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DPPH Scavenging Activity of *Cicerarietinum* seed Extract

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ABSTRACT

Legume seed sprouts are popular foods in globally. It is also reported that seed priming improve the antioxidant enzymes activity which decrease the adverse effects of Reactive Oxygen Species (ROS)[1] . Many antioxidants can be obtained from food sources such as sprouted grains, fruits and vegetables. Consumption of high amounts of antioxidant substances may have a positive impact on human health, particularly the prevention of cancer and inflammatory diseases. Seeds of Cicerarietinum are one of the ancient and widely consumed legumes in tropical and subtropical countries. Chickpeas are a rich source of zinc, folate and protein. They are also very high in dietary fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity. They can assist in lowering of cholesterol in the bloodstream and are effective in conditions like anemia, digestive system disorder, painful menstruation, skin and hair disease and sexual dysfunction[2] . In the present study an attempt has been made to evaluate the scavenging effect of Cicerarietinum seeds on DPPH assay.

Keywords: *Cicerarietinum,* DPPH, Anti-oxidants.

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INTRODUCTION

Many diseases are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance [3]. The antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radical [4]. However, there have been concerns about synthetic antioxidants such as butylatedhydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) because of their possible activity as promoters of carcinogenesis. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity [5]. Therefore, search for natural antioxidant has greatly been increased in the recent scenario.

Plant products including phenolics, flavonoids, tannins, proanthocyanidins, and various plant or herbal extracts have been reported to be radical scavengers and inhibitors of lipid peroxidation. Therefore, in view of the importance of these substances to health, phenolics have been proposed as health-promoting products in prophylactic medicines [6]

The plant Cicerarietinum belonging to family Fabaceae, largely cultivated in most parts of India. Seed is aphrodisiac, anthelmintic, tonic, enriches the blood, cures skin diseases, inflammation; more especially of ear, diuretic, halitosis, hepatosis, otitis, pharyngosis, pulmonosis and splenosis [7]

The present study aims to spectrophotometeric quantification of DPPH radical scavenging activity in the Ethanolic Extract of Cicerarietinum.

MATERIALS AND METHODS

Plant Material

Chickpeas seeds (Ciceraritenum) were purchased from the local market in Chennai and identified by The Director, National institute of herbal science, Anna nagar, Chennai.

Reagents required

1) 1, 1-Diphenyl-2-picrylhydrazyl (DPPH)
2) Ethanol
Reagent preparation DPPH= 200µM.

Preparation of Cicerarietinum Extract

Chickpeas seeds were obtained from the local market were shade dried and powdered using mechanical mixer. The plant extract was prepared using soxhlet apparatus, by maceration of 50 g of the chopped, dried buds of Cicerarietinum a mixture of 200 ml ethanol and 200 ml distilled water by shaking them for 48 h and pressing the solution out of the material using a filter press. The extraction solvent was then removed under reduced pressure until the extract was obtained as a dried gum. The final extracted material weighed 10g. Concentrations of the extract were prepared by dissolving final product in distilled water.

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was measured in terms of hydrogen-donating or radical scavenging ability, using the DPPH method [8, 9]. DPPH radical scavenging activity was done using the method of Yohozowa et al [10]. The reaction mixture containing 1.9ml of DPPH solution (200µM in ethanol) with different concentrations of the substance was shaken and incubated in dark for 20min at room temperature. The resultant absorbance was recorded at 517nm. The percentage inhibition was calculated using the formula

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$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} \times 100}{\text{Abs}_{\text{control}}}$$

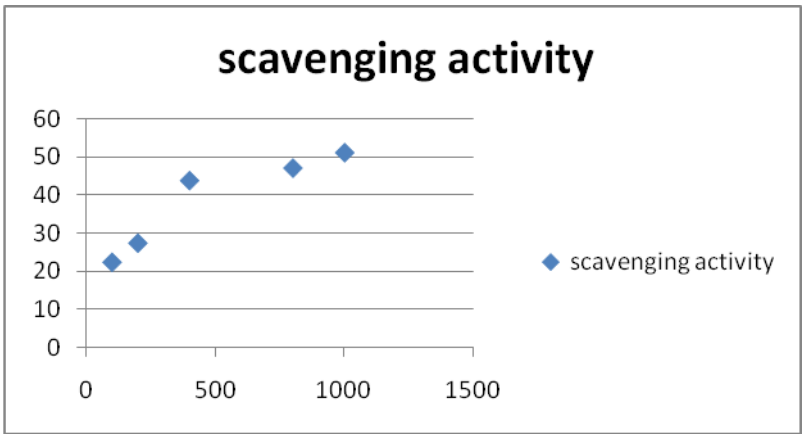
Ascorbic acid was used as a reference standard.

OBSERVATION

S.No	Sample taken(ml)	Conc (µg)	Reagent taken(ml)		Absorbance at 517nm		
1	0.10	1000	1.900	Incubation In dark for 30min	0.1640	0.1690	0.1752
2	0.08	800	1.920		0.1696	0.1806	0.2001
3	0.04	400	1.960		0.1852	0.1876	0.2116
4	0.02	200	1.980		0.2450	0.2496	0.2601
5	0.01	100	1.990		0.2570	0.2608	0.2890

RESULTS

Control: 1.9ml of DPPH + 0.1ml of DMSO =0.3470
Blank: 1.9ml of ethanol +0.1ml of DMSO
% Scavenging Activity= Control- Test/Test X 100
For 100µg concentration = 0.3470-0.2689/0.3470*100=**22.49%**
For 200µg concentration = 0.3470-0.2515/0.3470*100=**27.50%**
For 400µg concentration = 0.3470-0.1948/0.3470*100=**43.86%**
For 800µg concentration = 0.3470-0.1834/0.3470*100=**47.13%**
For 1000µg concentration = 0.3470-0.1694/0.3470*100=**51.18%**



The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. Extracts reduce the colour of DPPH due to the power of hydrogen donating ability [11]. DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers [12]. Antioxidants may guard against reactive oxygen species (ROS) toxicities by scavenging reactive metabolities and converting them to less reactive molecules.

The DPPH radical scavenging activity was recorded in terms of percentage Inhibition. It was observed from that Cicerarietinum (100 µg /ml) has minimum DPPH scavenging activity (**22.49%**) and Cicerarietinum (1000 µg /ml) has maximum DPPH scavenging activity (**51.18%**). The Results obtained were comparative to Curcumin standard. Higher Percentage Inhibition indicates better scavenging activity or antioxidant potential.

The given sample showed the dose dependent activity in scavenging the free radicals compared to that of the Curcumin standard.

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CONCLUSION

The Ethanolic Extract of Cicerarietinum exhibited significant antioxidant activity compared to Curcumin standard and the activity may be related to the flavonoids and phenolic compounds in this plant extract. Since reactive oxygen species are important contributors to several serious ailments, in the present study, the observed DPPH scavenging activity of the Ethanolic Extract of Cicerarietinum might be useful for the development of newer and more potent natural antioxidants.

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