Original Research Article

Ocimum Sanctum - A power bunch of antioxidants

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Abstract

Antioxidants are known to prevent cell damage by protecting them from excess oxidation. *Ocimum sanctum* (Tulsi) is known to have many antioxidants, therefore it has a powerful role in prevention of diseases caused by cell damage. Besides this Tulsi is very popular in the Indian community where it is used at the time of auspicious occasions. Therefore present research work was planned to find out the exact amount of certain types of antioxidant compounds in Tulsi, known as catalase and peroxidases. Both of these compounds are very important in many aspects. Catalase is an important member of the antioxidant system of all living organisms, which protects sensitive cell components from oxidative attack. In the present study level of catalase activity was studied in different parts of the tulsi plant viz; leaves, stem and roots. Highest activity was found in leaf, which corresponds to the higher number of the biochemical activities occurring in leaves and lowest activity was found in roots. The antioxidant profile of Tulsi makes it a promising tool to prevent many diseases as well as it can act as an anti-aging compound as well.

Keywords

Tulsi, *Ocimum Sanctum*, Antioxidants, Functional foods, Anti-carcinogenic

Introduction

Ocimum is from the Lamiaceae family that is known for aromatic herbs and shrubs. It has been used in Ayurveda for years as a prime herb. *Ocimum* is considered an adaptogen, maintains the body under balance and is beneficial in the condition of stress as well[1].

Health benefits from *Ocimum*

Many researches make it evident that *Ocimum* is helpful in stress reduction, immunity and stamina boosting, anti inflammatory, hypocholesterolemic, detoxifier, protects from radiation, protective role in gastric ulcer, antipyretic, good for digestion and rich in antioxidants and many more. It is also heart healthy, good for the liver and lungs and maintains blood pressure and blood glucose levels.

The *Ocimum* shows complex chemistry because of its uniqueness. It is packed with phytochemicals. These are antioxidant rich compounds that have antibacterial, antiviral, stress reliever and immunity boosting agents. It is evident from various researches that *Ocimum* has a pain killer like factor known as COX-2 inhibitor, as it has a significant amount of eugenol (1-hydroxy-2-methoxy-4-allylbenzene). *Ocimum* has also shown hypoglycemic and hypolipidemic effects. Positive role of tulsi has been observed in radiation poisoning as well as in cataracts[2].

Antioxidants

Free radicals cause cell damage in the body. Antioxidants are the compounds that scavenge free radicals from the body. These prevent excess oxidation in the cells. The cellular damage is responsible for many degenerative diseases. As *ocimum* is full with antioxidants so it has a promising role in protecting against free radicals and cell damage from oxidation. As a result of metabolism and several biochemical reactions a number of toxic molecules get accumulated in the body of living organisms. To detoxify them, all organisms have a well developed antioxidant system consisting of a number of enzymes viz, peroxidase, catalase, glutathione reductase, etc. Among these, catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide[3].

$2H_2O_2$ Catalase $2H_2O + O_2$

This reaction is called “Catalytic catalase is a heme protein. It contains ferriproto porphyrin (hematin) prosthetic group found in all living cells except certain bacteria. Its function appears to be the removal of the hydrogen peroxide, which is toxic. Keillin and Hartree[4] (1945) have observed that catalase can utilize hydrogen peroxide to oxidize methyl and ethyl alcohol to the corresponding aldehydes. On account of this reaction, Theorell[5] (1951) has suggested that catalase is an important peroxiding enzyme.

Catalase is present in microbodies. Microbodies are cytochemically defined by their catalase activity. This enzyme protects the cell from a potentially dangerous accumulation of hydrogen peroxide. A highly reactive reducing agent formed by flavin oxidase thus

$AH_2 + O_2$ $A + H_2O_2$

It is the flavin oxidase reaction where A is flavin, containing molecule such as oxidase. Catalase when supplied with the proper substrate, can act as a peroxidase, for cytochemical studies, diaminabenzidine (DAB), which turns brown when oxidized is used[6].

H_2O_2 + DAB $2\ H_2O$ + DAB

Oxidized reduction

It is difficult to isolate micro bodies by centrifugation because their membrane is fragile. But because they are richer in protein then other cell organelles of similar size, micro bodies are somewhat denser. Their catalytic activity (the enzyme makes up about ¼ of all the protein in the organelle) makes identification straight forward. Isolated microbodies are generally permeable to the diffusion of small molecule and ion. The pH optimum of the flavin oxidase is about 8.5 and those of catalase and other microbody enzymes are usually greater than 7. This implies that the intramicrobody pH is slightly basic.

Although catalase and flavin oxidase are of universal presence in microbodies, specialized enzymes composition occur in different tissues. Their three main classes of organelles are liver peroxisome, leaf peroxisome and seed glyoxysomes. These organelles appear to arise from pre existing microbodies, possibly by vesicles that are then filled with microbody products from the cytoplasm. In liver peroxisomes contain a wide variety of enzyme but the most important of them quantitatively is the flavin oxidase catalase system, which accounts for 20% of the oxygen consumption of liver tissue. Several flavin containing enzyme produce hydrogen peroxide in the peroxisome. Glucolate oxidase acts on glucolate or lactate[7].

Methodology

0.1 M phosphate buffer

0.1 M H_2O_2

0.7 N H_2O_2

0.01 $KMnO_4$

Fresh young leaves (Tulsi)

1. **Determination of catalase activity**
   - Grinded 500 mg of leaves in 20 ml of phosphate buffer
   - Filtered the homogenate
   - Centrifuged it at 15000 rpm for 15 minutes
   - Brought up the extract to the 25 ml with the phosphate buffer
   - Placed 1 ml of this extract in a test tube.
   - Added 2 ml of 0.1 M H_2O_2 and 3 ml phosphate buffer
   - For stop reaction, added 1 ml of 0.7 N H_2SO_4 after 5 minutes
   - Incubated for 5 min. At 27 ^oC and titrated the residual H_2O_2 against 0.01 N $KMnO_4$ [8]

2. **Determination of peroxidase activity**

Required

- Enzyme extract: using the sieve
- 0.05 M phyrogallol in 0.1 M phosphate buffer
- 1% H_2O_2

Procedure

- Pipetted out 3 ml of pyrogallol phosphate buffer and 0.1 ml of enzyme extract into a cuvette
- Added 0.1 ml of H_2O_2 and shook well
- Measured the absorbance after 3 min. at 420 nm
- Recorded the value of the blank with boiled enzyme extract
- Calculated the enzyme activity by subtracting the absorbance value of the blank from the sample

Results & Discussion

The results obtained for **catalase** activity are

25 x 0.85/2 x V/W

25 x 0.85/2 x 0.9/500

= 0.0191 enzyme unit/ml

Peroxidase -

Absorbance taken by spectrophotometer and the results obtained were 0.105 enzyme unit[9]. Sethi[10] et al. (2004) and Basak[11] et al. (2014) also documented the antioxidant effect of Tulsi leaves and its benefits on reducing blood sugar levels. Different types of scavenging agents were also reported to be there in tulsi leaves which played a good role as antioxidants, anti inflammatory, anti-cholinesterase and cytotoxic activities.

Conclusion

Catalase is an important member of the antioxidant system of all living organisms, which protects sensitive cell components from oxidative attack cell against the toxic effects of H_2O_2 produced as a byproduct of several biochemical reactions. Hydrogen peroxide is a powerful and potentially harmful oxidizing agent, causing the production of free radicals, which in turn can damage cell membrane or even DNA content of the cell. It is degraded through a disproportionation reaction catalyzed by catalase into water and oxygen.

In the present study level of catalase activity was studied in different parts of the tulsi plant viz; leaves, stem and roots. Highest activity was found in leaf which corresponds to the higher number of the biochemical activities occurring in leaves and lowest activity was found in roots., as they are allotted to perform only a few functions like absorption of nutrients. Therefore, less the biochemical activities, the less will be the production of toxic substances hence there will be a less developed antioxidant system as comparable to organs with large number of metabolic activities.

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