EISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 1546-1550
Accepted: 06-04-2018

Mudasir A Mir
Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

P Mani
Department of Biotechnology, Annai College of Arts & Science, Kumbakonam, Tamil Nadu, India

Zahoor Dar
Department of Biotechnology, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India

MV Rao
1Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu India

Correspondence
Mudasir A Mir
Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Jammu and Kashmir, India

Evaluation of antioxidant properties of Lavatera cachemiriana

Mudasir A Mir, P Mani, Zahoor Dar and MV Rao

Abstract
The free radicals are routinely produced in the body which brings many cellular cascades that can lead damage to the cells, tissues, cell function and macromolecules such as oxygen, lipids, proteins and DNA. Natural antioxidants are seen as promising agents to offer protection against oxidative stress related diseases. Lavatera cachemiriana plant species was undertaken to evaluate its In-vitro antioxidant potential through various assays like DPPH, reducing power, TAC, and ABTS. Results have demonstrated that methanolic roots extract have showed lower but statistically significant antioxidant activities against various free radicals as compared to reference standards. The free radical scavenging potential of L. cachemiriana reflects its use as an ethno-pharmacological medicine to prevent and treat different diseases.

Keywords: Antioxidant, free radical scavenging, Lavatera cachemiriana, oxidative stress

Introduction
The free radicals are routinely produced in cellular metabolism which brings many cellular cascades that can cause lead damage to the cells, tissues, cell function and macromolecules such as oxygen, lipids, proteins and DNA (Vijayalakshmi and Ranganathan, 2012; Pisoschi and Negulescu, 2011) [27, 18]. The oxidative stress has been associated with various pathological conditions such as aging, atherosclerosis, diabetes, cancer etc. (Ramkumar et al. 2007) [21]. Antioxidants such as phenolics, tannins, flavonoids are seen as promising agents to protect against oxidative stress related diseases (Basker and Negbi, 1999) [3] by retarding rate of oxidation or inhibit the propagation of controlled regulation of free radical formation (Babbar et al. 2011) [2].

Lavatera cachemiriana (Family-Malvaceae) being an ethno-medicinally important plant species is used to prevent and treat different diseases or ailments by the people of Kashmir Himalaya. It possesses important medicinal applications towards renal colic, common cold, mumps, throat problems, as a mild laxative, dandruff, skin irritation, antiseptic, anti-lipoxygenase, anti-bacterial, anti-cancer etc. (Kaul, 2010; Jeelani et al. 2013; Malik et al. 2011, Vidyarthi, 2010; Parveen, 2013; Dar et al, 2004) [11, 10, 15, 26, 17]. The over exploitation of this species poses a danger to its existence, thus determination of biological properties scientifically seems a good strategy to validate its ethno-medicinal properties. Hence, current investigation was carried out to study anti-oxidant activities in the root portion of Lavatera cachemiriana plant species.

Materials and Methods
Planting material collection, authentication and extraction
L. cachemiriana roots were collected from Gulmargh region of Jammu and Kashmir (10,020 feet above sea level) during the year 2012. Samples were deposited at the University of Kashmir herbarium (KASH-1726). The collected root samples were air dried at room temperature for 3 days and subjected to powder formation, extraction of phytocompounds was done using methanol as a solvent. The crude extract was filtered using Whatman No. 1 filter paper and the extract so obtained was stored in brown colour sterile bottles. These extracts were stored in the labeled sterile brown colour screw capped bottles at 5°C for subsequent use (Tiwari et al. 2011) [25].

In-vitro antioxidant assays
DPPH radical scavenging activity
DPPH radical scavenging of each plant extract was determined by method as described by Silva and Soysa, 2011 [23]. The radical scavenging activity was determined using below equation:

\[
\text{DPPH scavenging activity} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100
\]
\[
\% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}}) \times 100}{A_{\text{control}}}
\]

Where, A control represents absorbance of control at t=0 min and A sample represents absorbance of sample at t=30 mins. Gallic acid, ascorbic acid and Rutin were used as reference standards and calibration curve was used to determine IC\(_{50}\) values for each test solution i.e. conc. required to inhibit formation of DPPH radical by 50%.

**Reducing power assay**

Ferric chloride reducing power of all the sample extracts were determined by method previously described by Hazra et al. 2008. Gallic acid and ascorbic acid were used as reference standards and analysis of all extracts was performed in triplicates (n=3).

**Total antioxidant capacity (TAC) by phosphomolybdenum assay**

The total antioxidant capacity of all the extracts was determined based on method reported in the literature (Ganesana et al. 2010) [5]. Ascorbic acid was used as reference standard and analysis of each extract was performed in triplicates (n=3). The total antioxidant activity was determined using calibration curve of ascorbic acid obtained by dissolving it in different concentrations using methanol (62.5, 125, 250, 500, 100 µg/ml) and values are expressed as mg/g ascorbic acid equivalents.

**ABTS radical cation assay**

ABTS based In-Vitro antioxidant assay of all methanolic sample extracts were determined using ABTS radical cation Assay (Nenadis et al. 2004) [16]. Antioxidant activity of extracts were expressed as IC\(_{50}\) & Rutin was used as a standard.

\[
\text{Scavenging effect (} \% \text{)} = \frac{(Ab - At) \times 100}{Ab}
\]

Where Ab = Absorbance of blank
At = Absorbance of test

**Results and Discussion**

The generation of free radicals (reactive oxygen species-ROS) brings many reactions which can cause damage to the tissues, cell function and macromolecules such as oxygen, lipids, proteins and DNA (Pisoschi and Negulescu, 2011) [18]. The oxidative stress has been associated with various pathological conditions such as aging, atherosclerosis, diabetes, cancer etc. (Ramkumar et al. 2007) [21]. Nevertheless, antioxidants offers promising protection against oxidative stress related diseases (Alhakmani et al. 2012; Ramkumar et al. 2007) [21] by retarding rate of oxidation or inhibit the propagation of free radical formation (Pisoschi and Negulescu, 2011) [18] which eventually shows anti-cancer, hypolipidemic, anti-aging and anti-inflammatory activities (Shokrzadeh and Saravi, 2010) [22]. Considering the presence of significant quantity of phenol and flavonoid in methanolic extract, we took up only methanolic extract further for assessing in-vitro antioxidant activities of L. cachemeriana root extract. As per Hermans et al. 2007, [8] more number of experiments should be carried out to assess antioxidant potential of a compound as this produces a comprehensive prediction of its antioxidant potential. Therefore, current study used three in-vitro models to validate the antioxidant potential of L. cachemeriana methanolic root extract.

**DPPH radical scavenging activity**

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical (Deep purple colour) owing its delocalization of spare electrons which prevents its dimmerization and side reactions, as that happens with most of the free radicals (Nenadis et al. 2004; Rakimuthu et al. 2012) [16, 20]. DPPH radical scavenging activity of ethanolic extract was expressed as percentage inhibition, IC\(_{50}\) values were determined (Table 1, Fig. 1). The results were compared with three reference anti-oxidants i.e. gallic acid, ascorbic acid and rutin; results have showed IC\(_{50}\) value of 91.47±1.6 µg/ml for root extract which is significantly not different (P>0.05) from gallic acid (90.94±0.95 µg/ml).

The order of DPPH based antioxidant activity observed in current study is rutin> ascorbic acid> gallic acid> root extract. The IC\(_{50}\) values of rutin, ascorbic acid and sample have significant difference in the radical scavenging capacity (p<0.05). Furthermore, presence of various phytoconstituents within the root extract of L. cachemeriana could be responsible for its radical scavenging activities such as phenolics, flavonoids, alkaloids, quinones, tannin etc (Son and Sosa et al. 2013) [24]. The radical scavenging activity of root extract can be also attributed due to its highest flavonoid content (6.62±1.15 mg/g of catechol equivalents) because it has been reported that there exists a positive linear correlation between antioxidant activities with their total phenolic and flavonoid contents (Zheng and Wang, 2001) [28]. The extent of DPPH radical scavenging activity by an extract mostly depends on the concentration of phenolic and flavonoid compounds (Kiessoun et al. 2010) [12].

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Sample</th>
<th>Test Conc. (µg/ml)</th>
<th>IC(_{50}) (µg/ml)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LCR</td>
<td>150</td>
<td>50.411±1.3</td>
<td>91.47±1.6</td>
</tr>
<tr>
<td>2</td>
<td>Gallic acid</td>
<td>10</td>
<td>35.99±0.34</td>
<td>90.94±0.95</td>
</tr>
<tr>
<td>3</td>
<td>Ascorbic acid</td>
<td>40</td>
<td>40.42±2.7</td>
<td>94.22±1.6</td>
</tr>
<tr>
<td>4</td>
<td>Rutin</td>
<td>6.25</td>
<td>33.45±0.43</td>
<td>95.86±1.6</td>
</tr>
</tbody>
</table>

Table 1: DPPH radical scavenging activity and IC\(_{50}\) values of methanolic root extract of Lavatera cachemeriana and reference standards.
Reducing power assay

The highest reducing power was presented by ascorbic acid (0.940±0.03 at conc. 1000 µg/ml) followed by methanolic root extract (0.65±0.06 at conc. 1000 µg/ml) and lowest was shown by gallic acid as 0.335±0.45 at conc. 1000 µg/ml. The increase in the absorbance of the reaction mixture indicates higher reducing power (Prasad et al. 2012) [19] and reducing power was interpreted using IC50 value i.e. the concentration of the sample (Extract/standard) at which absorbance is 50% (Table-2, Fig. 2). It was observed that methanolic root extract has showed significantly greater reducing power (IC50 320±2.3 µg/ml) as compared to ascorbic acid and gallic acid with IC50 as 984±2.12 µg/ml and 580±1.12 µg/ml respectively (P<0.05). The reducing power is an indicator of anti-oxidant potential (Ganesana et al. 2010) [5] where in transformation of ferric-Fe (III) to ferrous-Fe (II) takes place in presence of an antioxidant by formation of Perl’s Prussian blue (Gulcin et al. 2003) [6] and this could be possibly due to transfer of hydrogen from phenolic compounds, also the position and number of hydroxyl groups of phenolic determine antioxidant activity (Huda et al. 2007) [9]. The presence of significant amount of flavonoid and phenolic content could be responsible for its reducing power activity as has been already reported in the earlier literature which mentions that a positive linear correlation exists between total phenol and flavonoid content versus reducing power (Zheng and Wang, 2001) [28]. However, reducing power assay alone does not fully characterize the antioxidant activity and needs to be validated by other methods.

Table 2: Absorbance and IC50 values of Lavatera cachemeriana methanolic root extract and standards by reducing power assay

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Gallic acid</th>
<th>Ascorbic acid</th>
<th>LCR</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0.3±0.034a</td>
<td>0.314±0.022a</td>
<td>0.439±0.11a</td>
<td>984±2.12</td>
</tr>
<tr>
<td>400</td>
<td>0.305±0.05a</td>
<td>0.48±0.07b</td>
<td>0.468±0.09b</td>
<td>580±1.12</td>
</tr>
<tr>
<td>600</td>
<td>0.31±0.12c</td>
<td>0.517±0.12c</td>
<td>0.749±0.06e</td>
<td>320±2.3</td>
</tr>
<tr>
<td>800</td>
<td>0.32±0.07c</td>
<td>0.585±0.08d</td>
<td>0.876±0.14d</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>0.335±0.45b</td>
<td>0.65±0.06e</td>
<td>0.940±0.03e</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD, n=3; and values with different letters shows significant difference at P<0.05 level

Total antioxidant capacity (TAC)

The results of total antioxidant capacity assay have shown that extract has promising TAC of 57 mg/g ascorbic acid at 1000 µg/ml (Fig.3B), a good correlation was observed between absorbance and sample concentration with (r²=0.97) for ascorbic acid and r²=0.94 for root extract. The addition of the extract leads reduction of Mo (VI) to Mo (V) and subsequent formation of green phosphate/Mo (V) complex at acidic pH (Prasad et al. 2012) [19]. The increase in the absorbance indicates the increase in total antioxidant capacity of the extracts (Rakkimuthu et al. 2012) [20] and it was observed in current study that methanolic root extract and ascorbic acid have showed absorbance of 3.79±0.1 and 2.172±0.16 at 1000 µg/ml (Fig. 3A & Table-3). It has been found that there is a significant difference in the absorbance values of root extract and ascorbic acid at all tested concentrations (P<0.05).
The total antioxidant capacity of extracts could be due to presence of phenolics, flavonoids, ascorbates, terpenes, reducing carbohydrates, tocopherols, carotenoids, other organic acids, pigments or the synergistic effect and redox interactions among these molecules (Leland and Seke, 2006; Pisoschi and Negulescu, 2011; Babbar et al. 2011) \[13, 18, 2\]. Earlier reports mentioned that there exists a statistically significant relationship between total antioxidant capacity and total phenol and flavonoid contents (Zheng and Wang, 2001) \[28\]. As per previous review (Pisoschi and Negulescu, 2011) \[18\], TAC is a recent novel way to estimate the relationship between diet and oxidative stress induced diseases and there exists a negative relationship between dietary TAC versus incidences of diseases such as gastric cancer or the levels of C-reactive proteins. The mechanism of antioxidant activity could be due to various ways such as chain initiation prevention, the binding of transition metal ion catalysts, peroxide decomposition, reducing capacity, free radical scavenging etc.

### Table 3: Absorbance values of Lavatera cachemiriana methanolic root extract by TAC phosphomolybdenum assay

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Ascorbic acid</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>2.41±0.07</td>
<td>1.148±0.07</td>
</tr>
<tr>
<td>400</td>
<td>2.71±0.17</td>
<td>1.286±0.2</td>
</tr>
<tr>
<td>600</td>
<td>2.98±0.12</td>
<td>1.370±0.09</td>
</tr>
<tr>
<td>800</td>
<td>3.25±0.5</td>
<td>1.523±0.08</td>
</tr>
<tr>
<td>1000</td>
<td>3.79±0.1</td>
<td>2.172±0.16</td>
</tr>
</tbody>
</table>

The results of ABTS based antioxidant assay has demonstrated IC\(_{50}\) value as 46.47±2.3 µg/ml which lower than the IC\(_{50}\) values of standard antioxidants i.e. ascorbic acid and rutin with IC\(_{50}\) as 9.59±2.1 µg/ml and 15.77±0.1 µg/ml respectively (Table 4 & Fig. 4). Thus, order of antioxidant potential against ABTS based on IC\(_{50}\) value can be arranged in ascorbic acid>rutin>LCR. The results have indicated that there exists a significant difference in ABTS radical scavenging activity by L. cachemiriana root extract compared to reference standards \(P<0.05\). ABTS [2, 2'-azino-bis (3-ethylbezothiazoline-6-sulfonic acid)] assay is relatively a recent one, which involves a more drastic radical, chemically produced which absorbs at 743 nm which gives bluish-green colour due to loss of an electron by the nitrogen atom upon oxidation by potassium persulphate or manganese dioxide. In the presence of an antioxidant, the nitrogen atoms would quench hydrogen atoms, resulting in the discoloration of the reaction mixture (Nenadis et al. 2004) \[16\]. ABTS assay is mostly used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids. The solubility in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS radical for the estimation of the antioxidants (Pisoschi and Negulescu, 2011; Nenadis et al. 2004) \[18, 16\]. The existence of antioxidant activity of root extract could be due to presence of phenolics, flavonoids, ascorbates, terpenes, reducing carbohydrates, tocopherols, carotenoids, other organic acids, pigments or the synergistic effect and redox interactions among these molecules (Pisoschi and Negulescu, 2011) \[18\]. The various phenolics, flavonoid and other non-polar compounds have been found in other species Malvaceae family which promising antioxidant potential (Kiessoun et al. 2010) \[13\].

### Table 4: ABTS radical scavenging activity and IC\(_{50}\) values of methanolic root extract of Lavatera cachemiriana and reference standards

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample</th>
<th>Test Conc. (µg/ml)</th>
<th>% Inhibition</th>
<th>IC(_{50}) values (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LCR</td>
<td>6.25</td>
<td>5.84±1.0</td>
<td>2.172</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5</td>
<td>28.07±6.8</td>
<td>1.523±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>43.66±1.2</td>
<td>1.370±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>63.74±4.50</td>
<td>1.148±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>99.4±1.5</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>99.8±0.9</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.625</td>
<td>18.4±0.55</td>
<td>26.58±0.09</td>
</tr>
<tr>
<td>2</td>
<td>Ascorbic acid</td>
<td>2.5</td>
<td>9.59±2.1</td>
<td>1.370±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>43.37±1.2</td>
<td>1.148±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>61.61±4.80</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>72.29±1.3</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.12</td>
<td>23.2±0.25</td>
<td>26.58±0.09</td>
</tr>
<tr>
<td>3</td>
<td>Rutin</td>
<td>12.5</td>
<td>48.5±1.5</td>
<td>15.77±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>89.2±1.7</td>
<td>1.370±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>93.5±0.5</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>96.5±1.7</td>
<td>0.625</td>
</tr>
</tbody>
</table>

(Values are mean ± SD, n=3; and values with different letters shows significant difference at \(P<0.05\) level).
Fig 4: IC₅₀ values of Lavatera cachemiriana methanolic root extract and reference standards by ABTS radical scavenging method. (Values are mean ± SD, n=3)

Conclusion
The present work attempted to evaluate In-vitro antioxidant properties of L. cachemiriana methanolic crude extract of roots. It was found that this plant species is rich in antioxidant compounds which are responsible to confer it radical scavenging properties. In conclusion, methanolic extract of root portion has demonstrated lower but statistically significant levels of antioxidant potential.

Conflict Of Interest
All the authors confirm that there is no conflict of interest.

References
2. Babbar N, Oberoi HS, Uppal DS, Patil RT. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Res. Intern. 2011; 44:391-396.