Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (5):28-32
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

In vitro antioxidant activity of plant extract of *Cressa Cretica*

Pryianka[1*], Sangh Partap[2], Mansi Verma[1] and Keshari Kishore Jha[1]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, Teerthankar Mahaveer University, Moradabad, Uttar Pradesh, India
[2]Department of Pharmaceutical Chemistry, Jamia Humdard University, New Delhi

---

ABSTRACT

The free radical scavenging activity of plant extract of Cressa cretica was studied on in vitro antioxidant models. The antioxidant activity was evaluated by determining the activity of hydrogen peroxide (H_2O_2) radicals scavenging and 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. In all these studies, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The extract was also shown to have high phenolic content, i.e. 99.09±0.10 µg/mg. These results clearly indicated that the plant extract of Cressa cretica could be a potential source of natural antioxidant and effective against free radical mediated diseases.

Keywords: Antioxidant, In vitro, *Cressa cretica*, Reactive oxygen species.

INTRODUCTION

Free radicals, often called reactive oxygen species (ROS), are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism[1]. They are generated as by-products of biological reactions or from exogenous factors. When ROS production is greater than the detoxification capacity of the cell, excessively generated ROS causes extensive damage to DNA, proteins, lipids, etc. and acts as a mediator of pro-inflammatory and carcinogenic events[2]. Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer and cardiovascular diseases[3]. In the treatment of such diseases, antioxidant therapy has gained an immense importance. These antioxidants interfere with the oxidative processes by reacting with free radicals, chelating catalytic metal ions and also by acting as oxygen scavengers [4]. The biological effects of dietary antioxidants have generated a lot of interests in the modern era due to their potent antioxidant activities, absence of side effects and economic viability[5]. Many scientists have tried to obtain dietary antioxidants such as ascorbate, tocopherol and carotenoids from fruits and vegetables as they could help in protecting cells from cellular damages induced by oxidative stress.

The plant, *Cressa cretica* Linn (family Convolvulaceae) known as Dhana is a common plant extract used throughout in India. Since time immemorial plant is used as anthelmintic, stomachic, tonic and for aphrodisiac purposes, enriches the blood and is useful in constipation, leprosy, asthma, and urinary discharges, in the treatment of diabetes and general debility. Dry leaves of *C. cretica* crushed with sugar are used as emetic in Sudan. It is also reported that the fruits of *Cressa cretica* are potential sources of edible oil[6]. *C. cretica* extensively is used to get relief from asthma and cough. The plant also possesses antifungal[7], antibacterial[8], antimicrobial and anti-inflammatory activities[9].

In view of the immense medicinal importance of the plant, the present investigator focused onto an exploration of free radical scavenging activity of this plant to determine and establish its role in various oxidative stress conditions generated by various reactive oxygen species.

MATERIALS AND METHODS

Chemicals and Reagents

All the drugs and chemicals used in the study were of analytical grade. 1,1-diphenyl-2-picryl hydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from Sigma Chemicals (St. Louis, MO, USA) and other chemicals used for evaluation of oxidative stress parameters were obtained from Sisco Research Laboratories (Mumbai).

Plant material

The plant of *Cressa cretica* was collected in the month of October 2010 from Banglore, India and were identified by by Dr. D.C Saini (Head and scientist, Birbal Shani Institute of Paleobotany) as *Cressa cretica* (Convolvulaceae). A voucher specimen was preserved in the herbarium (17849.) in the Birbal Shani Institute of Paleobotany for further references. The Plant was washed with tap water, dried in the shade and were then ground to a coarse powder and stored in an airtight container.

Preparation of extracts

The dried and coarsely powdered plant material was extracted with petroleum ether (60˚–80˚) by hot percolation in soxhlet apparatus. The defatted plant material was then extracted with methanol until it became colourless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. The last traces of the solvent were evaporated under reduced pressure in rotatory evaporator. Standard methods were used for preliminary phytochemical screening of the extract to recognise the phytoconstituents present in the extract (Harborne 1984). It was concluded that the extract contained terpenoids, steroids, flavonoids and tannins.

In vitro antioxidant activity

DPPH assay

The ability of the extracts to scavenge DPPH radicals (DPPH•) was determined according to the method prescribed[10] with minor modifications. A 50 µL aliquot of extract, in 50 mM Tris–HCl buffer (pH 7.4), was mixed with 450 µL of Tris–HCl buffer and 1.0 mL of 0.1 mM DPPH• in methanol. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm against corresponding blanks (0.01 mM DPPH in methanol) and ascorbic acid was used as standard. All the tests were performed in triplicate and the graph was plotted with ±SEM of three observations.

Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity

H_2O_2 scavenging activity of the extract was estimated by a previously prescribed method[11]. A solution of H_2O_2 (20 mM) was prepared in phosphate buffer saline (pH 7.4). Different concentrations of plant extract and standard ascorbic acid solution viz. 25–250 µg/mL in methanol (1 mL) were added to H_2O_2 solution (2 mL). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. For each concentration, a separate blank sample was used for background subtraction. The experiment was performed in triplicate.

Statistical Analysis

The results are expressed as mean±standard error (mean±SE) of three observations. The % inhibition of various radicals was calculated by comparing the results of the test with those of controls using the formula[12].

% inhibition = absorbance (control) – absorbance (test) /absorbance (control) X 100

RESULTS

Several concentrations ranging from 25–250 µg/mL of the plant extract were compared for their antioxidant activity in different in vitro models. It was observed that free radicals were scavenged by the extracts in a concentration dependent manner (within the predetermined concentration range) in all the models.

DPPH Radical Scavenging Activity

Free radicals scavenging activity of DPPH has been widely used to evaluate the antioxidant activity of natural products obtained from plant and microbial sources. In the DPPH scavenging activity model it was observed that the plant extract (25–250 µg/mL) significantly scavenged DPPH• in a concentration dependent manner. However, extract showed weak scavenging activity at lower concentrations; the higher concentrations (25–250 µg/mL) exhibited promising DPPH• scavenging activity ranging from 36.26% to 65.93% (Table 1). DPPH is a relatively stable free radical and the assay determines the ability of plant extract of *Cressa cretica* to reduce DPPH• to the corresponding hydrogen by converting the unpaired electrons to form pairs. This conversion is the action of the antioxidant.

Hydrogen Peroxide Radical Scavenging Activity

Plant extract also demonstrated H_2O_2 decomposition activity in a concentration dependent manner with an IC_{50} of 42.15µg/mL (Table 2). The decomposition of H_2O_2 by Plant extract might have partly resulted from its antioxidant and free radical scavenging activity.

Table 1: Antioxidant activity of plant extract of *Cressa cretica*

Concentration (µg/ml)	% Inhibition of Radicals		
	Percent inhibition of Ascorbic Acid	Percent inhibition of DPPH	Percent inhibition of H_2O_2
Control	-	-	-
25	35.57	17.06	5.90
50	40.75	24.32	19.71
100	52.02	43.44	37.58
150	60.95	51.60	51.35
200	68.30	62.44	55.76
250	78.78	72.37	63.83

Table 2: Free radical scavenging ability of plant extract of *Cressa cretica* and ascorbic acid.

Activity	IC_{50} (µg/mL)	
	Plant extract of *Cressa cretica*	Ascorbic acid.
DPPH	149.38	139.11
H_2O_2 scavenging	42.15	31.43

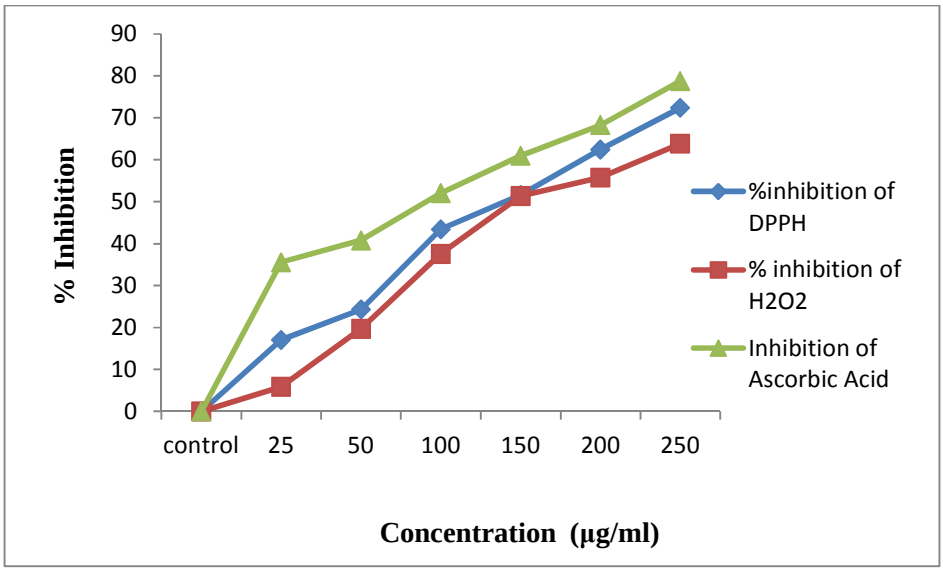

Fig 1: Inhibition Curve of DPPH and Ascorbic Acid

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radical can brings about many adverse reactions leading to extensive tissue damage. Lipid proteins are all susceptible to attack by free radical. Many plant species with antioxidant activities act as protective agents against these radicals. In the present investigation potent antioxidant activity of *Cressa cretica* extract was observed using different methods. However the efficacy of extract to scavenge the different radicals differed in each method depending upon the mechanism of free radical scavenging and assay methodology.

The result of DPPH scavenging activity assay in this study indicated that the plant was potently active. This suggested that the plant extract did contain compounds that could be capable of donating hydrogen to a free radical in order to remove the odd electron which is responsible for the radical's reactivity.

H_2O_2 is a weak oxidising agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly. Once inside the cell H_2O_2can probably react with Fe2+, and /or Cu2+ ions to form hydroxyl radical and this might be the origin of many of its toxic effects[13]. It is therefore biologically advantageous for cells to control the amount of H_2O_2 getting accumulated. Scavenging of H_2O_2 by the plant extract could be attributed to its phenolics which donate electron to H_2O_2 thus reducing it to water. The extract was capable of scavenging H_2O_2 in a concentration dependent manner.

It is well known that superoxide anions damage biomolecules directly or indirectly by forming H_2O_2, •OH, peroxy nitrite or singlet oxygen during aging leading to pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation[14]. The scavenging activity of this radical by the plant extract compared favourably with the standard reagent suggesting that the plant could also be a potent scavenger of superoxide radical. The probable mechanism of superoxide scavenging would be attributed to the inhibitory effects of *Cressa cretica* extract towards generation of superoxide in the in vitro reaction system.

CONCLUSION

The results obtained in the present study indicated that of *Cressa cretica* extract exhibited free radical scavenging activity against hydroxyl, peroxide and DPPH•. The overall antioxidant activity of *Cressa cretica* extract might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggested that of *Cressa cretica* could be a potential source of natural antioxidant that would have great importance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and associated oxidative stress related degenerative disease.

Acknowledgement
Authors are very thankful to Shri Suresh Jain, Honourable Chancellor of the Teerthanker Mahaveer University for his invariable encouragement and endowing us with facilities necessitated for successful completion of the study. Authors are also thankful to Dr. D.C Saini Head and scientist, Birbal Shani Institute of Paleobotany for authentication of plant material.

REFERENCES

[1] Tiwari, A. *Current Science*, **2001**; 81: 1179–1187.
[2] Kowaltowski, A. J. & Vercesi, A. E. *Free Radical Biology & Medicine*, **1999**; 26: 463–471
[3] Yamaguchi, F., Saito, M., Ariga, T., Yoshimura, Y. & Nakazawa, H. *Journal Of Agricultural And Food Chemistry*, **2000;** 48: 2320–2325.
[4] Buyukokuroglu, M. E., Oktay, M. & Kufrevioglu, O. I. *Pharmacological Research*,**2001;** 44: 491–495.
[5] Auudy, B., Ferreira, F., Blasina, L., Lafon, F., Arredondo, F., Dajas, R. *Et Al. Journal Of Ethanopharmacology*, **2003;** 84: 131–138.
[6] Gupta R., Jhoshi Y. C., *Ijppr*, **2006;** 44(5): 25-30
[7] Agha F., *Pak. J. Bot*. **2009;** 41(1): 2883-2892
[8] Parekh J., Chanda S.V., *Truk J Biol*.,**2008;** 32: Pp.63-71
[9] Sunita P., *Ijpsr*, **2011;** 2(4): 849-955
[10] Zeyep, T., Muberra, K. & Esra, K. *Journal Of Ethnopharmacology*, **2007;** 110: 539–547.

[11] Sroks, Z. & Cisowski, W. *Food And Chemical Toxicology*, **2003;** 41: 753–758.
[12] Shirwaikar, A., Rajendran, K. & Dinesh Kumar, C. *Indian Journal Of Experimental Biology*, **2004;** 42: 803–807.
[13] Halliwell, B. & Gutteridge, J. M. C. *Federation Of European Biochemical Societies* (*Febs*) *Letters*, **1981;** 128: 347–352.
[14] Yen, G. C. & Duh, P. D. *Journal Of Agricultural and Food Chemistry*, **1994;** 42: 629–632.