

Original Article

In-vitro anti-oxidant activity of leaves of *Cicer arietinum*

Supriya Mhamane¹, Priyanka Adat²

From, ¹Sahyadri College of Pharmacy, Methwade, Sangola, Maharashtra, ²Assistant Professor, Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, Maharashtra, India.

ABSTRACT

Background: *Cicer arietinum* also known as chickpea. Chickpea plant belongs to family *Fabaceae*. Chickpeas are an essential food plant in India, Africa and Central and South America. This study concludes the *Cicer arietinum* leave's anti-oxidant activity. It shows anti-oxidant activity and it is used to reduce oxidative stress. **Materials and Methods:** This work conceived the anti-oxidant activity of leave's of *Cicer arietinum* via DPPH scavenging activity and measuring the absorbance at 517nm. **Result:** The maximum peak of free radical scavenging activity by using ethanolic and hydro-alcoholic extract is observed at 400µg/ml. By comparing both the extract hydro-alcoholic extract shows maximum percentage inhibition. **Conclusion:** Thus, the high percentage inhibition of hydro-alcoholic extract which is 51.31% can be more effective as an antioxidant than the ethanolic extract.

Keywords: *Cicer arietinum*, DPPH, Ascorbic acid, Free radical scavenging activity, Oxidative stress.

All over the world, traditional medicine has a long history of serving people. As ancient as human civilization, there is use of natural products with therapeutic properties. Main sources of drugs for a long time are mineral, plant and animal products. In almost all ancient civilizations, there is an evidence of herbs being used in the treatment of diseases and for revitalizing body systems. Free radicals included in ROS (Reactive Oxygen Species) can cause liver cirrhosis, atherosclerosis, cancer, diabetes. To protect body from damage due to reactive oxygen species, antioxidants are used. In the diabetic state, oxidative stress is increased. Oxygen free radical activity can lead to peroxidation of lipids, which in turn activates glycation of protein, inactivation of enzymes and alterations in the structure and function of collagen, basement and other membranes and play a role in the long team of diabetes [1,7].

MATERIALS AND METHODS

Extraction method: In soxhlet apparatus, add 50gm of powdered drug and pour the solvent system. Pour solvent upto which three cycles were complete. Then start the heating mantle at 55°C and start water supply to condenser. The solvent pass from powder and along with constituents present in powder it settles in round bottom flask. Keep this process upto the solvent comes colourless [2,8].

In-Vitro Anti-Oxidant Activity

Oxidative stress: The imbalance between formation and accumulation of reactive oxygen species (ROS) in the body cells and tissues is called as oxidative stress [3].

Oxidative stress can lead to: Cancer, cardiovascular disease, neurologic disease, respiratory disease, rheumatoid arthritis, kidney disease, delayed sexual maturation, etc. [3,5].

Antioxidants:

Exogenous antioxidants: Vitamin E, flavonoid.

Prooxidant agents: Ascorbic acid, polyphenols, radiation [4].

Dpph Free Radical Scavenging Activity:

Principle: DPPH i.e. 2,2-diphenyl-1-picrylhydrazyl is a deep violet colored stable free radical. It has unpaired electron. Antioxidant donate electron to DPPH, so non radical of pale yellow color form from free radical of deep violet color. Due to this, absorbance get decreased which is observed by UV spectrophotometer at 517 nm wavelength [1,6].

Chemicals used in the assay: DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, ascorbic acid, ethanol.

Procedure: Methanolic solution of DPPH was used to test free radical scavenging action of *Cicer arietinum* leaves

Correspondence to: Supriya Mhamane, Korwali, Tal. Mohol, Dist. Solapur, Maharashtra, India. **Email:** supriyamhamane99@gmail.com. **Tel.:** +91 8080244609.

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extract. In this assay as a free radical, DPPH is used. Antioxidants reduce DPPH which is observed at 517 nm wavelength. In this assay, as a standard antioxidant, ascorbic acid is used. In this assay, reaction mixture is used, which contain 0.1 ml leaves extract of 6 different concentrations in 6 different test tubes and add 3.9 ml methanolic DPPH solution in each test tube as shown in (Figure 1). As a standard, 0.1 ml ascorbic acid was used and as a control 0.1 ml methanol was used instead of extract. At a room temperature for 30 minutes it is incubated. Observe the absorbance at 517 nm wavelength [1,6].



Figure 1: Dilution of Anti-oxidant Activity

Calculation: Percentage inhibition were calculated by using following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs.of control} - \text{Abs.of test}}{\text{Abs.of control}} \times 100$$

RESULTS

In-vitro anti-oxidant activity of standard (Ascorbic acid), having percentage inhibition was found to be 96.83%.

Table: *In-vitro* antioxidant activity of *Cicer arietinum*

Conc. (µg/ml)	Percentage Inhibition (Ethanolic) (%)	Percentage Inhibition (Hydro-alc.) (%)
25	5.26	5.17
50	7.09	6.5
100	7.27	8.01
150	7.61	10.57
200	9.62	12.99
400	22.92	51.31

DISCUSSION

Cicer arietinum plant includes plant constituents like alkaloids, glycosides, steroids, carbohydrates, amino acids, inorganic elements. These constituents are responsible for showing anti-oxidant activity. In this study, the antioxidant activity was determined using DPPH free radical scavenging activity in which the purple color of DPPH gets reduced to yellow color due to transfer of electrons. Due to this, decrease in value of absorbance depicts that constituents of leaves of *Cicer arietinum* are able to reduce free radicals.

Due to free radicals of oxygen, the number of reactive oxygen species (ROS) increases, so that the oxidative stress

increases this can lead to various diseases. To overcome it, the antioxidants are used. Among two samples ethanolic and hydro-alcoholic extract, the highest percentage inhibition was observed in hydro-alcoholic extract at 400µg/ml which was 51.31%. The percentage inhibition increases as concentration increases, which is as shown in table and graph. Ethanolic extract shows high percentage inhibition at 400µg/ml i.e. 22.92%. Thus it could be concluded that hydro-alcoholic extract shows good antioxidant property.

CONCLUSION

The conclusion of this study shows that, the ethanolic and hydro-alcoholic extract of leaves of *Cicer arietinum* shows anti-oxidant activity by DPPH free radical scavenging activity. The hydro-alcoholic extract shows high percentage inhibition as compared with ethanolic extract. Hydro-alcoholic extract at 400µg/ml shows high percentage inhibition i.e. 51.31%. It was observed as the concentration of extract increases from 25µg/ml to 400µg/ml The percentage inhibition also increases. From given Table, it can be concluded that hydro-alcoholic extract has the highest antioxidant activity or good scavenging activity.

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