Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (3):840-844
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

In vitro antioxidant activity of aqueous extract of seeds of *Cucumis callosus* (Rottl.) Cogn

Tara Chand[*1,2] Anil Bhandari[2], Bhupendra K. Kumawat[3], Ashok Sharma[1], Anil Pareek[4], Vijay K. Bansal[4]

[1] *Regional College of Pharmacy, Jaipur-302022, Rajasthan, India*
[2]*Faculty of Pharmaceutical Science, Jodhpur National University, Jodhpur-342001, Rajasthan, India*
[3] *Marwar Pharmacy College, Maulasar-341506, Rajasthan, India*
[4]*L M College of Science & Technology, Jodhpur-342003, Rajasthan, India*

ABSTRACT

The aim of present study was to estimate the in vitro antioxidant activity of aqueous extract of Cucumis callosus (Rottl.) Cogn (Cucurbitaceae) seeds which is commonly known as "Kachri" in Rajasthan (India) evaluated by using DPPH radical scavenging activity and hydrogen peroxide radical scavenging activity. The antioxidant activity is compared with ascorbic acid as standard. The IC_{50} values of Cucumis callosus and ascorbic acid were found 37.71 µg/ml and 16.72 µg/ml respectively for the DPPH radical scavenging activity while 94.71 µg/ml and 143.45 µg/ml respectively for hydrogen peroxide radical scavenging activity. Thus, aqueous extract of Cucumis callosus seeds possess potent antioxidant activity in hydrogen peroxide model and may be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases.

Key words: *Cucumis callosus*, Seeds, Antioxidant, Free radical scavenging, DPPH

INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result in increased radical activity and damage [1].

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, cancer, Parkinson's diseases, senility, mongolism, ageing process and diabetes mellitus. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant [1, 3]. Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen which include free radicals as well as non-free radical species (H_2O_2). Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid per-oxidation, protein damage and DNA stand breaking [2].
Cucumis callosus (Rottl.) Cogn *(Cucurbitaceae*) commonly known as "Kachri" in Rajasthan (India) has been claimed in traditional literature as a valuable against a wide variety of diseases. The herb is distributed throughout India in arid zones. The herb is much branched very common prostate, perennial herb; leaves are cordate, suborbicular, deeply palmately 5-7 lobed; flowers are yellow; fruits are smooth, obovoid-ellipsoid, green variegated stripes and fruiting in August-November. Fruit is traditionally used to prevent insanity to strong memory and

remove vertigo. The seeds (Fig 1) are useful in bilious disorder [4,5], diabetics, easy bowl syndrome, stomach pain, vomiting and constipation [6, 7]. Paste of root is applied on scorpion sting; decoction of root is given in indigestion, dropsy, and pulp of fruit used in abortion and to increase menses for women [8]. Hence, the present study was aimed at evaluating the free radical scavenging activity of the aqueous extract of seeds of *Cucumis callosus*.

Figure 1: Seeds of *Cucumis callosus* (Rottl.) Cogn *(Cucurbitaceae*)

MATERIALS AND METHODS

Plant material collection & authentication

The plant was collected around the Jaipur, Rajasthan (India) and authenticity of plant was confirmed from "Herbarium Department of Botany, University of Rajasthan, Jaipur, India. The herbarium No RUBL 20955 of the same was preserved in Herbarium Department of Botany, University of Rajasthan, Jaipur, India for further reference.

Chemicals

DPPH (1,1-Diphenyl-2-picryl hydrazyl) was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Ascorbic acid was obtained from Merck Ltd., Mumbai. Dimethyl Sulphoxide, methanol, hydrogen peroxide (H_2O_2), mannitol, potassium dihydrogen phosphate, potassium hydroxide, phosphate buffer saline (PBS, pH 7.4) were analytical grade and obtained from Ranbaxy fine chemicals and SD fine chemicals Ltd., India.

Extraction of plant seeds

The seeds were dried at room temperature in shade, powdered coarsely. The extraction of powdered seed was carried out by maceration process with water for 24 hrs to obtain aqueous extract. The aqueous extract was dried in hot air oven below 50^0C. The extract was then concentrated by distilling off the solvent by evaporation to a water bath [9,10] and stored in refrigerator.

DPPH radical scavenging activity

The antioxidant activity of the aqueous extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 1 ml of various concentrations of the extracts in methanol was added to 4 ml of a 4 mg/100 ml (0.004% w/v) methanol solution of DPPH. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer (Systronics, UV-2203, India) which was compared with the corresponding % inhibition of standard ascorbic acid concentrations (10-100 µg/ml). Extract concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration [2, 11-13]. Ascorbic acid (AA) was used as positive controls and all tests were carried on triplicates. The free radical scavenging activity (FRSA) was calculated by using the following equation:

$$\text{DPPH radical scavenging activity } (\%) = \frac{[\text{Abs (control)} - \text{Abs (sample)}]}{\text{Abs (control}} \times 100$$

Where, Abs (control): Absorbance of DPPH radical + methanol; Abs ((sample): Absorbance of DPPH radical + extract/standard; IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

Hydrogen peroxide-scavenging activity

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS at pH 7.4). Various concentrations of the extract and standards in methanol (1 ml) were added to 2 ml of hydrogen peroxide solutions in PBS. After 10 min, the absorbance was measured at 230 nm against a blank solution that contained extracts in PBS without hydrogen peroxide [11]. The percentage scavenging of hydrogen peroxide and standard compounds was calculated using the following formula:

$$\%\ \text{scavenged [Hydrogen peroxide]} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 was the absorbance of the control; A_1 was the absorbance in the presence of the sample and standards.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

The reduction capacity of DPPH radical which is induced by antioxidant was determine by the decrease in its absorbance. It is visually noticeable as a change in colour from purple to yellow.

Table 1: Free radical scavenging activity of aqueous extract of *Cucumis callosus* and ascorbic acid

Concentration (µg/ml)	DPPH (% Inhibition)		Hydrogen peroxide (% Inhibition)	
	Aqueous extract	Ascorbic acid	Aqueous extract	Ascorbic acid
10	29.5203	48.7085	26.70237	31.2165
20	36.5314	51.6605	28.9977	32.1347
30	47.9705	53.5055	30.83397	33.5884
40	53.8745	55.3506	33.97093	35.3481
50	59.7786	57.1956	36.5723	36.0367
60	64.9446	59.7786	38.17904	37.1844
70	69.7417	62.7306	42.84621	39.4797
80	75.6458	66.0517	45.83015	41.0099
90	84.5018	68.6347	48.89059	42.4637
100	86.7159	71.2177	52.63963	43.9939

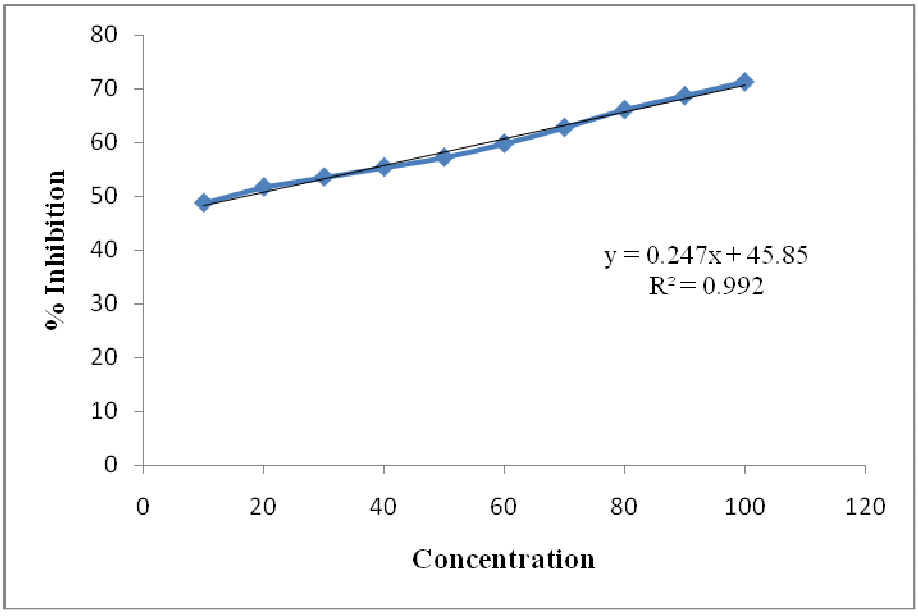

Figure 2: DPPH radical scavenging activity of standard IC_{50} = 16.72 µg/ml

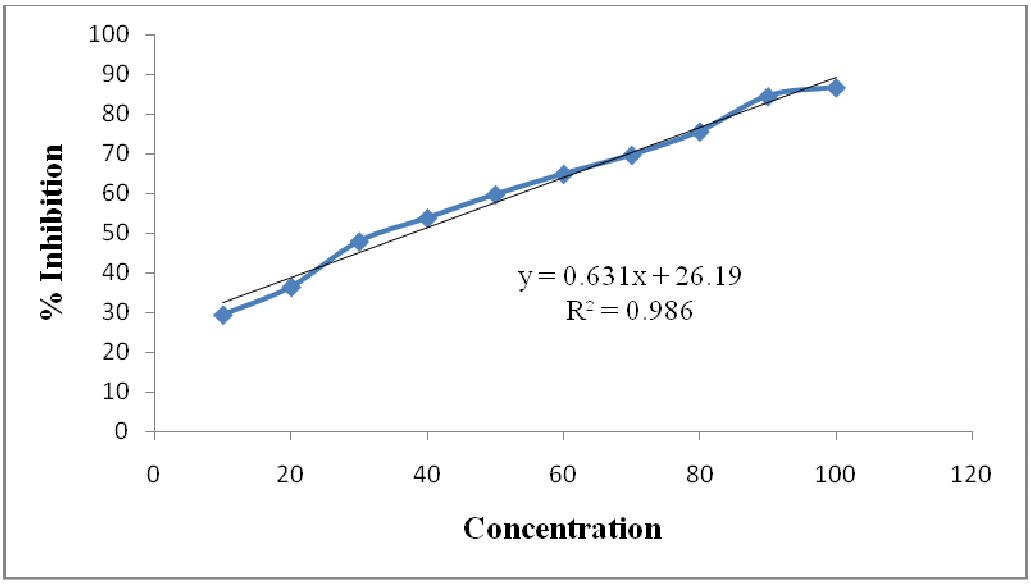

Figure 3: DPPH radical scavenging activity aqueous extract of *Cucumis callosus* seeds IC_{50} = 37.71 µg/ml

Hence, DPPH is usually used as a substance to evaluate the antioxidant activity. In the DPPH free radical scavenging assay, aqueous extract of *Cucumis callosus* seed was evaluated for their free radical scavenging activity compared with ascorbic acid as standard compound. The radical scavenging activity of *Cucumis callosus* extract increased with increasing concentrations respectively (Table 1 & Fig 3).

The scavenging effect increased with the increasing concentrations of test compound. The IC_{50} value for aqueous seeds extract was 37.71 µg/ml which was comparatively higher than the IC_{50} **(**16.72 µg/ml**)** of ascorbic acid (Table 2 & Fig 2). These results indicated that aqueous extract of *Cucumis callosus* seeds exhibited the ability to quench the DPPH radical which indicated that extract was antioxidant with radical scavenging activity.

Hydrogen peroxide free radical scavenging activity

The extract was capable of scavenging hydrogen peroxide in a concentration-dependent manner. The radical scavenging activity of *Cucumis callosus* extract increased with increasing in concentrations (Table 1 & Fig 5). The IC_{50} value for seeds aqueous extract was 94.71 µg/ml which was comparatively lower than the IC_{50} **(**143.45 µg/ml) of ascorbic acid (Table 2 & Fig 4). These results indicated that aqueous seeds extract of *Cucumis callosus* exhibited the effective antioxidant activity.

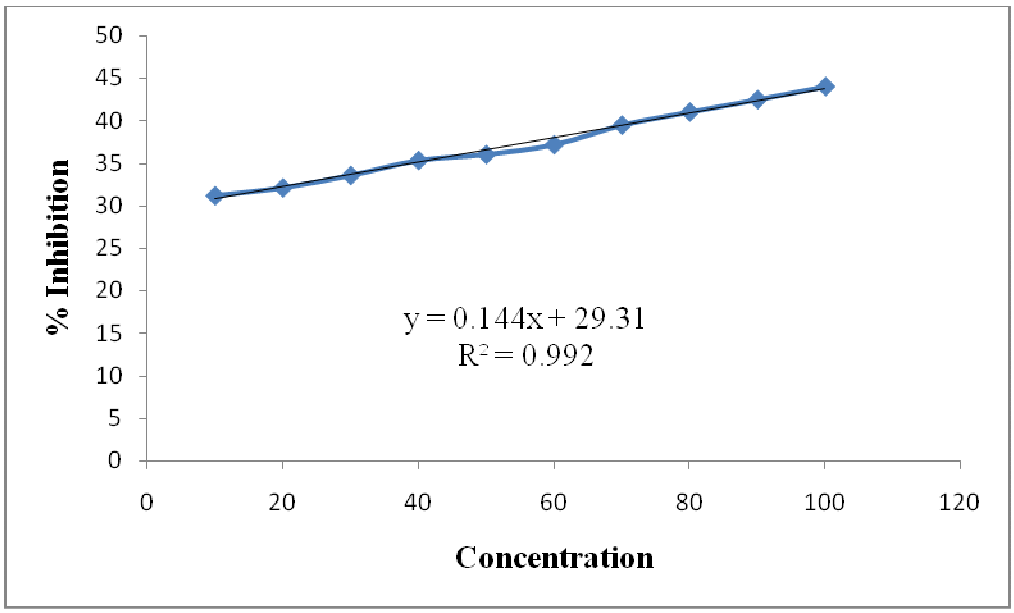

Figure 4: Hydrogen peroxide radical scavenging activity of standard IC_{50} = 143.45 µg/ml

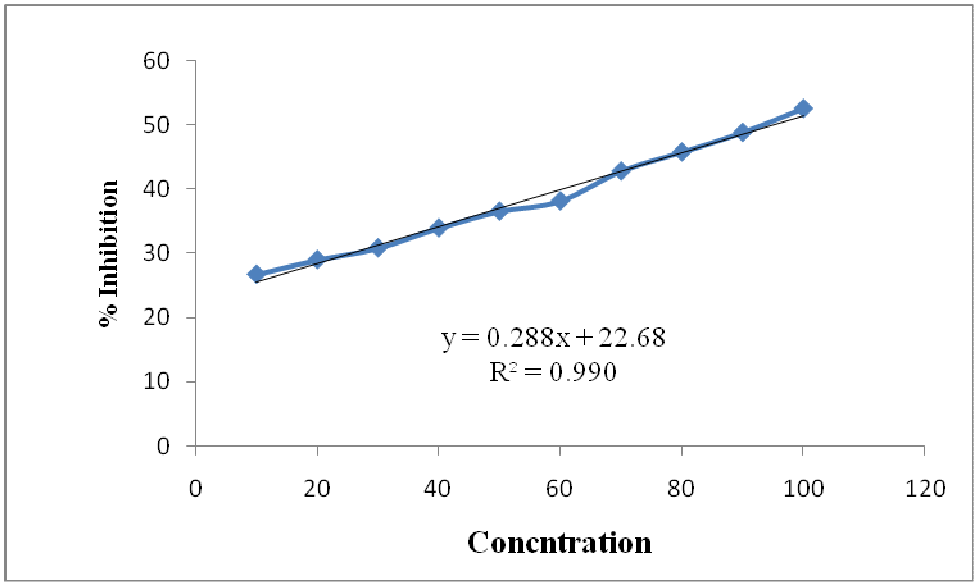

Figure 5: Hydrogen peroxide radical scavenging activity aqueous extract of *Cucumis callosus* seeds IC_{50} = 94.71µg/ml

Table 2: IC_{50} value (µg/ml) of aqueous extract of *Cucumis callosus* seeds & ascorbic acid

Compounds	DPPH	Hydrogen peroxide
Aqueous extract	37.71	94.71
Ascorbic acid	16.72	143.45

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that the aqueous seeds extract of *Cucumis callosus* (Rottl.) Cogn possesses the significant antioxidant activity. However, the components responsible for the anti oxidative activity are currently unclear. These finding suggest that this plant is a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative. Further studies are warranted for the isolation and characterization of antioxidant components and also *in vivo* studies are needed for understanding their mechanism of action as an antioxidant better.

Acknowledgement
The authors are highly thankful to management and staff, Regional College of Pharmacy Jaipur for providing necessary facilities and support to carry out this work.

REFERENCES

[1] V. Nagavani , Y. Madhavi, D. Bhaskar Rao, P. Koteswara Rao, T. Raghava Rao, *EJEAFChe, 9(9),* **2010**, 1471-1484..
[2] S.M. Patil, V.J. Kadam, R. Ghosh, *Int.J. PharmTech Res*.,**2009**,1(4), 1480-1484.
[3] M.A.R. Bhuiyan, M.Z. Hoque, S.J. Hossain, *World J. Agric. Sci.*, **2009**,5 (3), 318-322.
[4] A.H.M.M Rahman, M. Anisuzzaman, M.Z. Alam, A.K.M.R Islam, A.T.M.N. Zaman, *Res. J. Agric. & Biol. Sci.*, **2006**, 2(6), 299-302.
[5] K.R. Kirtikar, B.D. Basu. Indian Medicinal Plants, Lalit Mohan Basu Allahabad, India, **1984**, Vol. 1, 2.
[6] E.R.H.S.S.Ediriweera, W.D.Ratnasooriya, Ayu, newsletters Ayurved University, Sri Lanka, **2009**, 30, 373-391.
[7] M. Goyal, S.K. Sharma, *Ind. J Traditional Knowledge*, **2009**, 8(4), 581-585.
[8] A.R. Seliya, N.K.Patel, *Ethnobotanical Leaflets*, **2009**, 13, 865-872.
[9] C.K. Kokate, Practical Pharmacognosy. Vallabh Prakashan, New Delhi, **1997**, 4,109.
[10] J.B. Harborne, Methods of extraction and isolation. In: Phytochemical Methods. Chapman & Hall, London, **1988**.
[11] Pharmacopoeia of India, Vol II, Govt of India, Ministry of health, controller of publication , New Delhi, **1996**, A-53-54
[12] P.K. Basniwal, M. Suthar, G.S. Rathore, R. Gupta, V. Kumar, A. Pareek, D. Jain, *Nat. Prod. Rad.*, **2009**, 8(5), 483-487.
[13] A. Pareek, M. Suthar, G. S. Rathore, V. Bansal, T.C.Kumawat, *Phcog J*, **2002**, 2(10), 357-360.