Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 1546-1550
Received: 04-03-2018
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
[4]Department 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 cachemeriana*

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

Abstract
The free radicals are routinely produced by 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 cachemeriana (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, anti-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. cachemeriana 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 5^0C 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:

$$\% \text{ inhibition} = \frac{(A_{control} - A_{sample}) \times 100}{A_{control}}$$

Where, A control represents absorbance of control at t=0 min and Asample 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 acidand 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 IC50 & Rutin was used as a standard.

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

Where Ab = Absorbance of blank
At = Absorbance of test

Esults 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.

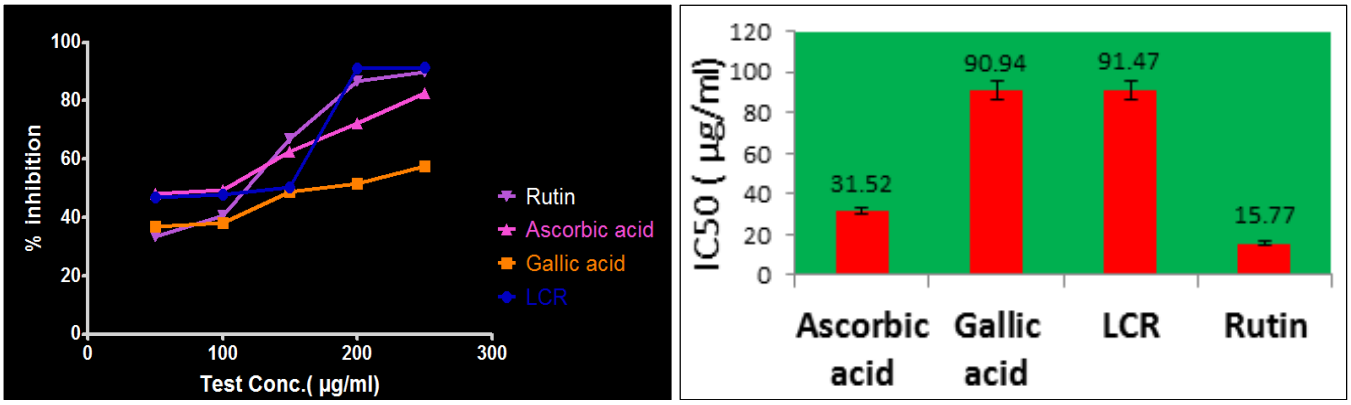
DPPH radical scavenging activity

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; Rakkimuthu *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 (Soni 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 1: DPPH radical scavenging activity and IC_{50} values of methanolic root extract of *Lavatera cachemeriana* and reference standards.

Sl No.	Sample	Test Conc. (µg/ml)	% inhibition	IC_{50} value (µg/ml)
1	LCR	50	*47 ± 1.5	91.47 ±1.6
		100	47.94 ± 2.45	
		150	50.411 ± 1.3	
		200	91 ± 1.21	
		250	91.47 ± 0.5	
2	Gallic acid	10	36.99 ± 0.34	90.94 ± 0.95
		20	38.06 ± 0.07	
		40	48.79 ± 0.25	
		80	51.64 ± 0.4	
		160	57.7 ± 1.35	
3	Ascorbic acid	20	48.22 ± 1.35	31.52 ± 0.38
		40	49.42 ± 0.27	
		60	62.64 ± 0.48	
		80	72.29 ± 1.3	
		100	82.75 ± 0.26	
4	Rutin	6.25	33.45 ± 0.03	15.77 ± 0.12
		12.5	40.7 ± 0.15	
		25	66.8 ± 0.9	
		50	86.6 ± 1.4	
		100	89.8 ± 0.8	



A B

Fig 1-A: DPPH radical scavenging activity (% inhibition) B) IC_{50} values of methanolic root extract of *Lavatera cachemeriana* and reference standards.

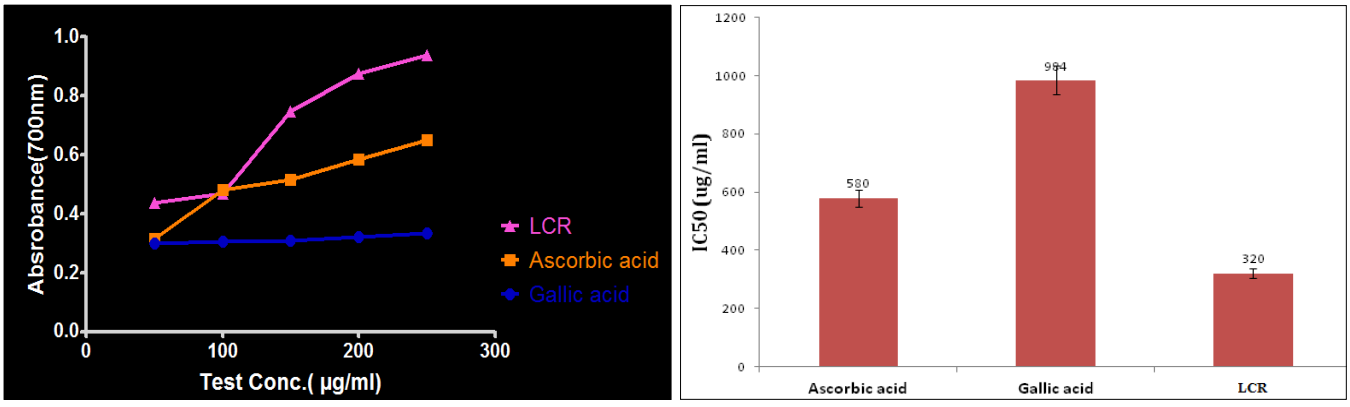
Reducing power assay

The highest reducing power was presented by ascorbic acid (0.940±0.03at 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 (IC_{50} 320±2.3µg/ml) as compared to ascorbic acid and gallic acid with IC_{50} 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 IC_{50} values of *Lavatera cachemeriana* methanolic root extract and standards by reducing power assay

Conc. (µg/ml)	Gallic acid	Ascorbic acid	LCR
200	0.3±0.034^{a}	0.314±0.02^{a}	0.439±0.1 ^{a}
400	0.305±0.05^{a}	0.48±0.07^{b}	0.468±0.09^{b}
600	0.31±0.12^{a}	0.517±0.12^{c}	0.749±0.06^{c}
800	0.321±0.07^{b}	0.585±0.08^{d}	0.876±0.14^{d}
1000	0.335±0.45^{b}	0.65±0.06^{e}	0.940±0.03^{e}
IC50 (µg/ml)	984±2.12	580±1.12	320±2.3

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



A B

Fig 2-A: Antioxidant activity and IC_{50} values of *Lavatera cachemeriana* methanolic root extract and standards by reducing power assay

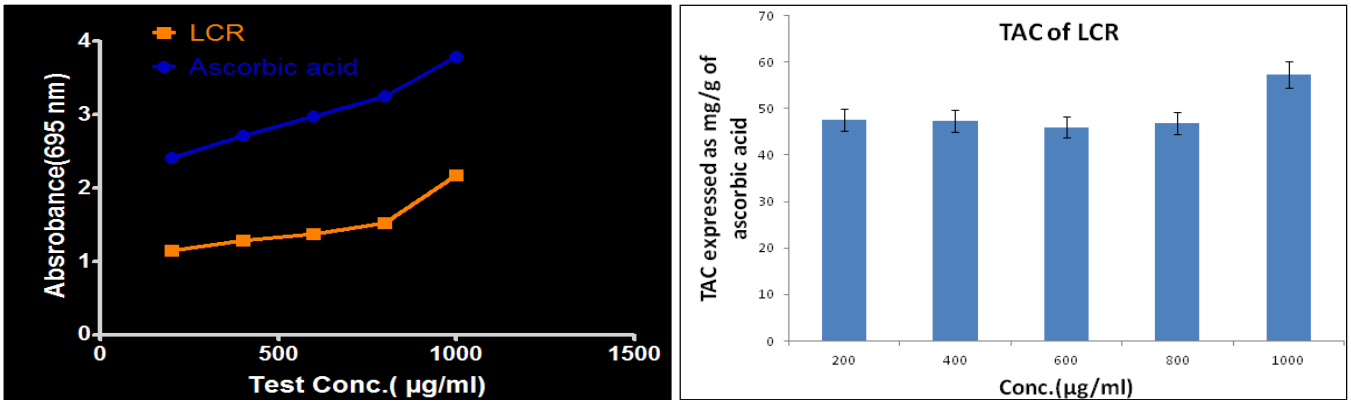
Total antioxidant capacity (TAC)

The results of total antioxidant capacity assay have shown that extract has promising TAC of 57 mg/g ascorbic acid at 1000 ug/ml (Fig.3B), a good correlation was observed between absorbance and sample concentration with (r^2=0.97) for ascorbic acid and r^2=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 ug/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 dietry 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 cachemeriana* methanolic root extract by TAC phosphomolybdenum assay

Conc. (ug/ml)	Ascorbic acid	LCR
200	2.41±0.07	1.148±0.07
400	2.71±0.17	1.286±0.2
600	2.98±0.12	1.370±0.09
800	3.25±0.5	1.523±0.08
1000	3.79±0.1	2.172±0.16



A B

Fig 3-A: Absorbance values shows total antioxidant capacity (TAC) B) IC_{50} values of *Lavatera cachemeriana* methanolic root extract by TAC phosphomolybdenum assay

ABTS radical cation Assay

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. cachemeriana* 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) [12].

The difference in the total antioxidant capacity of extracts could be assigned due to their difference in the concentrations of phenolic and flavonoid compounds. Also, the difference in total antioxidant activity values depending on the method used because of difference in the radicals used and the mechanisms of reaction (Lizcano *et al*. 2010) [14]. The antioxidant potential evaluated in the current study would help to understand the importance of this traditional medicinal herb and could pave the way for sustainable conservation and utilization.

Table 4: ABTS radical scavenging activity and IC_{50} values of methanolic root extract of *Lavatera cachemeriana* and reference standards

Sl. No.	Sample	Test Conc. (µg/ml)	% inhibition	IC_{50} values (µg/ml)
1	LCR	6.25	5.84±1.0	
		12.5	28.07±0.8	
		25	43.66±1.2	46.47 ± 2.3[a]
		50	63.74±0.50	
		100	99.4±1.5	
		150	99.8±0.9	
		0.625	18.8±0.55	
2	Ascorbic acid	1.25	21.305±0.49	
		2.5	26.58±0.09	9.59± 2.1[b]
		5	43.37±1.2	
		10	61.61±0.48	
		20	72.29±1.3	
		3.12	23.2±0.25	
		6.25	25.5±0.9	
3	Rutin	12.5	48.5±1.5	15.77±0.12[c]
		25	89.9±1.7	
		50	93.5±0.5	
		100	96.5±1.7	

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

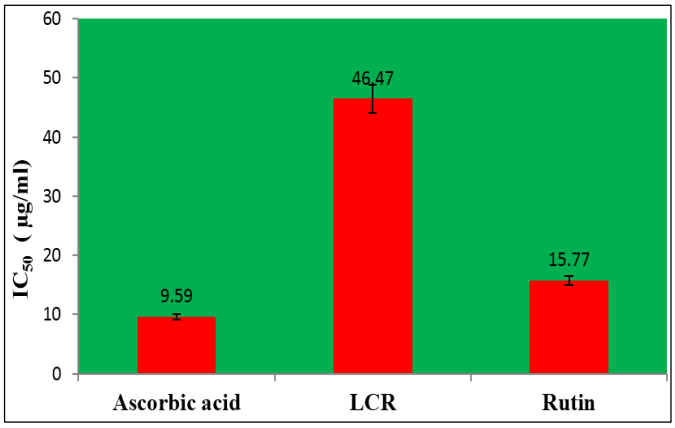


Fig 4: IC_{50} values of *Lavatera cachemeriana* methanolic root extract and reference standards by ABTS radical scavenging method. (Values are mean ± SD, n=3)

Conclusion

The present work attempted to evaluate *In-vitro* antioxidant properties of *L. cachemeriana* 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

1. Alhakmani F, Kumar S, Khab SA. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of Moringaoleifera. Asianpac J Trop Biomed. 2013; 8:623-627.
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.
3. Basker D, Negbi M. The uses of Saffron. Econ. Bot. 1999; 3:228-236.
4. Dar GH, Bhagat RC, Khan MA. Biodiversity of Kashmir Himalaya Valley book house, Srinagar Kashmir, J & K, 2002.
5. Ganesana B, Perumal P, Manickam VB, Mummudi S, Gotteti SD, Srikakolapu SR *et al*. Quantitative Estimation of Gallic Acid in Triphala Churnam Tablet by RP-HPLC. Der Pharma Chemica. 2010; 2:3.
6. Gulcin I, Oktay M, Kirecci E, Kufrevvioglu. Screening of antioxidant and antimicrobial activities of anise (*Pimpellaanisum* L.) seed extracts. Food. Chem. 2003; 83:371-382.
7. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of Spondiaspinnata. BMC Complement Altern Med. 2008; 8:63.
8. Hermans N, Cos P, Maes L, Bruyne TD, Berghe DV, Vlietinck AJ *et al.* Challenges and Pitfalls in Antioxidant Research. Curr. Med. Chem. 2007; 14:417-430.
9. Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS. Antioxidative activities of water extracts of some Malaysian herbs. ASEAN food J 2007; 14:61-68.
10. Jeelani SM, Wani MP, Kumari S, Gupta RC, Siddique MA. Ethno botany of some polypetalous plants from the Kashmir Himalaya. JMPR. 2013; 7:2714-2721.
11. Kaul MK. High altitude botanicals in integrative medicine-Case studies from Northwest Himalaya. IJTK. 2010; 9:18-25.
12. Kiessoun K, Souza A, Meda MTR, Coulibaly AY, Kiendrebeogo M, Meda AL *et al*. Polyphenol Contents, Antioxidant and Anti-Inflammatory Activities of Six Malvaceae Species Traditionally used to Treat Hepatitis B in Burkina Faso. Eur. J Sci. Res. 2010; 4:570-580.
13. Leland J, Seke C. Natural Products from Plants 2nd edn. Taylor & Francis group, 2006.
14. Lizcano LJ, Bakkali F, Ruiz-Larrea MB, Ruiz-Sanz JI. Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use. Food Chem. 2010; 119:1566-1570.
15. Malik AH, Khuroo AA, Dar GH, Khan ZA. Ethnomedicinal uses of some plants in the Kashmir Himalaya. Indian J Tradit. Knowl. 2011; 10:362-366.
16. Nenadis N, Wang LF, Tsimidou M, Zhang HY. Estimation of scavenging activity of phenolic compounds using the ABTS + assay. J Agric Food Chem. 2004; 15:4669-4674.
17. Parveen S. *In vitro* studies of some medicinal plants of western Himalayas *viz* Rheum emodi, Bergenialigulata, *Lavatera cashmiriana*. Ph.D. Thesis Submitted to University of Kashmir, Srinagar, 2013.
18. Pisoschi AM, Negulescu GP. Methods of Total antioxidant activity determination: A review. Biochem & Anal Biochem. 2011; 1:106.
19. Prasad SK, Kumar R, Patel DK, Sahu AN, Hemalatha S. Physicochemical standardization and evaluation of *In-vitro* antioxidant activity of Aconitum heterophyllum Wall. Asian Pac J Trop Biomed. 2012; 2:S526-S531.
20. Rakkimuthu R, Kavithakrishna R, Suganyadevi P, Aravinthan. Quantitaive phytochemical analysis and their antioxidant activity of *Cocculus hirsutus* (L.) Diels fruit. International Journal of Phytomedicine. 2012; 4:447-455.
21. Ramkumar KM, Rajaguru P, Ananthan R. Antimicrobial properties and phytochemical constituents of an antidiabetic plant Gymnema montanum. Adv Biol Res. 2007; 1:67-71.
22. Shokrzadeh M, Saravi SS. The chemistry, pharmacology and clinical properties of Sambucus ebulus: A review. J Med. Plants. 2010; 4:095-103.
23. Silva IK, Soysa P. Evaluation of phytochemical composition and antioxidant capacity of a decoction containing *Adenanthera pavonina* L. and *Thespesia populnea* L. Pharmacogn Mag. 2011; 727:193-199.
24. Soni A, Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J Pharmacogn Phytochem. 2013; 4:22-29.
25. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction. A Rev. IPS. 2011; 1:99-100.
26. Vidyarthi SOP. Forest Flora of Kashmir. Working Plan Circle, Jammu and Kashmir Forest Department, 2010, 91-92.
27. Vijayalakshmi R, Ranganathan R. Antioxidant potential of various extracts from whole plant of *Anisomeles malabarica* (Linn.) RBR. RJPBCS. 2012; 3:43-49.
28. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric. Food Chem. 2001; 49:5165-5170.