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INVESTIGATION OF IN VITRO ANTIOXIDANT POTENTIAL OF METHANOLIC EXTRACT OF BARK OF *PRUNUS CORNUTA* AND ROOT OF *RUMEX OBTUSIFOLIUS*

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ABSTRACT

In the present research work, in vitro, the antioxidant activity of methanolic extracts of bark of *Prunus cornuta* and root of *Rumex obtusifolius*, respectively, was assessed by DPPH free radical scavenging method. Total phenolic content (TPC) of methanolic extracts was also determined by spectrophotometric method using Folin-Ciocalteu reagent and calculated in µg/mL gallic acid equivalent (GAE). DPPH scavenging activity results show that the root extract of *Rumex obtusifolius* (IC_{50} = 17.15) and bark extract of *Prunus cornuta* (IC_{50} = 27.32) shows good antioxidant potential. TPC of root extract was found to be 178.56 µg/g GAE, and bark extract was 137.23 µg/g GAE. Antioxidant effect (%) determined by using phosphomolybdate assay and superoxide anion radical scavenging percentage further supports the potential antioxidant activity.

Keywords: Antioxidant, total phenolic content, DPPH, Folin-Ciocalteu reagent, phosphomolybdate assay

INTRODUCTION

Medicinal plants contain therapeutic agents that are used by natives to treat various diseases (Joshi *et al.*, 2016). They contain a wide variety of bioactive substances like saccharides, polyphenols, alkaloids, vitamins, tannins, terpenes, saponins, etc. having various biological functions (Segneanu *et al.*, 2017). Traditionally, medicinal plants are used to cure a wide range of health problems. Natural antioxidants (Xu *et al.*, 2017) like polyphenols, carotenoids, and vitamins seek researchers' attention to exploring the antioxidant activity of medicinal plants. Antioxidants are compounds that inhibit and delay the oxidation of cells by free radical species (Hunyadi, 2019), i.e., reactive nitrogen species (RNS), reactive oxygen species (ROS), reactive sulfur species (RSS), and others; produced in the body. Antioxidants acts by single electron transfer (SET) mechanism, hydrogen atom transfer (HAT) mechanism, and others to consume the free radicals species (Lu *et al.*, 2010).

Conventionally utilized natural antioxidants from leaves, fruits, vegetables, and many other plant materials/ species are exploited as antioxidant additives and dietary supplements (Halliwell, 2000). Generally, there is a lot of requirement to find more information regarding the antioxidant property of plant materials, and it has been demonstrated that the antioxidant property of plants may be due to their phenolic components, e.g., flavonoids (Schuler, 1990 & Cook and Samman, 1996). The use of traditional remedies is universal, and plants still present a great source of natural antioxidants that serve as bases for the development of new drugs. That is why; current research is now directed towards exploring naturally present antioxidants principally of plant origin.

Prunus cornuta (or Himalayan Bird Cherry) (Gaur, 1999) belongs to a family Rosaceae, is a deciduous or evergreen medium-sized tree with grey-brown to brown bark. Leaves are oblong to lance-shaped, 8-15 cm long, long-pointed, with a finely toothed margin. Small white flowers are borne in long drooping clusters, 10-15cm long and are up to 1cm across, with round petals and blunt sepals. Fruits are round cherries, about 8 mm, in a long raceme, initially red, maturing to dark purple and black. The fruit of this species often gets infected by an insect and becomes long and horn-like, from which comes its species name *cornuta*, meaning horn-like. The fruit is edible, and the leaves are used for fodder. It is found in the Himalayas, at altitudes of 2100-3500 meters. The flowering season is from April-June. With the vast potentiality of plants as sources for antimicrobial drugs regarding antibacterial and antifungal agents, a systematic investigation was carried out to screen the antibacterial and antifungal activity from *P. cornuta.* *Prunus* species have been reported as an antipyretic, refrigerant, and useful for thirst, leprosy, and leucoderma (Wang *et al.*, 2006). Some important organic compounds isolated from *Prunus cornuta* are Naringenin, Aromadendrin or Dihydrokaempferol, Cyanidin chloride, Leucocyanidin or Leucoanthocyanin, β-Sitosterol, ipuranol, Umbelliferone, 2,7,-Dimethyl-2*E*,4*E*-octadienedioic acid, Melilotoside 3' methyl ester, Prunasin or D-Mandelonitrile- -glucoside (4') or Prulaurasin Tetracosan-1,24-diol, Kaempferol (Bhatnagar and Sastri, 1960 & Austin *et al.*, 1969).

Rumex obtusifolius (Polygonaceae family) (Wang *et al.*, 2006), commonly called the broad-leaved dock

or bitter dock, is a perennial herbaceous flowering plant species. It is widely distributed throughout the world. It generally grows near meadows, waste ground, roadsides, ditches, and shorelines. It grows to a height of 50-130 cm and easily recognizable by large oval leaves with cordate bases and rounded tips. The leaves can grow to about 40 cm in length, and it contains small greenish flowers that change to red as they mature. This plant's stems are tough, often reddish, and un-branched until just below the inflorescence, and the stem leaves are alternate and are narrowly ovate-lanceolate. It blooms June through September. It shows numerous medicinal or therapeutic, and pharmacological properties. This plant is used to cure jaundice, rheumatism and its roots are used for dyeing purposes. Plants belonging to the Polygonaceae are known to produce a large number of biologically important secondary metabolites, such as anthraquinones, naphthalenes, stilbenoids, steroids, flavonoid glycosides, leucoanthocyanidins, and phenolic acids (Purohit *et al.*, 2005; Jang *et al.*, 2005; Mei *et al.*, 2009, Liang *et al.*, 2010; Gescher *et al.*, 2011; Demirezer *et al.*, 2001; El-Hawary *et al.*, 2011). It contains organic compounds such as Aloe-emodin, Sennoside A, Sennoside B, Procyanidin B2, Procyanidin B3, Procyanidin B7, Epicatechin-3-O-gallate-(4β→8)-epicatechin-3-O-gallate or procyanidin B2-3,3'-di-O-gallate, chrysophanol, beta-sitosterol, and other anthraquinones, steroids, phenolic compounds, naphthalenes, etc. (Wegiera *et al.*, 2007 & Spencer *et al.*, 2007). Therefore, this research work's objectives were to determine the amount of total polyphenolic compounds and investigate the *in vitro* antioxidant activity of the crude extracts of bark of *Prunus cornuta* and root of *Rumex obtusifolius* through DPPH radical scavenging method.

MATERIALS AND METHODS

Collection of Plant Material

Fresh plant materials of selected plants (*Rumex obtusifolius* and *Prunus cornuta)* were collected from the different regions of district Chamoli, Uttarakhand, India. These were washed thoroughly 2-3 times from tap water and then once with sterile distilled water and then air-dried under the shade at room temperature for few days. The dried material was powdered to obtain an ideal particle size using sterile clean mortar and pestle. The plants were authenticated from the Department of Botany, H.N.B. Garhwal University (A Central University), Srinagar Garhwal, Uttarakhand, India.

Preparation of plant extract

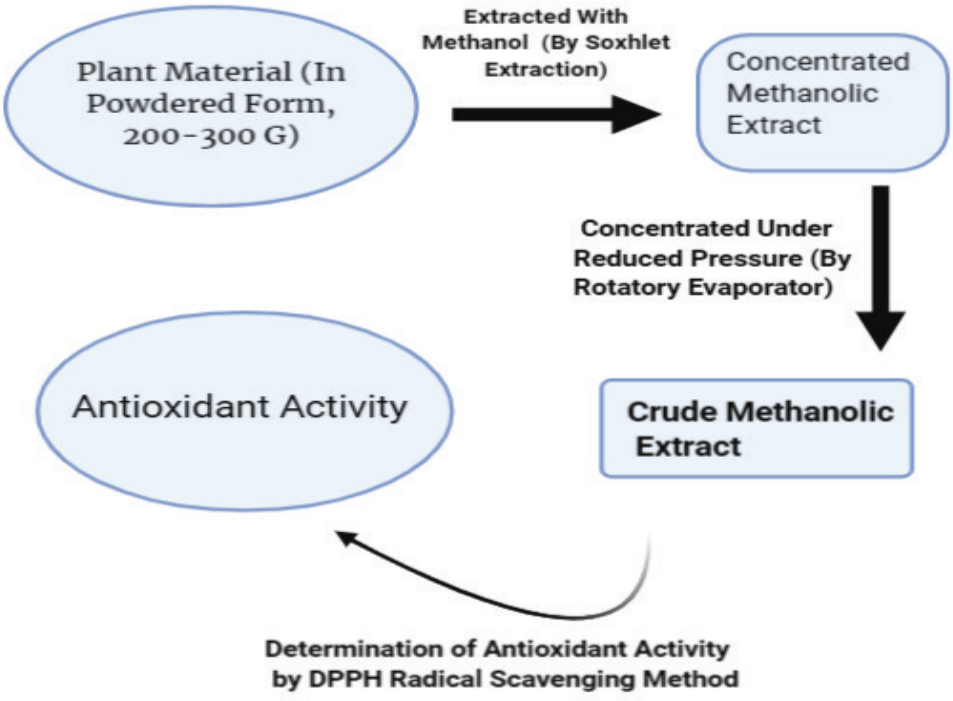
The soxhlet extraction process was employed for the preparation of extracts of dried material of selected plants using methanol as a solvent. The collected methanolic extract was further concentrated to obtain a crude methanolic extract of plant material using the Buchi-type rotatory vacuum evaporator. Finally, the natural section was organized and stored additionally used for the antioxidant assay. Fig.1. shows the scheme for the preparation of plant extract via the systematic approach.

Fig.1: Scheme of preparation of plant extract.

In vitro antioxidant activity

Determination of Antioxidant Activity by DPPH Radical Scavenging Method

The extract solution for the DPPH test (Fargere *et al.*, 1995) was prepared by re-dissolving 0.2 g each of the dried methanolic extract in 10 ml of the specific solvent in which the extract was prepared. The concentration of DPPH solution was 0.025 g in 1000 ml of methanol. Two ml of the DPPH solution was mixed with 40 µl of the extract and transferred to a cuvette. The reaction solution was monitored at 515 nm, after an incubation period of 30 minutes at room temperature, using a UV-Visible



Systronics spectrophotometer.

The DPPH free radical scavenging effect was calculated by absorbance of the solution by using the following equation:

$$\text{DPPH free radical scavenging effect } \% = \left[\frac{A_{0\,Min} - A_{30\,Min}}{A_{0\,Min}}\right] \times 100$$

A0 Min was the absorbance of the DPPH solution at zero time, and A30 Min was the absorbance of the DPPH solution after 30 minutes of incubation.

Ascorbic acid (0.5 mM) was dissolved in methanol and

Table 1: Antioxidant activity of methanolic extracts

Samples/Positive control	Antioxidant activity (IC_{50} Concentrations)	Total Antioxidant Capacity (Antioxidant effect %)
R2 @ 200 µg/ml	17.15	61.88
P1 @200 µg/ml	27.32	37.45
Ascorbic acid @5mg/ml	76.72	85.56

Table 2: TPC (µg/g gallic acid equivalents) of methanolic extracts

Samples/ Positive control	Total Phenolic Content (TPC) (µg/g GAE)
R2 @ 200 µg/ml	178.56
P1 @200 µg/ml	137.23
Ascorbic acid @5mg/ml	324

Table 3: % Inhibition of superoxide free radical of methanolic extracts

Samples/ Positive control	Superoxide anion radical scavenging activity (% Inhibition)
R2 @ 200 µg/ml	68.12
P1 @200 µg/ml	45.56
Ascorbic acid @5mg/ml	85.13

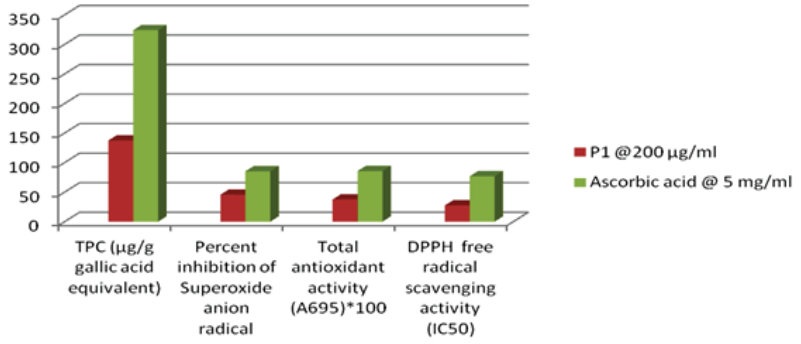


Fig. 2: Antioxidant activity of R2 sample by different conventional method

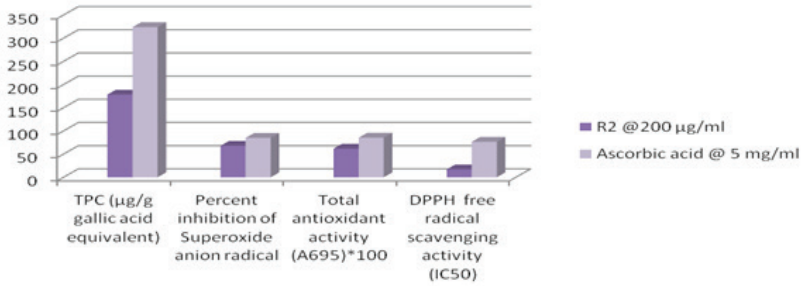


Fig. 3: Antioxidant activity of R2 sample by the different conventional method

used as a standard to convert the inhibition capability of plant extract solution to the Ascorbic acid equivalent. IC_{50} is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Determination of Total Antioxidant Activity (TAC)

The total antioxidant capacity of extracts and standard ascorbic acid was determined by the traditional determination method (Pan *et al.*, 2008). An aliquot (0.1M) of these fractions was mixed with 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Therefore, then the tubes were then capped and incubated at 95 °C for 90 minutes. After that, the samples were cooled at 25°C, and the absorbance was measured at 695 nm against the blank. The blank contained 1ml of reagent solution without the sample. The total antioxidant activity was expressed as an absorbance value at 695 nm. A higher absorbance value indicates the maximum antioxidant activity. Full antioxidant capacity was expressed in term of antioxidant effect (%) and calculated by the following equation:

$$\text{Antioxidant effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Determination of Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) of each extract was determined (Singleton and Rossi, 1965), and the phenolic content was expressed as µg/g gallic acid equivalents. In brief, a 100 µl aliquot of the sample was added to 2 ml of 0.2% (w/v) Na_2CO_3 solution. After two minutes of incubation, 100 µl of 500ml/l Follin-Ciocalteu reagent was added, and the mixture was then allowed to stand for 30 minutes at 25°C. The absorbance was measured at 750 nm using a UV-VIS Systronics spectrophotometer. The blank consist of all reagents and solvents but no sample. The Total Phenolic Content (TPC) was determined using the standard Gallic acid calibration curve and was expressed as µg/g Gallic acid equivalents.

Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activity was determined by the standard method with some modifications. The various extracts were mixed with 3ml of reaction buffer solution (pH, 7.4) containing 1.3 µM riboflavin, 0.02 M methionine, and 5.1 µM NBT. The reaction solution was illuminated by exposure to 30W fluorescent lamps for 20 minutes, and the absorbance was measured at 560 nm using Systronics UV-VIS double beam spectrophotometer. Ascorbic acid was used as the positive

control, and the reaction mixture without any sample was used as the negative control. The following equation calculated the Superoxide anion radical scavenging activity (%):

RESULTS AND DISCUSSION

Methanolic extract of samples, i.e., root of *Rumex obtusifolius* (sample R2) and bark of *Prunus cornuta* (sample P1) was investigated for their antioxidant activity, total antioxidant capacity (TAC), total phenolic content (TPC), and superoxide anion radical scavenging activity. *In vitro,* the antioxidant activity of extracts was determined by DPPH radical scavenging method. The total antioxidant capacity was determined by phosphomolybdate assay by using the ascorbic acid standard. Results are shown in Table 1. Results show that methanolic root extract of *Rumex obtusifolius* (sample R2) exhibits antioxidant activity (IC_{50} = 17.15) and bark extract of *Prunus cornuta* (sample P1) (IC_{50}=61.88). The antioxidant effect (%) value of extracts supports the result obtained from IC_{50} concentrations. Total phenolic content (TPC) of extracts expressed as µg/ml of gallic acid equivalent (GAE) was determined by spectrophotometric method, and its results are given in Table 2. Superoxide anion radical scavenging activity of methanolic extracts was determined and, the results are shown in Table 3 in terms of % inhibition.

Total phenolic content (TPC) for the R2 sample was found to be (178.56 µg/g GAE), and the P1 sample was found to be (137.23 µg/g GAE). Percentage inhibition of superoxide anion radical scavenging was found in a similar pattern as of TPC because % Inhibition of R2 sample (68.12%) was found greater than P1 sample (45.56%). All the antioxidant studies were done in reference of taken standard ascorbic acid at 5 mg/ml concentration. Results of *In vitro* antioxidant studies of both samples are shown separately in fig. 2 and 3.

CONCLUSION

The present research work is a successful attempt for the assessment of the antioxidant property of methanolic root extract of *Rumex obtusifolius* and bark extract of *Prunus cornuta,* and both samples exhibit potential antioxidant behavior. Sample R2 showed 68.12% superoxide inhibition, whereas 45.56% superoxide inhibition by sample P1. This superoxide inhibition of extracts further supported by the total phenolic content (TPC) of sample *R. obtusifolius* (178.56 µg/g GAE) and (137.23 µg/g GAE) for sample *P. cornuta*. Hopefully, this research work might motivate various researchers to further explore antioxidant applications of these plant extracts in various fields of medicine.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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