

ANTIOXIDANT POTENTIAL OF *CUSCUTA REFLEXA* AND *LATHYRUS ODORATUS*

Usman ul Haq¹, Muhammad Altaf Hussain¹, Aamir Sharif^{2*}

Abstract: In this study, the antioxidant activity of methanolic extracts of two medicinally important plants *Lathyrus odoratus* (*L. odoratus*) and *Cuscuta reflexa* (*C. reflexa*) collected from different districts of Azad Jammu and Kashmir were assessed. Methanolic extract of *Cuscuta reflexa* and *Lathyrus odoratus* have been analyzed for their free scavenging radical activity by utilizing 1, 1- diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. In a dose-dependent manner, all extracts showed antioxidant properties as compared to the standard drug i.e., ascorbic acid i.e. 2.65. The IC₅₀ value of *C. reflexa* collected from Bhimber (CRB) and Kotli (CRK) was 2.90 µg/ml and 1.12 µg/ml respectively, whereas the IC₅₀ value of *L. Odoratus* collected from Kotli (LOK) and Mirpur (LOM) was found to be 28.35 µg/ml and 12.95 µg/ml respectively as compared to IC₅₀ value of standard ascorbic acid that was 21.22 µg/ml. The total antioxidant activity also increased in a dose-dependent manner. The phytochemical analysis showed that these plants are rich in glycosides, alkaloids, tannins, saponins, anthocyanin, flavonoids, and phenolics. These results may show that these plant species have antioxidant properties and may act as a cardioprotective agents.

1. Department of Biotechnology, Mirpur University of Sciences and Technology, Azad Jammu and Kashmir-Pakistan
2. Department of Pathology, Sargodha Medical College, University of Sargodha-Pakistan

Corresponding author email:

aamirsharif95@gmail.com

Keywords: *Cuscuta reflexa*, *Lathyrus odoratus*, antioxidant activity, IC₅₀

INTRODUCTION

Oxygen free radicals and their secondary metabolites play a significant role in the development of different neurodegenerative diseases like Parkinson's syndrome, Alzheimer's disease, autism, arthritis, diabetes, cancer, cataracts, ischemia, aging and liver disorders (1-3). In a biological system, oxygen free radicals are normally produced during cellular metabolism and functional activities as a byproduct and have a vital role in ion transportation, cell signaling, gene expression, tissue injury, and apoptosis (4). The major sites of ROS production are endothelial cells, phagocytic cells, nuclei, endoplasmic reticulum, microsomes, and mitochondria (5). In order to reduce or inhibit the oxidative damage induced by ROS, cells exhibit various mechanisms which include free scavenging, metal chelating, and enzymatic

activities to neutralize these free radicals after their production. Moreover, when these mechanisms failed to cope with body needs, antioxidant supplementation may be helpful to reduce tissue damage caused by oxidative stress. Plants are a rich source of antioxidants (6). Antioxidants from natural resources increased the antioxidant capability of plasma and as a result reduce the risk of various ailments like stroke, heart disease, and cancer (7). Plants have secondary metabolites like flavonoids and phenolics which are considered effective free radical scavengers. They are present in all parts of plants like roots, seeds, fruits, leaves, and bark (8). These days, many synthetic antioxidants are in practice. Various side effects (9) like carcinogenesis and liver damage are reported in laboratory animals to which these antioxidants were given. Exploration

for natural antioxidants agent has become progressively significant due to health implications since synthetic antioxidants like tert-butyl hydroquinone (TBHQ), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) could be harmful and might have oncogenic effects (9). For this purpose, there is an increasing interest in finding cost-effective, less toxic, and more effective antioxidants from medicinal plants to replace synthetic antioxidants and eliminate these health concerns.

In this study, two medicinally important plants were used i.e. *Cuscuta reflexa* and *Lathyrus odoratus*. *Cuscuta reflexa* is a twining parasitic stem of the family *Convolvulaceae* (4). *Cuscuta reflexa* contains a number of phytochemicals like alkaloids, glycosides, tannins, volatile oil, cuscutin, quercetin, cerotic acid, flavonoids, sitosterol, carotenoids, kaempferol, dulcitol, coumarin, cetiparone, myricetin, rhamnoside, rutinoid, propionamide, reflexin, lycopene, amarbelin, stearic, palmitic acid, and hydroxyoleanane. Various studies show that *C. reflexa* exhibits hypoglycaemic, anti-spasmodic, free radical scavenging and antioxidant activity. The plant extract also shows anti-viral activity due to the combined effect of different constituents (2).

Lathyrus odoratus is a flowering plant belonging to the family *Fabaceae*. It has pinnate leaves with terminal tendrils. It is cultivated for food and forage as well as ornamental purposes. *L. odoratus* contains a number of phytochemicals i.e. alkaloids, glycosides, tannins, flavonoids, etc. due to these chemicals, it also exhibits antioxidant activity.

MATERIALS AND METHODS

Present research work was conducted from March 2016 to February 2017 in the Department of Biotechnology Mirpur University of Sciences and Technology (MUST) Mirpur, Azad Kashmir.

COLLECTION OF PLANT MATERIAL

Cuscuta reflexa Roxb stem and leaves of *Lathyrus odoratus* were collected from different localities of the Mirpur Division including Districts Mirpur, Bhimber, and Kotli. Parts of medicinal plants were washed and dried under shade. After drying, the plant sample was macerated to powdered form with a mixer grinder and stored in an airtight jar for further use.

EXTRACTION

The ground samples were soaked in organic solvents (Methanol) in a separate airtight flask for seven days in the dark. In *Cuscuta reflexa* Roxb. 100g dried stem powder was soaked in the 200 ml solvent while in 100g dried leaves powder of *Lathyrus* spp. 500 ml solvent was added. After seven days, the extracts were filtered with the help of filter paper and the filtrate was dried by a rotary evaporator. A solid mass of plant extract was preserved at 4°C for further investigation for potential antioxidant properties to evaluate.

RADICAL SCAVENGING ACTIVITY BY DPPH

The free radical scavenging activity was measured by using DPPH assay. The reduction of DPPH radicals was obtained by measuring the absorption at 517 nm. Ascorbic acid was used as standard. The scavenging action was measured by using the following formula: Percentage radical scavenging activity

$$= \frac{(A \text{ Control} - A \text{ Sample})}{(A \text{ Control})} \times 100$$

RESULTS

The antioxidant activity of the methanolic extract of *Cuscuta reflexa* and *Lathyrus odoratus* was measured by DPPH free radical scavenging assay and ascorbic acid was used as standard (Table 1). Percentage inhibition and half inhibitory concentration were calculated and results

were shown in Table 2 and 3 respectively. The graph was drawn against different concentrations of plant extracts i.e. 5µl, 10µl, 20µl and 30µl. In the antioxidants, graph concentration was taken on X-axis while percentage inhibition was taken on Y-axis.

The percentage inhibition of methanolic extract of *Cuscuta reflexa* collected from Bhimber was 92.7, 91.27, 90.36, and 89.97 respectively while the *Cuscuta reflexa* collected from Kotli showed percentage inhibition of 91.08, 90.82, 90.36 and 89.97 respectively as shown in Table 2. Methanolic extract of *Lathyrus odoratus* collected from Mirpur showed percentage inhibition of 79.81nm, 72.65nm, 70.05nm, and 68.09nm respectively. While methanolic extract of *Lathyrus odoratus* collected from Kotli values was recorded as 83.72, 83.07, 76.56 and 60.28 respectively (Table 2). The half maximum inhibitory concentration IC₅₀ of *Cuscuta reflexa* (CRK) was 1.122118

While IC₅₀ of CRB was 2.908277 as shown in table 3. The IC₅₀ of *Lathyrus odoratus* (LOM) was 12.95743 and IC₅₀ of *Lathyrus odoratus* of Kotli (LOK) was 28.35026 (Table 3).

The *Cuscuta reflexa* collected from Bhimber showed a slightly higher percentage of inhibition than those samples collected from Kotli. While the *Lathyrus odoratus* samples collected from district Kotli showed maximum percentage inhibition as compared to *Lathyrus odoratus* of Mirpur. The dosage correlation used in the antioxidant activity of methanolic extract of *Cuscuta reflexa* as the value of R² was 0.9387. While *Lathyrus odoratus* extract R² with increase in concentration was recorded as 0.8268 and 0.904.

DISCUSSION

Medicinal plants contain secondary metabolites, which are organic in nature and don't involve directly in reproduction, growth and development of an organism. But they often play a major role in plant defences

(7) and have the capability to inhibit or kill the growth of microorganisms (8).

Antioxidant compounds have the ability to trap free radicals; these compounds like flavonoids or peroxidase inhibit oxidative mechanisms. In the last few years, the attention towards natural antioxidants has gained importance due to oxidative stress production which is ultimately a major factor of the disorder. In the body, exogenous and endogenous factors contribute to the production of free radicals. Antioxidants are used for the neutralization of free radicals by various methods and prevent the body from different diseases like liver cirrhosis, aging, atherosclerosis, cancer, etc. Synthetic as well as natural antioxidants are available. Synthetic antioxidants like BHA have proven nowadays harmful effects on humans. Antioxidants from natural sources gain attention for their less harmful effects. So as an alternate source of antioxidant compounds, medicinal plants as a whole or their parts have been investigated.

Cuscuta reflexa and *Lathyrus odoratus* contain several compounds having antioxidant potential. Recent studies showed that *Cuscuta reflexa* is a rich source of flavonoids. Due to the presence of flavonoids, *Cuscuta* is considered a source of dietary antioxidants. Preliminary phytochemical studies of the stem of *L. Odoratus* indicate that the antioxidant activity is due to the presence of flavonoids and polyphenols.

Various methods are used to measure antioxidant activities, but the DPPH scavenging assay was the most used method. All samples show dose-dependent anti-DPPH radical activity. The IC₅₀ value was calculated from regression equations. According to the results, *Cuscuta reflexa* shows more antioxidant activity than *Lathyrus* spp (9). The antioxidant activity of *Cuscuta reflexa* Roxb. *Lathyrus odoratus* was observed and considerable variations.

observed between these two species. The lowest percentage inhibition was observed in LOK i.e. 60.28% (Table 2) and the highest percentage inhibition was observed in 92.70% in CRB (Table 2) graph 1,2 and 3 of antioxidant activities showed that antioxidant activity of extract increased in dose-dependent manner.

CONCLUSION

C. reflexa and *L. odoratus* showed strong antioxidant activity by inhibiting DPPH as compared to ascorbic acid. The result of this study shows that extract can be utilized as an antioxidant.

REFERENCES

1. Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Geier MR. A prospective study of transsulfuration biomarkers in autistic disorders. *Neurochemical research*. 2009;34(2):386.
2. Giles GI, Jacob C. Reactive sulfur species: an emerging concept in oxidative stress. *Biological chemistry*. 2002;383(3-4):375-88.
3. Siddhuraju P, Mohan P, Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food chemistry*. 2002;79(1):61-7.
4. Vajragupta O, Boonchoong P, Berliner LJ. Manganese complexes of curcumin analogues: evaluation of hydroxyl radical scavenging ability, superoxide dismutase activity and stability towards hydrolysis. *Free radical research*. 2004;38(3):303-14.
5. Vallyathan V, Shi X. The role of oxygen free radicals in occupational and environmental lung diseases. *Environmental Health Perspectives*. 1997;105(suppl 1):165-77.
6. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. *Free Radical Biology and Medicine*. 2004;7(36):827-8.
7. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables: diet and health implications. *HortScience*. 2000;35(4):588-92.
8. Mathew S, Abraham TE. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food and Chemical Toxicology*. 2006;44(2):198-206.
9. Ito N, Fukushima S, Haqlwara A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats.

Table 1: Antioxidant Activity of *Cuscuta reflexa* and Lathyrus Spp.

Serial No.	Extract name	Concentrations of extract				Blank
		5µl	10µl	20µl	30µl	
1	CRB	0.112	0.134	0.145	0.163	1.536
2	CRK	0.137	0.141	0.148	0.154	
3	LOK	0.25	0.26	0.36	0.61	
4	LOM	0.31	0.42	0.46	0.49	
5	Ascorbic acid	0.068	0.071	0.089	0.116	

Table 2: Percentage Inhibition of Antioxidant Activity

Serial no.	Extract name	Concentrations of extract			
		5µl	10µl	20µl	30µl
1	CRB	92.70	91.27	90.36	89.97
2	CRK	91.08	90.82	90.36	89.97
3	LOK	83.72	83.07	76.56	60.28
4	LOM	79.81	72.65	70.05	68.09
5	Ascorbic acid	95.57	95.37	94.20	92.44

Table 3: Half Maximal Inhibitory Concentration of Plant Extracts

Plants	5 µL	10 µl	20 µl	30 µl	IC50
CRB	92.7	91.27	90.36	89.97	2.908277
CRK	91.08	90.82	90.36	89.97	1.122118
LOK	83.72	83.07	76.56	60.28	28.35026
LOM	79.81	72.65	70.05	68.09	12.95743



