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ANTIOXIDANT POTENTIAL AND FREE RADICALS SCAVENGING ACTIVITY BY CICER ARIETINUM LINN.

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ABSTRACT

*Cicer Arietinum (*Leguminosae) is an annual herb, known as local Indian name 'Kala Chana'. Its root was extracted using solvents of different polarities and explored for *in-vitro* free radical scavenging activity. Preliminary assays of three different extracts of *Cicer Arietinum* root, scavenges 1, 1-diphenyl-2-picrylhydrazyl stable free radicals in concentration dependent manner. All studied extracts possess electron donating ability and reduction of ferric ion to ferrous in a cell free system at pH-7.4. It has also been found from total antioxidant capacity as assessed by reduction of molybdate showed Cicer arietinum root extracts to possess (standard) ascorbic acid equivalents per milligrams of the extracts. Hydro alcoholic root extract was found more effective when compared to alcoholic and water extracts in scavenging 1, 1-diphenyl-2-picrylhydrazyl, reducing ferric ion and molybdate reduction in antioxidant capacity. The significant correlations exist between extract concentrations and percentage scavenging activity of radicals in all models. These results clearly indicate that *Cicer Arietinum* root is effective free radical scavenger and chain breaking antioxidant.

KEY WORDS: **Antioxidant, Cicer arietinum, DPPH, Free Radical Scavenging, Kala Chana.**

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INTRODUCTION

Free radicals may be designated as molecular sharks that damage molecules in cell membranes, mitochondria, DNA and are very unstable, tend to rob electrons from the molecules in the immediate surroundings in order to replace their own losses [1]. Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals but also some non-radical derivatives of oxygen; these include hydrogen peroxide, hypochlorous acid and ozone [2]. Numerous disorders like rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases and gastrointestinal ulcerogenesis are reported as ROS mediated [3-4]. The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of ageing [5-6]. Screening of compounds which scavenge the free radicals, could lead to promising compounds. Most of the antioxidants used in therapy are derived from natural sources. About 28% drugs approved by the FDA between 1981 and 2002 are either natural products or chemicals derived from them[7-8]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects. Hence attention has been given to naturally occurring antioxidants. Therefore, identification of effective antioxidants and free radical scavengers from plants origin is an ideal strategy for new drug development. Hence, present study was designed to explore the antioxidant potential and free radical scavenging activity of *Cicer Arietinum Linn* (C. Arietinum) in a prospective way. *Cicer Arietinum Linn* belonging to the family of Leguminosae. It is an annual herb which is spreaded into Southern Europe, India, Egypt and Southern America. It is extensively cultivated in India mainly in Rajasthan, East Punjab, Haryana and Madhya Pradesh. Its black gram is native of India but the white species are commonly called as 'Kabuli Chana' which came to India in 18th century from European countries and Afghanistan[9]. It contains carbohydrates (in seeds), various proteins and minerals[10]. Proteins, tannins, steroids, flavonoidsand terpenoids in *Tagetes erecta* flower extracts encourage antioxidant studies[11]. In India it is often used as a crash diet and it is one of the most widely made recipes in Indian kitchen due to its good taste and nutritive values. Traditionally it is used as antibacterial, antifungal, antipyretic, antidiarrhoeal, inflammation and cold coughs etc.; In present study we aim to explore the antioxidant potential of different extracts (root) of *Cicer Arietinum Linn* using solvent of different polarity (alcoholic, hydro alcoholic and aqueous).

MATERIAL AND METHODS

Chemicals Details

1, 1-diphenyl-2-picryl hydrazyl (DPPH), o-phenanthroline, ferric chloride, ascorbic acid, and ethylene diamine tetra acetic acid (EDTA) were procured from Haryana Scientific & Engg. Corp., Rohtak. The Plant with the root of *Cicer Arietinum* was collected in February-March month from Kharainti, Meham Teh, Rohtak District, Haryana (India). The plant material was authenticated by Dr. Ashok Kumar Sharma, M.D. (Dravyaguna Vigyan), Prof.& Head of Department, Shri Baba Mastnath Ayurvedic Degree College, Asthal Bohar, Rohtak.

Extraction process

The shade dried plant roots were grinded and coarsely powdered material (100 g) was used for extraction. The different root extracts were prepared by hot continuous percolation method in a Soxhlet apparatus. Following extractions were collected separately and dried in vacuum system. Root extracted with 99% ethanol (root-AA, yield: 09.15%); root extracted with 70%

ethanol (root-HA, yield: 11.65%) and root extracted with water (root-W, yield: 12.25%), these extracts were condensed by re-distillation and dried in vacuum desiccators to obtain a final extract residue.

DPPH radical scavenging activity[12]

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability by using stable free radical DPPH (100 µM) in ethanol. The equal volumes of different extracts were incubated with DPPH in graded concentrations of (10 to 500 µg/ml) for 20 min. at room temperature by using a digital UV/VIS spectrophotometer (model 371E) absorbance was recorded at 517 nm. The experiment was performed in triplicate. Scavenging ability was measured by decreased DPPH absorbance in test sample when compared to standard DPPH solution. Results were expressed as percentage inhibition of DPPH by comparing with blank.

Percentage scavenging of free radical by test compound

$$\%\text{age Scavenging} = \frac{\text{Absorbance of control sample- Absorbance of test sample}}{\text{Absorbance of control sample}} \times 100$$

Reduction of ferric-ions[13]

The electron donating capability (reducing ability) was studied by ferric chloride reduction in cell free system. The reaction mixture contained 1.0 ml of phosphate buffer (pH 7.4), 100 µM ferric chloride and 0.5 ml of test compounds of different concentrations (10-500 µg/ml). After 3 mins. of incubation, EDTA (100 µM) and ortho-phenanthroline (300µM) were added, reaction was allowed for 10 min at room temperature by using a digital UV/VIS spectrophotometer (model 371E) absorbance was recorded at 510 nm. Ascorbic acid was used as standard equivalent to 100% reduction of ferric ions, comparative reduction of Fe^{3+} by *Cicer Arietinum* root extracts were calculated.

Percentage reduction of Ferric ion by test compound

$$\%\text{age Reduction} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of ascorbic acid}} \times 100$$

Total antioxidant capacity[14] ***[By ammonium molybdate reduction method].***

Total antioxidant capacity was measured in different concentrations of extracts were mixed with 3ml of reagent solution (0.6Msulphuric acid, 28mMsodium phosphate and 4mM ammonium molybdate), after 90 minutes incubation at 95^0C for, sample cool to room temperature by using a digital UV/VIS spectrophotometer (model 371E) absorbance of molybdate (V) formed was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid. Ascorbic acid was used as standard equivalent to 100% antioxidant capacity, comparative antioxidant capacity of *Cicer Arietinum* root extracts were calculated.

Percentage antioxidant capacity of test compound

$$\text{Percentage antioxidant capacity} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of ascorbic acid}} \times 100$$

RESULTS

DPPH radical scavenging activity

Root extracts of *Cicer Arietinum* scavenged the DPPH stable free radicals in a concentration dependent manner (10-500 µg/ml). All the extracts showed maximum scavenging activity at 500µg/ml. The root-HA showed maximum activity (73%), root-AA (64%), while root-W showed a maximum activity (53%) at concentration 500 µg/ml. The experiment was performed in triplicate. The interaction of *Cicer Arietinum* root extracts with DPPH radicals presented in Tab. and Fig.1a.

Table 1a
Interaction of *Cicer Arietinum*[a] root extracts with DPPH Radical.

Conc. (µg/ml)	DPPH Scavenging by root-HA		DPPH Scavenging by root- AA		DPPH Scavenging by root-W	
	Absorbance	%Scavenging	Absorbance	%Scavenging	Absorbance	%Scavenging
500	.183±.004	73	.246±.003	64	.322±.003	53
400	.204±.005	69.91	.277±.003	59	.352±.003	48
200	.263±.003	61.21	.406±.001	40.11	.475±.003	29.94
100	.403±.004	40.56	.578±.006	23.60	.566±.001	16.52
50	.520±.008	23.30	.604±.003	10.91	.638±.008	5.90
25	.599±.002	13.13	.624±.003	7.96	.642±.005	5.30
10	.632±.004	6.78	.643±.004	5.16	.650±.003	4.13
Control	.678±.002	00.00	.678±.002	00.00	.678±.002	00.00

[a] ***All values are mean (n = 3).*** ***Data are expressed as Mean ± S.D.***

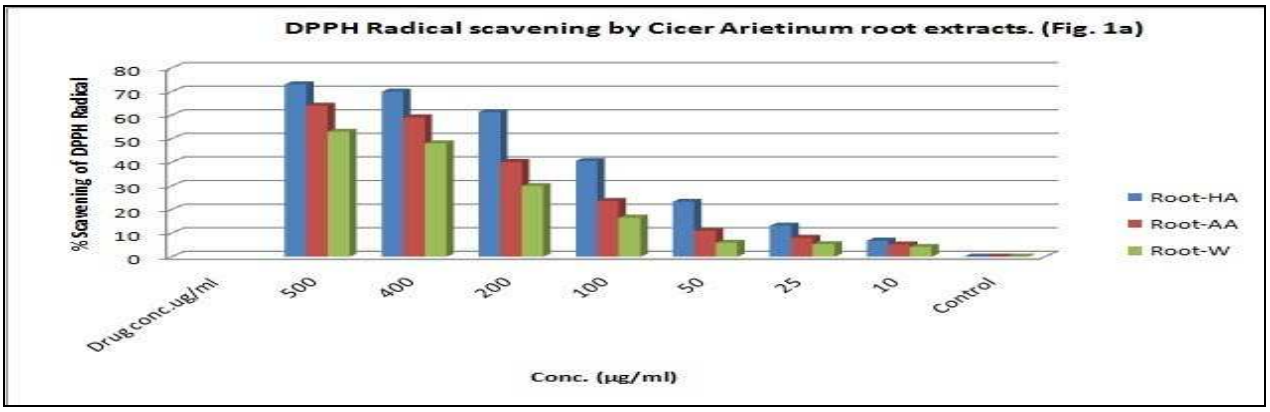

Figure 1a
Interaction of *Cicer Arietinum* root extracts with DPPH Radical.

Reduction of ferric ion

Reduction of ferric ion i.e. Fe^{2+}- Fe^{3+} couple is known to be involved in various free radical reactions. All of these extracts of root-HA, root-AA and root-W reduce Fe^{3+} in to Fe^{2+}at pH 7.4. In this study, reduction of Fe^{3+} into Fe^{2+} by ascorbate was taken as 100%. The root-HA and root-AA (500 µg/ml) caused a significant reduction of Fe^{3+} to (71.08%) and (60%) respectively, while the root-W (500 µg/ml) produced (51.36%). as shown in Tab. and Fig. 1b.

Table 1b
Reduction of ferric ions by *Cicer Arietinum*[a] root extracts.

Conc. (µg/ml)	Ferric ion reduction by root-HA		Ferric ion reduction by root- AA		Ferric ion reduction by root-W	
	Absorbance	%Reduction	Absorbance	%Reduction	Absorbance	%Reduction
Asc. acid (300µM)	.408±.002	100	.410±.003	100	.405±.002	100
500	.290±.002	71.08	.246±.004	60	.208±.004	51.36
400	.258±.001	63.24	.201±.006	49.02	.190±.004	46.91
200	.225±.002	55.15	.181±.004	44.15	.173±.003	42.72
100	.192±.002	47.06	.136±.003	33.17	.122±.003	30.12
50	.151±.001	37	.063±.002	15.37	.058±.002	14.32
25	.119±.003	29.17	.026±.002	6.34	.021±.002	5.19
10	.045±.002	11.03	.009±.002	2.20	.006±.001	1.48

[a] ***All values are mean (n = 3).*** ***Data are expressed as Mean ± S.D.***

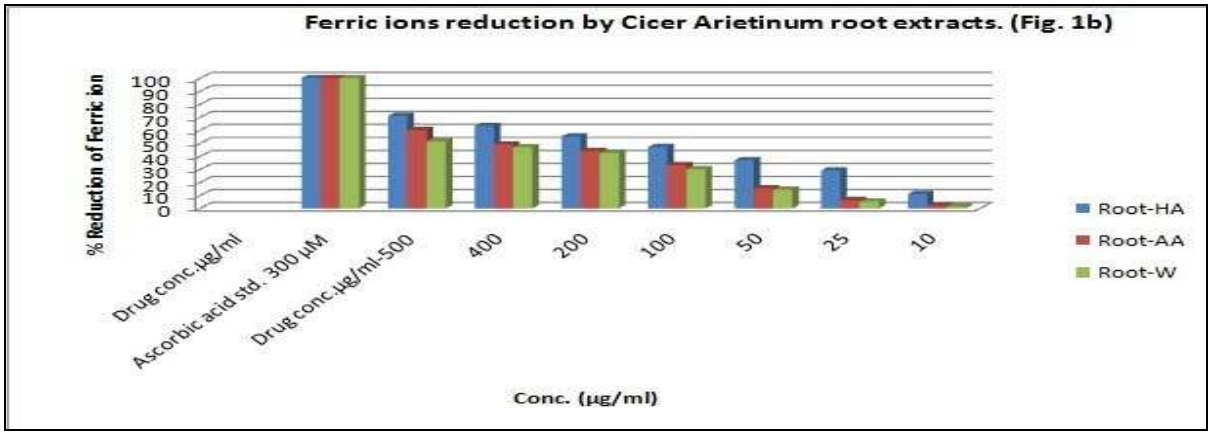

Figure 1b
Reduction of ferric ions by *Cicer Arietinum* root extracts.

Total antioxidant capacity [By ammonium molybdate reduction method].

The reduction of ammonium molybdate by *Cicer Arietinum* root extracts were studied, It has also been found from total antioxidant capacity as assessed by reduction of molybdate showed, extracts of root-HA and root-AA (600 µg/.3ml) to (73.40%) and (64.77%) respectively, while the root-W (600 µg/.3ml) to (40.05%) and ascorbic acid was taken as 100% reduction of molybdate as shown in Tab. and Fig. 1c.

Table 1c
Antioxidant capacity of *Cicer Arietinum*[a] root extracts.

Conc. (µg/ml)	Antioxidant capacity of root-HA		Antioxidant capacity of root- AA		Antioxidant capacity of root-W	
	Absorbance	% Antioxidant capacity	Absorbance	% Antioxidant capacity	Absorbance	% Antioxidant capacity
Asc.acid (300µM)	.880±.006	100	.880±.004	100	.884±.004	100
600µg/.3ml	.646±.006	73.40	.570±.004	64.77	.354±.005	40.05
300µg/.3ml	.490±.003	55.68	.297±.002	33.75	.209±.005	23.64

[a] ***All values are mean (n = 3).*** ***Data are expressed as Mean ± S.D.***

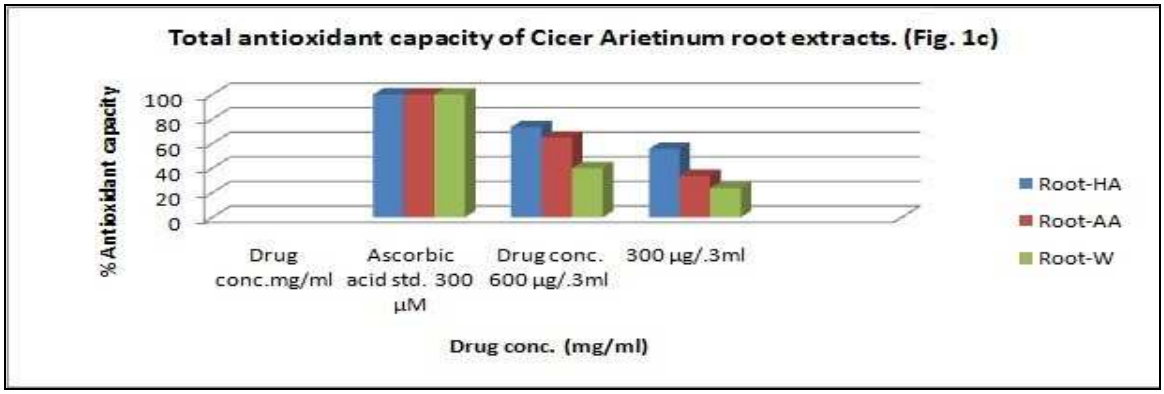

Figure 1c
Antioxidant capacity of Cicer Arietinum root extracts.

DISCUSSIONS

Free radicals have been a subject of significant interest among scientists in the past decade and their possible role in human diseases has gained importance nowadays[15-16]. Antioxidants neutralize toxin and volatile free radicals that are defined as atoms or groups of atoms having an unpaired electron[6]. These also include related reactive oxygen species (ROS) that leads to free radical generation, causes the cascading chain reaction in biological system. In a normal healthy organism or human body, the generation of pro-oxidants in the form of ROS is effectively kept in check by various levels of antioxidant defense. Antioxidants present in various dietary supplements offered their beneficial effects by neutralizing these ROS during various disease conditions. Lipids, proteins and DNA are all susceptible to attack by free radicals and cellular damage induced by oxidative stress has been implicated in the etiology of numerous diseases. DPPH radical is widely used as a model to investigate the scavenging potential of several natural compounds such as phenolic and anthocyanins or crude extract of plants[17]. The ability of root extracts of *Cicer Arietinum* to reduce DPPH radicals supports its free radical scavenging activity. Our study

indicates the proton donating property may be responsible for free radical scavenging activity of *Cicer Arietinum.* Antioxidant compounds for example, flavonoids, polyphenols and tannins reduce the Fe^{3+} to Fe^{2+} and are considered as chain breaking antioxidant for their proton donating activity[18]. *Cicer arietinum* root extracts reduce ferric ion at pH 7.4 which indicates its proton donating ability and supports its free radical scavenging activity. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. It was therefore to be expected that, *Cicer arietinum* root extracts have potent antioxidant and radical scavenging ability. This activity is believed to be mainly due to their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. It has also been found from total antioxidant capacity as assessed by reduction of molybdate showed *Cicer arietinum* root extracts to possess about (standard) of ascorbic acid equivalents per milligrams of the extracts. Hence present study revealed antioxidant property of *Cicer arietinum* root extracts.

CONCLUSIONS

Our study showed that hydro alcoholic root extract of *Cicer arietinum* is more effective antioxidant compared to (root-AA, root-W) in various *in vitro* assay systems. Cicer Arietinum contains carbohydrates, proteins, minerals, polyphenols and flavonoids.[10] Among these compounds, the flavonoids and phenolic compounds are probably responsible for its free radical and reducing property observed in this study. As compared to previous reports, our study showed that *Cicer Arietinum* hydro alcoholic root (root-HA) exerted a potent effect. Further studies are required for better understanding of these compounds and their effects on cellular function. Antioxidant properties of *Cicer Arietinum* root could be beneficial in pathological condition involving oxidative stress.

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