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Antioxidant Enzymatic Studies against the Oxidative Stress in Colocasia Esculenta

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Abstract: ***The antioxidant defense mechanism due to the presence of the antioxidant enzymes protects the plants against oxidative stress damages. Plants possess very efficient enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; mono dehydro ascorbate reductase, MDHAR; dehydro ascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S- transferase, GST) and non-enzymatic (ascorbic acid, ASH; glutathione, GSH; phenolic compounds, alkaloids, non-protein amino acids and a-tocopherols) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging. In this study, the Colocasia esculenta leaf extracts were subjected to enzymatic study for the detection of antioxidant enzymes which resulted in the positive response of the solvents of the leaves showing the occurrence of oxidation reaction taking place and prove to be an efficient protector against the reactive species.***
Keywords: Antioxidant enzymes, Defense mechanism, Reactive oxygen species, Colocasia esculenta, extracts.

I. INTRODUCTION

Cell growth, differentiation, progression, and death processes in the body involve the role of Reactive oxygen species (ROS). Higher amounts of ROS results in the aging process, cancer, ischemia, and low immune response and endocrine functions. As a defense against the release of ROS, several non-enzymatic and enzymatic antioxidant activities exist which secretes when there is an oxidative stress as a result of a pathologic event, the defense system promotes the regulation and expression of the antioxidant enzymes that helps in the protection of the body.

These antioxidant enzymes have potential in stabilizing, and deactivating the free radicals before they attack cellular components in the body. The mechanism used for the attack of free radicals is by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it stable. Antioxidant enzymes initiate the defense by interrupting the oxidizing chain reaction thereby causes minimal damage caused by free radicals.

A. Plant Antioxidants

Plants derive their energy from sunlight via photosynthesis, which generates free radicals and reactive oxygen species (ROS). Both the ultraviolet light from the sun and the reactive oxygen species generated during photosynthesis by plants would cause irreparable damage, in order to avoid this damage, as a defense mechanism, plants produce antioxidants to protect them. It is these same antioxidants such as vitamins, polyphenols, carotenoids, xanthophylls, and others in plants which make causes the ability to absorb and protect from the free radicals generated. These antioxidants play a vital role in the protection from both the free radicals and other sources of free radicals such as environmental stressors.

Excess of ROS leads to degradation of lipids, proteins and nucleic acids and thus may lead to oxidative damage of cells and in a consequence to overexpression of oncogenes, mutagens formation, induction of atherogenic activity, or inflammation. Oxidative stress is suggested to play a major role in pathogenesis of cardiovascular diseases, neurodegeneration, cancers, immune disorders, diabetes, aging, and others. Plants, especially dietary fruits and vegetables, are a rich source of antioxidants.

B. Importance Of Antioxidants In Plants

In the body, Reactive oxygen species (ROS), Reactive nitrogen species (RNS) and free radicals are generated through exogenous sources such as radiation, cigarette smoke, atmospheric pollutants, toxic chemicals, over nutrition, changing food habits, etc, intake of fruit and vegetables, overweight, obesity, and physical inactivity. Exogenous antioxidant from natural compounds , i.e., curcumin, baicalen, and resveratol prevent arthrosclerosis formation by exhibiting radicalscavenging effects. A number

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offlavonoids, including quercetin, morin, gossypetin, chrystin, myrecetin, rutin, catechin, and its derivatives and some oligomeric proanthocyanidins are reported to inhibit the oxidation of LDL in invitro studies.

Antioxidants in foods are from substances formed from reactions during processing; food additives isolated from natural sources.

Most natural antioxidants are from plants. Most plants contain compounds that possess antioxidant activity. They are polyphenols that occur in all parts of the plant - wood, bark, stems, leaves, fruit, roots, flowers, pollen and seeds. The antioxidant activities in these plants range from extremely slight to very great. Natural antioxidants may function (a) as reducing agents, (b) as free radical scavengers, (c) as complexers of pro-oxidant metals, and (d) as quenchers of the formation of singlet oxygen. The most common natural antioxidants are flavonoids (flavanols, isoflavones, flavones, catchins, flavanones), cinnamic acid derivatives, coumarins, tocopherols, and poly-functional organic acids.

II. MATERIALS AND METHODS

A. Specimen Collection

The plant samples were collected from Shettyhalli village, SakleshpuraTaluk, Hassan district, Karnataka and authenticated in Regional Ayurveda Institute for Metabolic Disorders, Govt. of India in order to confirm the plant species. The leaves as the sample were excised from the collected plant.



B. Plant Extraction

The leaves were subjected to extraction using water and ethanol as solvents at room temperature using Soxhlet apparatus where in the collected extracts were stored at 4 °C until use.

C. Antioxidant Enzyme Analysis

1) *Superoxide DISMUTASE:* Superoxide dismutase was generated by xanthine oxidase and detected by nitro blue tetrazolium (NBT) reduction method (Lie Fen Shyur, 2005). Reagents in this study are prepared with 50mM potassium phosphate potassium hydroxide buffer (pH 7.8). The reaction buffer should contain 50 µl of 0.6 mM NBT, 20 µl of 15 mM Na2EDTA (pH 7.4), 30 µl of xanthine oxidase solution, 150 µl of enzyme sample and 1.5 µl of potassium phosphate potassium hydroxide buffer is used as control. Reaction was initiated by the addition of xanthine oxidase at 25º C. The absorbance is read at 405 nm.
2) *CATALASE (CAT):* Catalase activity was estimated by the method of Chance and Machly (1955) with minor modification. The reaction mixture containing 50 mm sodium phosphate buffer (pH 7.0), 20 mm H_2O_2 and 1 ml enzyme sample. Decrease in absorbance was noted at 240 nm. The molar coefficient of H_2O_2 at 240 nm was taken as 43.6 M/cm. The enzyme activity was expressed as µmoles of H_2O_2 degrade minutes/gram.
3) *Ascorbic ACID Peroxidase (APX):* APX activity was determined according to Wang et al. (1991). APX extraction was performed in 1.5 ml of suspension solution including 50 mM Tris-HCl (pH 7.2), 2 % PVP, 1 mM Na2EDTA, and 2 mM ascorbate. Assay solution contained 50 mM potassium phosphate buffer (pH 6.6), 2.5 mM ascorbate, 10 mM, H2O2 and enzyme containing 100 µg protein in a final volume of 1 ml. The enzyme activity was calculated from initial rate of the reaction using the extinction coefficient of ascorbate (ε = 2.8 mM cm^{-1} at 290 nM).

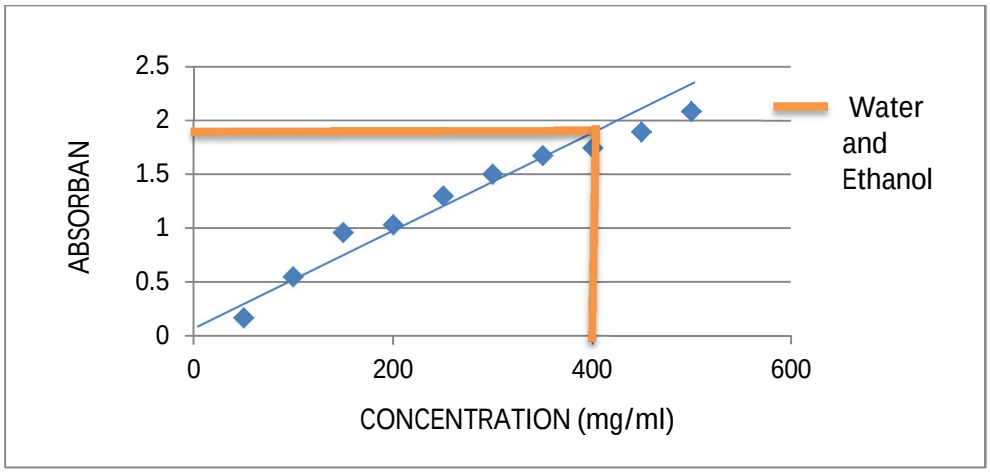
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III. RESULTS AND DISCUSSION

A. *Determination Of Catalase Activity (Cat)*

Table1: Catalase activity

Concentration (µg/ml)	Absorbance of std (H_2O_2)	Concentration of Catalase (mg/ml)	
		Water	Ethanol
50	0.1658	400	400
100	0.5492		
150	0.9611		
200	1.0299		
250	1.2970		
300	1.4966		
350	1.6713		
400	1.7433		
450	1.89		
500	2.0789		



Graph 1: Graph shows the catalase activity

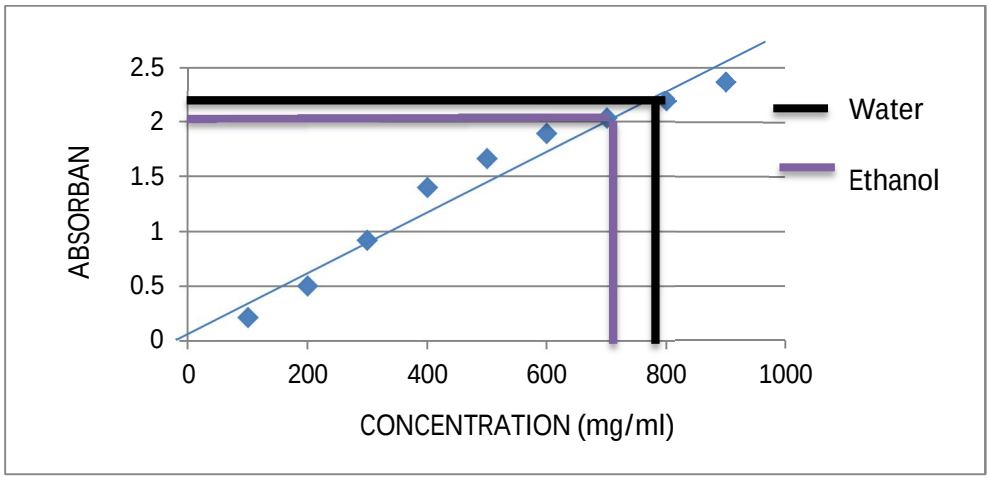
This enzyme rapidly destroys a vast majority of H_2O_2 produced in peroxisomes during photorespiration and formed as a result of mitochondrial electron transport In the above activity, Catalase was found to be the same in both the extracts of 400 mg/ml

B. *Estimation Of APX*

Table 2: APX activity

Concentration (µg/ml)	Absorbance of std	Concentratiom of APX (mg/ml)	
		Water	Ethanol
100	0.2171	795	720
200	0.503		
300	0.9185		
400	1.4022		
500	1.6671		
600	1.8930		
700	2.036		
800	2.1960		
900	2.369		

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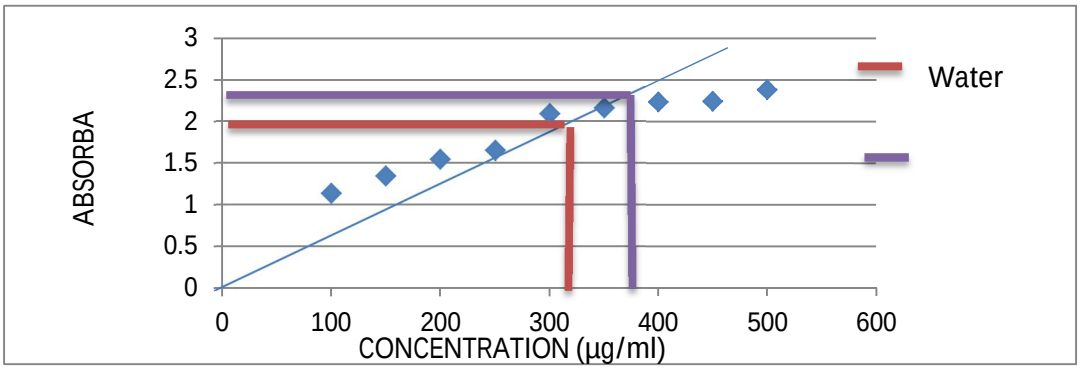
Graph 2: Graph shows the APX Content

APX has a higher affinity for H2O2 (mMrange) than CAT and POD (mM range) and it may have a more crucial role in the management of ROS during stress. In this study, APX was found to be higher in the water extract of 795 mg/ml than the ethanol extract of 720 mg/ml.

C. Determination Of Sod Activity

Table 3: SOD Activity

CONCENTRATION (µg/ml)	OPTICAL DENSITY AT 650nm	CONCENTRATION OF SOD (mg/ml)	
50	1.932	Water	Ethanol
100	1.139	310	390
150	1.346		
200	1.548		
250	1.653		
300	2.0911		
350	2.1665		
400	2.2399		
450	2.2458		
500	2.3876		



Graph 3: Graph showing SOD activity

SOD activity proved the presence in ethanol extract in higher quantity when compared to the water extract. SOD is one of the key enzymes involve in the cellular defense and considered as an important indicator of antioxidant capacity.

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IV. CONCLUSION

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. In this study, the selected plant has the activity which is capable of showing the enzymatic activity in 2 extracts such as aqueous and ethanol. Thus, the *Colacasia esculenta* is the promising source for the possession of natural antioxidants and can be used in the treatment of diseases associated with Oxidative stress.

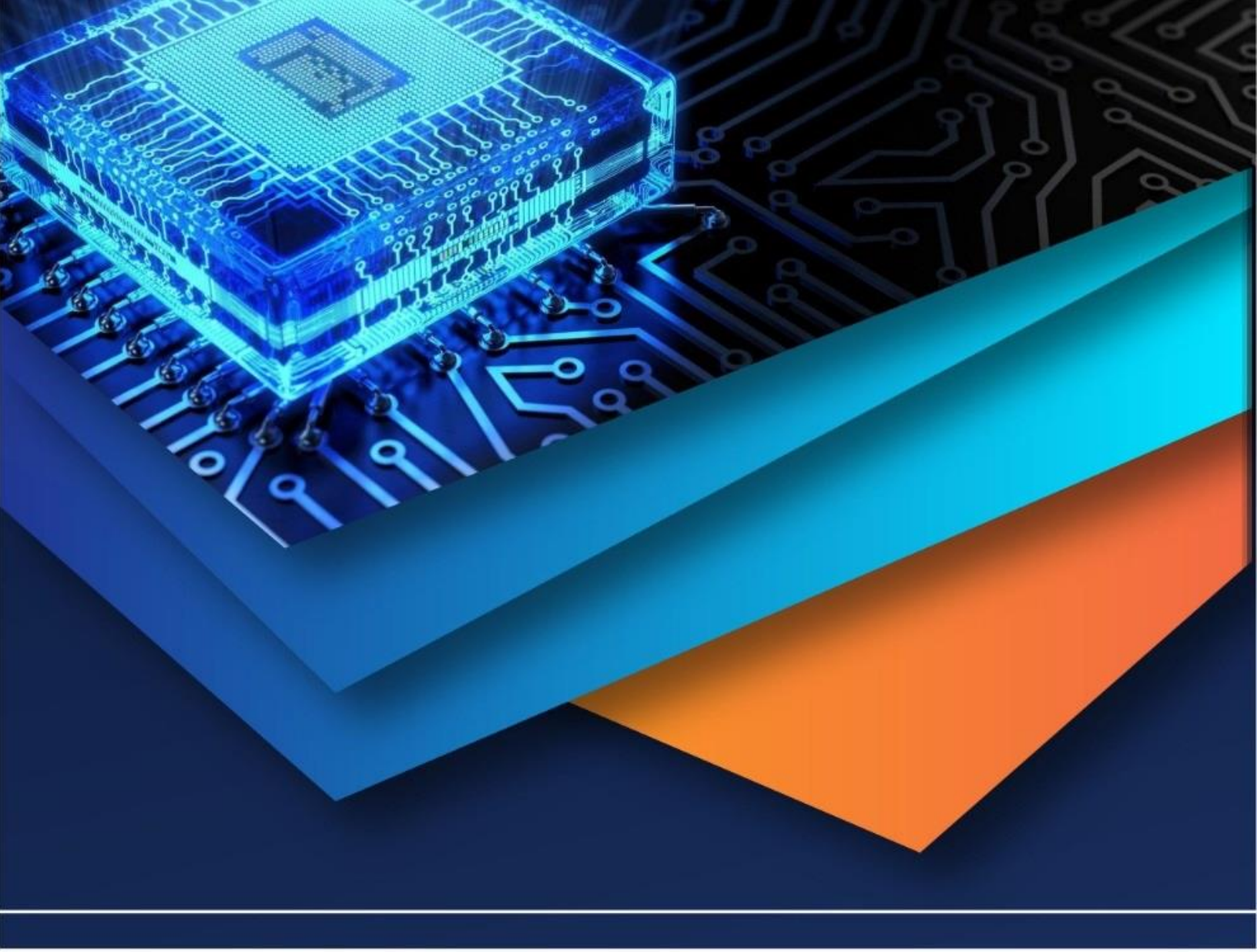
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