpm for 15 minutes. Volume was made up to 50ml in the solvent. Then the samples were transferred to microcentrifuge tube and stored at -20 ^{0}C for total phenolic content, total flavonoids and total antioxidant capacity determination.

2.3 Determination of Total Phenolics content

Reagents

1. Gallic acid (GA) standard solution (100 mg %):
   Stock solution: 100 mg GA dissolved in 100ml distilled water (D/W). Working solution: Took 1 ml stock solution and volume made up to 20ml with D/W.
2. Folin-Ciocalteu (FC) reagent (50%): 1:1 dilution with D/W.
3. Sodium carbonate (7.5%): Dissolved 7.5 g Na_2CO_3 in 100ml D/W.

Different known sample aliquots were taken and the volume was made up to 1.5ml with D/W. To this 0.5ml of Folin–Ciocalteau reagent was added. After that 10ml of 7.5% Na_2CO_3 was added and incubated at 37 ^{0}C for 60 minutes. After completion of this, the sample absorbance was taken at 750 nm. The results were expressed in mg Gallic acid equivalents/100g (mg GAE/100g) (Singleton *et al*., 1999) [6].

Std. Con Sample O.D Vol. made (ml) 100

$$\text{TFC (mg RE/100g)} = \frac{\text{Std. Conc}}{\text{Std. O.D}} \times \frac{\text{Sample O.D}}{\text{Aliquot (ml)}} \times \frac{\text{Vol. made (ml)}}{\text{Sample (g)}} \times \frac{100}{1000} \times \text{Dilution factor}$$

2.4 Total Flavonoids content (TFC)

Reagent

1. Routine standard solution (10mg %): Dissolved 10mg Rutine in 100ml methanol.
2. Sodium nitrite (5gm %): Dissolved 5gm $NaNO_2$ in 100ml D/W.
3. Aluminium chloride (10gm %): Dissolved 10gm $AlCl_3.6H_2O$ in 100ml D/W.
4. Sodium Hydroxide (1N): Dissolved 4gm NaOH in 100ml D/W.

Different known sample aliquots were taken and the volume was made up to 5 ml with distilled water. Then 0.5ml of 5% $NaNO_2$ was added in test tubes and were mixed after 5 minute, 0.6 ml of 10% $AlCl_3$ was added and again mixed. After 6 minute, 2 ml of 1N NaOH was added and mixed. Then 2.1 ml distilled water was added to make volume up to 10ml. After completion of this, the sample absorbance was taken at 510nm against blank.

Standard series of known concentration of Rutin (50-200µg) were made. For that 0.5, 1.0, 1.5, 2.0 ml aliquots were taken and volume was made up to 5ml with distilled water and treated same as sample. Absorbance was recorded at 510 nm and a calibration curve of absorbance versus concentration was plotted (Zhishen *et al*., 1999) [7].

$$\text{FRAP (mg TE/100g)} = \frac{\text{Std. Conc.}}{\text{Std. O.D}} \times \frac{\text{Sample O.D}}{\text{Aliquot(ml)}} \times \frac{\text{Vol. made (ml)}}{\text{Sample (g)}} \times \frac{100}{1000} \times \text{Dilution factor}$$

2.5 Ferric Reducing Antioxidant Power

Reagents

a. Trolox standard solution (10mg %):
b. Stock solution: Dissolved 10 mg Trolox in 100ml distilled water.
   Working solution: Took 1ml stock and made volume up to 10ml with distilled water.
c. Acetate buffer (0.2M) (pH 3.6): Mixed 92.6ml 0.2M glacial acetic acid and 7.4ml 0.2M sodium acetate. $3H_2O$. Set pH 3.6 and volume made up to 200ml with D/W.
d. Hydrochloric acid (40mM)
e. TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) (10mM): Dissolved 31.3mg TPTZ in 10ml 40mM HCl.
f. Ferric chloride (hexahydrate) (20mM) (Freshly prepared): Dissolved 0.05406gm $FeCl_3.6H_2O$ in 10ml D/W.
g. FRAP working reagent (Freshly prepared): Mixed 25ml acetate buffer, 2.5ml TPTZ and 2.5ml $FeCl_3.6H_2O$.

Different known sample aliquots were taken and made volume up to 0.3ml with distilled water. Then 1.8ml of FRAP working reagent was added and incubated at 37^{0}C for 10 minutes. Reading of the blue colored complex was taken at 593nm against blank.

Standard curve: Standard series of known concentration of Trolox (5-20µg) were made. For that 0.05, 0.1, 0.15, 0.2 ml aliquots were taken and volume up to 0.3ml was made with distilled water and treated same as sample. Absorbance was recorded at 593 nm and a calibration curve of absorbance versus concentration was plotted (Tadhani *et al*., 2009) [9].

$$\text{FRAP (mg TE/100g)} = \frac{\text{Std. Conc.}}{\text{Std. O.D}} \times \frac{\text{Sample O.D}}{\text{Aliquot(ml)}} \times \frac{\text{Vol. made (ml)}}{\text{Sample (g)}} \times \frac{100}{1000} \times \text{Dilution factor}$$

2.6 DPPH Radical Scavenging Activity

Reagents

1. Trolox standard solution (10mg %): 10 mg of trolox dissolved in100ml Distilled water.
2. DPPH solution: Dissolved 15.77 mg of DPPH in 200ml of methanol and set the O.D. 1.0 at 517nm.
3. Methanol

Different known sample aliquots were taken and volume was made up to 1 ml with methanol. Three ml of DPPH reagent was added to it and mixed the contents properly and incubated for 20 minutes at 37^{0}C. Reading of the resulting oxidized solution was taken at 517nm against methanol as blank.

Standard series of known concentration of Trolox (10-40µg) were made. For that 0.1, 0.2, 0.3, 0.4 ml aliquot were taken and made volume 1.0 ml with methanol and treated same as sample. Absorbance was recorded at 517 nm and a calibration curve of absorbance versus concentration was plotted Tadhani *et al*. (2009) [9].

The percent inhibition of activity = [(Ac – Ae)/Ac] x 100 (where, Ac = absorbance of control; Ae = absorbance of extract).

$$\text{DPPH (mg TE/100g)} = \frac{\text{Std. Conc.}}{\text{Y}} \times \frac{\text{X}}{\text{Aliquot (ml)}} \times \frac{\text{Vol. (ml)}}{\text{Sample (g)}} \times \frac{100}{1000} \times \text{Dilution factor}$$

X= Sample percent inhibition, Y= Standard percent inhibitioni

2.7 Statistical analysis

Means of standard deviation of different variables/parameters were calculated. Statistically data were analyzed for analysis of variance in a complete randomized design by OPSTAT software developed by Sheoran & Pannu (1999).

3. Result and Discussion

Table 1: Total phenolic content and Total flavonoids content of lotus stem powder and wheat flour

	Lotus stem powder
Total phenolic content (mg GAE /100g)	31.98±0.50
Total flavonoids (mg RE/100g)	72.13±0.36
Ferric reducing antioxidant power (mgTE/100g)	136.32±0.66
DPPH radical scavenging activity (mgTE/100g)	51.91±0.87

Values are mean ±SE of three independent determinations.
All values are mean ± standard deviation (n=3).

The total phenolic content of lotus stem powder was 31.98 mg GAE/100g. The values of total phenolic content obtained in present study are within the range (30.63 to 52.50 mg GAE/100g) as reported by Gnana and Estherlydia (2014) [17] for lotus stem cultivars. Yang *et al*. (2007) had reported 20.10 mg catechin equivalents/100g of total phenolic content of lotus stem powder.

Total flavonoid content of lotus stem was 72.13 mg RE/100g. The findings of present study are in close agreement with the values (77.80 mgRE/100g) reported by Gnana and Estherlydia (2014) [17] for lotus stem powder. The values of flavonoids for wheat obtained in present study falls in between the range 10.57 to 118.9 mg RE/100g reported by Thummakomma and Meda (2016) [13] and 11 to 75 mg/100g reported by Ivanisova *et al*. (2012) for cereals.

The ferric reducing antioxidant power (FRAP) of lotus stem was 136.32 mgTE/100g. These results are supported with values of lotus stem powder 75.91 to 100.43 mgTE/100g as reported by Yang *et al*. (2016) [16].

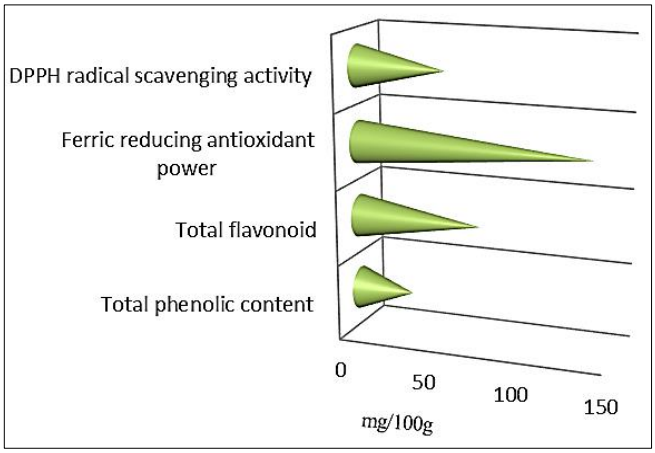

Fig 1: Antioxidant activity

The DPPH radical scavenging activity of lotus stem powder was 51.91 mgTE/100g. These results are in agreement with the values reported by Park *et al.* (2009) for lotus stem (21.98 to 54.27 mg TE/100g) whereas, slightly higher values (66.73 mgTE/100g**)** were found than results of present study by Yang *et al*. (2007).

4. Conclusion

It can be concluded from the results of present investigation that lotus stem powder are rich in polyphenols and antioxidant activity. Antioxidants fights oxidative-stress related diseases such as inflammation, cancer and skin diseases.

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