ORIGINAL ARTICLE

TIMES JOURNAL OF HEALTH & SOCIAL SCIENCES

Antioxidant Potential of Cuscuta reflexa and Lathyrus odoratus

Usman ul Haq1, Muhammad Altaf Hussain1, Aamir Sharif2*, Muhammad Akram3, Imtiaz Mahmood Tahir4, Wafa Abbaass3, Rida Zainab3, Idrees Khan3

Abstract: In this study, 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 property 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 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. 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 plants species have antioxidant property and may act as a cardioprotective agent.

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

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

3 Department of Eastern Medicine, Government College University Faisalabad-Pakistan

4 College of Allied Health Professionals, Government College University Faisalabad-Pakistan

Corresponding author email:
aamirsharif95@gmail.com

INTRODUCTION

Oxygen free radicals and its secondary metabolites play a signifi 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 by- product and have a vital role in ion transportation, cell signalling, 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 includes free scavenging, metal chelating and enzymatic activities to neutralize these free radicals after their production. Moreover, when these mechanisms failed to cope up with body need, 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 diseases and cancer (7). Plants have secondary metabolites like flavonoids and phenolics which are considered as the effective free radical scavengers. They are present in all parts of plants like roots, seeds, fruits, leaves and bark (8). In 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 the synthetic antioxidants like tert-butyl hydroquinone (TBHQ), butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) could be harmful and might have oncogenic effects (10). 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 to eliminate these health concerns.

In this study, two medicinally important plants were used i.e. *Cuscuta reflexa* and *Lathyrus odoratus*. *Cuscuta reflexa* is a twingling parasitic stem of 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, cumarin, cetiparone, myricetin, rhamnoside, rutinoside, propionamide, reflexin, lycopene, amarbelin, stearic, palmitic acid and hydrooxyoleanane. 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 (12).

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

MATERIALS AND METHODS

Present research work was conducted during March 2016 to February 2017 in 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 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 airtight jar for further use.

EXTRACTION

The grinded 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 were 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 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:

$$\text{Percentage radical scavenging activity} = \frac{(\text{A Control} - \text{A Sample})}{(\text{A Control})} \times 100$$

RESULTS

The anti-oxidant activity of 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 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 cereflexa* 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 IC 50 of CRB was 2.908277 as shown in table 3. The IC50 of *Lathyrus odoratus* (LOM) was 12.95743and IC 50 of *Lathyrus odoratus* of Kotli (LOK) was 28.35026 (Table 3).

The Cuscuta reflexa collected from Bhimber showed slightly higher percentage 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 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.

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	5µl	10µl	20µl	30µl	IC_{50}
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

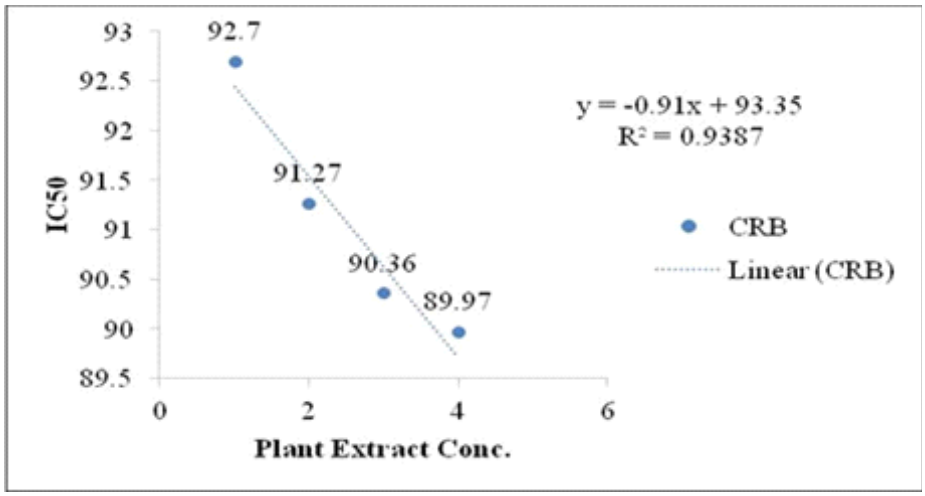

Fig. 1: Dosage effect (µl) and antioxidant activity of *Cuscuta reflexa* collected from Bhimber (CRB)

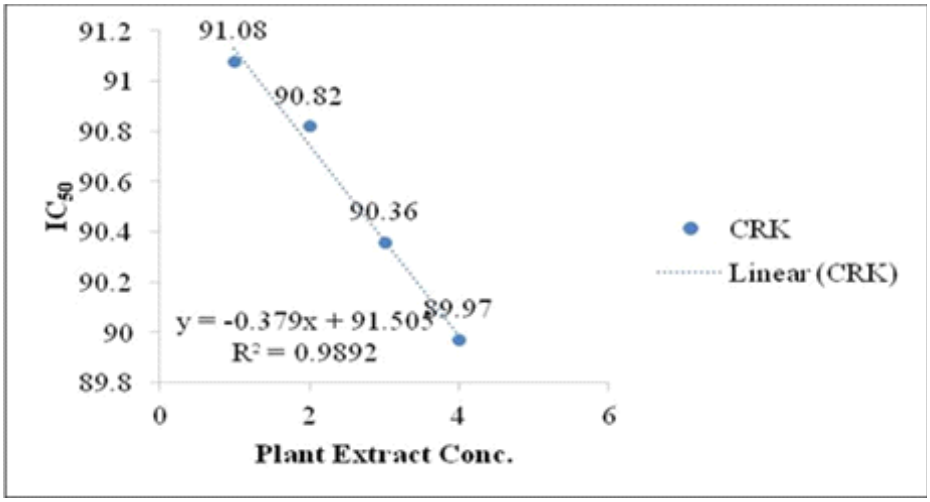

Fig. 2 Dosage effect (µl) and antioxidant activity of *Cuscuta reflexa* collected from Kotli (CRK)

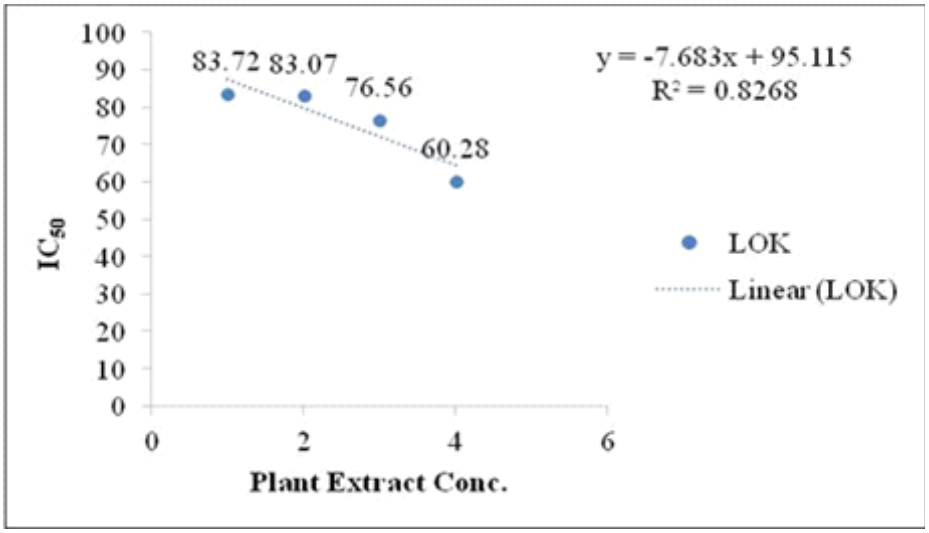

Fig. 3 Dosage effect (µl) and antioxidant activity of *Lathyrus odoratus* (LOK)

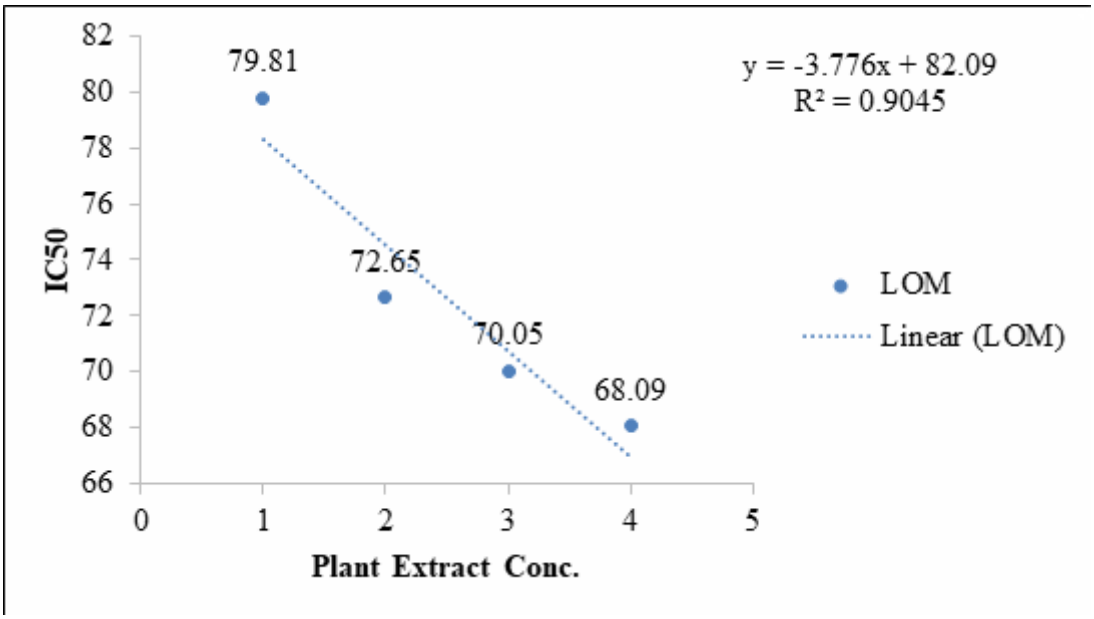

Fig. 4 Dosage effect (µl) and antioxidant activity of *Lathyrus odoratus* (LOM)

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 (17) and also have the capability to inhibit or kill the growth of microorganism (18).

Antioxidants compounds have its ability to trap free radicals; these compounds like flavonoids or peroxidase inhibit the oxidative mechanisms. In the last few years, the attention towards natural antioxidant has gained importance due to oxidative stress production which is ultimately a major factor of the disorder. In 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 and cancer etc. Synthetic as well as natural antioxidants are available. Synthetic antioxidants like BHA have proven now a days harmful effects for humans. Antioxidants from natural source gain attention for their less harmful effects. So as an alternate source of antioxidant compounds, medicinal plants as a whole or its 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 as a source of dietary antioxidant. Preliminary phytochemical studies of the stem of L. Odoratus indicate that the antioxidant activity is due to the presence of flavonoids and polyphenols.[50] Various methods are used to measure antioxidant activities, but DPPH scavenging assay was the most commonly used method. All samples show dose-dependent anti-DPPH radical activity. The IC value was calculated from regression equations. According to results Cuscuta reflexa show more antioxidant activity than Lathyrus spp (19). 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) The 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 easily available source of natural antioxidant. The phytoconstituents responsible for antioxidant activity of these plants are currently unidentified. Therefore, it is suggested further that activity guided isolation study should be performed.

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. Journal of the National Cancer Institute. 1983;70(2):343- 52.
10. Ghasemzadeh A, Jaafar HZ, Rahmat A, Wahab PEM, Halim MRA. Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (Zingiber officinale Roscoe). International Journal of Molecular Sciences. 2010;11(10):3885-97.
11. Gilani A-UH, Aftab K. Pharmacological actions of Cuscuta reflexa. International journal of pharmacognosy. 1992;30(4):296-302.
12. Khan AM, Qureshi RA, Ullah F, Gilani SA, Nosheen A, Sahreen S, et al. Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings. Journal of medicinal plants research. 2011;5(25):6055- 60.
13. Sani A, Mohammed I, Koita H. Phytochemical screening of the leaves of Lophira lanceolata (Ochananceae). Life Sci J. 2007;4:75.
14. Edeoga HO, Okwu D, Mbaebie B. Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology. 2005;4(7):685-8.
15. Kokate C. A text book of Practical Pharmalognosy. Vallabh Prakashan. 2005:107-11.
16. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia. 2011;1(1):98-106.
17. Harborne J. Phytochemical methods chapman and Hall. Ltd London. 1973;4:49-188.
18. TRADE HM. The American Heritage Stedman's Medical Dictionary: Houghton Mifflin; 2002.
19. Sharma S, Hullatti K, Kumar S, Tiwari B. Comparative antioxidant activity of Cuscuta reflexa and Cassytha filiformis. Journal of pharmacy research. 2012;5(1):441-3.