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ANTIOXIDANT POTENTIAL OF ROOT BARK OF *BERBERIS LYCIUM* ROYLE. FROM GALLIYAT, WESTERN HIMALAYA, PAKISTAN

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Abstract

This study was to compare the relative level of antioxidant activity of methanolic extract of root bark of *Berberis lycium* Royle., in different test systems of antioxidant determination i.e ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical cation assay, Phosphomolybdenum assay and reducing power assay. In all these assays the antioxidant activity of extract was compared to that of ascorbic acid. The antioxidant activity was highest in phosphomolybdenum assay followed by ABTS and reducing power assay respectively. In phosphomolybdenum assay extract showed maximum antioxidant activity of 84.55% and ascorbic acid showed 86.99% while in ABTS assay extract showed maximum antioxidant activity of 62.15% and ascorbic acid showed 88.3%. In reducing power assay increased concentration of sample and standard resulted in more reduction of ferric cyanide complex to ferrous form, thus showed increased antioxidant activity at higher concentration. In ABTS assay influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption were taken into account when determining the antioxidant activity. On the basis of these results *Berberis lycium* is identified as a best source of free radical scavenging compounds.

Introduction

It is well known that reactive oxygen species (ROS) such as $^{-}O_2$ (superoxide anion), H_2O_2 (hydrogen peroxide), and ^{-}OH (hydroxyl radical) can lead to various human diseases such as Alzheimer's disease, cancer, inflammation, aging, rheumatoid arthritis and atherosclerosis (Singh *et al*., 1989). Reactive oxygen species are formed during metabolism or through the action of ionizing radiations and they can interact with biomolecules and led to ionizing diseases including cancer (Wisemann *et al*., 1996, Shinwari & Gilani, 2003). Natural antioxidants prevent the formation of above reactive species-related disorders in human beings (Shinwari, 2010). Medicinal plants are rich source of natural antioxidants and have an appreciable role in the development of modern medicines as many diseases like cancer, hepatic diseases and arthritis have no complete cure in allopathy (Verpoorte, 2000, Sarwat *et al*., 2012). Thus medicinal plants have become an alternate health care system to solve the health problems of world in today's synthetic allopathic era (Khan, 2003; Shinwari & Qaisar, 2011). The use of medicines from plants in the form of local medicine dates back to 4000-5000 B.C. In Pakistan out of 2000 estimated species of medicinal plants 400 are extensively used in traditional medicine (Zaidi, 1998).

Natural antioxidants especially phenolics and flavonoids from medicinal plants are already exploited commercially either as antioxidant additives or as nutritional supplements (Glucin *et al.*, 2002). In contrary to natural antioxidants, Synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene have restricted use in food because of their carcinogenic effect. Although many other plant species have been investigated in search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable extracts attention has been directed towards the identification of plant materials, rich in antioxidant ability (Suja *et al*., 2005).

Berberis lycium Royle is a member of family Berberidiaceae. It is distributed in mountaneous ranges in Kashmir and North West Himalayon regions of Pakistan. Flowering season of *Berberis lycium* ranges from April-June. Plants of family Berberidaceae are famous for its medicinal importance and are included in Biritish and Indian pharmacopeias (Srivatava *et al*., 2006, Jan *et al*., 2011). Medicinal traces are hidden in its root, bark and black berries produced by it. It has antispasmodic property and is also used for treatment of Jaundice and internal wounds (Manan *et al*., 2007). *Berberis lycium* is well known as alternate host for black stem rust fungus (*Puccinia graminis* triticii) espescially of wheat and other cereales (Jin, 2010).

Berberis lycium Royle contains variety of alkaloids (Kharc, 2004) but its major alkaloid is berberine (Khosla *et al*., 1992) which is an isoquinoline alkaloid isolated, usually taken from roots and bark. Berberine and palmatin are alkaloids extracted from *Berberis lycium* were reported to be strong growth inhibitory and proappoptosic effect (Khan *et al*., 2010), reducing serum cholestrol in broilers (Chand *et al*., 2007). The aim of present study was to compare relative proportion of antioxidant activity of root bark of *Berberis lycium* Royle in different antioxidants determination systems.

Materials and Methods

Plant collection: The *Berberis lycium* root bark was collected during April-August 2010 from Murree and allied hills from Pakistan and the plant was identified by its vernacular name and later validated by Plant Taxonomy and Biosystematic Lab. Department of Plant Sciences, Quaid-i-Azam University, Islamabad. A voucher specimen was deposited for future reference at the Herbarium of Pakistan (ISL) Pakistan.

Extract preparation: The root bark (about 1.0 kg fresh) at maturity were collected and dried under shade to obtain dry sample. The dried samples were powdered in a Willy Mill

to 60-mesh size and used for solvent extraction. For extract preparation, 500 g of dried sample was extracted twice with 95% methanol at 25°C for 72 h. The extracts were filtrated through Whatman No. 1 and combined followed by concentration using a rotary evaporator under reduced pressure at 40°C. The dry extract obtained was weighed.

Antioxidant assays

ABTS Radical cation assay: The spectrophotometric analysis of ABTS+ radical scavenging activity was determined according to the method of Re *et al*., (1999) with some modifications. Briefly, ABTS solution (7 mM) was reacted with potassium persulfate (2.45 mM) solution and kept for overnight in the dark to yield a dark colored solution containing ABTS radical cation. Prior to use in the assay, dilution of ABTS radical cation was done with 50% methanol for an initial absorbance of about 0.700 (± 0.02) at 745 nm, with temperature control set at 30°C. Free radical scavenging activity was assessed by mixing 300 µl of test sample with 3.0 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly 1 min after mixing the solution, then up to 6 min. The final absorbance was noted. The percentage inhibition was calculated according to the formula:

$$\text{Scavenging effect (\%)} = [(\text{control absorbance-sample absorbance}) / (\text{control absorbance})] \times 100$$

The antioxidant capacity of test samples was expressed as EC_{50}, the concentration necessary for 50% reduction of ABTS. Data for each fraction was recorded in triplicate.

Phosphomlybednum assay: The antioxidant activity of samples was evaluated by Phosphomolybdenum assay using the method of Prieto *et al*., (1999) with some modifications. 0.4ml of sample solution was mixed with 4ml of reagent solution containing (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in vials. The vials were capped and then incubated in waterbath at 95°C for 90 mintues. After the incubation, samples were cooled to room temperature and absorbance of the mixture was measured at 765 nm against a blank. A typical blank solution containing 4ml of reagent solution and appropriate volume of the same solvent was used for the extract. Experiment was performed in triplicates.

Reducing power assay: The reducing power was determined according to the method of Oyaizu (1986). Extract solution (2 ml), phosphate buffer (2 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2 ml, 10 mg/ml) were mixed, and then incubated at 50°C for 20 min. Trichloroacetic acid (2 ml, 100 mg/l) was added to the mixture. A volume of 2 ml from each of the above mixtures was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) ferric chloride in a test tube. After 10-min reaction, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated a high reducing power.

Results and Discussion

The range of traditional applications of *Berberis lycium* in domestic medicine seems to be endless: Overall results of antioxidant activity revealed that root bark of *Berberis lycium* has greater potential to neutraliz the free radicals. Antioxidant potential of the methanolic extract of root bark of *Berberis lycium* root bark was examined using three different assays because evaluation of antioxidant properties of plants cannot be carried out accurately by single universal method.

ABTS radical cation is a common organic radical that has been used to determine the antioxidant activity of single compounds and complex mixtures (Zhou, Laux, & Yu, 2004). Decolorization of ABTS+ reflects the capacity of antioxidant species to donate electrons or hydrogen atoms to inactivate this radical cation. The reaction between the antioxidant and ABTS is essentially complete after 1 min, little further reaction taking place thereafter. In ABTS assay two factors were taken into account while determining the antioxidant activity that was duration of reaction after mixing ABTS working standard with extract and ascorbic acid and secondly it was noticed that concenteration of antioxidants present in the sample and standard also has affect in absorption of radical cation. It was found that concentration of antioxidant in the sample was inversely proportional to the absorbance of the radical cation produced by 2,2 ´-azino-bis-(3-ethyl benzothiozoline-6-sulfonate) (ABTS). As duration of reaction between ABTS and antioxidant compounds present in test samples was increased it leads to lesser absorption of radical cation thus showed increased antioxidant activity after longer duration, so antioxidant activity was heigher after 6 minutes than after 1 minute after mixing ABTS working solution with sample and standard . Antioxidant activity of methanolic extract of root bark of *Berberis lycium* and of ascorbic acid in ABTS assay is given in the Figs. 1 & 2 below against the standard Ascorbic acid.

Phosphomolybdenum method is utilized for spectrophotometeric quantitation of total antioxidant capacity. In the presence of antioxidant compounds molybdenum Mo (VI) is reduced to form green phosphate/Mo(V) complex. In Phosphomolybdenum method extract showed excellent antioxidant activity that was compareable to that of ascorbic acid. The concenterations of sample and standard ranged from15.625 µg/ml to 250µg/ml. Antioxidant activity of sample and standard at various concenterations is showen in the graph. The antioxidant activity of sample and standard at different concenterations were found in the order 250µg/ml >125µg/ml >62.5µg/ml >31.25µg/ml >15.625µg/ml that is given in Fig. 3. This good antioxidant activity might be attributed towards the presence of certain phytochemicals such as bioflavins and flavonoids (Wang *et al*., 1999).

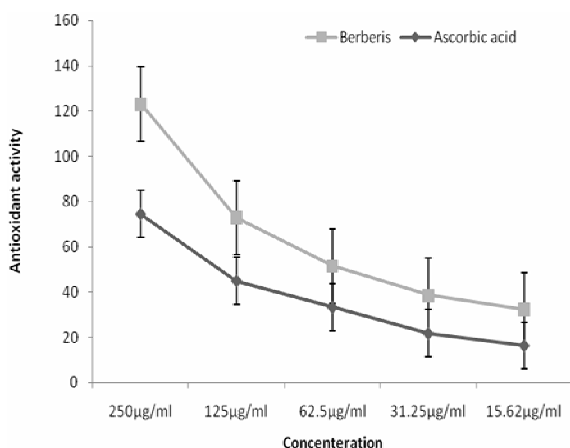

Fig. 1. Antioxidant activity of ascorbic acid and *Berberis lycium* after 1 minute of mixing ABTS working standard with sample analyte.

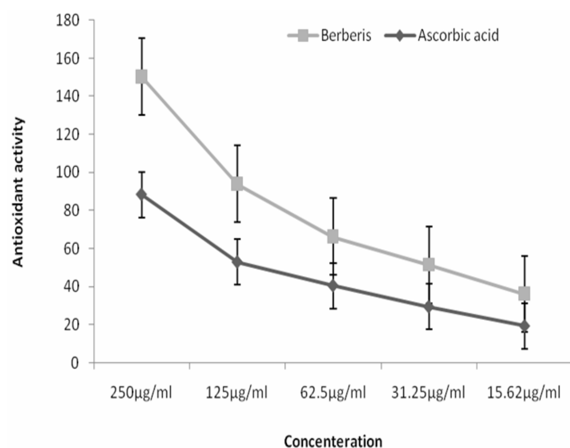

Fig. 2. Antioxidant activity of ascorbic acid and *Berberis lycium* after 6 minutes of mixing ABTS working standard with sample analyte.

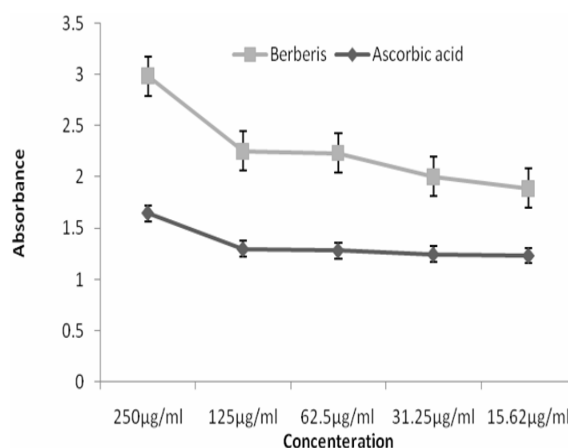

Fig. 3. Figure showing positive correlation between concentration and absorbance of ascorbic acid and *Berberis lycium*.

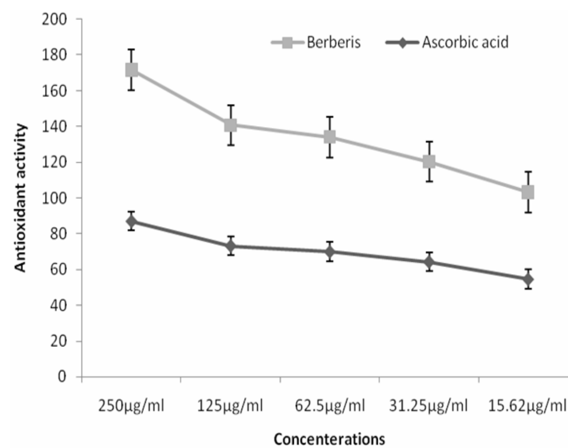

Fig. 4. Antioxidant activity of ascorbic acid and *Berberis lycium* in phosphomolybdenum assay at varying concentrations.

Compounds with reducing power indicate that they are electron donors, and have ability to reduce the oxidized intermediates that are formed as a result of lipid peroxidation processes thus they can act as primary and secondary antioxidants (Yen & Chen, 1995). Root bark of *Berberis lycium* Royle has considerable reduction potential when extract reacts with potassium ferricyanide (Fe^{3+}) it converts potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride and form ferric ferrous complex that has an absorption maximum at 700 nm. This assay is relatively simple and inexpensive. Reducing power assay does not however measures the thiole group containing reagents (Gupta *et al*., 2009). In reducing power assay concenteration of antioxidant in the sample was directly proportional to the reduction potential of the sample and standard thus showed higher absorbance and higher reduction potential at increased concentration. Absorbance of ascorbic acid and *Berberis lycium* at varying concentrations can be shown in Fig. 4.

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