Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: November 30, 2023

Citation: Balasubramanian K, Ramya N, et al., 2023. In-vitro Antioxidant and Radical Scavenging Properties of Acacia Ferruginea Plant Stem: A Phytochemical Study, Medical Research Archives, [online] 11(11). https://doi.org/10.18103/mra.v11i11.4714

DOI
https://doi.org/10.18103/mra.v11i11.4714

ISSN: 2375-1924

RESEARCH ARTICLE

In-vitro Antioxidant and Radical Scavenging Properties of *Acacia Ferruginea* Plant Stem: A Phytochemical Study

Nagesh Ramya[1], Kiruthika Balasubramanian[2#], Harish[1]

[1]Post Graduate, Department of Biochemistry, M S Ramaiah College of Arts, Science and Commerce, Bangalore, India.
[2]Secretary, New Jersey Academy of Science, Kean University, New Jersey, USA

#Corresponding author: medijoywithbk@gmail.com

ABSTRACT

Introduction: The study of free radical chemistry has received considerable attention in recent years. Our bodies produce free radicals through a variety of endogenous systems, exposed to various physiochemical or pathological conditions.

Objective: The study aimed to assess the in-vitro antioxidant properties of *Acacia ferruginea* plant stem and to screen for their phytochemical components.

Methodology: The stem was examined for enzymatic and non-enzymatic antioxidants, radical scavenging activity in three solvents with varying polarities, and phytochemical analyses.

Result: The stem of *Acacia ferruginea* possessed considerable levels of enzymatic and non-enzymatic antioxidants.

Conclusion: Although the three extracts had high scavenging properties, in-vitro assays did not show any significant DNA preventive properties. Phytochemical screening indicated the presence of alkaloids, flavonoids and phenols.

Introduction

The value of medicinal plants is growing globally, and as a result, there is a resurgence in interest in traditional medicine primarily because there is a current widespread belief that "green medicine" is safer and more reliable than expensive synthetic drugs as the deleterious side effects of many modern drugs along with the development of drug-resistant organisms have brought back attention to ethnomedicinal studies. [1-3] Many natural compounds extracted from plants have demonstrated biological activities, notably antibacterial, antifungal and antioxidant properties. [4]

Reactive Oxygen Species (ROS) or free radicals are reactive chemicals that have unpaired electrons in their outer orbitals which can induce oxidative damage to vital cellular and molecular compounds including DNA and proteins and can cause various diseases. [5-7] ROS, which are the by-products of regular cellular metabolism, can either harm or benefit living systems. The effects of oxidative stress on cellular metabolism can cause cell damage or death and it has been implicated in several diseases including cancer, atherosclerosis, and neurodegenerative diseases such as Parkinson's disease, Alzheimer's and Huntington's disease. [8-11]

The organism and human body have developed a variety of defence mechanisms to lower the levels of reactive oxidants and also to reduce free radical-induced damage. [12] The defence mechanism involves enzymes such as catalase, superoxide dismutase, peroxidase etc. in addition to the protective endogenous enzymatic antioxidants, the consumption of dietary antioxidants like tocopherol and carotenoids is of great importance. Phytochemicals serve as plant defence mechanisms. [13]

Acacia ferruginnea is familiarly known as Banni tree which is known for treating rabies, epilepsy, conjunctivitis and helminthiasis, the tee's leaves act as demulcent, alleviating mouth and throat irritation to protect the mucus membrane. Additionally, it has been recognised as an effective herbal remedy for chlorea.

This study was carried out to assess the potential of *Acacia ferruginea* plant stem to scavenge the free radicals and to characterise the enzymatic and non-enzymatic components to serve as antioxidants reducing the action of free radicals on biomolecules especially DNA preventing the diseases associated with the oxidative stress. The study of antioxidants also emphasizes the potential to expand our knowledge on how the medicinal plants and their bioactive components can lead to new antioxidant treatments and aid in fighting against the diseases affected by oxidative stress, advancing the modern medicines and health care significantly.

Despite the recognised medicinal value of the plant, there has been limited investigations conducted on the plant's biochemical composition therefore, this study aims to analyse the phytochemicals and its properties enhancing the scientific understanding of the same.

Materials and Methods

This project was designed to assess the enzymatic and non-enzymatic antioxidant contents *Acacia ferruginea* of the *Acacia ferruginea* plant stem. In addition, it emphasises how the stem acts as a radical scavenger.

Enzymatic Antioxidants

Catalase, Peroxidase and Glutathione S-transferase (GST), were the enzymatic antioxidants analysed for the study. Catalase activity was measured by the enzyme-catalyzed decomposition of hydrogen peroxide, a method given by Luck (1974)[14]. The peroxidase activity by the method of Reddy et al. (1995)[15] and the method proposed by Habig et al. (1974)[16] was adopted for assaying the activity of GST.

Non-enzymatic Antioxidants

The non-enzymatic antioxidant ascorbic acid was estimated in the stem of *Acacia ferruginea* by the method of Roe and Kuether (1943)[17]. Tocopherol by using Emmerie- Engel reaction explained by Rosenberg (1992)[18]. Total Carotenoids and Lycopene by Zakaria *et al*. (1979)[19] method. Total phenols, flavonoids, chlorophyll and reduced glutathione were measured by using methods proposed by Mallick and Singh (1980)[20], Cameron et al. (1943)[21], Witham et al(1971)[22] and Moron et al. (1979)[23]respectively.

Sample Preparation

The fresh stem of the *Acacia ferruginea* plant was obtained from the University of Agricultural Science, GKVK, Hebbal, Bangalore, and analyzed for its enzymatic and non-enzymatic antioxidant content. To estimate each parameter, fresh plants were purchased. They were cleaned under running water to remove any surface impurities before being patted dry between the layers of soft tissue paper.

Radical Scavenging Method

Preparation of Plant Extract

The plant with its fresh stem of *Acacia ferruginea* was collected washed in water to remove surface contaminants and dried on tissue paper. Then the stem was air-dried in shade without exposure to sunlight for 10 days. The completely dried stem was

then ground to a fine powder and weighed. The different solvent extracts were prepared by following the successive extraction procedure. The extracts were prepared by using three different solvents- hexane, ethyl acetate and isopropanol varying in their polarity. 10g of grounded powder of *Acacia ferruginea* stem was dissolved in 100ml of hexane solvent and placed in a shaker incubator for 24 hours. The next day the solution was filtered and the filtrate was hexane extract, stored for further analysis. The residue was air-dried, weighed and dissolved again in 100ml of ethyl acetate and the same procedure was continued for both ethyl acetate extract and isopropanol extract.

The free radical scavenging effects of the stem were analysed against hydrogen peroxide and hydroxyl radicals. The hydrogen peroxide was determined according to the method of Ruch et al. (1989)[24] and the scavenging capacity for hydroxyl radical was measured according to the method of Elizabeth and Rao (1990)[25]. Brown et al (1998)[26] was used to analyse the chelating property of *Acacia ferruginea*.

Agarose gel electrophoresis

The extent of damage to goat liver DNA by hydrogen peroxide in the presence and the absence of the extracts of *Acacia ferruginea* stem were studied by following the migration pattern of the treated DNA on agarose gels. The Goat liver DNA was isolated by the method of Marmur (1961)[27]. For electrophoresis 1% agarose gel was prepared and the samples were loaded in the well by mixing 2µl of 6x gel loading dye to 15 µl of DNA as control DNA. 15 µl of different plant extracts were mixed with 15 µl of DNA separately along with 6x gel loading dye and loaded in respective wells. DNA was induced to damage by generating free radicals by the addition of hydrogen peroxide and loaded in respective wells. DNA with extracts and hydrogen peroxide was mixed and loaded into a well for the migration pattern. The gel electrophoresis was carried out in 100volts.

Preliminary Phytochemical screening

The extracts of *Acacia ferruginea* stem were tested for the presence of various phytochemicals as described by Khandelwal (2002)[28]. Mayer's test, Dragendroff's test, and Wagner's test were used for alkaloid detection followed by the Lead Acetate and Ferric Chloride test for phenolics. To detect flavonoids Sodium hydroxide and Sulphuric acid tests were conducted respectively.

Results

Enzymatic and Non-enzymatic antioxidants

Antioxidants are substances that can neutralize ROS by different chemical reactions. The findings from the study of Enzymatic (CAT, POD, and GST) and Non-enzymatic (ascorbate, tocopherol, total carotenoids, lycopene, reduced glutathione, chlorophyll, total phenols, and flavonoids) antioxidants in the stem of *Acacia ferruginea* are shown in Tables 1 and 2 respectively. The results revealed that the stem of *Acacia ferruginea* possesses considerable levels of enzymatic and non-enzymatic antioxidant content.

Table 1: Levels of Enzymatic antioxidant in the stem of *Acacia ferruginea*

Enzymes	*Acacia ferruginea* stem
Catalase (U#/g)	17.74 ± 0.05
Peroxidase (U@/g)	5.56 ± 0.09
Glutathione s transferase (U$/g)	0.043 ± 0.005

The values are Mean ± Standard deviation of triplicates

1 Unit = Amount of enzyme required to decrease the absorbance at 240 nm by 0.05units/minute

@1 Unit = Change in absorbance at 430 nm /minute

$1 unit = nmoles of CDNB conjugated / minute

Table 2: Levels of Non-enzymatic antioxidants in the stem of *Acacia ferruginea*

Parameters	*Acacia ferruginea* stem
Ascorbic acid (mg/g leaf)	4.42 ± 0.02
Alpha Tocopherol (µg/g leaf)	228.30 ± 1.89
Carotenoids (mg/g leaf)	64.33 ± 0.57
Lycopene (mg/g leaf)	0.18 ± 0.004
Total Phenols (mg/g leaf)	67.43 ± 1.64
Flavonoids(mg/g leaf)	19.43 ± 0.12
Reduced Glutathione (nmoles/g leaf)	369.29 ± 0.40
Chlorophyll (mg/g leaf)	0.67 ± 0.001

The values are Mean ± Standard deviation of triplicates

Radical Scavenging Property

Antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. Figure 1 represents the radical scavenging ability of the three different extracts of *Acacia ferruginea* stem showed considerable hydrogen peroxide scavenging activity. The ethyl acetate extract of the stem showed strong hydrogen peroxide activity followed by isopropanol and hexane extracts. The hydroxyl radical scavenging results are represented in Figure 2. It was observed that the ethyl acetate extract had a more scavenging effect compared with that of hexane and isopropanol stem extracts. The chelating property of *Acacia ferrugenia* stem extract is graphically represented in Figure 3. The maximum per cent of inhibition was shown by hexane extract followed by ethyl acetate and isopropanol. Agents that chelate free iron can reduce ROS-mediated damage including radiation-induced damage.

Figure 1: Graphical Representation of Hydrogen peroxide radical scavenging effect of *Acacia ferrunginea* stem extracts

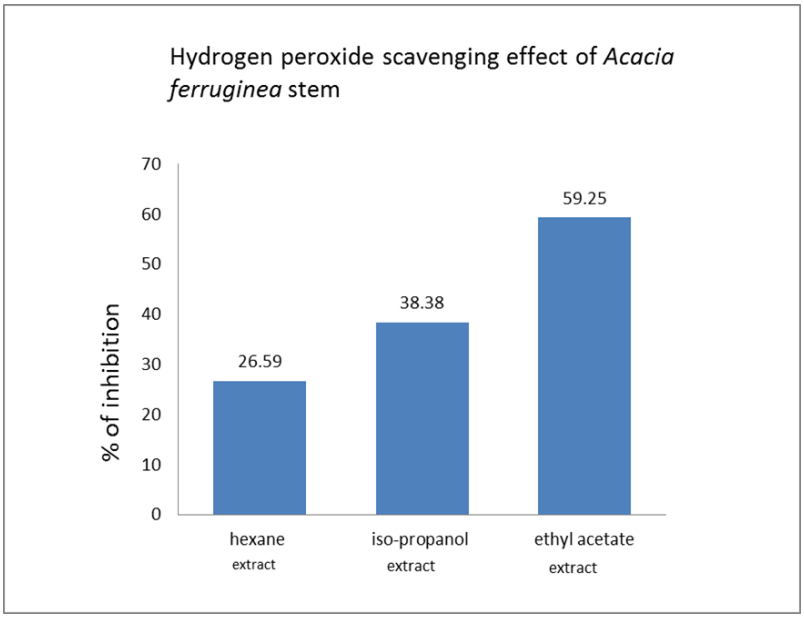


Figure 2: Graphical Representation of Hydroxyl radical scavenging effect of *Acacia ferrunginea* stem extracts

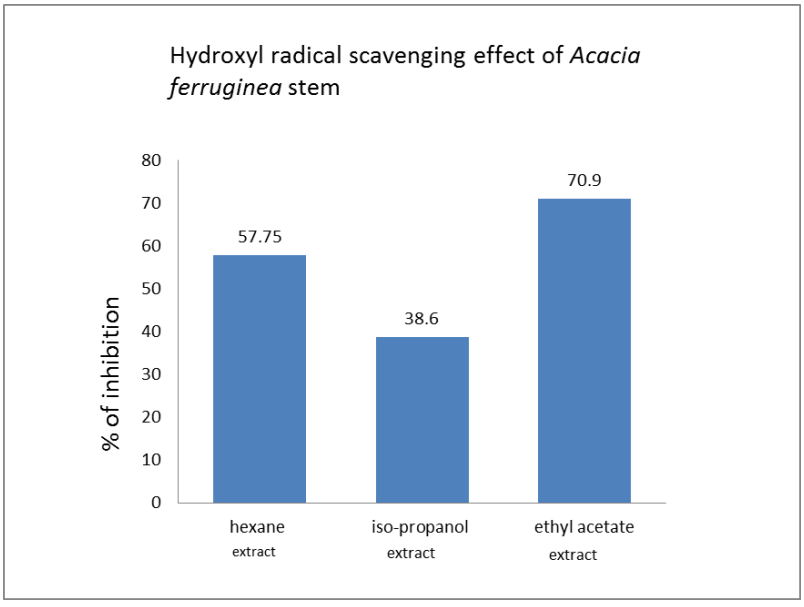
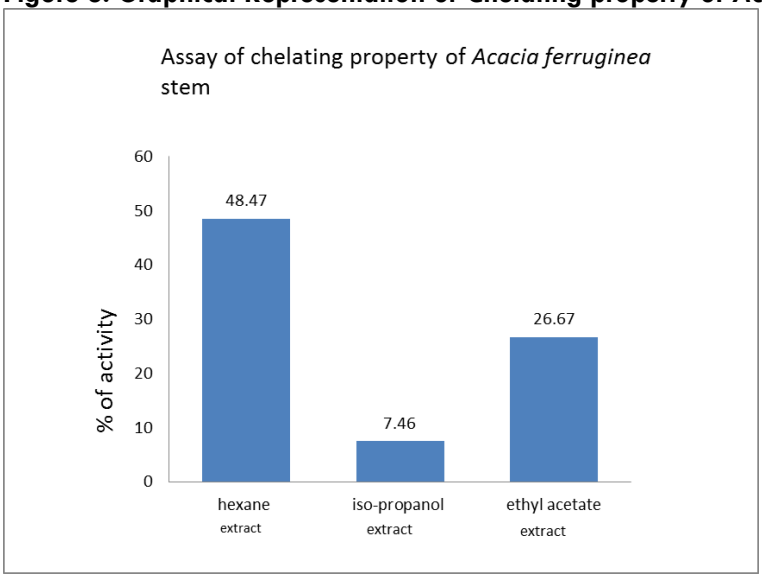


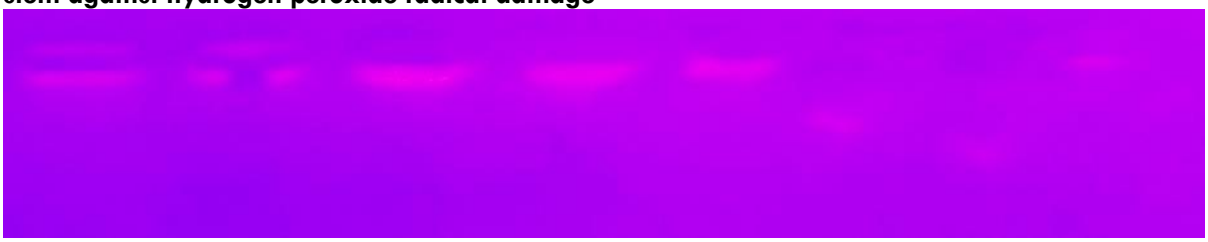
Figure 3: Graphical Representation of Chelating property of *Acacia ferrunginea* stem extracts



DNA protective action of Acaia ferruginea stems extract on the damage induced by hydrogen peroxide

The ethyl acetate extract of *Acacia ferruginea* stem showed minimal DNA protection against hydrogen peroxide radical damage. Hexane and Isopropanol extracts, on the other hand, did not exhibit much significant protection against hydrogen peroxide damage. The resultant pattern of agarose gel is represented in Figure 4.

Figure 4: Agarose gel electrophoresis- DNA protective ability of the three extracts from *Acacia ferruginea* stem against hydrogen peroxide radical damage



DNA treated with and without stem extracts of *Acacia ferruginea* against Hydrogen peroxide (H_2O_2) (Left-Right) W_1: Goat liver DNA, W_2: DNA+H_2O_2, W_3: DNA+ Hexane extract, W_4: DNA+ Isopropanol extract, W_5: DNA+ Ethyl acetate extract, W_6: DNA+ Hexane extract+ H_2O_2, W_7: DNA +Isopropanol extract +H_2O_2, W_8: DNA +Ethyl acetate extract +H_2O_2

Preliminary Phytochemical screening

In the present study, polyphenols, alkaloids and flavonoids were identified as the major active phytochemical components. The results of phytochemicals of the *Acacia ferruginea* stem of three different extracts are presented in Table 3.

Discussion

Medicinal plants have been globally used for their bioactive components and the potential of phytochemicals to provide therapeutic benefits to mankind. Studying the properties of antioxidants within medicinal plants like *Acacia ferruginea* helps in understanding the immediate effect and its significance in treating diseases. These antioxidants present in plants combat damage caused by free radicals. Several studies are going on around the world to identify antioxidant compounds that are pharmacologically potent with a low profile of side effects. Ayurveda, the oldest medical system in the world, provides lots of lead to find active and therapeutically useful compounds from plants.

The term "oxidative stress" is sometimes used to describe the oxidative damage that occurs when the critical balance between the production of free radicals and innate antioxidant defences is unfavourable. It has been associated with pathological conditions like cancer, inflammation, and cardiovascular disease. An interconnected network of antioxidant enzymes, including Superoxide dismutase and Catalase, protects cells from oxidative stress. Plants are also regarded as a rich source of non-enzymatic antioxidants like ascorbic acid, tocopherol, and uric acid. [29-30]

This study specifically focuses on assessing the potential of the *Acacia ferruginea* plant stem in scavenging free radicals, a critical part of preventing oxidative stress related diseases. *Acacia ferruginea* is a deciduous tree whose products have been used to treat pathologies such as haemorrhage, irritable bowel syndrome, leprosy and diarrhoea.[31] In the present study, 10 in-vitro methods were used to screen antioxidants, 3 in-vitro methods were used to study radical scavenging and phytochemical screening was also done. In addition to this DNA protection assay was also conducted, to see the protective effects of antioxidants present in the plant against damage caused by hydrogen peroxide radicals.

In the ancient system of medicine, several Indian medicinal plants have been widely used as rejuvenators, preventing infection, slowing the ageing process, and healing related disorders. Several such plants have already been highlighted e.g. *Emblica officinalis, Curcuma longa, Mangifera indica, Santhum album, Withania somnifera*, etc., for their rich enzymic and non-enzymic antioxidant activity. [32]

Among various species in the Piper plant family, the highest GSH activity was observed in *Piper nigrum* seeds and the highest catalase activity was observed in *Piper longum* seeds. Ascorbic acid is associated with better scavenging activities in-vivo compared to enzymatic antioxidants as they are present in both intracellular and extracellular fluids. [33]

Increased hydrogen peroxide radical causes the death of mesophyll and palisade parenchyma tissue in catalase-deficient plants. The free radical participates in resistance mechanisms, such as reinforcement of plant cell walls, phytoalexin production and resistance. [34-35] Catalase directly reacts with hydrogen peroxide and prevents the cell from damage by decomposing the radical. [36] Lipid peroxidation is caused by hydroxyl radicals and such damage causes a decrease in membrane fluidity. Hydroxyl radicals are the most common ROS produced during photosynthesis. [37-38]

Iron is considered to be an important contributor to the generation of reactive oxygen species. Polyphenolic compounds, including flavonoids, present in several medicinal plants have been reported to possess metal-chelating properties [39]. *Trichopus zeylanicus* is a rich source of polyphenols which have antioxidant and iron-chelating properties and can combat oxidative stress [40]. In the present study, polyphenols, alkaloids and flavonoids were identified as the major active components, which are probably responsible for the protective effects rendered by the *Acacia ferruginea* stem extracts.

The findings of this study not only help in understanding the effects of phytochemicals, and abilities of specific antioxidants in *Acacia ferrunginea* but also provides opportunities for further studies in medicinal applications. Understanding the interaction between antioxidants, free radicals and biomolecules provides valuable insight into mechanism in which medicinal plants provide protection against disease associated oxidative stress. Consequently, this study advances the development of new studies based on antioxidants.

Conclusion

The results have shown that *Acacia ferruginea* is a rich source of antioxidants and it also exhibited powerful antioxidant activity against various in vitro oxidative systems. Whereas, the DNA protective assays of the three extracts did not show much significant effect in protecting the DNA against hydrogen peroxide radicals. This could be due to the difference in the interaction between antioxidants and DNA in in-vivo and in-vitro conditions. However, the presented data is inconclusive and further studies are needed to understand the mechanism.

Evidence suggests that antioxidants are best acquired through whole-food consumption. Increasing the consumption of fruit and vegetables, whole grains, and soy, which are packed with antioxidants, is a practical strategy for consumers to optimize their health and reduce the risk of chronic diseases.

References

1. Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. *Int J Appl Sci Eng.* 2005;3(2):125–134.
2. Nair R and Chanda SV. Antibacterial activities of some medicinal plants of the western region of India. *Turk. J. Biol.* 2007; 31: 231-236.
3. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A.* 1993;90(17):7915-7922.
4. Ahmad P, Sarwat M, Sharma S. Reactive oxygen species, antioxidants and signaling in plants. *J. Plant Biol.* 2008;51:167-173.
5. Rahmatullah M, Jahan R, Khatun MA, Jahan F, Azad AK, Bashar AA, Miajee ZE, Ahsan SN, Ahmad NI, Chowdhury MH. A pharmacological evaluation of medicinal plants used by folk medicinal practitioners of Station Purbo Para village of Jamalpur Sadar Upazila in Jamalpur district, Bangladesh. *Am.-Eurasian J. Sustain. Agric.* 2010; 4: 170-195.
6. El-Missiry MA, Fayed TA, El-Sawy MR, El-Sayed AA. Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. *Ecotoxicol Environ Saf.* 2007;66(2):278-286.
7. Hadjigogos K. The role of free radicals in the pathogenesis of rheumatoid arthritis. *Panminerva Med.* 2003;45(1):7-13.
8. Naik AK, Tandan SK, Dudhgaonkar SP, et al. Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-L-cysteine in rats. *Eur J Pain.* 2006;10(7):573-579.
9. Buonocore G, Groenendaal F. Anti-oxidant strategies. *Semin Fetal Neonatal Med.* 2007;12(4):287-295.
10. Ramachandra Reddy A, Chaitanya KV, Vivekanandan M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J Plant Physiol.* 2004;161(11):1189-1202.
11. Quan LJ, Zhang B, Shi WW, Li HY. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J Integr Plant Biol.* 2008;50(1):2-18.
12. Vijayakumar M, Govindarajan R, Rao GM, et al. Action of Hygrophila auriculata against streptozotocin-induced oxidative stress. *J Ethnopharmacol.* 2006;104(3):356-361.
13. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999;12(4):564-582
14. Luck H. Catalase. In: Bergmeyer H.W., editor. *Methods of Enzymatic Analysis, section* 3. Academic Press; New York, NY, USA: 1963. pp. 885–894.
15. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyl adenine and dark treated graving rice (*Oryza sativa*) leaves changes in peroxidases activity. *Plant Cell Physiol.* 1995.26;987-994.
16. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem.* 1974;249(22):7130-7139.
17. Roe JH, Kuether CA. The determination of ascorbic acid in whole blood and urine through a 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J Biol Chlem.* 1943;147:399-407.
18. Rosenberg HR. *Chemistry and physiology of the vitamins.* New York: Interscience Publishers; 1992. pp. 452–453.
19. Zakaria M, Simpson K, Brown PR, Krstulovic A. Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes. *J Chromatogr.* 1979;176(1):109-117.
20. Mallick CP, Singh MB, *Plant enzymology and plant histoenzymology*, Kalaya. Pub. New Delhi. 1980. 281- 286.
21. Cameron GR, Mitton RF and Allan JW. Measurements of flavonoids in plant sample. Lancet. 1943. 179.
22. Witham FH Devlin RM Blaydes DF. *Experiments in Plant Physiology*. New York: Van Nostrand Reinhold; 1971.
23. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochim Biophys Acta.* 1979;582(1):67-78.
24. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis.* 1989;10(6):1003-1008.
25. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharmaceut.* 1990;**58**:237–240.
26. Brown DJ, Goodman J. A review of vitamins A, C, and E and their relationship to cardiovascular disease. *Clin Excell Nurse Pract.* 1998;2(1):10-22.
27. Marmur J. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. 1961. 3:208-218.

28. Khandelwal KR. Practical Pharmacognosy Techniques & Experiments. Edition 19th, Nirali Prakashan, Pune. 2008. pp. 146, 149-161.
29. Lai LS, Chou ST, Chao WW. Studies on the antioxidative activities of Hsian-tsao (Mesona procumbens Hemsl) leaf gum. *J Agric Food Chem*. 2001;49(2):963-968.
30. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 2010;4(8):118-126.
31. Sakthivel KM, Guruvayoorappan C. Acacia ferruginea inhibits inflammation by regulating inflammatory iNOS and COX-2. *J Immunotoxicol*. 2016;13(1):127-135.
32. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. *J Ethnopharmacol*. 2000;71(1-2):23-43.
33. Karthikeyan J, Rani P. Enzymatic and non-enzymatic antioxidants in selected Piper species. *Indian J Exp Biol*. 2003;41(2):135-140.
34. Vanacker H, Carver TL, Foyer CH. Early H(2)O(2) accumulation in mesophyll cells leads to induction of glutathione during the hyper-sensitive response in the barley-powdery mildew interaction. *Plant Physiol*. 2000;123(4):1289-1300.
35. Quan LJ, Zhang B, Shi WW, Li HY. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J Integr Plant Biol*. 2008;50(1):2-18.
36. Hung S-H, Yu C-W, Lin CH. Hydrogen peroxide functions as a stress signal in plants. *Bot Bull Acad Sin*. 2005;46:1–10.
37. Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food Chem Toxicol*. 2003;41(6):753-758.
38. Munné-Bosch S. The role of alpha-tocopherol in plant stress tolerance. *J Plant Physiol*. 2005;162(7):743-748.
39. Arora R, Gupta D, Chawla R, et al. Radioprotection by plant products: present status and future prospects. *Phytother Res*. 2005;19(1):1-22.
40. Tharakan B, Dhanasekaran M, Manyam BV. Antioxidant and DNA protecting properties of anti-fatigue herb Trichopus zeylanicus. *Phytother Res*. 2005;19(8):669-673.