ISSN XXXX XXXX © 2018 IJESC

Research Article Volume 8 Issue No.7

Enzymatic and Non Enzymatic Antioxidants of *Artocarpus heterophyllus* Ripe Fruits

Anila Jaya Let. J[1], Beena Lawrence[2], Michael Babu[3]
Assistant Professor[1], Associate Professor[2, 3]
Noorul Islam College of Arts and Science Kumaracoil, Tamilnadu, India[1]
Women's Christian College, Nagercoil, Tamilnadu, India[2]
Centre for Marine Science and Technology, Rajakkamangalam, Tamilnadu, India[3]

Abstract:
Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Traditional use of medicine is recognized as a way to learn about potential future medicine which was derived from ethno medical plant sources. Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. The present study was conducted to study the enzymatic and non enzymatic activity in *Artocarpus heterophyllus*. The leaves of *Artocarpus heterophyllus* were selected to access the enzymatic and non enzymatic antioxidant assays. The results revealed that in enzymatic assay poly phenol oxidase is higher and in non enzymatic assay the level of tannin is higher when compared to other in *Artocarpus heterophyllus* fruits.

1. INTRODUCTION

The use of medicinal plants especially in India, contributes significantly to primary health care. Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds for biological functions. Chemical compounds in plants mediate their effects on the human body through identical processes. Antioxidants are a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals leading to chain reaction that may damage cells. So the antioxidant deactivates the free radicals before they attack the cells (**Acedo. 1992**). Humans are exposed to free radicals in the environment through radiation and pollution. Free radicals are also produced naturally in the body through various metabolic reactions. These free radicals can lead to degenerative diseases as well as premature ageing (**Adeulu et al; 2008**). Antioxidants scavenge these free radicals and enable cells to rejuvenate or stabilize for the process of life. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppressant, neurodegenerative diseases and others. **(Harborne 1973**)

Artocarpus heterophyllus belongs to the family Moraceae, commonly known as Jackfruit. It is one of the most significant trees in tropical home gardens and the most wide spread and useful tree in the important genus *Artocarpus*. Jack fruit is a medium-size, evergreen tree that typically attains a height of 8-25m and a stem diameter of 30-80cm. The canopy shape is usually conical or pyramidal in young trees and becomes spreading and domed in older trees. The tree casts a very dense shade. Heavy side branching usually begins near the ground. All parts of the tree exude sticky white latex when injured. This species is monoecious, having male and female inflorescences on the same tree. Male and female spikes are borne separately on short, stout. Literature reveals that lot of pharmacological investigations have been carried on *Artocarpus species*. The reported pharmacological uses are the leaves of *Artocarpus heterophyllus* are used to treat fever, boils, ulcers, wounds and skin diseases.

The aim of the present study is to determine the enzymatic and non enzymatic antioxidants of *Artocarpus heterophyllus* ripe fruits.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The ripe fruits of *Artocarpus heterophyllus* were collected from Mekkamandapam, Kanyakumari District, Tamilnadu. It was identified and authenticated by a taxonomist. Ripe fruits of *Artocarpus heterophyllus* were rinsed under running tap water to eliminate dust. After that the samples were washed several times with distilled water and air dried. The dried samples were powdered and the powdered samples were kept in a clean, dried, air tight glass container to protect it from sunlight.

2.2. Enzymatic antioxidants

2.2.1. Assay of Superoxide Dismutase (SOD)

The assay of superoxide dismutase was done according to the method of **Dos et al 2000**. 1.4 ml of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer pH 7.4, 0.075 ml of 20 mM L-methionine, 0.04 ml of 1% Triton X 100, 0.075 ml of 10 mM hydroxylamine hydrochloride and 0.1 ml of 50 mM EDTA) was added to 100 µl of the sample extract and incubated at 30^0C for 5 minutes. 80 µl of 50 µM riboflavin was added and the tubes were exposed for 10 minutes in the fluorescent lamps. After the exposure time, 1 ml of Greiss reagent (mixture of equal volume of 1% sulphanilimide in 5% phosphoric acid) was added and the absorbance of the colour formed was measured

at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

2.2.2. Assay of Catalase (CAT)

Catalase activity was assayed by the method of **Sinha, 1972**. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1 ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity was expressed in terms of µmoles of H_2O_2consumed/min/mg protein.

2.2.3. Assay of Polyphenol Oxidase(PPO)

Assay of poly phenol oxidase activity was carried out according to the procedure of **Sadasivam and Manickam, 1996**. To 2.0 ml of plant extract and 3.0 ml of distilled water added 1.0 ml of cathecol solution (0.4 mg/ml) and the reactions mixture was quickly mixed. The enzyme activity was measured as change in absorbance/min at 490 nm.

2.2.4 Assay of Peroxidase

The assay was carried out by the method of **Addy and Goodman 1972**. The reaction mixture consisted of 3 ml of pyrogallol (0.05 M pyrogall in 0.1 M phosphate buffer (pH 7.0) and 0.1 ml of plant extract and the O.D. change was measured at 430 nm for every 30 seconds for 2 minutes.

2.3. Non-Enzymatic Antioxidants

2.3.1. Determination of Flavonoids

5 gm of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper no. 42 (125 mm). The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed (**Boham and Kocipai-Abyazani, 1994**).

2.3.2. Determination of Total Phenols

For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 minutes. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were left for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm (**Obadoni and Ochuko, 2001**).

2.3.3. Determination of Saponin

20 gm of each sample were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight (**Obadoni and Ochuko, 2001).**

2.3.4. Determination of Tannins

500 mg of the sample was weighed into a 50 ml flask. 50 ml of distilled water was added and stirred for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of filtered was pipetted out into a test tube and then mixed with 2 ml of 0.1 M $FeCl_3$. The absorbance was measured at spectrophotometer at 395nm within 10 min (**Van-Burden and Robinson, 1981**)

3. RESULT AND DISCUSSION

The enzymatic profile of *Artocarpus heterophyllus* ripe fruits is presented in Table 1 and non enzymatic assay in Table 2. It shows the levels and values of enzymatic antioxidant activity of superoxide dismutase, catalase, polyphenol oxidase and peroxidase and non enzymatic assays.

Table 1: Enzymatic antioxidants of *Artocarpus heterophyllus fruits*

No.	Parameters	ALF
1.	Superoxide Dismutase (unit/mg protein)	1.75±0.2746
2.	Catalase (µ moles of H_2O_2 consumed / min / mg protein)	30.724±0.1823
3	Polyphenol oxidase (µ moles / g tissues)	43±14.4049
4.	Peroxidase (µ moles / g sample)	1.606±0.1111

values are expressed as mean±SD (n = 5)

ALF*: ARTOCARPUS HETEROPHYLLUS* FRUITS

From the above enzymatic antioxidant assay, Polyphenol oxidase possess higher value as 43±14.4049 in *Artocarpus heterophyllus* fruits.

Table 2: Non enzymatic antioxidants of *Artocarpus heterophyllus* fruits.

No.	Parameters	ALF
1.	Saponin (g/100 g)	3.704±0.1213
2.	Flavanoids (g/100 g)	11.086±0.2050
3.	Total Phenol (mg/g)	0.656±0.4559
4.	Tannin (mg/100 g)	14.34±0.7635

values are expressed as mean±SD (n = 5)

ALF: *ARTOCARPUS HETEROPHYLLUS FRUITS*

From the above non enzymatic antioxidant assay the *Artocarpus heterophyllus* fruits showed high level of flavanoids 14.34±0.7635.

This study was correlated with the findings of **Muthukrishnan et al., 2014** on methanolic extract of *Tephrosia purpurea*. He states that oxidative damage has been suggested to be occurring as a consequence of reactive oxygen

produced as a byproduct of ETC in mitochondria. A number of studies have been suggested that ROS can affect critical events associated with many disorders **(Kuo et al., 2000**). It gets special attention due to many factors such as drought, cold, heat, herbicides and heavy metals because they harm the cell by raising the oxidative level through loss of cellular structure and function (**Lee et al., 2007**). Hence demands the detoxification agent like enzyme and non enzymatic antioxidants.

Catalase is a tetrahedrical protein constituted by four heme groups which Catalase the dismutation of hydrogen peroxide in water and oxygen (**Panchawat and Sisodia., 2010**). Vitamin C which includes ascorbic acid and its oxidation products dehydro ascorbic acid has many biological activities in human body have found that vitamin C can reduce level of CRP a marker of inflammation and possibly a predictor of heart disease (**Thevasundar and Rajendran., 2011).**

The antioxidant effect of plant extract is mainly due to the presence of phenolic compounds such as flavanoids, phenolic acids, tannins and phenolic diterpene. Phenolics are the largest group of phytochemicals and have been accounting for most of the antioxidant activity of plants or plant products. Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. So these findings of present study suggest that this plant have a potential source of natural antioxidant. Further studies are warranted for the isolation and characterization of antioxidant compounds and also *invivo* studies are needed for understanding their mechanism of action as antioxidants.

4. CONCLUSION

The present study confirmed that the fruits of *Artocarpus heterophyllus* showed promising antioxidant both enzymatic and non enzymatic. From the observations it can be concluded that the *Artocarpus heterophyllus* act as a good source of natural antioxidant and might be useful in treating the diseases associated with oxidative stress. Further research is necessary to find the relation between antioxidants and oxidative stress in this plant *Artocarpus heterophyllus.*

5. REFERENCES

[1] Acedo. J.R. Cytotoxic activity of methanolic extract of *Artocarpus heterophyllus*. *Journal of applied pharmaceutical science*; 1992; 01(07) 167-171

[2] Adeolu A Adedapo, Florence O Jimoh, Antony J Afolayan and Patrick J Masika. Antioxidant activities and phenolic content of the methanol extracts of stems of *Acokanthera oppositifolia*. *The official journal of international society for complementary medicine research* , 2008 8:54

[3] Addy SK, Goodman RN. Polyphenol oxidase and peroxidase in apple leaves inoculated with a virulent or avirulent strain for *Ervinia amylvora*. Ind Phytopath, 1972; 25:575-579.

[4] Blois MS. Antioxidant determination by the uses of a stable free radicals. *Nature,* 1958; 29:1199-2000.

[5] Boham AB, Kocipai-Abyazani MR. Flavonoids and condensed tannins from leaves of Hawiian *Vaccinium reticulatum* and *V.calycinum* (Ericaceae). *Journal of science* 1994; 48(4):458-463.

[6] Das K, Samanta K, Chainy GBN. A modified spectrometric assay of superoxide dismutase using nitrite formation by superoxide radicals. *Indian journal of biophysics and biochemistry*, 2000; 37:201-204.

[7] Dayal R and T.R.Seshadri. Colourless compounds of *Artocarpus heterophyllus*. *Indian journal of chemistry*,1974; 12:895-896

[8] Dyan FE, Cantrell CL, Duke SO. Natural products in crop protection. *Journal of medical chemistry*, 2009; 17:4022-4034.

[9] Gulcin. I, Zuberyr Hagut, Mahfuz Elmastas, Hassan Y and Aboul Enein. Radical scavenging and antioxidant activity of tannic acid. *Arabian journal of chemistry* 2010; 3(1): 43-53

[10] Halliwell B, Gutteridge JMC. Formation of a thiobarbituric acid reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. *FEBS Let*, 1981; 128(2):347-352.

[11] Harborne J.B. Modern techniques of plant analysis. *Journal of biology*, 4(79) 279-281.

[12] Janario G, Linley MSY, Yap L, Liansolazaro L, Robles J. Determination of sensitivity range of Biuret test for undergraduate Biochemistry experiments. *Journal of science and technology*, 2011; 2:77-83.

[13] Kuo W., Lin J and T K. Tang. Human glucose 6 phosphate dehydrogenase gene transform nin3t3 gene and induces tumour in nude mice. *International journal of cancer* 2000;85:857-864.

[14] Lee M.L., Yen M T., Mau J L. Antioxidant properties of various extracts from *Hypsizigus marmoreus*. *Journal of food chemistry* 2007; 104: 1-9.

[15] Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. *Global Journal of pur applied science*, 2001; 8:203-208.

[16] Panchawat S., Sisodia S.S. Invitro antioxidant activity of *Saraca asoca* stem bark extracts from various extraction processes. *Asian journal of pharmacology and clinical research* 2010; 3: 231-233.

[17] Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of agriculture food and chemistry* 2000; 48(8):3396-3402.

[18] Sadasivam S, Manickam A. 1996. Biochemical method: Polyphenol oxidase. New Delhi, New Age International (P) Limited, 2:108-110.

[19] Sinha AK. Colorimetric assay of catalase. Analytical Biochemistry, 1972; 47:389-394.

[20] Thevasundari S and Rajendran A. Antibacterial potential and phytochemical analysis of *Heterostemma tanjorense*. *World journal of science and technology* 2011; 11: 39-45.

[21] Van-Burden TP, Robinson EC. Formation of complexes between protein and tannin acid. *Journal of agriculture food and chemistry* 1981; 1:77-82