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In Vitro Antioxidant Activity of Alcoholic Extract of Seeds of *Cucumis Callosus* (Rottl.) Cogn

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ABSTRACT

The aim of present study was to estimate the *in vitro* antioxidant activity of alcoholic 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 assay. The antioxidant activity was compared with ascorbic acid as standard. The IC_{50} values of *Cucumis callosus* and ascorbic acid were found 41.99 µg/ml and 24.27 µg/ml respectively for the DPPH radical scavenging activity while 95.27 µg/ml and 153.35 µg/ml respectively for hydrogen peroxide radical scavenging activity. Thus, alcoholic 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

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INTRODUCTION

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids. 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, Parkinson's diseases, cancer, senility, mongolism, ageing process and diabetes mellitus[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 [2,8].

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 are traditionally used to prevent insanity to strong memory and remove vertigo. The fruits and seeds (Figure 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 to evaluating the free radical scavenging activity of the alcoholic extract of seeds of *Cucumis callosus*.

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

MATERIALS & METHODS

Plant material collection & authentication

The plant was collected around the Jaipur, Rajasthan (India) in September 2011 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. Methanol, hydrogen peroxide (H_2O_2), potassium dihydrogen phosphate, potassium hydroxide, phosphate buffer saline (PBS, pH 7.4) were analytical grade.

Extraction of plant seeds

The powder of seeds was subjected to hot continuous extraction in a soxhlet extractor, successively with different known solvents in increasing order of polarity *viz* petroleum ether (60-80^0 C), benzene, chloroform, alcohol. Finally, the powdered material was macerated with water for 24 hrs to obtain aqueous extract. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50^{0}C. Each extract was then concentrated by distilling off the solvent by evaporation to a water both [8,9,10,14] and stored in refrigerator.

DPPH radical scavenging activity

The antioxidant activity of the alcoholic extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. 01 ml of various concentrations of the extracts in methanol was added to 4 ml of methanolic solution of DPPH (0.004% w/v). 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 percentage inhibition of standard 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 or standard 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 & DISCUSSION

DPPH free radical scavenging activity

The reduction capacity of DPPH radical which is induced by antioxidant was determined by the increasing in its percentage inhibition. It is visualised as a change in colour from purple to yellow. The DPPH is usually used as a substance to evaluate the antioxidant activity. In the DPPH free radical scavenging assay, alcoholic extract of *Cucumis callosus* seeds was evaluated for their free radical scavenging activity compared with ascorbic acid as standard compound. The radical scavenging activity of *Cucumis callosus* extract was increased with increasing concentrations respectively (Table 1 & Figure 3).

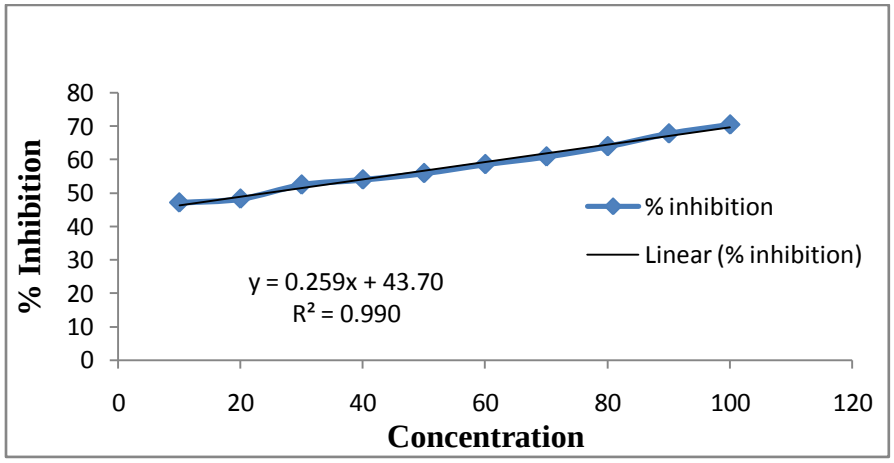

Figure 2: DPPH radical scavenging activity of standard

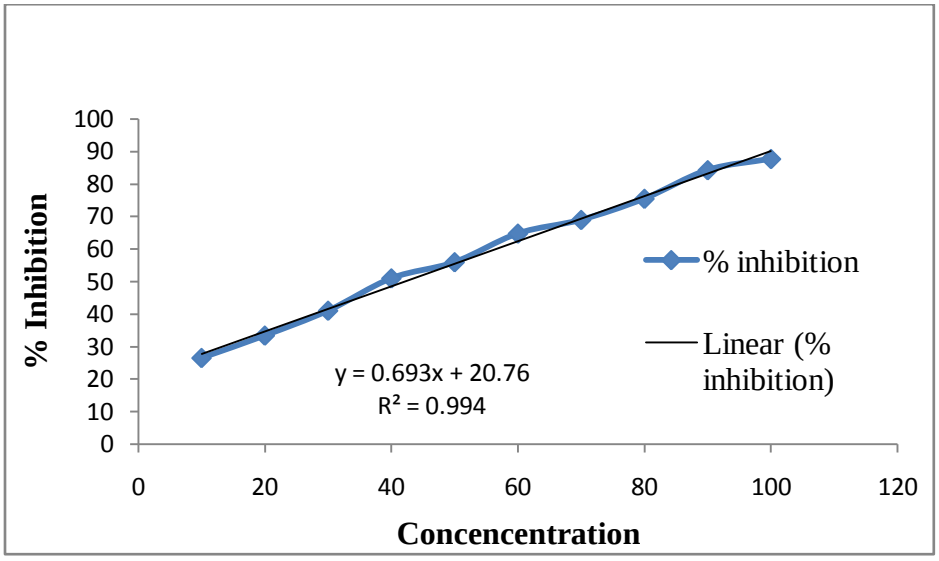

Figure 3: DPPH radical scavenging activity alcoholic extract of *Cucumis callosus* seeds

The scavenging effect increased with the increasing concentrations of test compound. The IC_{50} value for alcoholic seeds extract was 41.99 µg/ml which was comparatively higher than the IC_{50} **(**24.27 µg/ml**)** of ascorbic acid (Table 2 & Figure 2). These results indicated that alcoholic extract of *Cucumis callosus* seeds produced antioxidant activity.

Hydrogen peroxide free radical scavenging activity

The radical scavenging activity of *Cucumis callosus* extract increased with increasing in concentrations (Table 1 & Figure 5). The IC_{50} value for alcoholic extract of seeds was 95.27 µg/ml which was comparatively lower than the IC_{50} **(**153.35 µg/ml) of standard (Table 2 & Figure 4). These results indicated that alcoholic extract of seeds of *Cucumis callosus* exhibited the effective antioxidant activity.

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

Concentration (µg/ml)	DPPH (% Inhibition alcoholic	DPPH (% Inhibition ascorbic acid)	Hydrogen peroxide (% Inhibition alcoholic extract)	Hydrogen peroxide (% Inhibition ascorbic acid)
10	26.4368	47.1264	26.61043	31.5951
20	33.3333	48. 7259	28.98773	32.362
30	40.9962	52.4904	30.90491	34.2025
40	50.9579	54.023	33.12883	35.1994
50	55.9387	55.9387	35.96626	36.1963
60	64.751	58.6207	37.57669	37.0399
70	68.9655	60.9195	43.17485	39.1871
80	75.4789	63.9847	44.93865	40.2607
90	84.2912	67.8161	50.07669	41.7945
100	87.7395	70.4981	52.14724	43.5583

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

Compounds	DPPH	Hydrogen peroxide
Alcoholic extract	41.99	95.27
Ascorbic acid	24.27	153.35

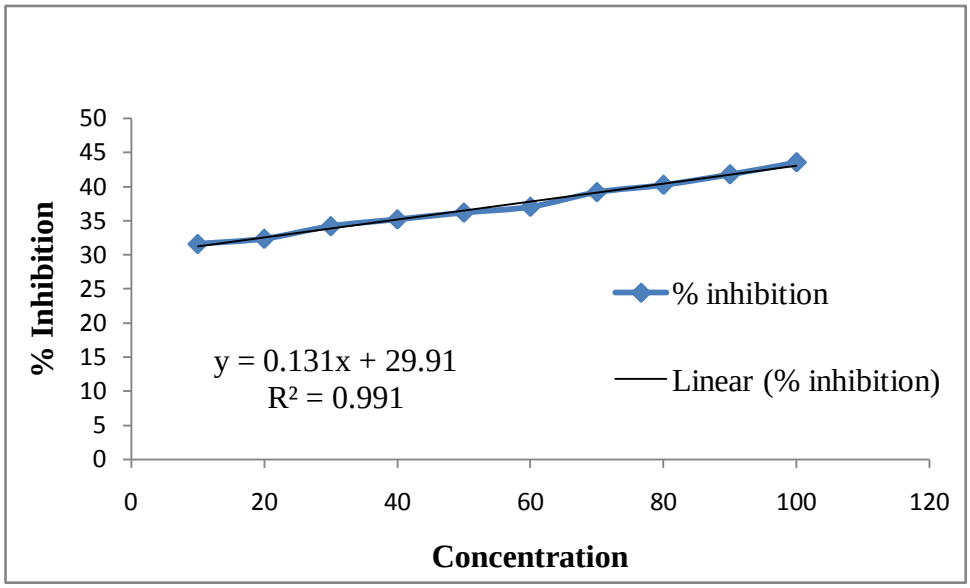

Figure 4: Hydrogen peroxide radical scavenging activity of standard

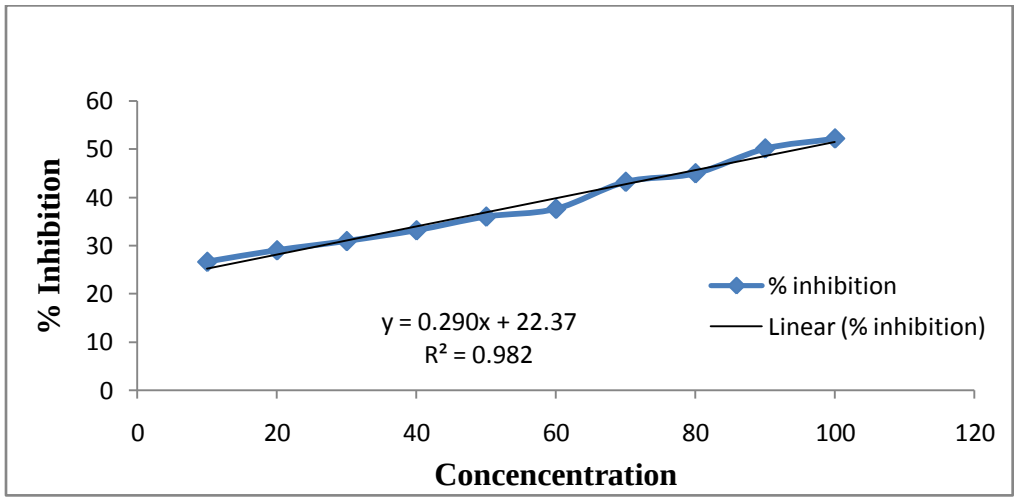

Figure 5: Hydrogen peroxide radical scavenging activity alcoholic extract of *Cucumis callosus* seeds.

CONCLUSION

On the basis of the results obtained in the present study, it was concluded that the alcoholic extract of seeds of *Cucumis callosus* (Rottl.) Cogn possesses the significant antioxidant activity. These finding suggest that this plant is a potential source of natural antioxidant . 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.

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REFERENCES

1. Nagavani V, Madhavi Y, Bhaskar Rao D, Koteswara Rao P, Raghava Rao T. Free radical scavenging activity and qualitative analysis of polyphenols by RP-HPLC in the flowers of *couroupita guianensis* abu. EJEAFChe 2010; 9(9): 1471-1484.
2. Patil SM, Kadam VJ, Ghosh R. In vitro antioxidant activity of methanolic extract of stem bark of gmelina arborea roxb. (verbenaceae). Int.J. PharmTech Res.2009;1(4): 1480-1484.
3. Bhuiyan MAR, Hoque MZ, Hossain SJ. Free radical scavenging activities of *Zizyphus mauritiana*. World J Agric Sci 2009;5 (3): 318-322.
4. Rahman AHMM, Anisuzzaman M, Alam MZ, Islam AKMR, Zaman ATMN. Res J Agric Biol Sci 2006; 2(6): 299-302.
5. Singh MP, Panda H. Medicinal Herbs with their formulations Delhi: Daya publishing house; 2005: 311-312.
6. Ediriweera ERHSS, Ratnasooriya WD. Ayu *newsletters Ayurved University*, Sri Lanka. 2009; 30: 373-391.
7. Goyal M, Sharma SK, Traditional wisdom and value addition prospects of arid foods of desert region of North West India. Ind J Traditional Knowledge 2009; 8(4): 581-585.
8. Tarachand, Bhandari A, Kumawat BK, Sharma A, Bansal VK Pareek A, Phytochemical investigation of seed of *Cucumis callosus* (Rottl.) cogn. Res J Biol Chem Sci 2012; 3(2): 570-576.
9. Kokate CK. Practical Pharmacognosy. New Delhi: Vallabh Prakashan; 1997: 4,109.
10. Harborne JB. Phytochemical Methods A guide to modern techniques of plant analysis. 3th ed., London: Chapman & Hall; 1998: 4-6.
11. Government of India. Ministry of health and family welfare. India Pharmacopoeia Vol II. The Controller of Publication, New Delhi; 1996:A-53-54.
12. Basniwal PK, Suthar M, Rathore GS, Gupta R, Kumar V, Pareek A, Jain D. *In-vitro* antioxidant activity of hot aqueous extract of *Helicteres isora* Linn. Fruits. Nat Prod Rad 2009; 8(5): 483-487.

13. Pareek A, Suthar M, Rathore GS, Bansal V, Kumawat TC. *In vitro* antioxidant studies of Lagerstroemia speciosa leaves. Phcog J 2010; 2(10): 357-360.
14. Kumawat BK, Gupta M, Tarachand, Singh Y. Preliminary phytochemical investigation on leaves of *Balanites Aegyptiaca* (L.) Delile. Res J Biol Chem Sci 2012; 3(2): 762-768.

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