In Vitro Antioxidant Activity Of Methanolic Extract Of *Rhodiola Imbricata* Edgew.

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Abstract

Rhodiola imbricata (Roseroot) is a perennial plant distributed in South Asia viz., Pakistan, Nepal, China and India. In India it is endemic to Trans Himalayan cold desert of Ladakh. It is a well known medicinal plant in the Amchi system of medicine (Tibetan system of medicine) being used in various ailments like anti-stress, radio-protective, anticancer, anti-inflammatory agent, adaptogen etc. The aim of this study was to assess the in vitro potential of methanol extract of *Rhodiola imbricata* roots. The DPPH activity of the extract (0.1–1.2 mg/ml) was increased in a dose dependent manner, which was found in the range of (39.55-70.76%) as compared to ascorbic acid (46.78 –81.47%). The IC_{50} values of methanol extract in DPPH radical, nitric oxide, hydroxyl radical were obtained to be 0.33, 0.47, 0.58 mg/ml, respectively. However, the IC_{50} values for the standard ascorbic acid were noted to be 0.42, 0.43, 0.51 mg/ml, respectively. Measurement of total phenolic content of the methanol extract of *R. imbricata* was achieved using Folin–Ciocalteau reagent containing 185.7 mg/g of phenolic content, which was found significantly higher when compared to reference standard gallic acid. The results obtained in this study clearly indicate that *R. imbricata* has a significant potential to use as a natural anti-oxidant agent.

Keywords: Antioxidant; DPPH; Hydroxyl radical; Nitric oxide radical; *Rhodiola imbricata*.

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INTRODUCTION

Research on relationships between antioxidants and prevention of non-communicable disease, such as cardiovascular disease, cancer and diabetes has been increasing sharply in recent years. Free radicals have been claimed to play a key role, affecting human health by causing severe diseases, such as cancer and cardiovascular diseases by cell degeneration. These free radicals can be generated during normal body function, and can be acquired from the environment. Interestingly the body possesses defence mechanism against free radical-induced oxidative stress, which involves preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Majority of the diseases/disorders are mainly linked to oxidative stress (1). The most common reactive oxygen species (ROS) include superoxide (0_2), hydrogen peroxide (H_2O_2), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion (ONOO). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (2). Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc. Whereas non-enzymatic antioxidants are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids etc (3). All these act by one or more mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants. This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area. Synthetic antioxidants

like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic (4). In recent years, the use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value. Epidemiological and *in vitro* studies strongly suggest that food containing phytochemicals with antioxidants have potentially protective effects against many diseases, including cancer, diabetes and cardiovascular diseases (5).

Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (6). Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic. etc. (7), they were also suggested to be a potential iron chelator (8, 9).

Rhodiola imbricata (Roseroot, Artic root, or Shrolo), belonging to the stone crop family *Crassulaceae*, is an important food crop and medicinal plant in Trans-Himalayan cold desert (10, 11). It is a popular medicinal plant in India, Tibet, China Nepal and Pakistan (12). *Rhodiola imbricata* is mainly known for its number of physiological functions including neurotransmitter levels, central nervous system stimulating activity, cardiovascular function, depression decreasing, enhancing work performance and learning, eliminate fatigue, immunomodulatory properties, and to prevent high-altitude sickness (13–15). Considering the importance of this area, present study was focused on some important *in-vitro* evaluation of antioxidant activity and quantification of total phenolic compounds responsible for free scavenging activity.

MATERIAL AND METHODS

Collection and Identification of Plant material

Roots of *R. imbricata*, were collected from Trans-Himalayan region (Chang-La Top, altitude 17500 ft. Above Mean Sea Level), in India in the month of June. The collected roots were identified and authenticated by a scientist Dr. OP Chaurasia, Medicinal and Aromatic Plant Division, Defence Institute of High Altitude Research (DRDO), C/o 56 APO, India. A Voucher specimen (Specimen no: A - 3) has been deposited at the Herbarium of our division. The plant samples were washed thoroughly to remove clay and dirt from them. The roots were cut into small pieces and shade dried at room temperature for 15 days, finely powdered and used for extraction.

Preparation of root extract

R. imbricata root powder was successively extracted by methanol with the help of Soxhlet apparatus till the residue remains colourless. The obtained extract was concentrated using rotary evaporator under vacuum and reduced pressure at 40°C and the residue was used for further studies.

Quantification of total polyphenolic compounds

Total polyphenols were determined by the Folin–Ciocalteu procedure (16). Aliquots (0.1 ml) of test-solution were transferred into the test tubes and volumes brought up to 0.5 ml by water. After addition of 0.25 ml Folin–Ciocalteu reagent and 1.25 ml 20% aqueous Na_2CO_3 solution, tubes were vortexed and absorbance of blue-coloured mixtures recorded after 40 min at 725nm against blank, containing 0.1 ml of extraction solvent. The amount of total polyphenols was calculated from the calibration curve of gallic acid standard solutions, concentration of total phenols was expressed as mg/g of dry extract.

DPPH radical scavenging activity

The free radical scavenging activity of the sample was measured *in vitro* by DPPH (1, 1 diphenyl 2, picryl hydrazyl) assay (17). The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. 0.1mM solution of DPPH in methanol was prepared and 3.0 ml of this solution was added to 40.0 µl of extract solution in water at different concentrations (200–1200µg/mL). The mixture was incubated at room temperature for 30 minutes and the absorbance was measured at 515 nm against corresponding blank solution. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{cont} - A_{sample}) / A_{cont} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of the extract/standard.

The antioxidant activity of the extract was expressed as IC_{50}, which the concentration (in µg/ml) of extract inhibits formation of DPPH radicals by 50%.

Nitric oxide radical-scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside