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TOTAL ANTIOXIDANT CAPACITY IN AQUEOUS EXTRACTS OF SOME COMMON FRUITS

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ABSTRACT

The present study was aimed to evaluate total antioxidant capacities of eleven different commonly consumed aqueous fruit extracts viz. *Ananas comosus, Artocarpus heterophyllus, Carica papaya, Citrullus vulgaris, Citrus sinensis, Malus domestica, Manilkara zapota, Musa paradisiaca, Phyllanthus emblica, Psidium guajava* and *Pyrus communis*. The total antioxidant capacity was assessed using phosphomolybdenum method at different concentrations. Total antioxidant capacity was found to be highest in Phyllanthus emblica (197.5µg of ascorbic acid/mg extract) and lowest in *Artocarpus heterophyllus* (1µg of ascorbic acid/mg extract). The present research programme underlies the total antioxidant potentials of fruits.

INTRODUCTION: Free radicals were a major interest for early physicists and radiologists and much later found to be a product of normal metabolism. Today, we know well that radicals cause molecular transformations and gene mutations in many types of organisms. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. In healthy individuals, free radical production is continuously balanced by natural antioxidative defence systems. Disruption of the balance between reactive oxygen species (ROS) production and elimination, due, among other things, to aging, leads to the process called oxidative stress.

As a consequence, ROS are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, cataracts, chronic inflammation, and neurodegenerative diseases, such as Alzheimer's or Parkinson's disease [1-2]. ROS and free radicals are also considered as inducers of lipid peroxidation and cause the deterioration of foods. Although organisms have endogenous antioxidant defences produced during normal cell aerobic respiration against ROS, other antioxidants are taken from the diet, both from natural and synthetic origin [3].

Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important in the prevention of many diseases [4]. Thus, synthetic antioxidants are widely used in the food industry. However, because of their toxic and carcinogenic effects, their use is being restricted. Thereby, interest in finding natural antioxidants, without undesirable side effects, has increased remarkably [3]. Fruits are examples of a dietetically important group of foodstuffs. These components of human diet are not adequately replaceable by any other products. The consumption of fruits and vegetables has been inversely associated with morbidity and mortality from degenerative diseases [5-6], and is associated with low incidences and mortality rates of cancer and heart disease [7-8]. It is not known which dietary constituents are responsible for this association, but antioxidants appear to play the major role in the protective effects of plant foods [9-11]. Fruits and vegetables contain considerable amounts of active components such as polyphenols, carotenoids and vitamins which are considered as potent scavengers of free radicals and reactive oxygen species [12].

In view of huge importance of fruits as antioxidant sources, in the present research programme, a comparative evaluation of antioxidant property of eleven different commonly utilized fruits in every household was investigated in order to identify their extent antioxidant capacities.

MATERIALS AND METHODS:

Plant Materials: Eleven different commonly consumed fruits were selected. Samples of ripened fruits were purchased fresh from a local market of Shivamogga - Bhadravathi, Karnataka, when they were most available, during the year of 2009. The fruits comprised of *Ananas comosus* (Pineapple), *Artocarpus heterophyllus* (Jackfruit), *Carica papaya* (Papaya), *Citrullus lanatus* (Watermelon), *Citrus sinensis* (Sweet Orange), *Malus domestica* (Apple), *Manilkara zapota* (Sapota), *Musa paradisiaca* (Banana), *Phyllanthus emblica* (Indian Gooseberry), *Psidium guajava* (Guava) and *Pyrus communis* (Pear). The fruit samples were authenticated by the taxonomist from the Dept of Botany, Sahyadri Science College, Shivamogga, Karnataka.

Extraction: After selection, each fresh fruit was washed under running tap water followed by washing with distilled water to remove the surface impurities. Exactly 500g of peeled fruit pulps were weighed. The fruit pulps were minced using a mixer grinder and finely macerated. After

homogenization, it was extracted in 500ml chloroform water (1.25ml $CHCl_3$ and volume is makeup to 500ml distilled water) for 7 days in dark under room temperature with intermittent shaking. After 7 days, the whole extracts are filtered using muslin cloth at first and then through filter paper. The filtrate is maintained in dark. To the mark, 300ml fresh solvent was added and refluxed for 90min followed by filtration and finally both the filtrate were mixed together and concentrated. The yield of crude extracts were noted and stored in desiccators for maximum of 3 days; later preserved in a deep freezer (-20^{o}C) for further use.

Qualitative phytochemical analysis: The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in aqueous extracts of eleven different fruit extracts [13-14].

Evaluation of Total Antioxidant Capacity:

General Chemicals and Instruments: All chemicals and solvents used in the study were of analytical grade. Sulphuric acid, sodium phosphate, ammonium molybdate was procured from Sd Fine chem. Ltd, India. UV-Vis Spectrophotometer (Elico SL 159, India), centrifuge (Remi RM12C, India), low deep freezer (Modern Industrial Corporation, India), vacuum rotary evaporator (Shivam Instruments, India), weighing balance (Sartorius, India) and pH meter (Systronics, India) were the instruments used for the study.

The total antioxidant capacity by phosphomolybdenum method was measured by spectrophotometeric method of Prieto *et al.* 1999, [15]. At different concentration ranges, aqueous extracts were prepared and combined in an eppendorf tube with 1ml of reagent solution (0.6M H_2SO_4, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90min at 95^{o}C. The mixture was cooled to room temperature and the absorbance was read at 695nm against blank. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicates and values were expressed as equivalents of ascorbic acid in µg / mg of extract.

RESULTS AND DISCUSSION:

Qualitative Phytochemical Analysis: The preliminary qualitative phytochemical analysis revealed that all the eleven aqueous fruit extracts showed the presence of carbohydrates, proteins, amino acids, glycosides, flavonoids, tannins & polyphenols. In addition to these phytochemicals, *Phyllanthus emblica, Citrullus lanatus, Manilkara zapota, Psidium guajava* and *Musa paradisiaca* revealed the presence of saponins whereas saponins were entirely absent in *Carica papaya, Ananas comosus, Citrus sinensis, Malus domestica, Pyrus communis* and *Artocarpus heterophyllus.* However, alkaloids were confirmed in *Carica papaya, Ananas comosus, Citrus sinensis, Musa paradisiaca, Malus domestica,* and *Artocarpus heterophyllus* among the eleven fruits tested (**Table 1**).

Total Antioxidant Capacity: Total antioxidant capacity by phosphomolybdenum method is based on the reduction of Mo VI to Mo V by the sample analyte and the subsequent formation of green phosphate/Mo V complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. In aqueous fruit extracts, total antioxidant capacity was found to be high in *Phyllanthus emblica* followed by *Psidium guajava*, *Carica papaya*, *Citrullus lanatus*, *Citrus sinensis*, *Musa paradisiaca*, *Malus domestica*, *Manilkara zapota*, *Ananas comosus*, *Pyrus communis* and *Artocarpus heterophyllus* and the values were 197.50, 70.00, 52.00, 48.90, 5.40, 5.29, 4.49, 4.29, 3.45, 1.45 and 1.00 µg of ascorbic acid/mg of extract respectively (**Fig. 1**).

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ELEVEN AQUEOUS FRUIT EXTRACTS

TESTS	FRUIT EXTRACTS										
	Ananas comosus	*Artocarpus heterophyllus*	*Carica papaya*	*Citrullus lanatus*	*Citrus sinensis*	*Malus domestica*	*Manilkara zapota*	*Musa paradisiaca*	*Phyllanthus emblica*	*Psidium guajava*	*Pyrus communis*
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	+	-	-	+	+	+	+	-
Alkaloids	+	+	+	-	+	+	-	+	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+
Tannins and Polyphenols	+	+	+	+	+	+	+	+	+	+	+

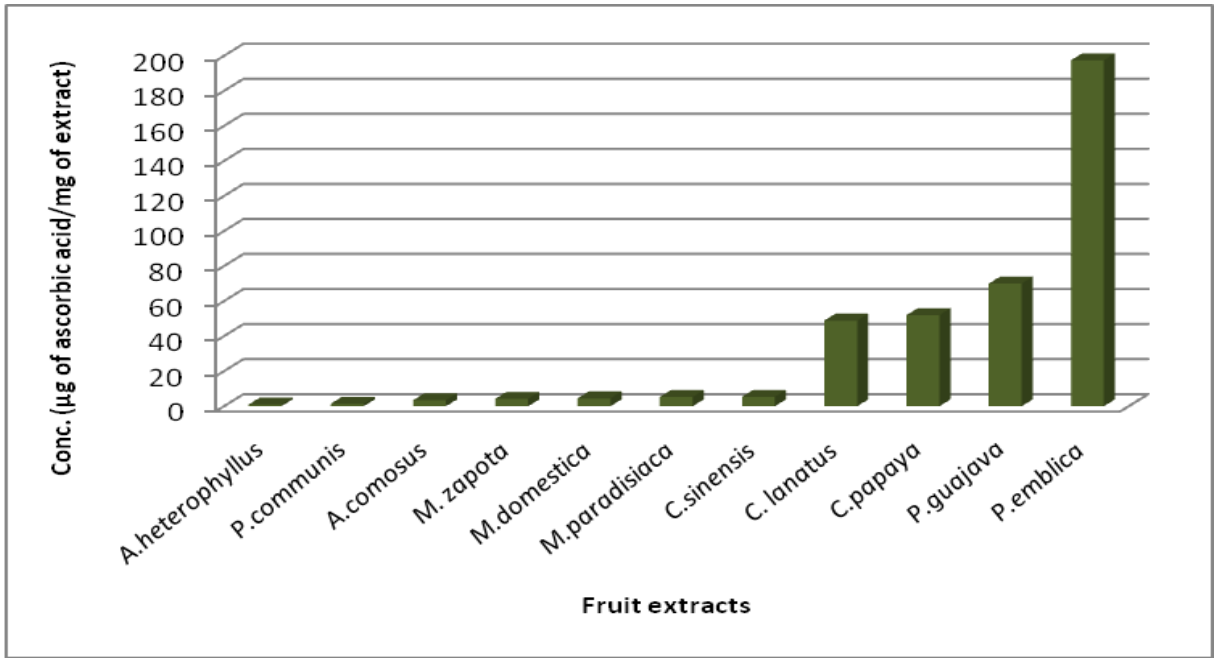

FIG. 1: TOTAL ANTIOXIDANT CAPACITY OF ELEVEN AQUEOUS FRUIT EXTRACTS (EQUIVALENTS OF ASCORBIC ACID)

On the basis of results of total antioxidant capacity, fruit extracts can be placed in the following order. *Phyllanthus emblica > Psidium guajava > Citrullus lanatus > Carica papaya > Citrus sinensis > Musa paradisiaca > Malus domestica > Ananas comosus > Manilkara zapota > Pyrus communis > Artocarpus heterophyllus*. The majority of the antioxidant capacity of a fruit may be derived from the active compounds such as polyphenols - flavonoids and tannins. The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [12]. The flavonoids, a large family of low molecular weight polyphenolic compounds, include the flavones, flavonols, flavonones, isoflavones, flavan-3-ols and anthocyanins [16]. Many flavonoids may help to

provide protection against the oxidation at the cellular level as antioxidants by interfering in enzyme activity, chelating of redox-active metals and effective scavengers of hydroxyl and peroxyl radicals as well as quenching superoxide radicals and singlet oxygen [17].

Tannins are widely distributed in nature and are present in almost all plant foods and some beverages. Tannins are known to inhibit lipid peroxidation and lipoxygenases *in vitro*, and information has been accumulated over the past few years demonstrating their ability to scavenge radicals such as hydroxyl, superoxide, and peroxyl, which are known to be important in cellular pro-oxidant states [18]. In addition to the role of various secondary metabolites vitamins too play a pivotal role in conferring antioxidant capacity.

Vitamin E is considered to be an efficient chain-breaking antioxidant that produces a relatively nonreactive chromanoxyl radical [19]. Vitamin C is a hydrophilic antioxidant, and is considered to be a poor antioxidant within the lipophilic plasma membrane [20]. However, vitamin C plays a valuable role in the regeneration of vitamin E and thereby acts to reduce the rate of oxidative consumption of vitamin E [21-22]. β- Carotene is another hydrocarbon carotenoid and quencher of singlet oxygen at a low partial pressure of oxygen [23].

The present research programme underlies the total antioxidant potentials of fruit extracts, even though extent varies from case to case. The capacity particularly Indian gooseberry, guava and papaya needs to be specially highlighted as these have clearly excelled over other fruits for their antioxidant merit. The investigation thus supports the plethora of investigations on the health benefits of fruits in prevention of degenerative diseases in ensuring longevity.

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