Immunomodulatory, Antioxidant potential and phytochemical study of some wild berries of North-Western Himalayan region: A Comparative study

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Abstract
In this study, the comparative analysis of some wild edible berries viz., *Berberis lycium, Duchesnea indica, Rubus fruticosus, Viburnum grandiflorum* and *Ziziphus jujube* were made for immunomodulatory, antioxidant and phytochemicals activities. Methanol extracts of *Rubus fruticosus* fruit, *Berberis lycium* leaves and *Viburnum grandiflorum* fruit demonstrated promising antioxidant potential as compared to *Ziziphus jujube* and *Duchesnea indica*. Immunomodulatory studies for these extracts were also determined by evaluating Humoral antibody (Hab) and Delayed type hypersensitivity response (DTH) in immune suppressed mice. It was found that methanol extracts of *Rubus fruticosus* fruit, *Berberis lycium* leaves, *Viburnum grandiflorum* fruit have considerable enhancing effect on Humoral antibody and DTH response as compared to *Duchesnea indica* leaves and *Ziziphus jujube* fruit. The results depicted that *Rubus fruticosus, Berberis lycium, and Viburnum grandiflorum* exhibited relatively better antioxidant and immunomodulatory potential than *Ziziphus jujube and Duchesnea indica*. Future experimentation on this species could explore their chemical compositions for better understanding of their biological activities.

Keywords: Berries; Immunomodulation; Antioxidants; Phyto-chemicals.

1. Introduction
It is known that over production of reactive oxygen species (ROS) such as O$_2^-$ (superoxide anion), H$_2$O$_2$ (hydrogen peroxide), and -OH (hydroxyl radical), formed during metabolism or through the action of ionizing radiations, can interact with biomolecules which led to various diseases such as Alzheimer, cancer, inflammation, aging, rheumatoid arthritis and atherosclerosis [1, 3]. Natural antioxidants prevent the formation of above reactive species-related disorders in human beings [4]. 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 [5, 6]. Thus medicinal plants have become an alternate health care system to solve the health problems of world in today’s synthetic allopathic era [7, 8]. Natural antioxidants especially phenolics and flavonoids from medicinal plants have been already exploited commercially either as antioxidant additives or as nutritional supplements [9]. In contrary to natural antioxidants, Synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) 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 [10].

Fruits are well known for their antioxidant potential which can help to protect the human body against cellular oxidation reactions. These benefits have stimulated research to investigate the total antioxidant capacity of fruits and vegetables [11]. Fruits, including berries, are one of the most important sources of phenolic compounds in our diets. Berry fruits are well known for containing phytochemicals (commonly referred to as phytonutrients) [12, 13]. Berry phenolics are best known for their ability to act as antioxidants. They include flavonoids (anthocyanins, flavonols and flavanols), tannins [condensed tannins (proanthocyanidins) and hydrolyzable tannins (ellagitannins and gallotannins)], stilbenoids and phenolic acids. Besides this, berry phytochemicals are known to regulate the activities of metabolizing enzymes; modulate nuclear receptors, gene expression and subcellular signaling pathways and repair DNA oxidative damage etc [14].

In our study, we targeted the wild growing plants of unexplored areas of Jammu and Kashmir i.e. Doda and Bhadarwah regions. Doda is located at 33.13°N 75.57°E with an average elevation of 1,107 metres (3631 feet) above sea level and does not have a uniform climate. In summer, the average temperature may rise upto as high as 30.4 ºC whereas in winter, it may fall upto - 1.9 ºC. Bhadarwah is located in the foothills of middle Himalayas at 32.98°N 75.72°E with an average elevation of 1,613 metres (5,295 feet) above sea level. The climate of Bhadarwah is different from that of Doda. There is a lot of snowfall during the winter season and the summer is as pleasant as Kashmir. In the present study, five wild, edible berry plants viz., *Berberis lycium, Duchesnea indica, Rubus fruticosus, Viburnum grandiflorum* and *Ziziphus jujube* were selected. All these plants are of ethnobotanical importance and have not been explored so far in this region. Fruits of these plants are edible while different parts of these plants are also used by the local folklore for treating various diseases like dysentery, diarrhoea,
jaundice, cough, fever, liver disorders etc.\textsuperscript{[15,16]. In view of the ethnobotanical importance and lack of scientific reports, we selected these plants from Doda-Bhadarwah region to evaluate their potential as natural antioxidants and phytochemical analysis of extracts of aerial parts of selected berry plants and also immunomodulatory responses.

2. Materials and methods

2.1. Collection of plant material

Fruits and leaf parts of the selected five plants viz., \textit{Berberis lyricum}, \textit{Duchesnea indica}, \textit{Rubus fruticosus}, \textit{Viburnum grandiflorum} and \textit{Ziziphus jujube} (without disease and mechanical injury) were collected from the hilly regions of Doda and Bhadarwah, Jammu and Kashmir, India (Table 1).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant</th>
<th>Part</th>
<th>Site of Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\textit{Berberis lyricum}</td>
<td>Fruit, Leaf</td>
<td>Bhadarwah</td>
</tr>
<tr>
<td>2</td>
<td>\textit{Duchesnea indica}</td>
<td>Fruit, Leaf</td>
<td>Bhadarwah</td>
</tr>
<tr>
<td>3</td>
<td>\textit{Rubus fruticosus}</td>
<td>Fruit, Leaf</td>
<td>Doda</td>
</tr>
<tr>
<td>4</td>
<td>\textit{Viburnum grandiflorum}</td>
<td>Fruit, Leaf</td>
<td>Doda</td>
</tr>
<tr>
<td>5</td>
<td>\textit{Ziziphus jujube}</td>
<td>Fruit, Leaf</td>
<td>Doda</td>
</tr>
</tbody>
</table>

2.2. Preparation of crude extracts

The plant materials (fruits and leaves) were shade dried and then ground into fine powder. The powder was used for the preparation of extracts. A total of 100 g of powdered plant material was extracted with 500 ml of methanol at 40ºC by continuous stirring for 6h. The whole process was repeated thrice. The extracts were pooled, filtered and evaporated, initially using rotary vacuum evaporator and finally using a lyophilizer\textsuperscript{[17].

2.3. Phytochemical analysis

2.3.1. Total phenolic content (TPC)

Total phenolic content in the extracts were determined by Folin Ciocalteau method\textsuperscript{[18]. In brief, 0.5ml of extract solution (1mg/ml) was mixed with 0.5ml of 1 N Folin–Ciocalteau reagent. The mixture was kept for 5 min, followed by the addition of 1ml of 20% Na2CO3. After 10 min. of incubation at room temperature, the absorbance was measured at 730 nm using UV-VIS spectrophotometer. Gallic acid was used as reference to estimate the concentration of phenolic compounds and was expressed in mg GAE/g of the extract.

2.3.2. Total flavonoids

Total flavonoid content was determined by aluminium chloride colorimetric assay, as described by Zhishen et al with some modifications\textsuperscript{[19]. 400 μl extract (1mg/ml) was mixed with 2.5ml of distilled water and 300μl of 5%NaNO3. After 5 min, 300μl of 10% AlCl3, 2ml of 1% NaOH and 1ml of distilled water were added to the mixture. The absorbance of the reaction mixture was measured at 512 nm, quercetin as standard. The total flavonoids content was expressed in mg QE/g of extract.

2.4. Free radical scavenging potential

2.4.1. DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay

In this assay, free radical scavenging activity was determined according to the method of Blois\textsuperscript{[20]. With modifications. A total of 1 ml from a 0.5mM methanol solution of the DPPH radical was mixed to 300 μl sample (1 mg/ml) and to this 0.1 M sodium acetate buffer (pH 5.5) was added. The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a double beam UV-VIS spectrophotometer. Radical scavenging activity (RSA) was expressed in percentage and calculated using the formula:

\[ \% \text{RSA} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \]

Result was presented as IC\textsubscript{50}, the concentration of extract required to scavenge 50% of the DPPH radical.

2.4.2. Ferric ion reducing antioxidant power (FRAP Assay)

FRAP activity was measured according to the method of Benzie and Strain\textsuperscript{[21]. Briefly, Acetate buffer (300 mM pH 3.6), TPTZ (2, 4, 6-tripyridyl-s-triazine) 10mM in 40mM HCl, FeCl\textsubscript{3}.6H\textsubscript{2}O (20 mM). The working FRAP reagent was prepared by mixing the three solutions in the ratio of 10:1:1 at the time of use. 200 μl test sample (1 mg/ml) was mixed with 3 ml of working FRAP reagent and absorbance was measured at 593 nm.

2.4.3. Ferrous ion chelating (FIC)

The chelating effect on ferrous ions of the prepared extracts was estimated by the method of Singh and Rajini\textsuperscript{[22]. Briefly, 500μl of each extract (1 mg/ml) and 2500 μl of methanol were added to 60 μl of 2 mM FeCl\textsubscript{3}. The reaction was initiated by the addition of 120 μl of 5 mM ferrozine into the mixture, which was then left at room temperature for 10 min before determining the absorbance of the mixture at 562 nm. The ratio of inhibition of ferrozine-Fe\textsubscript{2+}complex formation was calculated using the equation:

\[ \% \text{inhibition} = \left( \frac{\text{abs. of control} - \text{abs. of test sample}}{\text{abs. of control}} \right) \times 100 \]

2.4.4. Reducing power assay

The reducing power of the samples was assessed with slight modifications in the assay\textsuperscript{[23]. Different dilutions of extracts/fractions were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6). 2.5 ml of 1% potassium ferricyanide (K\textsubscript{3}Fe \[CN\] \textsubscript{6}) was added and the mixture was incubated at 50ºC for 20 min. After incubation, trichloroacetic acid was added to the mixture. The mixture was then centrifuged at 1036 × g for 10 min. The upper layer of the solution (2.5 ml) was taken and mixed with 2.5 ml of distilled water. To this, 2.5 ml of 0.1% ferric chloride solution was added and the absorbance was measured at 700 nm. The extract/fraction concentration providing 0.5 of absorbance (EC\textsubscript{50}) was calculated from the graph of absorbance at 700 nm against extract/fraction concentration.

2.5. Immunomodulatory Studies

Effect on humoral and cellular response in immune suppressed Swiss albino mice (\textit{Mus musculus})

Swiss albino mice (\textit{Mus musculus}) 10–12 weeks old, with 20–25 g bodyweight, and male Charles Foster rats (\textit{Rattus norvegicus}) 10–12 weeks, with 100–150 g body weight, in groups of six were employed for study. In every experiment, one group of animals was used as a vehicle control while another received a standard drug Azathioprine (Aza). The test sample was freshly prepared as a homogenized suspension in
1% w/v acacia gum administered orally daily once a day for the duration of the experiment.

(1) Antigen (SRBC): Fresh sheep red blood cells (SRBC) collected aseptically from jugular vein of sheep were stored in cold sterile Alsever’s solution, washed three times with pyrogen free sterile normal saline (0.9% NaCl w/v), and adjusted to a concentration of 5 × 10⁹ cells/mL for immunization and challenge at the required time schedule.

(2) Humoral Antibody Response (Hab): Groups of six mice each were immunized by injecting 0.2mL of 5 × 10⁹ SRBC/mL intraperitoneally (i.p.) on day 0 and challenged 7 days later by injecting an equal volume of SRBC i.p. Blood samples were collected on day +7 (before challenge) for primary antibody titre. Hemagglutination antibody titres were determined following the microtitration technique described by Nelson and Mildenhall [24]. The value of the highest serum dilution causing haemagglutination was taken as a titre. BSA saline alone served as a control.

(3) Delayed Type Hypersensitivity Response (DTH): The method of Doherty was followed to assess SRBC induced DTH response in mice [25]. Mice were immunized by injecting 20 μL of 5 × 10⁹ SRBC/mL subcutaneously into the right hind foot pad. Seven days later, the thickness of the left hind foot was measured with a spheromicrometer (0.01mm pitch) and was considered as a control. These mice were then challenged by injecting the same amount of SRBC intradermally into the left hind foot pad. The foot thickness was measured again at 0, 4, and 24 hr after challenge.

2.6 Statistical analysis
All experiments were carried out in triplicate. Data values are expressed as mean ± standard deviation.

3. Results and discussion

3.1. Extraction Yield
The extraction yield of crude methanolic extracts obtained from fruits and leaves of selected berry plants viz., Berberis lycium, Duchesnea indica, Rubus fruticosus, Viburnum grandiflorum and Ziziphus jujube is summarized in Table 2. Results showed that the yield was higher in fruit parts of all the plants as compared to their respective leaf parts except in Ziziphus jujube. Highest yield was obtained from the leaf of Ziziphus jujube (35.02%) whereas lowest yield was obtained from the leaf of Rubus fruticosus (6.24%).

3.2. Phytochemical analysis

Total phenolic content: Results indicated that leaves of all the berry plants possessed higher phenolic content as compared to their respective fruit parts (Table 3). The concentration of phenolic compounds was calculated using standard gallic acid graph. Leaf extract of Berberis lycium possessed highest phenolic content (188.64 mg GAE/g dry wt.) followed by leaf extracts of Rubus fruticosus (177.02 mg GAE/g dry wt.), Viburnum grandiflorum (172.43 mg GAE/g dry wt.), Ziziphus jujube (162.70 mg GAE/g dry wt.) and Duchesnea indica (162.43 mg GAE/g dry wt.) respectively. However, the fruit extract of Rubus fruticosus was found to contain highest phenolic content with the value of 162.16 mg GAE/g dry wt. as compared to the extracts of all the other fruits.

Total flavonoids: The flavonoid content in the extracts was calculated using standard quercetin graph. Results showed that the flavonoid content in the leaf parts of all the berry plants except Ziziphus jujube was higher than their respective fruit parts (Table 3). Leaf extract of Viburnum grandiflorum possessed highest flavonoid content (350 mg QE/g dry wt.), followed by Berberis lycium (340 mg QE/g dry wt.), Rubus fruticosus (280 mg QE/g dry wt.), Duchesnea indica (70 mg QE/g dry wt.) and Ziziphus jujube (70 mg QE/g dry wt.) respectively.

Analysis of total phenols and flavonoids in all the berry plants revealed that the leaves possessed higher content of phenols and flavonoids as compared to fruits. It is well established that phenols and flavonoids present in berry plants contribute to several biological activities like antioxidant, anti-carcinogenic, anti-angiogenic, antimicrobial and anti-aging [26-28]. Plant polyphenols vary from place to place and also with genotypes and growing seasons [29]. Changes in the plant phytochemistry occur during the maturation of fruit or other plant tissues [30]. Many studies have established that the concentration of phytochemicals may vary from plant to plant or even in different organs of the same plant at different ripening stages [31].
The total antioxidant activity of plant extracts/fractions cannot be evaluated by using one single method due to the different mechanisms of oxidative processes that produce different types of free radicals. Owing to multifaceted aspects of free radicals and antioxidants behavior, different assays were employed to assess the antioxidant activity of extracts/fractions of the selected berry plants viz., Berberis lycium, Duchesnea indica, Rubus fruticosus, Viburnum grandiflorum and Ziziphus jujube. These assays included 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, chelation power, ferric reducing antioxidant power (FRAP) and reducing power.

2, 2-diphenyl-1-picrylhydrazyl (DPPH): In the present study, DPPH radical scavenging activity of crude methanolic extracts obtained from fruit and leaf parts of selected berry plants was assessed. The assays were carried out in triplicates and the results were expressed as mean inhibitory concentration (IC50) which is defined as the concentration of substrate necessary to scavenge 50% of DPPH free radicals.

Extracts exhibited the scavenging activity in a dose-dependent manner. The percentage inhibition of extracts was assessed at five different concentrations (30, 50, 100, 150 and 200 μg/ml) respectively. The DPPH radical scavenging activity of the extracts is summarized in Table 4. Results showed that all the extracts exhibited the scavenging activity in a dose-dependent manner. The percentage inhibition of extracts was assessed at five different concentrations (30, 50, 100, 150 and 200 μg/ml) and was found to increase with an increase in concentration. In Rubus fruticosus, both fruit as well as leaf extracts exhibited highest DPPH radical scavenging activity with IC50 values of 30.78 μg/ml and 56.31 μg/ml respectively. In Berberis lycium, leaf extract showed better scavenging activity (IC50 = 83.29 μg/ml) than the fruit extract (IC50 = 238.52 μg/ml). Similar results were observed in Viburnum grandiflorum where leaf extract had better scavenging activity (IC50 = 125.82 μg/ml) than the fruit extract (IC50 = 294.57 μg/ml). In Duchesnea indica and Ziziphus jujube, both fruit and leaf extracts exhibited relatively low DPPH radical scavenging activities. The DPPH radical scavenging activity of crude methanolic extracts of fruit and leaf part of Rubus fruticosus, leaf part of Berberis lycium and leaf part of Viburnum grandiflorum was close to the standards used.

3.3. Free radical scavenging potential

The total antioxidant activity of plant extracts/fractions cannot be evaluated by using one single method due to the different mechanisms of oxidative processes that produce different types of free radicals. Owing to multifaceted aspects of free radicals and antioxidants behavior, different assays were employed to assess the antioxidant activity of extracts/fractions of the selected berry plants viz., Berberis lycium, Duchesnea indica, Rubus fruticosus, Viburnum grandiflorum and Ziziphus jujube. These assays included 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, chelation power, ferric reducing antioxidant power (FRAP) and reducing power.

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Thus, fruit extract of Rubus fruticosus showed moderate scavenging activity and the result was in accordance with previous reports where other species of Rubus genus like Rubus ellipticus (fruit) also showed ferrous metal ion chelation activity. Earlier reports also confirm low ferrous ion chelating activity in the fruits of Ziziphus jujube. Some studies on Berberis sp. Like Berberis crataegina, Berberis vulgaris and Berberis integerrima have shown moderate chelation activity. Some species of Viburnum i.e. Viburnum tinus and Viburnum luzonicum have been reported to possess significant chelating activity. In contrast to the previous reports regarding the metal ion chelating activity, our results suggest lack of chelating activity in Duchesnea indica and Viburnum grandiflorum. This variation in chelation activity may be due to many factors like variation in plant polyphenols from place to place and also with genotypes and growing seasons.

Ferric reducing antioxidant power (FRAP) assay: In the present study, the standard curve of ferrous (Fe2+) sulphate was plotted using different concentrations (100-1500 μM) and the results were expressed as μM Fe(II)/g dry weight of the plant material (Table 4). The FRAP value of extracts ranged from 33.4 μM Fe(II)/g to 955.4 μM Fe(II)/g dry weight of the plant material as indicated in Table 4. According to their antioxidant power, all the extracts were divided into four groups as per the classification given by Wong et al., 2006; extremely high (>500 μM Fe(II)/g), high (100-500 μM Fe(II)/g), medium 10-100 μM...
Fe(II)/g) and low (<10 μM Fe(II)/g). Our results showed strongest antioxidant capacity to reduce ferric ions was shown by *Rubus fruticosus* extracts (fruit = 955.4 μM Fe(II)/g dry wt. and leaf = 935.4 μM Fe(II)/g dry wt.) and leaf extract of *Viburnum grandiflorum* (909.4 μM Fe(II)/g dry wt.). Leaf extract of *Berberis lycium* and fruit extract of *Viburnum grandiflorum* exhibited high antioxidant activity with FRAP values of 319.4 μM Fe(II)/g dry wt. and 361.4 μM Fe(II)/g dry wt. respectively, whereas fruit and leaf extracts of *Duchesnea indica*, *Ziziphus jujube* and fruit extract of *Berberis lycium* showed moderate antioxidant activity (Table 4). Hence, these results are in agreement with those reported earlier [40, 41].

A range of blackberry genotypes harvested in different seasons and different regions of United States and Mexico showed varied range of ferric reducing antioxidant potential from low to high values [42]. Siriwoharn and Wrolstad showed *Rubus laciniatus* and other *Rubus* sp. hybrid for potential ferric reducing antioxidant capacity (Methanolic extract of *Viburnum tinus* fruit displayed high FRAP value which is in accordance with our results on *Viburnum grandiflorum* fruit extract [37]. According to Ozgen et al., fruits of *Berberis vulgaris* showed good ferric reducing antioxidant potential. Many cultivars of *Ziziphus mauritiana* (Indian jujube) have shown ferric reducing antioxidant potential [43].

**Reducing power assay:** The reducing power of crude methanolic extracts of all the five plants (fruit and leaf) was assessed. The reducing power of extracts to change ferric ions into ferrous ions was measured as the change in yellow colour of the test solution to various shades of green and blue depending on reducing power of each antioxidant extract. Therefore, the Fe2+ ion can be monitored by measuring the formation of Perl’s Prussian blue colour at 700 nm. The increasing absorbance indicates increase in reducing ability of the extract. The presence of reductants in plant extracts causes the reduction of Fe3+/ferrous cyanide complex to ferrous form. The assays were carried out in triplicates. The extract concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance at 700 nm against extract concentration. The reducing activity of the extracts is summarized in Table 4. Increase in absorbance (reducing power) of the extracts was observed in a dose dependent manner. Low reducing activity was observed in the extracts. The EC50 value of all the extracts ranged from 428μg/ml to 1657.66 μg/ml. On comparing the reducing power of extracts with the standard antioxidant, ascorbic acid (EC50 = 103.2μg/ml), it was observed that fruit extract of *Rubus fruticosus* and leaf extract of *Duchesnea indica* with EC50 values of 428μg/ml and 429μg/ml possessed moderate reducing activity. Least activity was observed in the fruit extract of *Berberis lycium* (EC50 = 1657.66 μg/ml).

Previous studies suggest that species of *Rubus* genus are known for their reducing activities. *Rubus ellipticus* (Yellow Himalayan Raspberry) showed strong reducing activity [33]. Root bark of *Berberis lycium* possesses reducing activity [34]. Several species of *Berberis* i.e. *Berberis vulgaris*, *Berberis croatica* and *Berberis tinctoria* have been reported for their reducing activity [35]. However our results showed low reducing activity in fruit and leaf parts of *Berberis lycium*. In the present study, *Ziziphus jujube* extracts displayed low reducing activity whereas Li et al., reported *Ziziphus jujube* (Chinese jujube) cultivars from China for their reducing activities. This variation can be due to changes in plant phytochemistry from place to place, organ to organ and different growing seasons.

**Table 4.** Free radical scavenging activities of crude methanol extracts of *Berberis lycium*, *Duchesnea indica*, *Rubus fruticosus*, *Viburnum grandiflorum* and *Ziziphus jujube*.

<table>
<thead>
<tr>
<th>Assays</th>
<th>Berberis lycium</th>
<th>Duchesnea indica</th>
<th>Rubus fruticosus</th>
<th>Viburnum grandiflorum</th>
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<tr>
<td></td>
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<tr>
<td>DPPH assay (IC50) μg/ml</td>
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<td>83.29</td>
<td>712.7</td>
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<td>Cpetition power (%)</td>
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<td>10.91</td>
<td>4.51</td>
<td>4.33</td>
<td>28.22</td>
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<td>FRAP (μMFe(II)/g dry wt.)</td>
<td>87.4±</td>
<td>319.4±</td>
<td>33.4±</td>
<td>81.4±</td>
<td>955.4±</td>
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<td>Reducing power (EC50) μg/ml</td>
<td>1657.6</td>
<td>530.0</td>
<td>560.8</td>
<td>429.0</td>
<td>428.0</td>
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</table>

**3.4 Immunomodulatory studies**

After screening of extracts (fruit and leaves) for the potent efficient antioxidants from above data (Table 4), *Berberis lycium* leaves, *Duchesnea indica* leaves, *Rubus fruticosus* fruit, *Viburnum grandiflorum* fruit and *Ziziphus jujube* fruit were selected for carrying out the immunomodulatory studies. The effect of methanol fractions of selected (screened) plants on humoral and cell mediated immune response in immune suppressed mice were studied (Table 5). After 7 days of oral administration of fractions to the immune suppressed mice showed some interesting results. The methanol fractions of the plants were observed to enhance the humoral and cell mediated immune response in immune suppressed mice (Table 5). The humoral antibody titre of *Rubus fruticosus* at the concentration of 100mg/ml was observed to be 108% which was more than that of standard drug, Levamisole (100%). Also, *Rubus fruticosus* showed noteworthy positive DTH response at 100mg/ml (133%). *Berberis lycium*, *Viburnum grandiflorum*, *Duchesnea indica* and *Ziziphus jujube* also showed potent abrogative effect on humoral antibody and delayed type hypersensitivity response in immune suppressed bab/lc mice. The phytoconstituents responsible for significant antioxidant and immunomodulatory activities of these plants are yet to be researched upon in order to recognize their other biological potentials. Therefore, it can be deduced from the study that all the methanolic fractions of *Berberis lycium*, *Duchesnea indica*, *Rubus fruticosus*, *Viburnum grandiflorum* and *Ziziphus jujube* possesses significant immune stimulating potential. These observations are suggestive of possible therapeutic usefulness in immune compromised patients.
Table 5: Effect of active methanol fractions of selected plants and parts on humoral and cell mediated immune response.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample</th>
<th>Conc. mg/kg P.O</th>
<th>Antibody Titre Mean ± S.E.</th>
<th>% Activity</th>
<th>DTH Mean ± S.E.</th>
<th>% Activity</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>4.66 ± 0.22</td>
<td>-29.0</td>
<td>0.35 ± 0.05</td>
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<td>Levamisole</td>
<td>2.5</td>
<td>6.5 ± 0.22</td>
<td>+100</td>
<td>0.51 ± 0.09</td>
<td>+133.33</td>
</tr>
<tr>
<td>4</td>
<td><em>Berberis lycium</em> (leaves)</td>
<td>100</td>
<td>5.83 ± 0.16</td>
<td>+64</td>
<td>0.51± 0.09</td>
<td>+133.33</td>
</tr>
<tr>
<td>5</td>
<td><em>Duchesnea indica</em> (leaves)</td>
<td>100</td>
<td>4.96 ± 0.26</td>
<td>+16</td>
<td>0.44 ± 0.17</td>
<td>+75</td>
</tr>
<tr>
<td>6</td>
<td><em>Rubus fruticosus</em> (fruit)</td>
<td>100</td>
<td>6.66 ± 0.21</td>
<td>+108</td>
<td>0.51± 0.09</td>
<td>+133.33</td>
</tr>
<tr>
<td>7</td>
<td><em>Viburnum grandiflorum</em> (fruit)</td>
<td>100</td>
<td>5.06 ± 0.21</td>
<td>+22</td>
<td>0.47 ± 0.07</td>
<td>+100</td>
</tr>
<tr>
<td>8</td>
<td><em>Ziziphus jujube</em> (fruit)</td>
<td>100</td>
<td>4.89 ± 0.17</td>
<td>+13</td>
<td>0.45 ± 0.02</td>
<td>+82</td>
</tr>
</tbody>
</table>

4. Conclusion

Berries play an important role in benefitting human health beyond basic nutrition. They are a rich source of phytochemicals and reduce the risk of a number of diseases caused as a result of oxidative stress. Their effects on health are largely attributed to their antioxidant properties. While commercially available synthetic antioxidants are not preferred for pharmacological use due to number of side effects and toxicological concerns, plants as a source of natural antioxidants can prove to be a better substitute. Thus, more interests have focussed on identifying plant extracts for use as dietary antioxidant supplements.

In the present study, five wild growing, edible berry plants viz., *Berberis lycium*, *Duchesnea indica*, *Rubus fruticosus*, *Viburnum grandiflorum* and *Ziziphus jujube* were selected from Doda-Bhadarwah region of Jammu and Kashmir. Fruits and leaves of all these plants were collected for investigation. Fruits of these plants are edible while leaves are used by the local folklore in the treatment of a number of diseases like dysentery, diarrhoea, jaundice, cough, fever, liver disorders etc. In addition to their medicinal importance, different parts of these plants are used in the dietary regime by local people. For example, leaves of *Berberis lycium*, *Rubus fruticosus* and *Ziziphus jujube* are used as tea substitute by the locals. In view of the ethnobotanical importance and lack of sufficient scientific reports of these plants in this region, these plants were selected for their antioxidant and phytochemical analysis. Different assays were employed for assessing the antioxidant potential of fruit and leaf parts of these plants i.e. DPPH radical scavenging assay, chelation power assay, ferric reducing antioxidant power assay and reducing power assay. Crude methanol extracts were prepared from dried fruits and leaves of these berry plants and then analyzed for their antioxidant and phytochemical constitution. It was found that methanol extracts of leaf part of *Berberis lycium*, fruit and leaf part of *Rubus fruticosus* and leaf part of *Viburnum grandiflorum* showed significant antioxidant potential which correlates with their phenolic and flavonoid content.

The methanol fraction obtained from crude methanol extract of these plants were observed to harbour considerable antioxidant potential and, in case of immune modulating studies, all these plants methanolic fractions were observed to show remarkable immune suppressive as well as immune stimulating potential, which could prove to be of immense value in autoimmune diseases as well as in immune compromised patients. However, the phytoconstituents responsible for their significant activity are still unknown. The results obtained in this work provide the basis for designing future experimentation on this species for better understanding of its antioxidative and immunomodulatory system and discovering the phytochemicals responsible for these properties.

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6. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

7. References


