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In vitro Evaluation of Antioxidant Potential of Leaves of *Opuntia dillenii* Haw.

Raushanara Akter[1*], S. M. Raquibul Hasan[1], Md. Mokarram Hossain[1], Taksim Ahmed[2], Muntasir M. Majumder[1], Masuda Akter[1]

Department of Pharmacy, Stamford University Bangladesh[1]
51 Siddeswari Road, Dhaka-1217
Department of Statistics, Jahangirnagar University [2]
Savar, Dhaka-1342

***Corresponding Author**
Raushanara Akter
Assistant Professor
Dept. of Pharmacy
Stamford University Bangladesh
Contact no.: +8801674899392
E-mail: raushanarabd@yahoo.com

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ABSTRACT

Antioxidant potential of the 80% methanol extract of the leaves of *Opuntia dillenii* was evaluated using 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) and nitric oxide radical scavenging, reducing power, total phenol and total flavonoid content determination assays. Preliminary phytochemical screening of the extract was also carried out, which revealed that the extract possesses flavonoids, steroids, alkaloids and tannins. The extract showed significant antioxidant activities in all antioxidant assays compared to the reference antioxidant in a dose dependent manner. In DPPH radical scavenging activity, the IC_{50} value of the crude extract was found to be 15.71µg/mL while the IC_{50} value for the reference ascorbic acid was 10.84 µg/mL. Again, the extract showed remarkable nitric oxide scavenging potential and good reducing power. Moreover, the methanol extract was found to contain high amount of phenols and flavonoids, expressed as gallic acid and rutin equivalents respectively. Based on the findings of the present study, we conclude that the methanol extract of the leaves of *O. dillenii* possesses remarkable antioxidant potential which may be attributed to the high amount of phenols and flavonoids present in the extract.

Key Words: *Opuntia dillenii,* Antioxidant, Reactive oxygen species, DPPH, Nitric oxide radical

INTRODUCTION

The presence of free radicals in the body causes oxidative stress leading to cell and tissue damage and has been implicated in various diseases, for example cancer, diabetes and heart diseases (Kumarasamy et al., 2007; Halliwell B. 1996). Many medicinal plants contain large amounts of antioxidants such as polyphenols, which have an important role in preventing a variety of stress-related diseases and aging which are closely related to the active oxygen and lipid peroxidation (Noguchi and Niki, 1999). Antioxidants have been used for the prevention and treatment of free radical-related disorders (Middleton et al., 2000). However, there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) due to their possible activity as carcinogen (Barlow, 1990).So there has been a renewed scientific interest to find naturally occurring antioxidants for use in foods to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Velioglu et al., 1998). *Opuntia dillenii* belongs to the family *Cactaceae* is a succulent shrub native to America and West Indies (Trenary, 1997). Commercially it is cultivated in Italy, Spain, Mexico, Brazil, Chile, Argentina. This plant is used as laxative, stomachic, carminative and antipyretic. It cures burning, leucoderma, urinary compliants, tumors, piles, anaemia, ulcers and the enlargement of spleen (Kirtikar and Basu, 1935). The leaf is applied in ophthalmia. The flowers cure bronchitis and asthma. Most species of *Opuntia* contain a range of alkaloids in ample quantities, notably substituted phenetylamines. Identified compounds of medical significance include 3-methoxytyramine, candicine, hordenine, N-methyltyramine and tyramine (Trenary, 1997). Literature review indicated that no study on the antioxidant activity of the leaves of O. dillenii has so far been undertaken. Taking this in view and as part of our ongoing search on Bangladeshi medicinal plants (Hasan et al., 2008), the present study aimed at evaluating the antioxidant potential of methanol extract of the leaves of *O. dillenii*.

MATERIALS AND METHODS

Chemicals

1, 1-diphenyl, 2-picryl hydrazyl (DPPH), ferric chloride and trichloroacetic acid were obtained from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine chem. Ltd., Biosar, India. Potassium ferricyanide and Sodium nitroprusside from May and Backer, Dagenham, UK.

Plant Material

For the present investigation, the leaves of *Opuntia dillenii* were collected from Baridhara Nursery, Baridhara, Dhaka, Bangladesh in July 2007 and was identified by experts of Bangladesh National Herbarium Mirpur, Dhaka, where a voucher specimen (Accession no: 32413) has been deposited. The leaves were separated from the plants and were dried in the hot air oven for 4 days.

Extraction

The dried leaves were coarsely powdered and extracted with a mixture of methanol: water (8:2 v/v) by Maceration. The solvent was completely removed and obtained dried crude extract which was used for investigation.

Phytochemical Screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

TESTS FOR ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Activity

The free radical scavenging capacity of the extracts was determined using DPPH (Hasan et al. 2006). A methanol DPPH solution (0.004% w/v) was mixed with serial dilutions (1 µg to 500 µg) of *O. dillenii* extract and after 10 min, the absorbance was read at 515 nm using a spectrophotometer (HACH 4000 DU UV – visible spectrophotometer). Ascorbic acid was used as a standard. The inhibition curve was plotted and IC_{50} value was calculated.

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging can be estimated by use of the Griess Illosvoy reaction (Garrat 1964). In this study, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and either *Opuntia dillenii* extract (10 µg to 160 µg) or positive control (BHT, 0.5 mL) was incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture was mixed with 1 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completion of diazotization. Then, naphthyl ethylene diamine dihydrochloride (1 mL) was added, mixed and allowed to stand for 30 min at 25°C. A pink colored chromophore was formed in diffuse light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Vitamin C was used as the positive control.

Reducing power assay

The reducing power of *O. dillenii* was determined according to the method previously described (Oyaizu, 1986). Different concentrations of *Opuntia dillenii* extract (100 µg – 1,000 µg) in 1 mL of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments were taken was expressed as mean ± standard deviation.

Total phenol and total flavonoid content determinations

The total phenolic content of plant extracts was determined using Folin–Ciocalteu reagent (Yu et al., 2002). Plant extract (100 µL) were mixed with 500 µL of the Folin–Ciocalteu reagent and 1.5 mL of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid.

The flavonoid content was determined using a method previously described by Kumaran and Karunakaran (2007) using rutin as a reference compound. One millilitre of plant extract in methanol (50-250 µg/mL) was mixed with 1mL aluminium trichloride in ethanol (20 mg/mL and a drop of acetic acid, and then diluted with ethanol to 25 mL. The absorption at 415nm was read after 40 min. Blank samples were prepared from 1ml of plant extract and a drop of acetic acid, and then diluted to 25 mL with ethanol. The absorption of standard rutin solution (0.5 mg/mL) in ethanol was measured under the same conditions. All determinations were carried out in duplicates. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula: $X= (A \times m_0)/ (A_0 \times m)$, where X is the flavonoid content, mg/mg plant extract in RE, A is the absorption of plant extract solution, A_0 is the absorption of standard rutin solution, m is the weight of plant extract in mg and m_0 is the weight of rutin in the solution in mg.

RESULTS AND DISCUSSION

Table 1: Result of phytochemical screening of methanol extract of the leaves of *Opuntia dillenii.*

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
ME of *O. dillenii*	+	++	-	++	-	++	+

ME: Methanol extract; (+): Present; (-): Absent

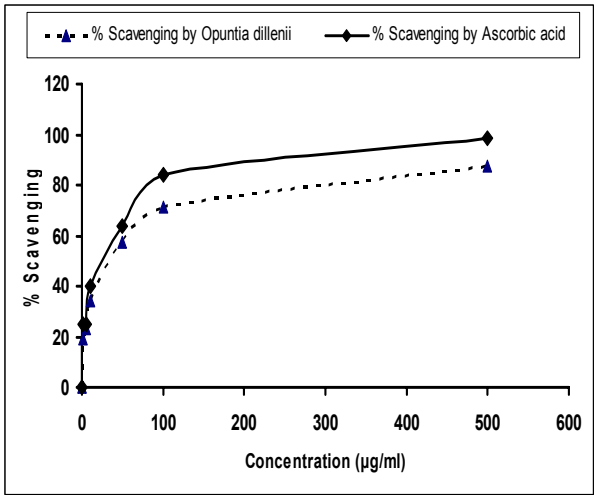

Figure 1: DPPH radical scavenging activity of methanol extract of the leaves of *O. dillenii.* Values are the average of duplicate experiments and represented as mean ± SD

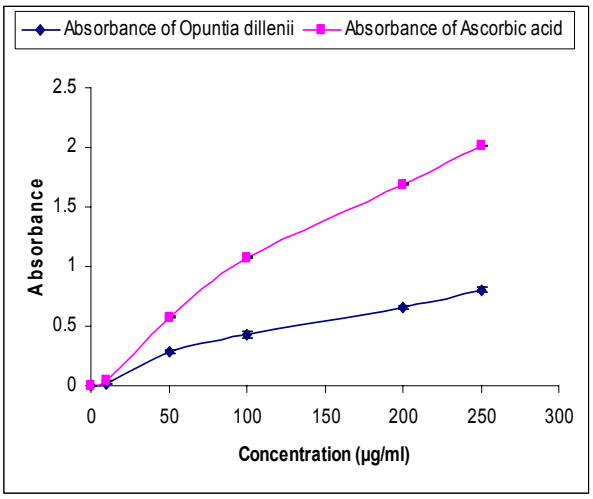

Figure 2: Reducing power of methanol extract of the leaves of *O. dillenii.* Values are the average of duplicate experiments and represented as mean±

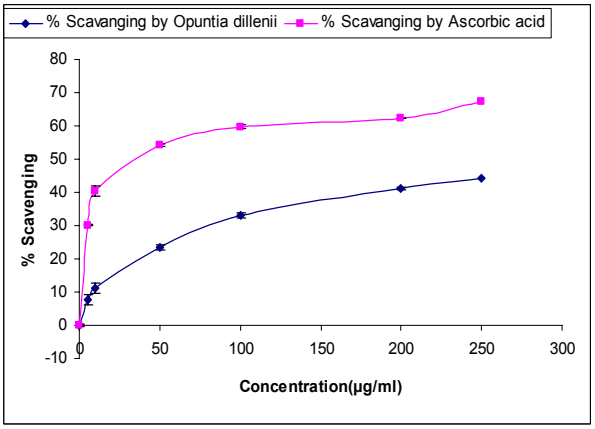

Figure 3: Nitric Oxide scavenging activity of methanol extract of the leaves of *O. dillenii.* Values are the average of duplicate experiments and represented as mean± SD.

Table 2: Total phenol and total flavonoid contents of methanol extract of the leaves of *O. dillenii.*

Extract	**Total phenol** (in mg/g , Gallic acid equivalents)	**Total flavonoid** (in mg/g, Rutin equivalents)
Methanol extract of *O. dilenii*	31.15±2.61	59.34±2.87

Values are the average of duplicate experiments and represented as mean± SD.

Preliminary phytochemical screening of methanol extract of the leaves of *O. dillenii* revealed the presence of various bioactive components like flavonoids, alkaloids, steroids, tannins and saponins (Table 1). In DPPH and nitric oxide radical scavenging assays, the plant extract showed dose dependent scavenging of DPPH and nitric oxide radicals in a way similar to that of the reference antioxidant ascorbic acid. In DPPH and nitric oxide radical scavenging assays, the IC_{50} values of the extract were 15.71µg/mL and 25.63µg/mL respectively as compared to the IC_{50} values of 10.84µg/mL and 11.68µg/mL respectively for ascorbic acid, which is a well known antioxidant. DPPH radical scavenging is a popular method for screening the free radical scavenging activity of compounds or antioxidant capacity of plant extracts. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants (Kumarasamy et al., 2007). The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Moreover, both nitric oxide and superoxide anion cause ischemic renal injury. The toxicity and damage caused by $NO\cdot$ and $O2^{\cdot -}$ is multiplied as they react to produce reactive peroxynitrite ($ONOO^-$), which leads to serious toxic reactions with biomolecules (Moncada et al.,1991; Radi *et al.,* 1991). Suppression of $NO\cdot$ released may be partially attributed to direct $NO\cdot$ scavenging, as all concentrations of *O. dillenii* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. However, the plant extract displayed good reducing power which was found to rise with increasing concentrations of the extract. The reducing ability of a compound generally depends on the presence of reductants (Duh, 1999), which have been reported to exhibit antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Gordon, 1990). So, it can be assumed that the presence of reductants (i.e. antioxidants) in *O. dillenii* extract causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, the Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 2 shows the reductive capabilities of the plant extract compared to ascorbic acid. Again, methanol extract of the leaves of *O. dillenii* was found to contain large amounts of phenols and flavonoids as evident from total phenol and total flavonoid content determinations. Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables (Deighton et al., 2000, Vinson et al., 1998). It has been reported that phenolic compounds with *ortho*- and *para*-dihydroxylation or a hydroxy and a methoxy group are more effective than simple phenolics (Frankel et al., 1995). Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants the extract of *O. dillenii* needs their characterization.

CONCLUSION

In light of the results of the present study, it can be concluded that the plant extract possesses remarkable antioxidant potential. However, further studies are needed to understand the underlying mechanism of antioxidant action and to isolate the compound (s) responsible for such activity.

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