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Assessment of A Few Non Enzymatic Anti Oxidants in Selected Fruits

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ABSTRACT

The therapeutic benefits of the plant are often attributed to their antioxidant properties. The fruits and vegetables play an important role in providing antioxidants to the body. Antioxidants are needed to protect against free radicals that is produced in the body.Free radicals Free radicals damages the cells and play an important role in aging process and disease progression. The free radicals attack and damage the healthy cells and their structure and function are lost.This cell damage contributes to aging and degenerative diseases.The anti oxidant protection system is helpful in protecting the cells from reactive oxygen species.Plant derived materials are increasingly used for antioxidant activity. Plant metabolites function as protectants of cells.Antioxidants protect the body against oxidative stress by neutralizing free radicals. Plants contain rich amount of polyphenols which are very potent natural antioxidants. The present study was designed to evaluate the relative contribution of different polyphenols such as total phenolics, flavonoids and flavonol contents and their antioxidants activities. Now a days it is a trend to search for natural products to replace the synthetic ones. The present work was carried out with an objective to assess the non enzymatic antioxidants in the selected fruits(*Ficus carica, Emblica officinalis, Cephalandra indica* and *Terminalia chebula).* Among different plants studied *Emblica officinalis* fruits was found to contain more ascorbic acid, reduced glutathione, polyphenol, carotenoides and lycopene.The *C.indica* had least non enzymatic antioxidants. *T. chebula* extract had maximum α – tocopherol.

KEY WORDS: Antioxidants, *F. carica, E. officinalis, C. indica* and *T. chebula*

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INTRODUCTION

Globally a positive trend has blossomed in favour of traditional and integrative health sciences both in research and practices. Medicinal plants form a large group of important flora that are used for their therapeutic values. Antioxidants are important in the prevention of human diseases. Naturally occurring antioxidants in leafy vegetables and seeds such as ascorbic acid ,vitamin E and phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases. Much of the protective effect of fruits and vegetables has been attributed to phytochemicals, which are the non-nutrient plant compounds such as the carotenoids, flavonoids, isoflavonoids, and phenolic acids. Thousands of phytochemicals have been identified in foods, yet there are still many that have not been identified. Different phytochemicals have been found to possess a range of activities, which may help in protecting against chronic disease. The phytochemicals may inhibit cancer cell proliferation, regulate inflammatory and immune response, and protect against lipid oxidation[1,2] .A major role of the phytochemicals is protection against oxidation. We live in a highly oxidative environment, and many processes involved in metabolism may result in the production of more oxidants. Humans, and all animals, have complex antioxidant defense systems, but they are not perfect and oxidative damage will occur. Both cardiovascular disease and cancer are thought to be particularly the results of oxidative stress, which can lead to damage of the larger biomolecules, such as DNA, lipids, and proteins. It has been estimated that there are 10,000 oxidative hits to DNA per cell per day in humans[3].

Ficus carica is a huge tree of the family Moraceae. The different parts of the tree namely bark, root, leaves , fruit and latex are used as remedy for various illnesses. It produces unique fruit which is an inverted flower. The species of Ficus are rich in polyphenolic compounds and flavanoids. These compounds have strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases[4]. The fruits and leaves are used as laxative, stimulant, antitussive, emollient, emmenagogue and resolvent[5,6] .

Emblica officinalis Gaertn., known as amla, a member of Euphorbiaceae is extensively found all over India, as well as in Srilanka, Malaya, China, Pakisthan and Bangladesh. The fruits of the plant have been used in Ayurveda as a potent Rasayana which is used to promote health and longevity by increasing defense against disease. *Emblica officinalis* fruit extract has shown protective action against alcohol induced oxidative stress to the cells as evidenced by the lowered plasma transaminases, ALP, LDH and GT enzyme activities and elevated levels of the enzymic and non-enzymic antioxidants. In addition lowered cholesterol level and elevated HDL level demonstrate that *Emblica officinalis* fruit extract offers protection against cardiovascular risk. The active tannoid principles, poly phenolic

compounds and vitamin C present in AEEO could be contributed for the above mechanism[7].Indian gooseberry (*Emblica officinalis* Gaertn.) (Euphorbiaceae) has a distinguished history in Ayurveda medicine and has ascribed a number of medicinal properties and as a dietary supplement, its use is increasing in Western countries. It is thought that its beneficial properties are a function of its antioxidant potency.

Cephalandra indica of the family Cucurbitaceae is commonly called as little gourd. It grows abundantly all over India, Tropical Africa, Australia, Fiji and throughout oriental countries. The plant is used extensively in Indian traditional system of medicine like Ayurveda and Unani[8].The various parts of the plant are used by indigenous people to get relief from diabetes mellitus. The leaves are found to depress the activity of glucose 6 phosphatase and also has antioxidant activity[9]. *Cephalandra indica* roots extracted with hexane , chloroform, methanol, ethanol and water exhibited strong reducing power and total antioxidant capacity[10].

Terminalia chebula belonging to the family combretaceae is an indigenous drug which forms a composition of various herbal formulations. It is reported to be antioxidant, hepato protective, antimicrobial[11,12],adaptogenic and anti-inflammatory[13]. Qercetin is an important component of T.chebula[14].

Reactive oxygen species (ROS) are produced during metabolic and physiological processes. As a result of those, harmful oxidative reactions may oxidative/antioxidative balance shifts towards the oxidative status. Consequently, oxidative stress develops[15,16]. Natural products such as herbs, fruits and vegetables become popular in recent years due to public awareness and increasing interest among consumers and scientific community[17]. Natural products which contain antioxidant properties such as phenolics, include flavonoids and phenolic acids [18],carotenoids and vitamins[19]. Epidemiological evidence has been provided that constituents in natural products show many biological and pharmalogical activities, including antioxidative, anti imflammatory and antiviral effects[20].Flavones, flavonols and proanthocyanidins are well known specific compounds associated with antioxidant activity in plants[21]. Phenolics can be classified into two groups, polyphenols and simple phenols which contain phenolic acids[22].Most of the antioxidant properties in plants are also due to polyphenol, phenolic acid, flavonoid and vitamin C. The plants with high antioxidant activities also have high total phenolic and flavonoid content. Oxidative stress is caused by an imbalance between preoxidants and antioxidants. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions[23]. Awareness of the importance of natural heritage and biodiversity is also growing. India is a gold mine of treasures with traditional and practical knowledge

of herbal medicines[24,25].The present work was carried out with an objective to assess the non enzymatic antioxidants in the selected fruits(*F. carica, E. officinalis, C. indica* and *T. chebula).*

METHODOLOGY

To estimate the ascorbic acid the sample was homogenised in 4% TCA and harvested, the supernatants were treated with a pinch of activated charcoal and incubated at 37^0 C for 15 minutes. Reaction mixture was centrifuged at 6000 rpm for 10 minutes to remove the charcoal residue. Supernatant was used to quantify vitamin C[26]. For α –tocopherol[27] the sample was mixed slowly with 0.1 N sulphuric acid and incubated at room temperature for overnight. The reaction mixture was filtered through Whatman No 1 filter paper and filtrate is used for estimation.For reduced glutathione[28] equal volumes of tissue homogenate and 20% trichloroacetic acid were mixed. The precipitated fraction was centrifuged and to 0.025ml of supernatant 2ml of 0.6mM 5,5'- dithiobis(2-nitro benzoic acid) reagent was added. The final volume was made upto 3ml with phosphate buffer(0.2 M,pH 8.0). The colour developed was read at 412nm against blank.The polyphenol was estimated by Folin ciocalteu [29] procedure. The samples were prepared and 200 l was introduced into test tubes.One ml of Folin ciocalteu reagent and 0.8 ml of sodium carbonate(7.5%) were added. The tubes were mixed and allowed to stand for 30 minutes. Absorption at 765 nm was measured. The total carotenoides[30] and lycopene [31] in the sample are extracted in petroleum ether. The total carotenoids are estimated in UV/visible spectrophotometer at 450nm and same extract was read at 503 nm for lycopene. The results obtained were subjected to statistical analysis of one way ANOVA.

RESULTS AND DISCUSSION

It is evident from Table 1 that the highest values of ascorbic acid (1.241mg/g), reduced glutathione (0.013mg/g), polyphenol (0.129mg/g), carotenoides (0.677mg/g) and lycopene (0.019mg/g) were observed in the fruit extract of *E.officinalis.* Fruit extract of *C.indica* was found to be the poorest source of the non-enzymatic antioxidants namely ascorbic acid (0.017mg/g), reduced glutathione (0.006mg/g), polyphenol (0.013mg/g), carotenoides (0.020mg/g) and lycopene (0.007mg/g). Maximum amount of α – tocopherol (0.545mg/g) was present in the extract of *T.chebula* and minimum (0.139mg/g) in the extract of *E.officinalis.*

Table 1: Non Enzymatic Anti Oxidants In The Plant Samples(mg/g)

S.No	Plant Screened	Non Enzymatic Anti Oxidants In The Plant Samples(mg/g)					
		Ascorbic acid	α – tocopherol	Reduced glutathione	polyphenol	carotenoides	Lycopene
1	*F.carica*	0.529	0.163	0.008	0.029	0.253	0.002
2	*E.officinalis*	1.241	0.139	0.013	0.129	0.677	0.019
3	*C.indica*	0.017	0.387	0.006	0.013	0.020	0.007
4	*T.chebula*	0.679	0.545	0.009	0.088	0.035	0.011
	SED	0.0008	0.0015	0.0006	0.0014	0.0011	0.0012
	CD(0.05)	0.0020	0.01126	0.0013	0.0031	0.0025	0.0027

The GSH antioxidant system consist of an array of non-enzymatic and enzymatic reactions pathways involved in the neutralization of reactive free radical species. GSH is an non-enzymatic mode of defence against the free radicals[32].

Green pepper fruits have vitamin C content between 52.8-115.5 mg in 100 g fresh weight[33,34]. Plant cells contain both enzymatic and non-enzymatic antioxidants [35,36,37]. Ascorbic acid or vitamin C, which is the most abundant water-soluble non-enzymatic antioxidant in plants, has the ability to scavenge a wide range of ROS such as

superoxide anion, singlet oxygen and hydrogen peroxide[38]. Ascorbate provides the first line of defense against damaging reactive oxygen species (ROS), and helps protect plant cells from many factors that induce oxidative stress, including wounding, ozone, high salinity, and pathogen attack. Ascorbate works in cooperation not only with glutathione (Halliwell-Asada cycle), but also maintains the regeneration of α - tocopherol, providing synergic protection of the membranes[39].

A study showed that extracts prepared from the leaves of Ficus carica L have antioxidant capacity. Antioxidant capacity results are consistent with total flavonoid and phenol contents. The α - tocopherol content of the n-hexane extract was found to be 3.2788%, whereas it was calculated as 0.0570% on the dry-weight basis of the leaves[40].

A study was carried out to evaluate the relative contribution of different polyphenols such as total phenolics, flavonoids and flavonol contents and their antioxidants activities. For this purpose the total phenolics, flavonoids and flavonol contents of some medicinal plants were determined in the aqueous extracts of leaves of *Trichosenthes dioica*, fruits of *Moringa olifera* and *Ficus bengalensis* as well as seeds of *Emblica officinalis*. Total antioxidant activity of these extracts was monitored by Free Radical Absorbing Power (FRAP) assay. They observed more phenolic content in leaves of T.dioica compared to the fruits and seeds of *M. oleifera* and *E.officinalis* and aerial roots of *F.bengalensis*. The highest antioxidant activity was also found in E.officinalis compared to other plant samples used in the study. These results indicated that E.officilnalis seeds could be used as potential antioxidant supplement[41].

The study investigated the chemistry and antioxidant properties of four commercial *E. officinalis* fruit extracts in order to determine if there are any qualitative-quantitative differences. All extracts produced positive responses in the total phenol, total flavonoid and total tannin assays. The presence of predominantly (poly)phenolic analytes, e.g. ellagic and gallic acids and corilagin, was confirmed by RP-HPLC coupled with photodiode array detection. Despite ascorbic acid being a major constituent of E. officinalis fruits, the furanolactone could not be identified in one of the samples. The extracts demonstrated varying degrees of antioxidative efficacy. The extract designated IG-3 was consistently amongst the most effective extracts in the iron(III) reduction and 1,1-diphenyl-2-picrylhydrazyl and superoxide anion radical scavenging assays while the extract designated IG-1 demonstrated the best hydroxyl radical scavenging activity. All extracts appeared to be incapable of chelating iron(II) at realistic concentrations[42].

CONCLUSION

Plant based products have been in use for medicinal, therapeutic and other purposes right from the dawn of history. Fruits and vegetables contain significant levels of biologically active components that impart health benefits beyond basic nutrition. The study shows that *E.officinalis* contain more quantity of non enzymatic antioxidants which exhibits the benefits of consumption of the raw fruit.

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