

Research Article

In Vitro Antioxidant Activity of Different Leaf Extract of *Celosia argentea* L.

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ABSTRACT

Celosia argentea L. is belonging to the family Amaranthaceae. This is commonly used as a leafy vegetable in most of the Asian countries. The whole plant used as medicine to cure diabetes, leucorrhoea, anti-inflammatory, etc. The present study was carried out to find out the antioxidant potential of different leaf extracts, of *celosia argentea* L. by using various in vitro assays. Among the different extracts of *celosia argentea* L. the methanol extract showed greater antioxidant activity compared to petroleum ether and aqueous extracts.

Keywords: *Celosia argentea*, leucorrhoea, anti-inflammatory, methanol extract.

INTRODUCTION

Antioxidant compounds in food are playing an important role as a health protecting factor. Dietary antioxidants are the supplements that may delay or reduce the effects of oxidative stress and phenolic compounds are the phytochemicals that are widely present in the plant kingdom exhibiting several bioactivities¹ and can be classified in natural antioxidants that take an important place in our diet which absorb and neutralize free radicals by donating a hydrogen atom from their hydroxyl groups². Nowadays most of the clinical studies revealed that increasing intake of fresh fruits and leafy vegetables in daily diet lowers the risk of many human diseases especially degenerative ailments linked to ageing process. Consumption of leafy vegetables is a major source of vitamins and micronutrients needed for the normal functioning of the body physiology. They are rich source of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron, phosphorus and other phytochemicals^{3,4}. *Celosia argentea* L. is an annual herb belonging to the family Amaranthaceae, grows as a weed during rainy season throughout India and other tropical countries. The whole plant is used to treat diarrhea, piles, bleeding nose, gynaecologic disorders and anti-inflammatory⁵. In India, the plant is well known for the treatment of mouth ulcer, blood diseases and used as an aphrodisiac⁶. The flower and seed is astringent, hemostatic, ophthalmic and parasiticide. It is widely used in Indian folk medicine for the treatment of diabetes mellitus^{7,8}.

MATERIALS AND METHODS

Collection of plant material

The aerial part of the plant *Celosia argentea* L., were collected from Periyanaicken Palayam, Coimbatore, Tamil

Nadu, between the months of August and October in the year 2014. The plant material was identified and authenticated by the Department of Botany, Avinashilingam University, Coimbatore, Tamil Nadu, India.

Extraction of plant material

The leaves were cleaned, dried in the shade and pulverized in a mechanical grinder, passed through a 40 mesh sieve and stored in an airtight container. 30 grams of powdered dried samples were successively extracted with petroleum ether (300ml), Methanol (300ml) and aqueous (300ml) by using soxhlet apparatus until the discolourisation of the solvents.

Enzymatic Assay

Estimation of Catalase Activity

Each sample was Homogenized in a blender with M/150 phosphate buffer (assay buffer diluted 10 times) at 1 – 4°C and centrifuged. Stirred the sediment with cold phosphate buffer, allowed to stand in the cold with occasional shaking and then repeated the extraction once or twice. The extraction should not take greater than 24 hours. The combined supernatants were used for the assay. Read against a control cuvette containing the enzyme solution as in the experimental cuvette, but containing H₂O₂ free phosphate buffer (M/150). Pipette out 3 ml of H₂O₂ – phosphate buffer into the experimental cuvette, added 0.01 – 0.04 ml of the sample and mixed with a glass or plastic rod flattened at one end. Noted the time (Δt) required for a decrease in absorption from 0.45 to 0.40. This value was used for the calculations. If 't' is more than 60 seconds, the measurements have to be repeated with a more concentrated solution of the sample.

Estimation of Peroxidase Activity

One part of each sample was macerated with 5 parts (w/v) of 0.1 M phosphate buffer (pH 6.5) in homogenate at 500

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rpm for 15 minutes and used the supernatants as the enzyme source. All procedures were carried out at 0 – 5°C. Pipette out 3 ml of 0.05 M pyrogallol solution and 0.02 ml of enzyme extract in a test tube and adjusted the spectrophotometer to read '0' at 430 nm. Then added 0.5 ml of 1% H₂O₂ in the cuvette and recorded the change in absorbance for every 30 seconds up to 3 minutes.

Estimation of Polyphenol Oxidase Activity

Take 0.5 ml of the plant sample and made up to 20 ml with the medium containing 50 µM Tris – HCl (pH 7.2), 0.4 M sorbitol and 10 µM NaCl. Centrifuged the homogenates at 2000 rpm for 10 minutes and used the supernatant for the assay. Then add 2.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.3 ml of catechol solution (0.01 M) into the cuvette and set the spectrometer at 495 nm. The enzyme extracts (0.2 ml) was added and started recording the change in absorbance for every 30 seconds upto 5 minutes.

Estimation of Glutathione – S – Transferase Activity

The enzyme activity was determined by monitoring the change in absorbance at 340 nm in a spectrophotometer. 0.1 ml both substrates (GSH and CDNB) were taken in 0.1 M phosphate buffer pH 6.5 at room temperature to make a volume of 2.9 ml. The reaction was started by adding 0.1 ml of sample to this mixture and the readings were recorded against distilled water blank for a minimum for 3 minutes. The complete assay mixture without the sample served as the control to monitor non-specific binding of substrates. Care was taken to ensure that final concentration of ethanol in mixture was always less than 4 per cent.

Non enzymatic assay

Estimation of Ascorbic Acid (Vitamin C)

Take 1 g of the sample and homogenized in 4 per cent TCA. Made up to 10 ml and centrifuged at 200 rpm for 10 minutes. The supernatant obtained was treated with a pinch of activated charcoal, shaken well and kept for 10 minutes and again centrifuged to remove the charcoal residue. Noted the volume of clear supernatant obtained. 0.5 and 1 ml aliquots of this supernatant were taken for the assay. The assay volume was made upto 2.0 ml with 4 per cent TCA. 0.2 to 1.0 ml of working standard solution containing 20 – 100 µg of ascorbic acid respectively was pipetted out into clean dry tubes and the volumes was made up to 2.0 ml with 4 per cent TCA and add 0.5 ml of DNPH reagent to all the tubes followed by 2 drops of 10 per cent thiourea solution. The test tube was incubated at 37°C for 3 hours. The osazone formed was dissolved in 2.5 ml of 85 per cent sulphuric acid, in cold, drop by drop with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After the incubation for 30 minutes at room temperature the absorbance were recorded at 540 nm.

Estimation of α-Tocopherol (Vitamin E)

The sample was homogenized in a blender. 2.5 g of the homogenized tissue is taken into a conical flask. 50 ml of 0.1 N sulphuric acid is added slowly without shaking. Stopped and allowed to stand overnight. The next day contents of the flask were shaken vigorously and filtered through Whatman No. 1 filter paper discarding the initial

10-15 ml of filtrate. Aliquots of the filtrate were used for the estimation. Into three stoppered centrifuged tubes (test, standard and blank) pipette out 1.5 ml of sample extract, 1.5 ml of standard and 1.5 ml of water respectively. To the test and blank added 1.5 ml of ethanol and to the standard, added 1.5 ml of water. Added 1.5 ml of xylene to all the test tubes stoppered, mixed well and centrifuged. Transferred 1.0 ml of xylene layer into another stoppered tube, taking care not to include any ethanol or protein. Added 2 ml of 2,2'-dipyridyl reagent to each tube stoppered and mixed. Pipetted out 1.5 ml of the mixture into the spectrophotometer cuvette and read extinction of the test and standard against the blank at 460 nm. The in turn, beginning with the blank, added 0.33 ml of ferric chloride solution. Mixed well and after exactly 15 minutes read test and standard against the blank at 520 nm. The amount of vitamin E can be calculated using the formula,

$$\frac{\text{Amount of } \alpha\text{-tocopherol in } \mu\text{g}}{\text{Reading at 520 nm} - \text{Reading at 460 nm}} \times 0.24 \times 15 = \text{Reading of standard at 520 nm}$$

Estimation of Polyphenol

Weighed exactly 0.5 to 1.0 g of the plant sample and ground it with a mortar and pestle in 10 times volume of 80 per cent ethanol. Centrifuged the homogenate at 10,000 rpm for 20 minutes and saved the supernatant. Re-extracted the residue with five times the volume of 80 per cent ethanol, centrifuged and pooled the supernatants and then evaporated the supernatant to dryness. Dissolved the residue in a known volume of distilled water and pipetted out different aliquots (0.2 – 2.0 ml) into test tubes. Make up the volume in each tube to 0.3 ml with water. Add 0.5 ml of Folin-Ciocalteau reagent. After 3 minutes, added 2.0 ml of 20 per cent sodium carbonate solution to each tube. Mixed thoroughly placed the tubes in a boiling water bath for exactly 1 minute, cooled and measured the absorbance at 650 nm against a reagent blank.

RESULTS AND DISCUSSION

The different solvent extracts of *Celosia argentea* L. leaf (petroleum ether, methanol and aqueous) were taken for the analysis of enzymatic and non-enzymatic antioxidants and depicted in table 1 and 2. In the present study the highest activity of catalase, peroxidase, Polyphenol oxidase Glutathione-s-transferase and Glutathione peroxidase was observed in methanol extract followed by Petroleum ether and aqueous. Catalase is a tetrahedral protein constituted by four heme groups which catalyze the dismutation of hydrogen peroxide in water and oxygen⁸. Phenol oxidases are copper proteins catalyze the aerobic oxidation of certain phenolic compounds to quinines polyphenol oxidase is one of the major enzymes that have a role in biosynthesis of lignin and defense against water stress by scavenges H₂O₂ in chloroplasts⁹. The glutathione peroxidase and catalase were found to be important in the inactivation of many environmental mutagens¹⁰. The significant enzymatic and non-enzymatic antioxidant activity in *Andrographis echiooides* and *Boerhavia diffusa*

Table 1: Enzymatic Antioxidant Activities of *Celosia argentea* L.

Solvents	Catalase (@ / g)	Peroxidase (# / g)	Polyphenol oxidase (\$ / g)	Glutathione - s- transferase (* / g)	Glutathione peroxidase (~ / g)
Petroleum ether	9.52± 1.08	10.2± 1.51	52.3 ± 0.88	140.52± 2.88	25.20± 1.44
Methanol	10.94 ± 1.58	12.37 ± 2.14	61.0 ± 0.01	147.20 ± 1.33	28.61 ± 2.47
Aqueous	9.25± 0.05	8.32± 1.22	58.3 ±1.08	132.10± 2.68	26.52± 2.8
SED	0.523	0.27	0.64	5.74	0.83
P<.01	0.183	0.065	0.01	0.076	0.26

The values are expressed in mean ± SD.

Units: @ / g – Amount of enzyme to decrease the optical dense by 0.5 units at 240 nm. # / g – Change of absorbance / minutes at 430 nm. \$ / g – Amount of catectiol oxidase / laccase which transforms / μ mole of dihydric phenol to quinine / minute. * / g – μ moles of CNNB conjugated / minute. ~ / g – μ moles of GSH utilized / minute.

Table 2: Non - Enzymatic Antioxidant Activities of *Celosia argentia* L.

Solvents	Ascorbic acid (μg / g)	α-tocopherol (μg / g)	Polyphenol (μg / g)
Petroleum ether	60.5 ± 0.88	50.0 ± 1.22	6.9 ± 1.42
Methanol	62.0 ± 0.16	51.72±1.89	7.37 ± 2.37
Aqueous	58.2 ± 1.44	49.2 ± 1.28	6.5 ± 1.22
SED	0.62	0.56	0.578
P<.01	0.008	0.18	0.83

The values are expressed in mean ± SD

were also reported¹¹. The importance of Ascorbic acid (vitamin C) as an antioxidant is indispensable in biological system. This vitamin is reputed for scavenging the harmful free radicals produced in the body and also enhance the antioxidant defense mechanism in body. It has been reported that a dose of 1 g of vitamin C per day is sufficient to counteract the ill effects of low density lipoprotein¹². The present study shows that the significantly highest amount of ascorbic acid and α-tocopherol observed in methanol extract of *C. argentea* as 62.0 ± 0.10 mg / g and 51.72 ± 1.89 mg / g respectively. Phenolic are aromatics secondary plant metabolites widely spread throughout the plant kingdom and associated with colour, sensory qualities, nutritional and antioxidant properties of food¹³. The antioxidant activity of phenolic compound is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers' heavy metal chelators and hydroxyl radical quenchers¹⁴. In the present study the total polyphenol content of *C. argentea* was absorbed in methanol, when compare to petroleum ether and aqueous extracts. Similar reports were already seen in some common Indian leafy vegetables¹⁵.

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