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Short Note

Antioxidant Activity of *Urtica hyperborea* from Cold Arid Desert Ladakh

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The aim of the study was to evaluate the antioxidant and free radical scavenging properties, total phenolic contents and phytochemical constituents of *Urtica hyperborea* Jacq. ex Wedd. growing in the high altitude cold desert region of Ladakh, Jammu and Kashmir, India. The antioxidant activity of the whole plant was investigated with four different methods such as 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS), Reducing power assay and Ferric reducing antioxidant property (FRAP). All the assays carried out found that the plant has antioxidant potential. The total phenol content (TPC) and total flavonoid content of the extract was determined as 42 ± 7.54 mg GAE/ g dry wt. and 4.0 ± 2.31mg QE/g dry wt. respectively. The study has validated the medicinal potential of *U. hyperborea* and revealed that the consumption of the plant could be beneficial as it possesses antioxidant activity.

Key words: Antioxidant activity, phytochemicals, polyphenols, *Urtica hyperborea*

The Reactive Oxygen Species formed may cause cellular and sub-cellular damages by peroxidation of membrane lipids, by denaturing cellular proteins, and by breaking DNA strands, disrupting cellular functions (Patra et al. 2008). Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and/or activators of antioxidative defense enzyme systems to suppress the radical damages in biological systems (Prior et al. 2005). Most of the antioxidants in use commercially [e.g., butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)] are synthetic and some of them have been suspected of causing or promoting negative health effects therefore, some restrictions are placed on their applications and there is a trend to substitute them with naturally occurring antioxidants (Galvez et al. 2005; Nakiboglu et al. 2007). Plant originated polyphenols and flavonoids have powerful anti-oxidant activities and are able to scavenge a wide range of reactive oxygen species, including hydroxyl radicals, peroxyl radicals, hypochlorous radicals, superoxide radicals inhibiting lipid peroxidation in human tissues (Sugihara et al.1999).

Urtica hyperborea Jacq. ex Wedd. **(**Family: Urticaceae) is an erect perennial herb commonly known as 'Stinging Nettle*'* in English and *'Zozot'* in Bodhi language. The plant is extensively used in the study area Ladakh, Jammu and Kashmir, India as anti-dysentric, carminative, antiseptic and stimulant in the Amchi system of medicine. The young and dry leaves of this plant are cooked to make a local dish *'Thukpa'* (Pal Murugan et al. 2010) which is considered as very nutritious food and given during post natal care of women. The plant is high in vitamins and minerals and makes an excellent spinach substitute and can be added to soups and stews. The leaves of the plants have stinging hairs, causing irritation to the skin which is neutralized during cooking. The plant is used for the treatment of rheumatism and joint pain in the Tibetan system of traditional medicine (Chaurasia *et al*. 2007). Considering the importance of the medical properties of *U. hyperborea* the present investigation was carried out to analyze the antioxidative capacity and to quantify the phenols and flavonoids.

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Materials and Methods

Collection and extraction of plant sample

Urtica hyperborea was collected from natural habitats and the herbal garden, DIHAR of Ladakh during May, 2010. The plant samples were air-dried and ground into uniform powder using pestle and mortar. The methanol extracts were prepared by dissolving 5 g of dried powder in 85% methanol. The solvents were concentrated using rotavapor.

Quantification of polyphenols

Total phenol content in the extracts was determined by the modified Folin-Ciocalteu method and was calculated from the calibration curve in terms of gallic acid equivalents ($y = 0.002x + 0.039$, $R^2 = 0.982$).

Determination of antioxidant activity

DPPH radical scavenging activity

The hydrogen atom- or electron donor ability of the corresponding extract and some pure compounds were measured from the bleaching of the purple coloured methanol solution of 2, 2'-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay used stable radical DPPH as a reagent (Cuendet et al. 1997). 5 ml of 20,40,60,80 and 100 mg L^{-1} of the extracts in methanol were added to 5 ml of 0.002% DPPH solution and inhibition of free radical DPPH in percent (I%) was calculated as $I\%=[(A_{blank} - A_{sample})/ A_{blank}] \times 100$ Where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. Extract concentration providing 50 percent inhibition was calculated using the graph by plotting inhibition percentage against extract concentration. Ascorbic acid (AA) and the synthetic antioxidant reagent butylated hydroxy toluene (BHT) were used as positive controls and all the tests were replicated thrice.

ABTS radical scavenging activity

For ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical) assay, the method of (Re et al.1997) was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS+ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. ABTS+ solution was freshly prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the $ABTS^{+}$ solution and the percentage inhibition [%] was calculated: $I\%=[(A_{blank} - A_{sample})/ A_{blank}] \times 100$ where A_{blank} is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract/standard. All the tests were replicated thrice and the extract concentration providing 50 percent inhibition was calculated using the graph by plotting inhibition percentage against extract concentration.

Reducing power assay

Whole plant extracts (200-1000 ppm) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%), and the mixture was incubated at 50^{o}C for 30 min. 2.5 ml of trichloroacetic acid (10%) was then added to the mixture and was centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power (Yen and Chen, 1995).

Total antioxidant activity (FRAP assay)

A modified method of Benzie and Strain (1996) was adopted for the FRAP (Ferric reducing antioxidant property) assay. The stock solutions included 300 mM acetate buffer (3.1 g CH_3COONa and 16 ml CH_3OOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37 °C before assay. Plant extract (150 ìL) was allowed to react with 2850 ìl of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results are expressed in ìM Fe (II)/g dry mass using the following equation based on calibration curve $y = 0.773x - 0.072$, $R^2 = 0.991$ and compared with that of butylated hydroxy toluene (BHT), butylated hydroxyanisole (BHA) and ascorbic acid.

Statistical analysis

The experimental results were expressed as average ± Standard Deviation from three replicates. Decolorisation was plotted against the sample extract concentration and a linear regression curve was established in order to calculate IC_{50} (µg/ml), which is the amount of sample required to decrease the absorbance of the free radical by 50%. Statistical analysis were performed using SPSS 11.5

Results and Discussion

Total phenol content

Phenolic compounds are a class of antioxidant agents which are a major group of compounds that contribute to the antioxidant activities of plant materials because of their scavenging ability on free radicals due to their hydroxyl groups (Djeridane et al 2006). Flavonoids are a group of polyphenolic compounds with known properties of free radical scavenging, antibacterial and anti-inflammatory action (Balasundram *et al.* 2005). Total phenol content observed in *U. hyperborea* was 42 ± 7.54 mg GAE/g dry wt. and total flavonoid content was 4.0 ± 2.31 mg QE/g dry wt.

Antioxidant activity

The antioxidant activity of *U. hyperborea* was observed in the present study by *in vitro* assays

Table 1. Ascorbic acid Equivalent Antioxidant Capacity (AEAC) determined by DPPH, ABTS and RPA (mg g^{-1} dry wt.) and Ferric Reducing antioxidant property (FRAP) (µM Fe (II) g^{-1} dry wt.) for *Urtica hyperborea.*

Plant Extract	DPPH	ABTS	RPA	FRAP
Urtica hyperborea	6.18 ± 1.55	11.06 ± 2.03	43 ± 2.30	560.59 ± 18.72

such as DPPH, ABTS, FRAP and reducing power assays to evaluate the free radical scavenging activity and antioxidant capacity of the extract (Table.1). The antioxidant activity of methanol extract of *U. hyperborea* was examined by comparing with the activity of known antioxidants such as ascorbic acid and BHT with inhibition of DPPH radical. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity (Koleva et al.2002). The antioxidant activity of the extracts increased with an increasing amount of extract. The methanol extract was able to reduce the stable free radical DPPH to the yellow coloured diphenylpicrylhydrazine with an IC_{50} of 83.37 ìg/ml, while AA was 4.67 ìg/ml and the BHT was 14.34 ìg/ml. The results of DPPH activity is showed in (Fig.1). The antioxidant activity (IC_{50}) of *U. dioica* was reported as 70.8 ìg/ml (Pourmorad et al.2006). However, these contradictory results are most likely due to differences in species and experimental conditions.

The result of IC_{50} values of ABTS of synthetic antioxidants like BHT, AA and *U. hyperborea* were higher than DPPH assay results (Fig.1). Earlier reports on honey and potato (Teow et al. 2007) samples also showed that the average Antioxidant Activity (AOA) values based on AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) determined by ABTS assay were two to three times higher as compared

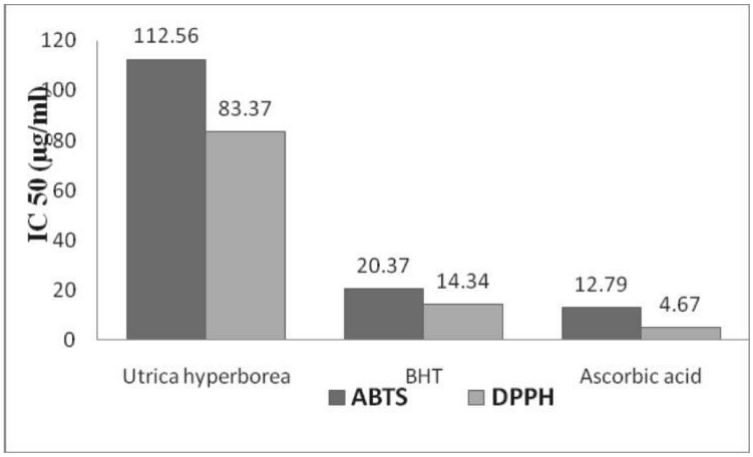

Fig.1 IC $_{50}$ µg/ml (50% free radical scavenging) of *Urtica hyperborea* Extracts, BHT and AA determined by DPPH and ABTS method

to DPPH assay and the present study also showed two to three times higher average AOA values for ABTS in comparison with DPPH (Table. 1).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability of a compound generally depends on the presence of reductones, which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Thaipong et al. 2006). The reducing power of *U. hyperborea* increased with increasing concentrations of the sample. Reducing power of *U. hyperborea* and standard compounds exhibited the following order: Ascorbic Acid > methanol extracts > â-carotene (Fig 2).

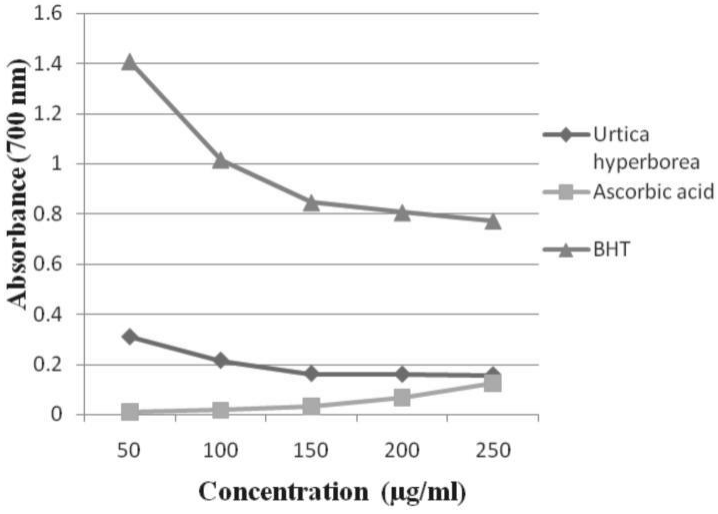

Fig 2. ABTS scavenging activity of various concentrations of methanolic extract of *Urtica hyperborea* Ascorbic acid and BHT.

The total antioxidant potential was determined by ferric reducing antioxidant power (FRAP) assay of the methanolic extract of *U. hyperborea* against a standard curve of ferrous sulphate. The total

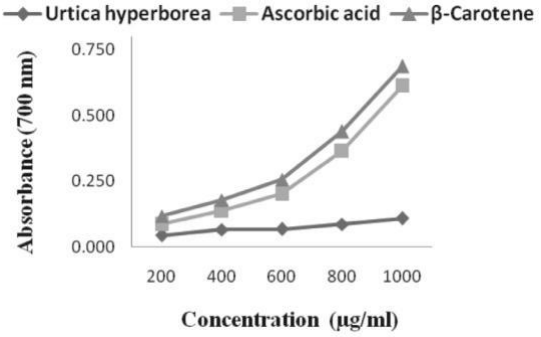

Fig 3. Reducing power of various concentrations of methanolic extract of *Urtica hyperborea* Ascorbic acid and â-Carotene.

antioxidant capacity was 560.59 ± 18.72 µM equivalents of Fe (II) g^{-1} dry wt. The higher FRAP values indicate higher antioxidant capacity. The present study gave the FRAP values in the following order: Ascorbic Acid > BHA > BHT > *Urtica hyperborea.*

Conclusion

The results of this study showed that *U. hyperborea* has strong radical scavenging and reducing activity. Traditionally local people of Ladakh were using *U. hyperborea* since long time and it may serve as a good antioxidant source for this region. The preliminary phytochemical investigation and total phenols and flavonoids determination indicates the presence of antioxidant activity. Further studies will also be needed to evaluate its potential in various animal models and the isolation and identification of the potent antioxidant principles.

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