Research Article

In vitro free radicals scavenging activity of stems of *Cuscuta reflexa*

Patil Amol*, Patil Vikas, Chaudhari Kundan, Patil Vijay, Chaudhari Rajesh

*TVES's College of Pharmacy, Faizpur, Tal. Yawal Dist. Jalgaon-425503 (Maharashtra)

For correspondence: Patil Amol ,MotheWaghode Tal: Raver Dist: Jalgaon ,425502 (Maharastra) M.H.

E-mail: amolpatil311@gmail.com, amolppatil2003@rediffmail.com

Received on:17-08-2008; Accepted on :19-11-2008

ABSTRACT

Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of many disease. *Cuscuta Reflexa* plant reported to have *in vitro* antioxidant activity (non-enzymatic hemoglobin glycosylation), antibacterial activity, onset of puberty and ovarian steroidogenesis The aim of the present study to evaluate the free radicals scavenging activity by using DPPH radical scavenging assay and reducing power assay of methanolic extract of *Cuscuta Reflexa* (MECR). The DPPH assay results were expressed as IC50 value. Ascorbic acid which was used as a standard showed an IC50 9.22µg/ml, whereas, the methanolic extract of *Cuscuta Reflexa* (MECR) showed antioxidant activity with IC50 value 359.48µg/ml. The reducing power of MECR was found to be increase with increasing amount of extract concentration. All the concentrations of MECR showed significant antioxident activities when compared to control and these differences were statistically significant ($p < 0.001$)

Key Words: *Cuscuta Reflexa,* antioxidant activity, DPPH assay, Ascorbic Acid

INTRODUCTION

Oxygen is a highly reactive atom that is capable of becoming a part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function.

Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids. Many herb species, especially those belonging to the *Lamiaceae* family, such as sage, oregano, and thyme, show strong antioxidant activity. A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts. *Cuscuta Reflexa* belonging to family Convolvulaceae, has a bitter sharp taste and traditionally used for expectorant anthelmintic, purgative,diuretic, pains and also useful in jaundice[1,2]. Stems are specially used for bilious disorder. *Cuscuta Reflexa* plant reported to have in vitro antioxidant activity (non-enzymatic hemoglobin glycosylation)[3], antibacterial activity[4], onset of puberty and ovarian steroidogenesis[5] *Cuscuta Reflexa* has reported to have phenolic compounds[6]. Still no DPPH free radical scavenging assay and reducing power assay has been carried out on this plant .Our aim to evaluate free radical scavenging activity of methanolic extract of *Cuscuta Reflexa* by using DPPH free radical scavenging assay and reducing power assay.

MATERIAL AND METHODS:

Plant material

The stems of *Cuscuta Reflexa* was collected from local habitat. The plant specimens were authenticated by Dr. Harshad M. Pandit, Botany Department, Gurunanak Khalsa College, Mumbai.

Preparation of extracts

The stems were cut into small pieces and dries at room temperature. The dried stems were subjected to size reduction to coarse powder by using dry grinder. This powder is packed into soxhlet apparatus and extracted with methanol. The extract is evaporated to dryness at 40°C (yield: 3% w/w) [7]. A phytochemical screening of residue revealed the presence of alkaloids, phenolic compounds and tannins, flavonoids, proteins [8].

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY:

Chemicals

DPPH solution (200µM) Phosphate buffer (0.2M, pH 6.6) (Potassium dihydrogen phosphate 0.2M): (NaOH 0.2M): Potassium ferricyanide (1%), TCA (10%), Ferric chloride (0.1%).

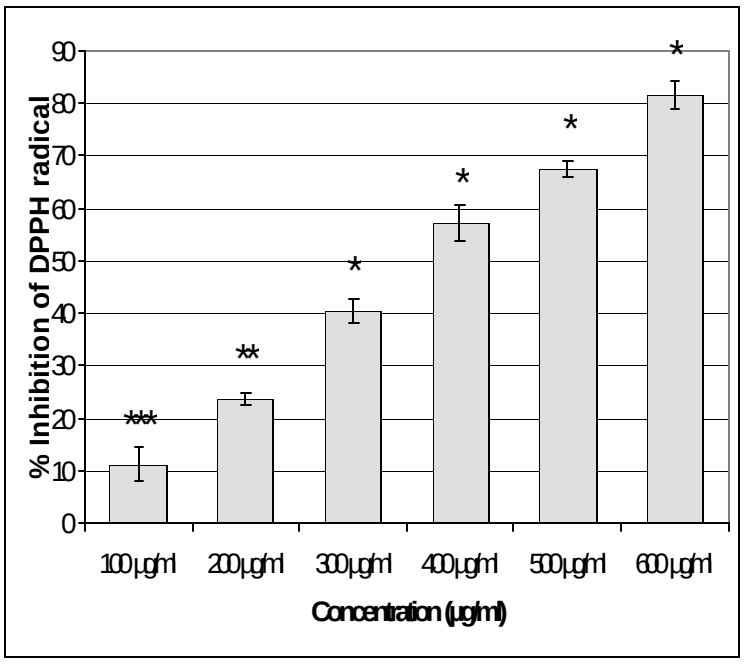
1. DPPH Free Radical Scavenging Assay:

1gm extract powder was dissolved in 1 ml of 90% methanol solution to obtain 1000 mg/ml sample solution. 1000 mg/ml solutions were series diluted into concentration ranging from 100-600µg/ml (i.e. 100, 200, 300, 400, 500 and 600µg/ml). 200µM solution of DPPH in ethanol was prepared and 1.5 ml of this solution was added to 1.5 ml of extract solution at different concentrations (100-600 µg/ml). Ascorbic acid was used as the standard control, with concentrations ranging from 2-20µg/ml (i.e. 2, 5, 10, 15 and 20µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. The absorbance of DPPH solution decreases when kept in contact with antioxidant test sample and free radical scavenging activity is inversely proportional to the absorbance of DPPH solution[9]. Percent inhibition of DPPH free radical scavenging activity was calculated using the following formula,

$$\text{DPPH Scavenged (\%)} = \frac{(\text{Acont} - \text{Atest})}{\text{Acont}} \times 100$$

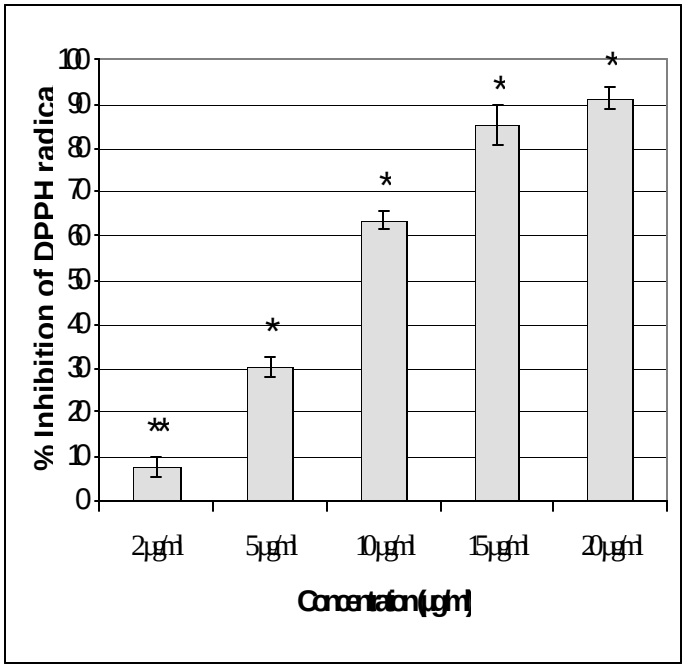
Where Acont is the absorbance of the control reaction. Atest is the absorbance in the presence of the sample of the extracts

Figure 1: DPPH radical scavenging assay of MECR



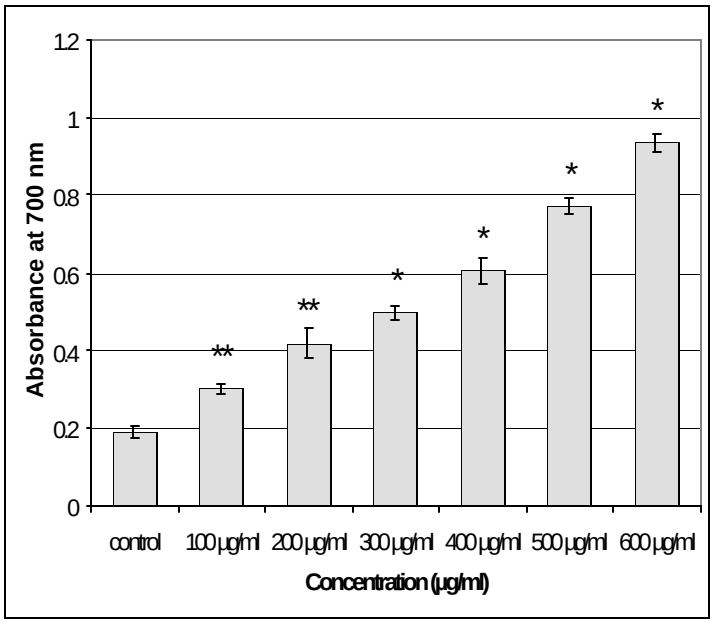
Values are expressed as a mean ± standard error of mean of 3 observations.,* Represents statistical significance: $p < 0.001$, when compared with control, n = 3,** Represents statistical significance: $p < 0.05$, n = 3.,*** Represents no statistical significance: $p > 0.05$, n = 3

Figure 2: DPPH Free radical scavenging activity of L-ascorbic acid



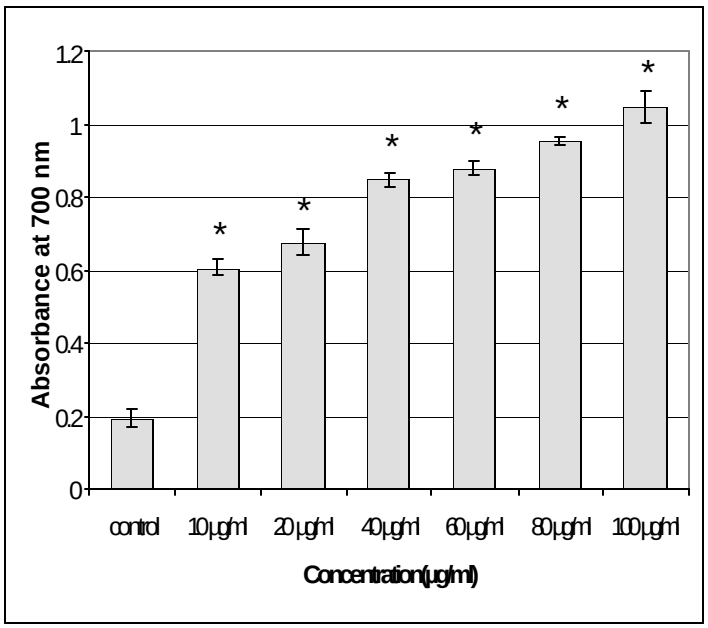
Values are expressed as a mean ± standard error of mean of the 3 observations.,*Represents statistical significance: $p < 0.001$, when compared with control, n = 3,** Represents no statistical significance: $p > 0.05$, n = 3.

Figure 3: Reducing power of MECR. at 700 nm



Values are expressed as a mean ± standard error of mean of the 3 observations. Represents statistical significance: $p < 0.001$, n = 3, ** Represent statistical significance: $p < 0.05$, n= 3

Figure 4: Reducing power of L-ascorbic acid at 700 nm



Values are expressed as a mean ± standard error of mean of the 3 observations.* Represents statistical significance: $p < 0.001$, n = 3

2. Determination Of Reducing Power:

The total reducing power of MECR was determined according to the method of Oyaizu[10,11]. Different concentrations of MECR (100, 200, 300, 400, 500 and 600µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 × g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl$_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm using a UV-Visible spectrophotometer. Increasing absorbance at 700 nm was interpreted as increasing reducing activity.

Ascorbic acid was used as the standard control with concentrations 10, 20, 40, 60, 80 and 100 µg / ml.

RESULTS AND DISCUSSION:

DPPH radical scavenging assay of MECR

MECR significantly inhibited the DPPH free radical at the concentrations ranging from 100-600µg/ml showing highest inhibition i.e. 81.70% at 600µg/ml (Figure No.1) The IC_{50} value obtained was found to be 359.48µg/ml. Ascorbic acid was used as reference standard for the DPPH free radical scavenging assay; it significantly inhibits DPPH free radical at the concentrations ranging from 2-20 µg/ml, showing highest % inhibition i.e. 91.26% at 20µg/ml (Figure No.2). The IC_{50} value obtained was found to be 9.22µg/ml.

Reducing Power Assay

For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of the MECR using the method of Oyaizu. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant. Reducing power of the selected diluted extract found to be significant ($p < 0.001$) (Figure No:3).The antioxidant activity has been reported to be concomitant with development of reducing power. The reducing power of MECR was found to be increase with increasing amount of extract concentration. All the concentrations of MECR showed significant activities when compared to control and these differences were statistically significant ($p < 0.001$)

It has been studied that damages caused by free radical induced oxidative stress is the major causative agent of many disorders including cancer, tissue injury, and rheumatoid arthritis [12]. neurodegenerative diseases, aging [13]. Oxidative stress major pathological factor for many disease. Natural antioxidants have also some advantages over synthetic ones. They can be obtained easily and has lesser side effects and cheaply available. The present study shows antioxidant activity of *Cuscuta Reflexa,* The antioxidant activity may be due to phenolic compounds in *Cuscuta Reflexa* extract.

However, the exact components responsible for the antioxidant activity of MECR are not clear. Therefore, further work is necessary to isolate and characterize those constituents.

ACKNOWLEDGEMENT:

The authors are thankful to principal and management of the college for providing research lab facilities.

REFERENCES:

1. Narayan das Prajapati kumar, Agro's dictionary of medicinal plants, 2003, published by agrobios, page no.99
2. Kirtikar Basu, Indian medicinal plants vol.8, 2001 Page no. 2401-2402.
3. Yadav SB; Tripathi V; Singh RK; Pandey HP, Antioxidant activity of *Cuscuta reflexa* stems, Indian Journal of Pharmaceutical Sciences. 2000 Nov-Dec; 62(6): 477-480
4. Pal D.K., M. Mandal , G.P. Senthilkumar , A. Padhiari Antibacterial activity of *Cuscuta reflexa* stem and Corchorus olitorius seed Fitoterapia 77 (2006) 589–591
5. Gupta M., Mazumdera U.K., Pal D.K. , Onset of puberty and ovarian steroidogenesis following administration of methanolic extract of *Cuscuta reflexa* Roxb. Stem and Corchorus olitorius Linn. seed in mice, Journal of Ethno pharmacology 89 (2003) 55–59
6. Christiane Löffler, Antje Sahm, Victor Wray, Soluble phenolic constituents from *Cuscuta reflexa* and *Cuscuta platyloba*, Biochemical Systematics and Ecology, Volume 23, Issue 2, March 1995, Page no. 121-128,
7. Ghosh Dipankar and Laddha K. S., Herbal drug extraction: An Update Chemical Weekly, 2005, Feb.8.
8. Khandelwal K.R., Practical Pharmacognosy Techniques and experiments, Nirali Prakashan, Pune. 2001 8th edn. Page no. 149-156.
9. Yerra Rajeshwar, G.P. Senthilkumar, Malwa Gupta, Studies on in vitro antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds, European bulletin of drug research, Volume No. 1 , 2005.
10. Oyaizu, M. Studies on products of browning reaction: Antioxidative activities of browning reaction prepared from glucosamine. Jap Journal of Nutrition (1986) 44: 307–315.
11. P.Y.Y.Wong and D.D.Kitts Chemisty of Buttermilk Solid Antioxidant activity, J Dairy Sci (2003), 86: 1541-1547.
12. Catherine W. Lukhoba, Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda, Journal of Ethnopharmacology, (2005) 99: 165-178.
13. S. Khlfi, Y.El Hachimi et al., In vitro antioxidant properties of *Salvia verbenaca* L. hydromethanolic extract, Indian Journal Pharmacology, (August 2006) ,Vol-38, Issue 4: 274-280.

Source of support: Nil, Conflict of interest: None Declared