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Enzymatic and non-enzymatic antioxidants in *Chickpea (Cicerarientinum L.)*

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Abstract

Chickpea (Cicerarietinum L.) belong to the family leguminosae. Young chick pea leaves are also eaten as a cooked vegetable and could be a useful source of dietary nutrients. Enzymic parameters like peroxidase and polyphenoloxidase are selected. Experimental part include screening of non-enzymatic parameters like proteins, reducing sugar, polyphenols and ascorbic acid. From this study we can conclude that the mature leaves are rich source of non-enzymatic antioxidants. From the result of the studies on non-enzymatic antioxidants, it is observed that polyphenols are less than other parameters. Two stages of development of leaves were included for the study of antioxidants from enzymatic and non-enzymatic status in order to ascertain if a difference existed in the levels of enzyme activity in stage of development. This result showed that the leaves in both stages possessed considerable activity of the non-enzymatic and enzymatic parameters. These findings suggest that potential use of Cicerarientinum leaves to battle the innumerable diseases and disorder linked with oxidative damages. From the result of present study, it is apparent that leaves of Cicerarientinum L. possess considerable levels of both antioxidants and it is advisable to consume the leaves at mature stage. To summarize the observations of the studies on the enzymatic and non-enzymatic antioxidants, it can be inferred that the full mature leaves have rich source of enzymatic and non-enzymatic parameters except polyphenols.

Keywords: Cicerarietinum, Antioxidants, Chick pea, enzymatic and non enzymatic.

Introduction

In human body free radicals are formed which leads to cellular damages in pathobiological conditions, lung damages, inflammatory diseases, antherosclerosis and aging. Antioxidants play an important role in reducing oxidative damage due to free radicals. Enzymatic parameters like peroxidase, polyphenol oxidase, catalase, super disroxidemutase and non-enzymatic parameters like polyphenols, reducing sugar, starch and vitamin C have their role as antioxidants. Leafy vegetables and seeds are the natural source of antioxidants. They are rich in ascorbic acid, vitamin E and polyphenolic compounds. All these parameters have their counter effect in reducing the damage at cellular level, cancer cardiovascular disease, diabetes, immune deficiency and aging.

Antioxidant compounds acts as a free radical scavengers. They scavenge the harmful effects of free radicals and thus have their significant role in inhibiting diseases like cancer[1]. The important path for production of free radicals in foods, drugs and in living system is an oxidative process[2].

Since ancient times, the plants are the great source of phytochemicals. The screening of such phytochemicals found to be very effective in various treatments of many diseases and preservation of foods from the toxic effects of oxidants. The phytoconstituents of many plant herbs have antimicrobial and antioxidant activities[3].

Vitamin C, Vitamin E and carotenoids of the plants are the sink of large amounts of antioxidants[4]. Antioxidant parameters obstruct the oxidation process and react with free radicals and acts as an oxygen scavengers[5,6].

As the plants are rich in polypohenols, they have their defense action against development of cancers, cardiovascular diseases, and diabetes. Proteins stop lipid oxidation. The role of ascorbic acid is well known for maintenance of the vascular system and the reduction of formation of fatty acids in the arteries through regulation of collagen.

Chickpea (*Cicerarietinum* L.) belonging to the family Leguminosae. Chickpea contains 19-21% protein and 60% carbohydrate, so it provides an excellent quality of dietary protein. So seeds and leaves of Chickpea are eaten as a cooked vegetable. In Mediterranean countries it is cultivated principally as a legume crop, since it is well adapted to semi-arid condition[8].

In India, cooked seeds are mostly consumed. It is highly rich in proteins, minerals, fibers[9]. It occupies a very important place in human nutrition. Extensive research work done on source of natural antioxidants from crude extracts and natural phytochemical compounds which have antioxidant properties[10]. Researches done on medicinal aspects of plants and vegetables leads to the conclusion that the phytochemical contents of plants

have antioxidant activity which can cure oxidative stress in biological system[11].

The plants are the great source of secondary metabolites like polyphenol, lectins, and oligosaccharides. These secondary metabolites are considered as anti-nutrients, simultaneously advising health benefits[12,13].

These compounds have been found to remove free radicals; chelates metal catalysts, activate antioxidative enzymes, reduce alpha tocopherol radicals and inhibit oxidases[14].

Polyphenolic compounds not only effectively prevent the oxidation of foods but they also act as a protective factor against oxidative damage in the human body[15,16].

Recently, natural antioxidants which can inhibit some of the natural key enzymes, like alpha-amylase and alpha-glucosidase etc. linked to post prandial hyperglycemia have attracted lot of interest as a potential approach for curing type 2 diabetes mellitus[17,18].

Oxidative stress can also modify DNA, proteins and small cellular molecules, and it is thought to have a significant role in the occurrence of diseases, such as cancer, arteriosclerosis, cardiovascular diseases, diabetes mellitus and neurological disorders[19,20].

In recent years, it has been observed that there are many protein hydrolysates like fish protein[21], soybean[22], rice bran[23], and milk[24] which have their role in purification of antioxidative peptides.

Many research paper encountered that the antioxidative activity of protein hydrolysates and isolated peptides prepared from natural resources, in some cases, is similar or higher than that of commonly used synthetic antioxidants, such as butylatedhydroxytoluene (BHA), butylatedhydroxylanisole (BHT) and propyl gallate[25].

It has been reported that Chick pea protein hydrolysate acts against fungi, angiotensin I-converting enzyme (ACE) inhibition, metal-chelating ability, antioxidant activity, and reduction of antigenic activity[27-29]. Chick peas are rich in dietary protein due to amino acids and low level of antinutritional factors[26].

Polyphenols one of the major constituent of foods. Polyphenols of plant origin and are major antioxidants in the human diet. These compounds haveantioxi-dant, apoptotic, antiaging, anticarcinogenic and anti-in-flammatory activities, cardiovascular protection, and improvement of endothelial function. Polyphenols also stop angiogenesis and cell-proliferation activity[30].

Dry legumes are a good source of bioactive polyphenols and also contribute to polyphenol intake from other foods. The polyphenolic contents of the legumes have the antimutagenic, apoptosis-related and antiproliferative effects. The abundance of phenolic compounds in such legumes as the common bean (*Phaseolus vulgaris*), faba (broad) bean (*Viciafaba*), beach pea (*Lathyrus maritimus*), mung bean (*Vignaradiata*), lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*) implies that they may be significant food sources of active antioxidants. Chickpea contains relatively low phytic acid content, compared to other legumes[31].

Cancer and inflammatory diseases can be prevented by consuming sprouted grains, fruits, which are the great source of antioxidants[32].

It has been recognized that oxidative stress causes induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others[33].

Methodology

Naturally grown plants were collected from the botanical garden, Fergusson College Campus, Pune, India. The leaves were collected fresh for the assay of each parameter. Enzymic parameters are peroxidase, polyphenol oxidase, Non-enzymatic parameters used here are ascorbic acid, polyphenols, reducing sugars, total carbohydrates, starch and proteins.

The parameters were analyzed at the vegetative & fruiting stage. The enzymic, non-enzymic and organic constituents were examined using protocols such as for enzymic antioxidants; peroxidase[34] and polyphenol oxidase[34]. Similarly for non-enzymic antioxidants such ascorbic acid[35], polyphenols[36], reducingsugar[37] andproteins[38].

Screening of enzymatic parameters

a) Peroxidase (Vidyasekhran and Durairaj, 1973)**:**

Weigh 1gm. plant material.

▼

Homogenize in chilled mortar & pestle in 5ml of 0.1M phosphate buffer (pH 7.0).

▼

Centrifuge at 15000rpm for 20 min at 4^0 C.

▼

Discard the residue. Use the upper liquid as an enzyme source within 2-4 hrs.

▼

1.8ml of 1M phosphate buffer (pH 7.0) = 1ml freshly prepared 10mM Guaicol solution + 0.1ml of 12.3mM H_2O_2.

▼

Measure OD at 436nm.

▼

Absorbance has to be increased by 0.05. Take a note of the stop watch and memodown the time in minutes to surge the transmission density by 0.1.

Formula: Enzyme activity (units/litre) =
3.18×0.1×100/6.39×1×t×0.1

b) Polyphenol Oxidase (Vidyasekhran and Durairaj, 1973)**:**

Weigh 1 gm. plant material
▼
Homogenize in cool mortar and pestle in 5ml 0.1M phosphate buffer (pH 7.0)
▼
Centrifuge at 10000 rpm for 20 min at 4^{o} C.
▼
Discard the residue. Use the upper liquid as an enzyme source within 2-4 hrs.
▼
2ml phosphate buffer (pH 6.5) + 0.5ml extract + 1ml 0.01M catechol.
▼
Read the transmission density at 412nm at every 30 sec.
▼
Determine the absorbance per min.

Enzyme unit= K*t
K for catechol oxidase is 0.272

Screening of non-enzymatic parameters

a) Ascorbic Acid:
Weigh 0.5gm plant material & Centrifuge.
▼
Crush in 4% oxalic acid and make up a known total volume.
▼
Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V_2ml).
▼
Dissolve 100mg ascorbic acid in 100ml of 4% oxalic acid and dilute 10ml of this solution to 100ml with 4% oxalic acid.
▼
Pipette out 5ml of working solution, add 10ml 4% oxalic acid and titrate against dye solution (V_1ml).]

b) Polypenols:
Crush 1gm of plant material into 5ml 80% ethanol & centrifuge it.
▼
Take the supernatant into a beaker & re-extract the material by adding 5ml of 80% ethanol.
▼
Mix both the supernatants. Evaporate the material by keeping it in a hot water bath to 1ml.
▼
Add 5ml water to it and use it for estimation.
▼
Pipette out 0.2 & 0.4ml of extract into different test tubes. Make up the volume in each test tube to 3ml with water.
▼
Add 0.5ml of Folin-Ciocalteau reagent.
▼
Incubate for 3min.
▼
Add 2ml 20% Na_2CO_3 to each test tube.
▼
Mix thoroughly. Place in a boiling water bath for 1 min.
▼
Cool & measure the absorbance at 650nm against reagent blank.
▼
Prepare a standard graph using different concentrations of catechol.

c) Reducing Sugars:
Crush 1gm of plant material into 5ml 80% ethanol & centrifuge it.
▼
Take the upper liquid into a beaker & re-extract the material by adding 5ml of 80% ethanol.
▼
Mix both the supernatants. Evaporate the material by keeping it in a hot water bath to 1ml.
▼
Add 5ml water to it and use it for estimation.
▼
Pipette out 0.2 & 0.4ml of extract into different test tubes. Make up the volume in each test tube to 3ml with water.
▼
Add 3ml DNSA reagent to each test tube.
▼
Boil in water bath for 5 min.
▼
Add 1ml Rochelle reagent to each test tube.
▼
Measure the absorbance at 510nm.
▼
Prepare a standard graph using different concentrations of glucose.

d) Proteins (Lowrey method, 1951):
Weigh 0.5gm plant material and crush into 10ml phosphate buffer.
▼
Centrifuge and use the supernatant as protein estimation.
▼
Pipette out 0.1ml & 0.2ml of sample in two test tubes.
▼
Make up the volume 1ml by adding water.
▼
Add 5 ml Reagent C to each test tube. Incubate for 10 min in dark.
▼
Add 0.5ml of Reagent D, mix well and allow standing for 30min in dark. Blue colour is developed.
▼
Measure the absorbance at 660nm.

Results and discussion

The highest activity of non-enzymatic parameters like ascorbic acid, polyphenols, reducing sugars and proteins were recorded in mature leaves. Similar observations were recorded[39]

Peroxidase can be observed as the enzyme partaking 3 kinds of enzyme happenings, namely IAA oxidase, peroxidase and polyphenol oxidase. It catalyzes the oxidation happening of aextensivediversity of electron benefactor with the assistance of H_2O_2 and thereby forages the endogenous H_2O[40]

Table-1: Enzymic and non-enzymic oxidants organic constituents in *Solanumnigrum* Linn.

Parameters stage	Vegetative stage Tender leaves	Vegetative stage Mature leaves
Peroxidase (Units/g)	41.67	55.55
Polyphenol Oxidase (Units/g)	0.004	0.003
Protein (mg/g)	10.5	24.6
Polyphenol (mg/g)	1.7	1.7
Reducing sugar (mg/g)	7.6	7.75
Ascorbic acid (mg/g)	306.6	420

Peroxidase, 1 unit = change of absorbance min^{-1} at 430nm. Poly Phenol Oxidase, 1 unit = amount of enzyme which transforms one micromole 1of dihydric phenol to quinine.

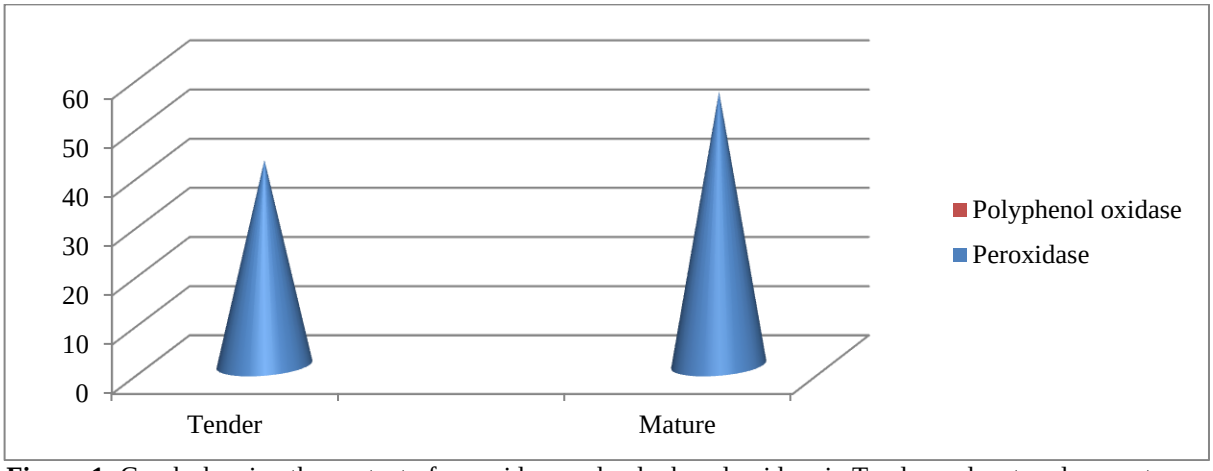

Figure-1: Graph showing the content of peroxidase and polyphenol oxidase in Tender and mature leaves stages.

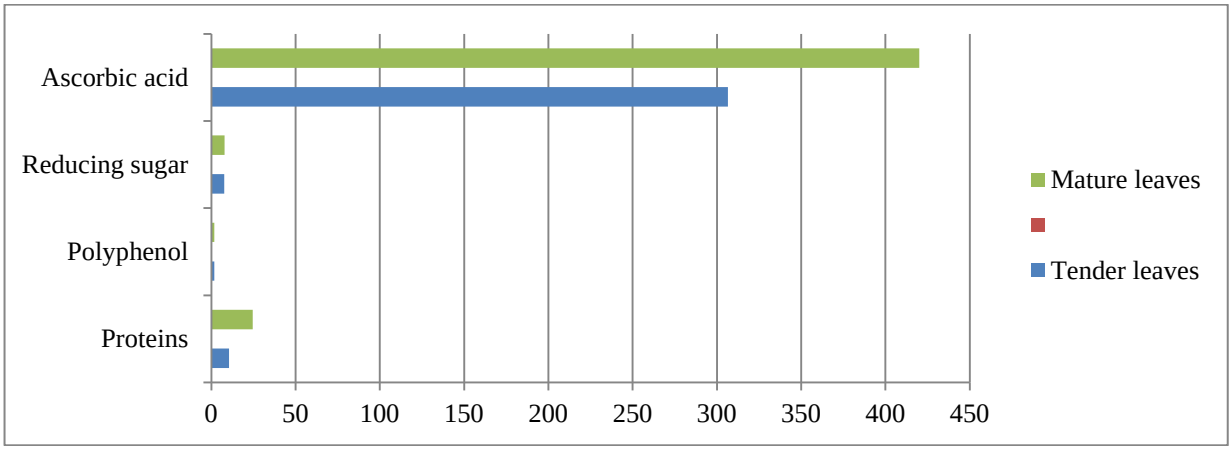

Figure-2: Graph showing the content of Proteins, Polyphenol, Reducing sugar and Ascorbic acid.

Conclusion

Young chick pea leaves are also consumed as a prepared vegetal delicacy and might be a valuable foundation of nutrients prescribed in many diet plans[41]. From this study we can conclude that the mature leaves are rich source of non-enzymatic antioxidants. From the result of the studies on non-enzymatic antioxidants, it is observed that polyphenols are less than other parameters.

Peroxidase and polyphenol oxidase are amongst the maximum studied enzymes in fruits and spuds[42]. Numerous reports suggests their role in antioxidation. A Polyphenolic compound has been evolved as a means to render Protection and defend to plant against pathogen and has been now Shown have antioxidant roles.

Phenolic compounds have their role in Cellular defense mechanism in antherogenesis and cancer[43]. Polyphenols are reported to be promising in treatment of lymphocyte of malignancy. Phenolic compounds have also been shown to exhibits cellular defense mechanism in antherogenesis and cancer[44]. The role of carbohydrates and proteins is well documented in human diet. The role of proteins and carbohydrates is well documented in human diet.

The ascorbic acid is an significant aquatic solvable antioxidant and theatres a noteworthy role in upholding the water soluble oxidation reduction latent in anthropological tissue[45,46]. It has been recognized to be an operative forager of free radicals and has described to decrease carcinogenic nitrosamines to inactive products[47,48].

Two stages of development of leaves were included for the study of enzymatic and non-enzymatic antioxidants status in instruction to ascertain if a difference existed in the levels of enzyme activity in stage of development. This result showed that the leaves in both stages possessed considerable activity of the non-enzymatic and enzymatic parameters. These findings suggest that potential use of *Cicer* leaves to battle the innumerable diseases and disorder linked with oxidative damages.

From the result of present research it is apparent that leaves of *Cicer* possess considerable levels of both enzymatic and non-enzymatic antioxidants and it is advisable to consume the leaves at mature stage. To summarize the observations of the studies on the enzymatic and non-enzymatic antioxidants, it can be inferred that the full mature leaves have rich source of enzymatic and non-enzymatic parameters except polyphenols.

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