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Evaluation of antioxidant activities of *Hippophae rhamnoides* Linn leaves extracts

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ABSTRACT

Objective: To investigate the antioxidant activity of aqueous, methanol, ethanol, acetone, ethyl acetate, chloroform and *n*-hexane extracts of *Hippophae rhamnoides* (*H. rhamnoides*) leaves.
Methods: Antioxidant activity was evaluated by using *in-vitro* antioxidant assays model 1, 1'-diphenyl-2-picrylhydrazyl radical-scavenging activity. The antioxidant activities were compared with standard antioxidant agents such as ascorbic acid.
Results: The antioxidant activities (% inhibition) of all the tested extracts were increased in the order *i.e.* menthol > ethanol > aqueous > acetone > chloroform > ethyl acetate > *n*-hexane. The methanol extract EC_{50} (μg/mL) value was compatible with vitamin C (standard). The antioxidant activity of *H. rhamnoides* leaves extracts increased in a dose dependent manner.
Conclusions: It was observed that *H. rhamnoides* was a potential resource of antioxidants and thus could put off numerous radical linked diseases.

1. Introduction

Plants are precious gifts of Allah to the human beings on this planet. Plants are always known to provide shelter, food, medicine, *etc*. for human beings as well as other living organisms. Medicinal plants hold some pharmacologically active compounds which have been used from the ancient times for the cure of different diseases.

All over the world medicinal plants are abundantly available and are more focused than ever because they have the ability to produce many benefits to human society, especially to cure different types of diseases. World Health Organization reported that above eighty percent inhabitants of the world depended on conventional medicines for various types of ailment. A number of chemical compounds are present in plants which have possessed the therapeutic properties and cause a specific physiological action on the human body. Phenolic compounds, alkaloids, tannins and flavonoids are the primarily significant bioactive compounds present in plants[1]. The alkaloids have antimicrobial, anticancer, cytotoxic and antimalarial properties, while flavonoids possess high antibacterial activity and are more effective in the treatment of inflammation, allergy, cancer, viral infection and hypertension. Tannin has shown high activities against viral and bacterial infection as well as act as strong antioxidant[2]. Based on ethno-pharmacological knowledge the phytochemical research was usually considered a useful methodology in the innovation of novel antimicrobial compounds from higher plants[1].

Hippophae rhamnoides Linn (*H. rhamnoides*) commonly known as Seabuckthorn is a medicinal plant, grown in large areas of Asia and Europe. Its different components have long been utilized for the cure of many diseases including pain, rheumatoid arthritis and colds. It has possessed many types of active compounds, which have latent applications in human health. The seeds, leaves and fruits hold high amounts of useful substances such as flavonoids, carotenoids, phenolic compounds and vitamins with antibacterial, antifungal and free radical scavenging activities[3]. Iron, magnesium, calcium, sodium, potassium, phosphorus and silver were found in seabuckthorn seeds[4]. The methanol and chloroform: methanol extracts of *H. rhamnoides* twigs, have been reported[5] the presence of glycoside, terpenoids, steroids, flavonoids, reducing sugar and tannins, while alkaloids, coumarins and saponins were absent. It has been investigated scientifically that *H. rhamnoides* extracts from berries, oil and leaves have been found various pharmacological activities such as anti-inflammation, immune-modulation, radio protective and tissue regeneration[6].

Antioxidants protect human beings from harmful effects due to continuous synthesis of reactive oxygen species and related

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protein damage, DNA stand breaking and lipid peroxidation[7]. Greater part of the world population, especially in third world countries, depend on the folk system of medicine for a number of diseases. Hundreds of plant genera are used medicinally, providing major sources of powerful and potent drugs[8]. Many health problems, especially heart attack, cancer, AIDS, hepatitis, skin and gastrointestinal tract diseases, are proliferating rapidly in the whole world particularly in underdeveloped countries and their treatments by medicine (synthetic) are unaffordable by poor people or adversely affected. The active secondary metabolites from plants, especially *Hippophae,* is a natural therapy for curing these health problems. The current research work was designed to evaluate the antioxidant activities of different *H. rhamnoides* leaves extracts.

2. Materials and methods

2.1. Collection of H. rhamnoides leaves

The fully matured healthy leaves of *H. rhamnoides* were collected from Pakistan Council of Scientific and Industrial Research, Skardu Gilgit Baltistan, Pakistan. The leaves were slightly washed to remove any dust, shade dried and powdered with a laboratory mill. The crushed leaves were kept in an air-tight plastic bag till used.

2.2. H. rhamnoides leaves extraction

Fifty grams powder of *H. rhamnoides* leaves was extracted in 250 mL of water, ethanol, acetone, methanol, ethyl acetate, chloroform and *n*-hexane for 48 h. These extracts were then filtered under vacuum in the course of No.1 Whatman filter paper into a Buchner flask. The extracts were concentrated in rotary evaporator and transferred in a sterilized beaker for heating on water bath at 50 °C to obtain dried residue. The resultant crude extract was transferred into airtight sample bottles and kept at 4 °C until used.

2.3. Antioxidant activity of H. rhamnoides leaves extracts

Antioxidant activities of *H. rhamnoides* L. leaves extracts were measured by 1, 1'-diphenyl-2-picrylhydrazyl (DPPH) scavenging procedure[9]. The stock solutions of each crude extracts were prepared by dissolving separately in a known amount of dry extract in methanol (95%). The working solutions (10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL, 70 µg/mL and 80 µg/mL) of each extracts were prepared from the stock solution using suitable dilution in each case. Vitamin C was used as standard using the above same concentrated solution as used in case of extracts. 0.004% of DPPH was prepared in methanol and 1 mL of this solution was mixed with 1 mL of sample solution and standard solution separately. DPPH and methanol (95%) with 1:1 mL was used as blank. These solutions were kept in a dark room for 20 min and absorbance was measured at 517 nm by using UV-visible spectrophotometer (UV-1700, Shimadzu, Japan). The scavenging inhibition (I %) was calculated by following formula.

Inhibition percentage (I %) = (A – B) / A × 100

Where, A is the absorbance of blank, B is the absorbance of the Samples.

2.4. Statistical analysis

All experiments were carried out in triplicate. Values are presented as mean ± SD (n = 3). The EC_{50} values were determined by using computer program SPSS.

3. Results

DPPH radical scavenging activity of *H. rhamnoides* leaves extracts was presented in Figure 1. These extracts showed a concentration dependent activity in comparison with ascorbic acid (standard). At concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL, the scavenging activity (%) of vitamin C was 37.73 ± 0.46, 53.40 ± 0.23, 68.07 ± 0.33, 80.81 ± 0.46, 82.74 ± 0.56, 83.02 ± 0.05, 83.15 ± 0.18 and 84.55 ± 0.47, respectively. At the concentration of 10 µg/mL, *n*-hexane showed the least radical scavenging activity of (15.26 ± 1.24)%, followed by ethyl acetate extract with an activity of (18.07 ± 0.47)%, while the highest activity was showed by methanol extract [(36.33 ± 0.47)%]. At the concentration of 20 µg/mL, the lowest and hightes activities were showed by ethyl acetate extract [(26.50 ± 0.47)%] and methanol extract [(50.34 ± 0.42)%], respectively. At the concentraction of 30 µg/mL, *n*-hexane extract showed the lowest activity of (34.92 ± 0.46)%, and methanol extract showed the highest activity of (62.33 ± 0.24)%. At all the different concentrations, methanol extract exhibited the highest activity compared with other extracts. The antioxidant activities (% inhibition) of all the tested extracts were increased in the order *i.e.* menthol > ethanol > aqueous > acetone > chloroform > ethyl acetate > *n*-hexane.

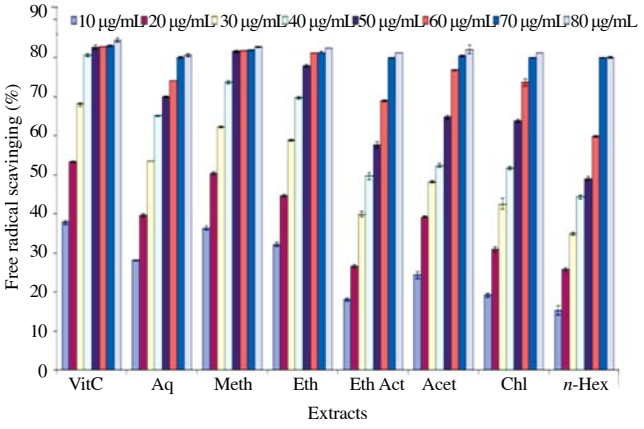

Figure 1. Antioxidant activity (DPPH) of *H. rhamnoides* leaves extracts. VitC: vitamin C; Aq: Aqueous extract; Meth: Methanol extract; Eth: Ethanolic extract; Eth Act: Ethyl acetate extract; Acet: Acetone extract; Chl: Chloroform extract; *n*-Hex: *n*-Hexane extract.

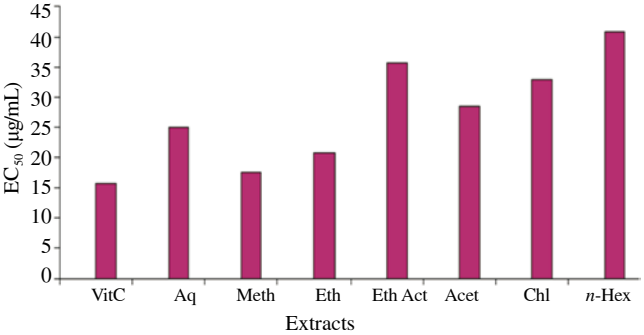

Figure 2. EC_{50} (µg/mL) of *H. rhamnoides* leaves extracts. VitC: vitamin C; Aq: Aqueous extract; Meth: Methanol extract; Eth: Ethanolic extract; Eth Act: Ethyl acetate extract; Acet: Acetone extract; Chl: Chloroform extract; *n*-Hex: *n*-Hexane extract.

The EC_{50} (µg/mL) values (Figure 2) of ascorbic acid, aqueous, methanol, ethanol, ethyl acetate, acetone, chloroform and *n*-hexane were found to be 15.75, 25.01, 17.72, 20.75, 35.70, 28.62, 32.85 and 40.86 respectively. The methanol extract EC_{50} (µg/mL) value was compatible with vitamin C (standard).

4. Discussion

The compounds which were holdup or slow down the oxidation of other molecules through stop the beginning or proliferation chain reaction of oxidation are known as antioxidant[10]. Medicinal plants have been performing a central role for drug innovation all over the world. Synthetic antioxidants are produced nowadays but the major drawback with these antioxidants is the adverse effect when used *in vivo*[11]. The uses of artificial antioxidant were restricted due to its carcinogenic nature. Therefore, the importance of non-synthetic antioxidants rises. Non-synthetic antioxidants are vitamin C, carotenoids, phenolic compounds (flavonoids, phenolic acids and tocopherols) and nitrogen compounds (amines, chlorophyll derivatives, alkaloids and amino acids)[10]. Plants have polyphenols, which are natural antioxidant because of its ability to donate H^+ or e^- and capturing free radicals[12]. Different extracts of *H. rhamnoides* pomace showed that the activity was concentration dependent and maximum was 70% in methanolic extract[13]. *H. rhamnoides* stem and roots methanol extract showed strong DPPH activities[3]. The *H. rhamnoides* pomace extracts reducing power rise as the concentration of the extracts increased and maximum was 70% in methanolic extract[14]. Flavonoids (37.50 mg/mL) from seabuckthorn exhibited an outstanding DPPH scavenging activity, which inhibit 46.5% of DPPH in two minutes[15]. DPPH radical scavenging activity RC_{50} (µg/mL) of *H. rhamnoides* methanolic extract was 5.0, while fractions of hexane, ethanol, butanol and aqueous were 3.0, 1.5, 2.5 and 9.5 respectively. Similarly stem methanolic extract RC_{50} (µg/mL) was 2.7 and fractions of hexane, ethanol, butanol and water were 3.2, 1.0, 1.4 and 3.0 respectively[3]. The current study showed that antioxidant activity was a dose dependent and methanolic extract showed the maximum activity, which was in close agreement of the most cited above research work.

Our findings revealed that *H. rhamnoides* leaves were a rich source of antioxidants. Further leaves extracts could be a strong accepted source of antioxidant and used as a curative agent to prevent or slow down oxidative stress linked with degenerative diseases. The leaves can be used in health foods for therapeutic and additive purposes, and therefore can be used as antioxidant additives or as nutritional supplements.

Conflict of interest statement

We declare that we have no conflict of interest.

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