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Research Paper

Enzymatic Antioxidant Activity Of The Fruit-Pulp Extract Of Cassia fistula L.

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Abstract
Enzymatic antioxidant activity of the ethanolic extract of the fruit pulp of Cassia fistula L. was investigated by carrying out ascorbate oxidase, polyphenol oxidase, catalase, ascorbate peroxidase, glutathione s-transferase and superoxide dismutase enzyme activity assays. Enzyme activities of the crude extract were measured using standard spectrophotometric method. The results showed that Cassia fistula L. crude extract had good activity in all the enzymatic procedures tested. The activity levels of enzymatic antioxidants ascorbate oxidase, catalase, glutathione s-transferase and superoxide dismutase of the plant were found to be 6.746 ± 0.035; 7.57 ± 0.055 6.761 ± 0.067; 5.40 ± 0.65 (units/g dry tissue) respectively. The value for polyphenol oxidase is 30.17 ± 1.76 µmoles /g dry tissue

Key Words: *Cassia fistula L., Enzymatic, Antioxidant, Ascorbate oxidase, Polyphenol oxidase, Catalase, Glutathione s-transferase, Superoxide dismutase.*

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I. INTRODUCTION

Humans and animals have been using plants as an important source of materials in the maintenance of health for thousands of years.[6] They consume plants and plant products as a food, spices and condiments, as drink or as herbal medicine. Plants as natural chemical factories are a source of medicine for various kinds of ailments. A World Health Organization report estimates that up to 80 percent of people rely mainly on traditional remedies from herbs for their various health needs owing to better cultural acceptability, non-noticeable side effects and better compatibility with the human body.[3] In another report, it is stated that about 25% of all medicinal drugs in developed countries are said to be based on plants and plant products while the estimate in the developing as well as underdeveloped countries is about 75%.[58] The significant level of bioactive natural constituents present in dietary plants such as fruits, seeds, roots, vegetables and spices are beneficial to human health beyond basic nutrition and thus help in the management of oxidative stress induced damage to cells, tissues and organs.[43]

About 30% of the total dry weight of typical plant cell is constituted of proteins. Excluding inert materials, such as the cell wall and starch, which can account for up to 70% of the dry weight of some cells, proteins and amino acids represent about 60 to 70% of the dry weight of the living cell. Microtubules and microfilaments which are cytoskeletal structures are composed of protein. There are also storage forms of proteins, particularly in seeds. The major function of proteins in metabolism is to serve as enzymes, which are biological catalysts that greatly speed up the rates of biochemical reactions, thereby making life possible. Enzymes participate in these reactions but remain fundamentally unchanged in the process.[5, 12, 61]

Enzymes regulate almost all life processes. Typically, cells have several thousand different enzymes, involved in a wide variety of actions.[42] One of the most important features of enzymes is their specificity, which confers on them the ability to distinguish between very similar molecules, and their catalytic efficiency, which is far above those of ordinary catalysts.[14] As remarkable as the stereo specificity of enzymes is, it allows them to distinguish not only between enantiomers (mirror-image stereoisomers), but between apparently identical atoms or groups of atoms.[62]

Human beings do not produce enough endogenous enzymes for the digestion of food and other metabolic activities[5]. Naturally grown raw fruits, vegetables, seeds, nuts and grains contain enough enzymes in themselves to breakdown food and release their nutrients. These enzymes in the plants complement the amount of enzymes produced in the body so as to digest the food completely and mediate in metabolism.[15]

Living organism and its enzymes are inhabited by a vital principle of life energy distinct from the caloric energy liberated from food by enzyme action. Enzymes are the life element and are biologically recognized as their action can be measured in terms of enzyme activity.[22]

The three classes of enzymes are: metabolic enzymes, which run living systems; digestive enzymes, which are responsible for the digestion of food; and food enzymes from raw foods, which initiate food digestion.[24]

There are some far reaching consequences when there is a deficiency of plant enzymes or none at all as a result of destroying the enzymes by over cooking. These consequences can also arise when the raw plant foods that contain the enzymes are not properly chewed to break up the cells and release the enzymes. The plants may be planted in nutrient-deficient soil, which makes the plants deficient in enzymes.[5, 23]

Enzymes that are plant based are usually consumed as food in their raw form, thereby preserving the integrity of the enzymes themselves. Plant-based digestive enzyme function over a broad scope of pH levels, usually between 3.0 and 9.0, which matches that of the human gastrointestinal environment, making them compatible for supporting comprehensive metabolic health. Important enzymes present in plants include protease, amylase, lipase and Cellulase.[13, 48]

Naturally occurring antioxidants in plant cells also include enzymatic and peptide defense mechanisms comprising numerous enzymes (superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase) and various compounds of low molecular weight (ascorbate, glutathione, tocopherols, carotenoids, phenols).[16,17]

The various mechanisms by which the action of antioxidants are brought about include: preventing the formation of reactive species, scavenging free radicals, chelating metal ions, repairing or removing damaged tissues and by effecting the biosynthesis of antioxidant defense enzymes.[1, 10]

Natural antioxidants are associated with herbs and spices which are exclusively of plant origin as product developers use plant extracts as replacement for synthetic antioxidants. Recent researches have shown that many herbal products have the ability to enhance the activity of antioxidative enzymes such as superoxide dismutase and catalase in a dose-dependent manner and also enhance cell viability by providing protection against oxidative stress-induced damages to cells and tissues.[13, 17, 21]

Protease is involved in the break down protein that can be present in meat, fish, poultry, eggs, cheese and nuts. Amylase assist in the breakdown and subsequent absorption of carbohydrates. Lipase aids the digestion of fat. When a diet includes lipase-rich foods, it eases the production burden on the gall bladder, liver and pancreas.[18, 37] Cellulase is present in many fruits and vegetables, and it breaks down food fibers, thereby increasing their nutritional value. The presence of Cellulase in plant-based sources is important, because it is not naturally present in the human body. Fruits and vegetables are an ideal source for enzymes.[25, 26] They are enzyme-rich and easily consumed without needing to be cooked or processed, ultimately preserving the full functionality of the enzymes. By using plant biotechnology several enzymes can be produced from plants as well algal resources.[19, 20]

The endogenous systems include Superoxide dismutase (SOD), an enzyme with extremely high activity, widely distributed in all tissues that catalyzes the removal of superoxide anions from tissues. The reaction involves two superoxide anions and two protons: The complete transfer of an electron from one superoxide anion to another brings about a dismutation reaction, producing hydrogen peroxide, which is also a toxic product and must be removed.[27, 28] The enzyme superoxide dismutase exist in two isozymes: one that contains zinc and copper (located in the cytosol) and the other that has manganese (located in mitochondria). The ions Zn, Cu, and Mn are essential and indispensable components of SOD for oxidative stress protection. Superoxide dismutase plays an important role in therapeutic approaches for treatment of various diseases.[29]

The decomposition of hydrogen peroxide is catalyzed by catalase, a tetrahedral active hemoprotein present in almost all cells, particularly liver, kidney, bone marrow, and blood cells. Phagocytes are rich in this enzyme.[30] The enzyme produces water and two oxygen atoms from two molecules of hydrogen peroxide. It is found predominantly in peroxisomes, which are small organelles containing oxidases that produce hydrogen peroxide.[31] Although at very low levels, catalase can also be found in cytosol, mitochondria, and microsomes. Little or no catalase activity has been described in patients with a genetic defect known as acatalasemia. It is interestingly to state that, this deficiency does not cause major clinical symptoms, likely due to compensation by other enzymes with complementary activity as catalase.[32]

Polyphenol oxidase (benzene diol: oxygen oxidoreductase) and plant ascorbate oxidase are extracellular, multicopper enzymes family that use molecular oxygen to oxidize various aromatic and nonaromatic compounds by a radical-catalyzed reaction mechanism.[33, 34] They catalyze the four electron reduction of molecular oxygen to water with one-electron oxidation of reducing substrate, without producing

hydrogen peroxide. Although these enzymes preferably act on phenolic compounds, their substrate spectrum is large, and the range of substrates oxidized varies from one enzyme to another.[35, 36]

Ascorbate peroxidase functions as hydrogen peroxide detoxification and glutathione regeneration via ascorbate-glutathione pathway. The enzyme is able to scavenge hydrogen peroxide produced by superoxide dismutase using ascorbate as an electron donor.[53]

Peroxidases are heme-containing enzymes which are able to bring about the oxidation of organic and inorganic compounds using hydrogen peroxide as co-substrate.[51]

Glutathione peroxidase is selenium-containing enzyme which catalyzes the reduction of hydrogen peroxide and organic hydroperoxides (ROOH), using glutathione (GSH) as a hydrogen donor. In these reactions, each glutathione gives an electron from its SH group, which becomes a thiol radical. Two of these radicals form oxidized glutathione (GSSG) with a disulfide bridge.[40, 41, 44]

Because of the role of selenium in the activity of glutathione peroxidase, this micronutrient mineral is considered an antioxidant. Glutathione peroxidase is found in cytosol and mitochondria where it functions in maintaining low concentrations of hydrogen peroxide in tissues, converting fatty acid peroxides to hydroxyl derivatives, and reversing the oxidation of sulfhydryl groups in proteins and other compound.[45, 46, 47]

Oxidized glutathione is reduced by the action of glutathione reductase, an NADPH-dependent enzyme. Other peroxidases contribute to the removal of hydrogen peroxide. They are hemoproteins very common in plants and are also present in leukocytes and milk. They catalyze the reaction: where AH_2 represents different hydrogen donor substrates used by peroxidases. The enzyme of neutrophil granulocytes uses NADPH as a hydrogen donor. Other molecules synthesized in the body that possess thiol groups, and can act as electron donor/acceptor, are thioredoxin and lipoic acid.[39, 44]

Since plants provide protection against free radicals, there is increased attention to the antioxidant activity of plant extracts and plants have to themselves counteract stress caused by oxygen, they present a potential source of natural antioxidants. Hence, screening of medicinal plants for their antioxidant potential is essential.[8, 53, 54]

II. Materials and Methods.

Plant collection, identification and extraction

Cassia fistula pods were collected from trees growing within the permanent site campus of Kwara State Polytechnic, Ilorin and identified at the Department of Biology of the Institution.

The pods were mechanically broken and 0.5gr of the manually scooped- out gelatinous fruit-pulp was ground with 8ml freshly prepared solution made of 50 mM potassium phosphate buffer (pH 7.0) and 1% polyvinylpolypyrolidone.[11,52] The homogenate was centrifuged for 30 min and supernatant was used for enzymes assays.

Enzyme Assays.

Ascorbate oxidase (E.C.1.10.3.3)

Ascorbate oxidase activity was determined.[3, 56] 10 µl enzyme extract was added to 1.0 ml of reaction mixture containing 20 mM potassium phosphate buffer (pH 7.0) and 2.5 mM ascorbic acid. Decrease in absorbance was observed for 3 min at 265 nm due to ascorbate oxidation and calculated using extinction coefficient, 14 $mM^{-1}cm^{-1}$.

Polyphenol oxidase (E.C. 1.10.3.1)

Polyphenol oxidase enzyme activity was determined by measuring the initial rate of Quinone formation as indicated by an increase in absorbance at 410nm at 15 seconds interval at 30°C by using catechol as substrate.[2, 57] The reaction mixture contained of 0.1ml crude enzyme extract, 3.9ml of 100mM phosphate buffer (pH 7.0) and 1.0ml of 50mM catechol. The initial velocity was calculated from the slope of the absorbance vs. time curve. One unit (U) of PPO activity is defined as the amount of the enzyme that increased the absorbance by 0.001 per minute under the conditions of the assay.

Catalase ((EC 1.11.1.6)

The reaction was initiated as soon as 0.1 ml of crude enzyme extract was added to the reaction mixture made up of (3 ml) 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. Alterations in absorbance of the reaction mixture were recorded at 240 nm every 20 s. One unit of enzyme activity was defined as an absorbance change of 0.01 unit per minute.[7, 9, 56, 57]

Glutathione S-transferase (EC 2.5.1.18)

The assay is based on the GST-catalyzed reaction between glutathione and a GST substrate, 1-chloro- 2, 4-dinitrobenzene (CDNB). The activity of the enzyme is determined by the rate of production of reduced glutathione and CDNB, which is proportional to the increase in absorbance at 340nm over time.[2, 5]

100 µl crude enzyme extract is added to the reaction mixture containing 100 µl of GSH, 100 µl of CDNB and 2.7ml phosphate buffer. The absorbance was recorded at 340nm against blank over four minutes, collecting data

every 30 seconds. The assay mixture without the enzyme serves as the control to monitor non-specific binding of the substrates.[56, 57] One unit of GST will conjugate 1.0µM of CDNB per minute at 25^{0}C, based on the actual extinction coefficient of CDNB at 340nm is 9.6$Mm^{-1}cm^{-1}$.

Superoxide dismutase (EC 1.15.1.1)

The assay mixture contains 0.5 ml of plant extract, 1 ml of 125 mM sodium carbonate, 0.4 ml of 25 µM NBT and 0.2 ml of 0.1 mM EDTA. The addition of 0.4 ml of 1 mM Hydroxylamine hydrochloride initiated the reaction.[56, 57] Absorbance was read at 560 nm in a spectrophotometer at an interval of 1 min for 5minutes. Units of SOD were expressed as amount of enzyme that is able to inhibit the reduction of NBT by 50%. The specific activity was expressed as units per mg of protein.

STATISTICAL ANALYSIS

All enzyme activity was assayed in triplicate and the results expressed as mean of obtained values±SD.

III. RESULTS AND DISCUSSION

Table 1 shows the level of the antioxidant enzymes Ascorbate oxidase, Polyphenol oxidase, Catalase, Glutathione S-transferase and Superoxide dismutase present in the sample of the fruit pulp extract of Cassia Fistula.

Enzyme	Value/ Units
Ascorbate oxidase	6.746 ± 0.035 units/g dry tissue
Polyphenol oxidase	30.17 ± 1.76 / µmoles /g dry tissue
Catalase	7.57 ± 0.055 units/g dry tissue
Glutathione S- transferase	6.761 ± 0.067 units/g dry tissue
Superoxide dismutase (SOD)	5.40 ± 0.65 units/g dry tissue

Table 1 Specific activity of antioxidant enzymes in the fruit-pulp extract of Cassia fistula.

The values are expressed as Mean ± SD (n=3)

The obtained values are 6.746 ± 0.035, 7.57 ± 0.055, 6.761 ± 0.067and 5.40 ± 0.65 units/g dry tissues for Ascorbate oxidase, Catalase, Glutathione S transferase and Superoxide dismutase respectively. Polyphenol oxidase level was found to be 30.17±1.76 µmoles/g tissue in the tested sample.

Protection of cells and tissues from the damaging effects of ROS/RNS has been an important issue of concern to scientists in recent years. Understanding the mechanism of action of various antioxidant compounds has also been an ever increasing knowledge.[42,49] The crucial role played by oxygen derived pro-oxidants and antioxidants in both normal metabolism and several clinical disease states is also of great importance.[50] Antioxidants exhibit pro-oxidant activity depending on the specific set of conditions mainly, dosage and redox conditions of these antioxidant components in the cell.[59] Most importantly, antioxidants have been shown to reduce free-radical damage to normal tissues leading to diminished toxicity while the non-oxidative cytotoxic mechanisms of drugs remain unaffected by antioxidant supplementation.[60]

The generation of toxic free radicals which are responsible for oxidative stress in mammalian cells is a normal metabolic activity in living systems. These reactive oxygen species (ROS/RNS) are known to have effect in cell growth, differentiation, progression, and apoptosis[51]. This action has been shown to be responsible for neurodegenerative diseases including cancer, ischemia, and failures in immunity and endocrine functions. In order to counteract their toxicity, the body is equipped with efficient protective mechanisms to safeguard against the accumulation of these reactive free radicals.[10, 12] The concentrations of these free radicals are maintained at very low levels at the normal oxygen tension of the atmosphere under normal conditions due to the action of endogenous antioxidant systems and exogenous compound components of food. The consumption of food with their enzymes intact and the supplementation of cooked foods with enzyme capsules can slow down the abnormal and pathological aging process.[15] Diet or in general life style has been implicated in antioxidant enzymes expression level, so that ROS production is controlled and maintained at lower levels. This has been shown to be beneficial or even indispensable in processes such as intracellular signaling and defense against microorganisms.[10]

The assessment of diet and nutrition status in cancer risk has been a major focus of research as well as public health policy of developed countries. Diet has been shown to play a significant role in the etiology and prevention of cancers. It is interesting to note that studies have shown that the incidence of cancer can be reduced substantially by dietary modification.[33, 34, 55]

As much as the various existing antioxidants are often seen as a single functional entity, they cannot replace each other as they have different chemical and physiological characteristics that is responsible for their mechanism of free radical scavenging to ensure that all parts of the cell, organs and tissues are given maximum protection from oxidative stress.[35, 36] Dietary antioxidants also exist in various forms, with polyphenols and carotenoids being the largest groups of compounds. These have different functions and are produced by plants under stress to protect plant cells against oxidative damage.[38, 40]

The mechanisms by which the enzymes responsible for catalyzing the rate determining step in the biosynthetic pathways of the various secondary metabolites like polyphenols and carotenoids are transcribed and translated has been elucidated by recent advances in molecular genetics. The genes for these enzymes are turned-on by environmental stress. On the other hand, the genes coding for antioxidant enzymes are constitutively expressed as they are not secondary metabolites.[40, 47]

Free radicals affect the production, stability, or function of proteins by adversely altering the activity of transcription factors in the nucleus. Generally, the reduced transcription factor binds to deoxyribonucleic acid (DNA) to promote transcription, whereas an oxidized transcription factor will not be able to bind to DNA and thus preventing transcription. Furthermore, the oxidation of proteasomes adversely affect the stability of protein rendering them inactive and degradable, thereby maintaining or increasing the level of proteins.[61,62,63]

The following three different strategies can also be employed to modify the function of proteins and molecules through the action of reactive species: (1) Thioredoxin is a protein, whose activity can be altered directly by oxidation. (2) A chaperone protein can be targeted by oxidation usually inhibiting protein activity. Further oxidation causes the protein to dissociate from its inhibitor and thus becoming active. (3) The oxidation of Phosphatases and kinases alter their activity through posttranslational modifications.[4, 62]

Sufficient proof of causal relationship between dietary modification and the incidence of neurodegenerative diseases has been obtained using different epidemiological designs. Thus offering the prospect for initiating primary and secondary preventive measures for control and prevention of these diseases.[4, 6, 15]

IV. CONCLUSION

The result obtained identified the fruit pulp of Cassia fistula as a good source of important enzymatic antioxidants that can complement human nutrition as an enzymeless diet has been implicated in many chronic health conditions that may arise from conditions of oxidative stress.

This justifies the inclusion of various parts of this plant in many herbal preparations.

References

[1]. Shi, C., Y. Dai, X. Xu, Y. Xie and Q. Liu. The purification of polyphenol oxidase from tobacco. Protein Expression and Purification; 2002; 24(1): 51-55

[2]. Deepaa Manohan and Wong Chen Wai. Characterization of Polyphenol Oxidase in Sweet Potato (Ipomoea Batatas L). Journal for the Advancement of Science and Arts; 2012; Vol. 3, No. 1.

[3]. Umesh Kumar, Prakash Veeru and Meenakshi Mishra. Assessment of antioxidant enzymes and free radical scavenging activity of selected medicinal plants. Free Radicals and Antioxidants; 2012; Vol.2 / Issue 3

[4]. Wong D.W.S. Food enzymes: Structure and mechanism. Chapman & Hall, New York, USA. ISBN-13:978-0412056918.1995; pp. 85-98.

[5]. 5. Edward Howell and Maynard Murray. Enzyme Nutrition (The Food Enzyme Concept) Avery Publishing Group Inc. Wayne, New Jersey. ISBN 978089592216.1985; pp. 35-45.

[6]. Iris F.F., Benzie and Sissi Wachtel-Galor. Herbal Medicine Biomolecular and Clinical Aspects-2nd Edition Taylor & Francis Group, LLC. ISBN 978-1-4398-0713-2. 2011; pp. 11-25.

[7]. Sinha A.K. Colorimetric assay of catalase. *Anal. Biochem*. 1972; 47: 389

[8]. Kuo Y.H., Lee P.H., Wein Y.S. Four new compounds from the seeds of *Cassia fistula*. *J. Nat. Prod*. 2002; 65: 1165–1167

[9]. Sariri Reyhaneh, Fatemeh Shams Moattar, Parichehr Yaghmaee, Masoud Giahi. Enzymatic and Non-enzymatic Antioxidants of Calamintha officinalis Moench Extracts. Journal of Applied Biotechnology Reports. 2016; Volume 3, Issue 4, 489-494

[10]. Antonio Blanco, Gustavo Blanco. Antioxidants: In Medical Biochemistry. 2017; pp.230-250. ISBN 978-0-12-803550-4. Academic Press.

[11]. Haida Z, Hakiman M. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. Food Sci Nutr. 2019. 7:1555–1563.

[12]. Pisoschi Aurelia Magdalena, Aneta Pop, Carmen Cimpeanu, and Gabriel Predoi. Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review. Oxidative Medicine and Cellular Longevity Volume 2016; Article ID 9130976, 36 pages http://dx.doi.org/10.1155/2016/9130976

[13]. Butler M, Robertson A, Cooper M. Natural product and natural product derived drugs in clinical trials. Nat Prod Res 2014; 31(11):161261.

[14]. Thangarajan S and Velliyur K. Enzymatic and Non enzymatic Antioxidant properties of Tylophora pauciflora-an In-Vitro Study - Asian J Pharm Clin Res, Vol 6, 2013; Suppl 4, 68-71

[15]. Ergul Belge Kurutas. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state -Kurutas Nutrition Journal. 2016; 15:71

[16]. Ashish Rawson, Ankit Patras, Dave Omah, Rocio Campos-Vega and Mohammad B. Hossain. Antioxidant Activity of Phytochemicals and Their Method of Analysis in (eds.) Advances in Food Science and Nutrition. 2014; pgs 153–256, Edited by Visakh. P. M, Laura B. Iturriaga and Pablo Daniel Ribotta © Scrivener Publishing LLC)

[17]. Bennett R.N, Wallsgrove R.M. Secondary metabolites in plant defense mechanisms. New Phytol. 1994; 127(4):617–33.

[18]. Curtis H, Noll U, Störmann J, Slusarenko A.J. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (Allium sativum L.) against plant pathogenic bacteria, fungi and oomycetes. Physiol Mol Plant Pathol. 2004; 65(2):79–89

[19]. VanEtten H.D., Mansfield J.W., Bailey J.A., Farmer E.E. Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". Plant Cell. 1994; 6(9):1191–2.

[20]. Wijnsma R, Go JTKA, Weerden INv, Harkes PAA, Verpoorte R, Baerheim-Svendsen A. Anthraquinones as phytoalexins in cell and tissue cultures of Cinchona spec. Plant Cell Rep. 1985; 4(5):241–4

[21]. Nidhi Saxena, Shrivastava P.,and Saxena R. Preliminary Physico-phytochemical study of the fruit of a medicinal plant Cassia Fistula L. Int. J. Chem. Sci. 2011; 9(1), 223-228 ISSN 0972-768X

[22]. Theeshan Bahorun, Vidushi S Neergheen, Okezie I Aruoma. Phytochemical constituents of Cassia fistula- African Journal of Biotechnology Vol. 4. (13), 2005; pp. 1530-1540,

[23]. Roy, J., Galano, J.-M., Durand, T., Le Guennec, J.Y., Lee, J. C. Physiological role of reactive oxygen species as promoters of natural defenses. FASEB J. 2017; 31, 3729–3745

[24]. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002; 7: 405-410.

[25]. Sarvajeet S.G. and Narendra T. Reactive oxygen species and antioxidant machinery in a biotic stress tolerance in crop plants. Annual Rev. Plant Physiology and Biochemistry. 2010; 3: 1-22.

[26]. Solomon E.I., Sundaram U.M., Machonkin T.E. Multicopper oxidases and oxygenases. Chem. Rev. 1996; 96: 2563- 2605.

[27]. Weis W. Ascorbic acid and electron transport. Ann NY Acad Sci. 1975; 258: 190-200.

[28]. Michiels C., Raes M.,Toussaaint O., Remacle J. Importance of selenium glutathione peroxidase, catalase and Cu/Zn-SOD for cell survival against oxidative stress. Free Radical Bio. Med. 1994; 17: 235-248.

[29]. Gomathi D, Kalaiselvi M, Ravikumar G,Uma C. Evaluation of enzymatic and non-enzymatic antioxidant potential of Evolvulus alsinoides L. Asian J Pharm Clin Res. 2012; 5: Suppl 2: 159-163

[30]. Pric A, Lucas P.W., Lea D.J. Age dependent damage and glutathione metabolism in ozone fumigated barley: A leaf section approach. J. Exp. Bot. 1990; 41: 1309-1317.

[31]. Ruegesgger A, Schmutzz D, Brund C. Regulation of glutathione biosynthesis by cadmium in Pisum sativum L. Plant Physiol. 1990; 93: 1579-1584.

[32]. Kayang H. Tribal knowledge on wild edible plants of Meghala Indian -A, Northeast India. J. Traditional Knowledge. 2007; 6 suppl 1: 177-181

[33]. Maxwell S.R.J. Prospects for use of antioxidant therapies. Drugs 1995; 49: 345-361.

[34]. Sies H. Strategies of antioxidant defence. Eur. J. Biochem. 1993; 215: 213-219

[35]. Mohammad Behrouzi Varjovi, Mostafa Valizadeh and Ali Bandehagh. Primary Antioxidant Enzymes and Their Important Role in Oxidative Stress in Plants and Mammalian. Biological Forum – An International Journal. 2015; 7(1): 148-154

[36]. Al-Shahrani M.M., Zaman G.S., Amanullah M. Measurement of Antioxidant Activity in Selected Food Products and Nutraceuticals. J Nutr Food Sci. 2013; 3: 205. doi:10.4172/2155-9600.1000205

[37]. Seon-Joo Park, Mi-Ok Kim, Jung Hoan Kim, Sehyun Jeong, Min Hee Kim, Su-Jin Yang, Jongsung Lee, and Hae-Jeung Lee. Antioxidant Activities of Functional Beverage Concentrates Containing Herbal Medicine Extracts. Prev. Nutr. Food Sci. 2017; 22 (1):16-20

[38]. Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs aging. 2001; 18: 685-716.

[39]. Block G., Patterson B., Subar A. Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. Nitr cancer. 1992; 18: 1-29.

[40]. Willett W.C. Balancing life-style and genomics research for disease prevention. Science. 2002; 296: 695-698.

[41]. Eberhardt M.V., Lee C.Y., Liu R.H. Antioxidant activity of fresh apples. Nature. 2000; 405: 903-904.

[42]. Shiv, K. Free radicals and antioxidants: Human and food system. Advances in Applied Science Research. 2011; 2(1): 129-135

[43]. Hamid, A. A., Aiyelaagbe, O.O.,Usman, L. A., Ameen, O. M. and Lawal, A. Antioxidants: Its medicinal and pharmacological Properties. African Journal of Pure and Applied Chemistry. 2010; 4(8): 142-151

[44]. Jorge L. & Maria E. G. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutation Research. 2009; 674: 137–147

[45]. Apel K., Hirt H. Reactive oxygen species: Metabolism, Oxidative Stress, and Signal Transduction. Annual Rev. Plant Biol. 2004; 55:373-399.

[46]. Hickman G. Prevention of cancer: Vegetables and Plants: A review. *Comp Biochem. Physiol*. 1989; 93: 201-212.

[47]. Dharmendra K., Gupta José M., Palma Francisco J., Corpas. Antioxidants and Antioxidant Enzymes in Higher Plants. ISBN 978-3-319-75087-3 ISBN 978-3-319-75088-0 (eBook) https://doi.org/10.1007/978-3-319-75088-0 Springer International Publishing AG. 2018; pp. 30-45.

[48]. Corpas F.J., Gupta D.K., Palma J.M. Production sites of reactive oxygen species (ROS) in plants. In: Gupta D.K., Palma J.M., Corpas F.J. (eds.) Reactive oxygen species and oxidative damage in plants under stress. Springer. 2015; pp 1–22.

[49]. Barroso F.J., J.B., Palma J.M., Rodríguez-Ruiz M. Plant peroxisomes: a nitro-oxidative cocktail. Redox Biol. 2017; 11:535–542.

[50]. Manju Singh, Divya Shrivastava and Raosaheb Kale. Antioxidant Potential of *Asparagus adscendens.* http://dx.doi.org/10.5772/52983. 2012; pp. 30-35.

[51]. Sujogya Kumar Panda. Assay Guided Comparison for Enzymatic and Non-Enzymatic Antioxidant Activities with Special Reference to Medicinal Plants http://dx.doi.org/10.5772/50782. 2012; 380-390.

[52]. Hakiman M., and Maziah M. Non enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea* accessions. Afr. J. Biotechnol. 2009; 3(3): 120-131.

[53]. Diallinas G, Pateraki I, Sanmartin M, Scossa A, Stilianou E. Melon ascorbate oxidase: cloning of a multigene family, induction during fruit development and repression by wounding. Plant Mol. Biol. 1997; 34: 759-770.

[54]. Chance B. and Machly C. Assay of catalase and peroxidases. Methods Enzymol. 1955; 11:764-775.

[55]. Reddy K.P., Subhani S.M., Khan PA, Kumar K.B. Effect of light and benzyl adenine on dark treated growing rice (*Oryza sativa*) leaves-changes in peroxidase activity. Plant Cell. Physiol. 1995; 26:987-994.

[56]. Aebi H. Catalase *In-Vitro*. Methods Enzymol. 1984; 105:121-126.

[57]. Luck H. Methods in enzymatic analysis. ISBN: 9780323161374. Academic Press. New York. 1974; pp.170-180.

[58]. World Health Organization (WHO). National Policy on Traditional Medicine and Regulation of Herbal Medicines. 2005. Report of WHO global survey. Geneva.

[59]. Maria H. and Geoffrey B. Fincher. Plant Enzyme Structure. Explaining Substrate Specificity and the Evolution of Function Plant Physiol. 2011; Vol. 125, 54-57.

[60]. Tilburt, J. C., and T. J. Kaptchuk .J. Herbal medicine research and global health: An ethical analysis. Bull World Health Organ. 2008; 86(8):594–9.

[61]. Mathews, C. K., and Van Holde, K. E. Biochemistry, 3rd Ed. Addison Wesley Longman, San Francisco. ISBN 0-8053-3066-6. 2000; pp. 280-290.

[62]. Creighton, T. E. Proteins: Structures and Molecular Principles. Second Ed. ISBN-13:978-0716770305, ISBN-10-:071677030X.W. H. Freeman, New York. 1983; pp. 150-170.

[63]. Delgoda, R.; Murray, J.E. Chapter 7—Evolutionary Perspectives on the Role of Plant Secondary Metabolites. In *Pharmacognosy*; Badal, S., Delgoda, R., Eds.; Academic Press: Boston, MA. USA. 2017; pp. 93–100.