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Evaluation of Antioxidant activity of commonly consumed Fruits of South India

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Abstract

Consumption of natural foods play important role in preventing health disorders due to oxidative stress caused by free radicals. Fruits are the major ingredients in the Indian diet. However, information on the antioxidant activity of fruits used in Indian diet is inadequate. The main objective of this study is to evaluate the antioxidant activity of some commonly used fruits by Indians such as *Manilkara zapota* (Sapota), *Carica papaya* (Papaya), *Musa acuminate* (Banana) and *Psidium guajava* (Guava). Antioxidant activity is assessed by DPPH (2,2-diphenyl-1-picryl-hydrazyl) and HRSA (Hydroxyl radical scavenging activity) methods. The present study revealed that the crude methanol extracts of *Psidium guajava* (Guava) showed the maximum scavenging activity, where as *Musa acuminate* (Banana) showed minimum scavenging activity in both DPPH method and in HRSA methods. Scavenging activity of selected fruits revealed that regular usage of *Psidium guajava* is better than *Carica papaya, Manilkara zapota* and *Musa acuminate* in descending order, in the regular Indian diet to overcome the effects of free radicals and be healthy

Keywords: Antioxidant activity, DPPH method and Hydroxyl radical scavenging activity, Methanol, fruits.

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INTRODUCTION

Fruits are a part of regular diet in the Telangana region. There are several studies which focused on antioxidant activity in different fruits. (Lin *et al*, 2002; Cespedes *et al*, 2010; Huang *et al*, 2012; Carolins *et al*, 2014). Intake of antioxidants has become inevitable to maintain good health and prevent many non-infective disorders. Free radicals that are generated in the body during endogenous metabolic process are captured by antioxidants, which became an important defense mechanism to overcome oxidative stress (Valko *et al*, 2006; Oliveria *et al*, 2009; Liu *et al*., 1995). Free radicals are reactive molecule with unpaired electrons which react with and damage different types of biomolecules like lipids, proteins, carbohydrates, DNA, RNA etc. and may leads to different problems like cancer, oxidative stress, tissue damage, rheumatic arthritis, ageing, circulatory diseases, neuro degenerative diseases, alteration of DNA and several other pathological diseases. Antioxidants taken through diet play a vital role in controlling free radicals damage and can present illness mainly oxidative stress (Scalbert *et al*, 2000; Juliana *et al*, 2015). It has been reported that fruits are the rich source of a wide range of antioxidants such as ascorbic acid, carotinoids, phenolics, flavanoids. These have shown effective scavenging activity on oxidants and inhibit lipid peroxidation (Frei 1991; Lim *et al*., 1992; Vinson *et al*., 1995; Roberts *et al*., 2003). The objective of this study was to screen some fruits used in regular diet like *Manilkara zapota* (Sapota), *Carica papaya* (Papaya), *Musa acuminate* (Banana) and *Psidium guajava* (Guava) to find new potencial sources of natural antioxidants. Antioxidant properties of the fruits are assessed through DPPH and Hydroxyl scavenging methods.

MATERIALS AND METHODS

Collection of fruits

Fresh fruits of *Manilkara zapota* (Sapota), *Carica papaya* (Papaya), *Musa acuminate* (Banana) and *Psidium guajava* (Guava) were obtained from the local

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market in Hanamkonda city, Warangal, Telangana, India.

Preparation of the extracts

All the fruits collected were cleaned with distilled water and grinded separately in an electric blender. 20grams of each sample were soaked in 50ml methanol and kept on a shaker at 120rpm for 24h. Then the extracts were filtered. The filtrates were allowed to evaporate in Petri dishes and collected the crude extract which are used for further assays.

DPPH free radical scavenging assay

The antioxidant activity of different plants was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. In 200µl aliquots of each plant extract at different concentrations was mixed with 1.8ml of methanolic DPPH solution (0.5mM). The reaction mixture was allowed to stand at room temperature for 30 minutes and absorbance was measured at 517nm using UV-VIS spectrophotometer. Methanol without extract was used as blank and the solution consists of DPPH only as control. Ascorbic acid was used as standard. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula:

$$= \left\{\frac{A\ Control - A Sample}{A\ Control}\right\} \times 100$$

Where,

$A_{control}$ is the absorbance of DPPH and methanol solution without extract and

A_{sample} is the absorbance of extract.

Hydroxyl Radical Scavenging Assay

Hydroxyl Radical Scavenging capacity of an extract is directly related to its antioxidant activity. This method involves invitro generation of hydroxyl radicals using Fe 3^+ /ascorbate/EDTA/H_2O_2 system using Fenton reaction (Klein *et al*., 1968, 1969). Scavenging of this hydroxyl radical in the presence of antioxidant is measured. In one of the methods the hydroxyl radical formed by the oxidation is made to react with DMSO (Dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed produces intense yellow color with Nash reagent (75gms of ammonium acetate, 3ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and made up to one liter with distilled water).The intensity of yellow color formed is measured at 412nm in spectrophotometer against the reagent blank. The activity is expressed as % hydroxyl radical scavenging.

A volume of 1ml of a known diluted extracts (10 mg/ml of the methanol leaf extracts), were taken and 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5ml of EDTA (0.018%) and 1ml of DMSO (0.85% v/v in 0.1M phosphate buffer, pH 7.4) were added. The reaction was initiated by adding 0.5ml of 0.22 % ascorbic acid. Test tubes were capped tightly and heated in a water bath at 80 – 90^0 C for 15 minutes. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5 % w/v). 3ml of Nash reagent was added to all the tubes and left at room temperature for 15 minutes for color development which indicates the radical scavenging activity. The intensity of yellow color formed was measured in spectrophotometer at 412nm against blank (Thabrew *et al.,* 1998). The experiment was repeated in triplicates and L-ascorbic acid was used as the standard. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of hydroxyl radical generation and is calculated as:

$$= \left\{\frac{A\ Control - A Sample}{A\ Control}\right\} \times 100$$

RESULTS AND DISCUSSION

The antioxidant capacity of the fruits was evaluated by two different methods using 1,1-diphenyl-2-picrylhydrozyl (DPPH) radical–scavenging and HRSA method. The fruit extracts had different antioxidant capacities in relation to the method of estimation. The antioxidant capacity of the different fruit extracts for each assay is shown in Table-1.

DPPH radical scavenging assay

The DPPH system is a stable radical generating procedure. The DPPH assay is a simple method to measure the ability of antioxidants to trap free radicals. It is well known that the DPPH has ability to capture free radicals is due to the delocalization of the unpaired electron all over the molecule (Yokozawa et al, 1998).

The fruit extract of Musa acuminate and Carica papaya showed promising free radical scavenging effect of DPPH compared to *Manilkara zapota* and *Psidium guajava.* The free radical scavenging effect effect of DPPH in concentration dependent up to 1 µg/ml. The free radical scavenging effect of DPPH radicals was found to have <0.25 µg/ml IC_{50} in *Carica papaya* and also in *Psidium guajava* (Table-1 and Figure-1). Comparatively, *Psidium guajava* extract is found to exhibit significant antioxidant activity.

The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods. The DPPH scavenging activity was found to be dose dependent. This radical scavenging activity of plants extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability.

Hydroxyl Radical Scavenging Assay

The results from Figure-2 and Table-1 revealed that the best extract that exhibited Hydroxyl radical scavenging activity was *Psidium guajava,* followed by *Carica papaya* and, to a lesser extent, *Musa acuminate* and *Manilkara zapota.* The scavenging rates of *Psidium*

Table-1. Antioxidant activity of fruit extracts of different plants

Fruit extract	Antioxidant activity					
	DPPH radical scavenging assay			Hydroxyl Radical Scavenging Assay		
	0.25 µg/ml	0.5 µg/ml	1 µg/ml	0.25 µg/ml	0.5 µg/ml	1 µg/ml
Manilkara zapota	28.02±0.01	34.07 ±0.06	38.03±0.02	21.03±0.2	29.04±0.6	31.36±0.8
Carica papaya	59.53±.005	65.71 ± 0.08	69.36 ± 0.17	41.26 ± 1.8	47.26 ± 0.6	59.26 ± 1.6
Musa acuminata	24.56±0.04	29.34±0.07	36.77±0.09	11.56±0.12	20.89±0.18	32.53±0.11
Psidium guajava	65.5± 0.02	79.15± 0.04	85.45± 0.07	53±1.8	61.11±1.3	69.78±1.4

Figure-1. Graph showing % inhibition of hydroxyl radical generation through DPPH radical Scavenging Assay

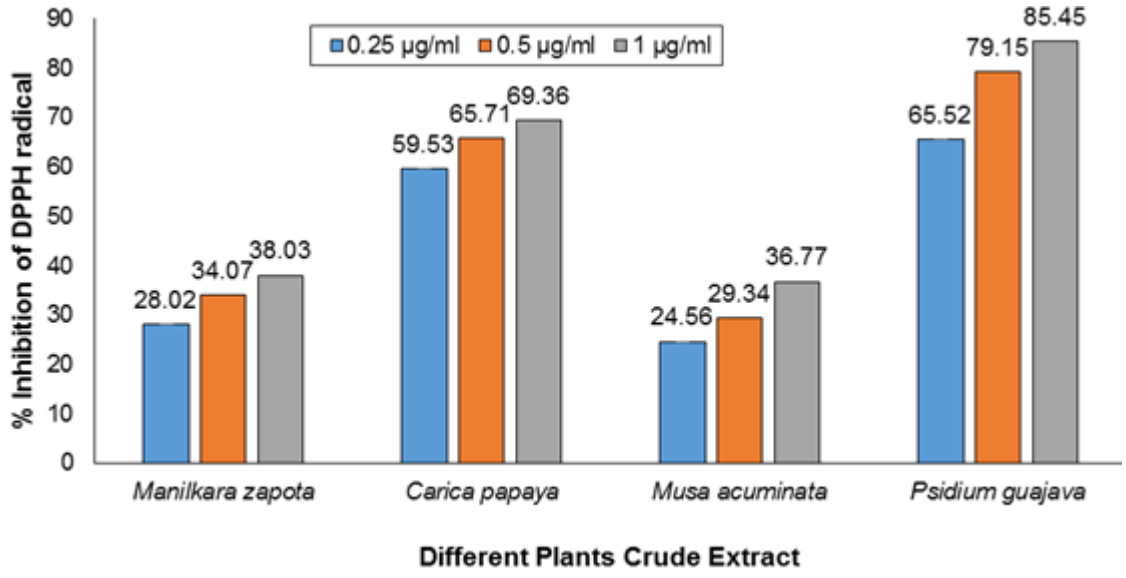
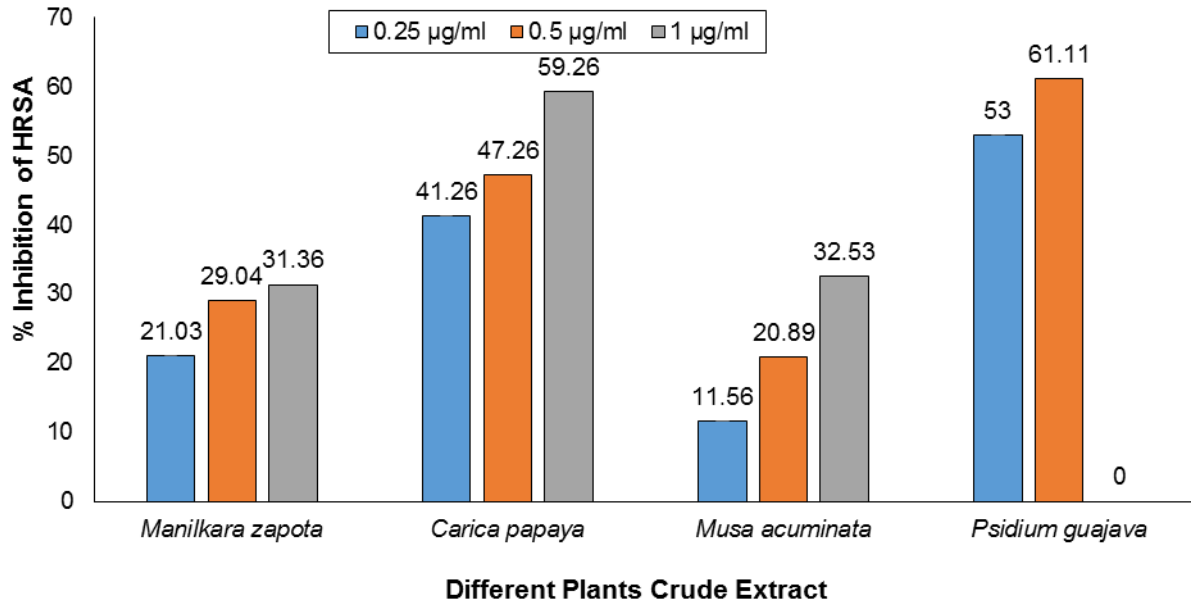


Figure-2. Graph showing % inhibition of Hydroxyl Radical Scavenging Assay



guajava to HRSA were 53, 61 and 69% at concentrations of 0.25, 0.5 and 1µg/ml. The percentages were 41, 47 and 59% in the case *Carica papaya* at concentrations of 0.25, 0.5 and 1µg/ml, respectively.
The high molecular weight phenolics have more ability to quench free radicals and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group substitution than the specific functional groups was reported by (Hagerman et al, 1998). Higher concentrations of the extracts were more effective in quenching free radicals in the system. Phenols and flavonoids contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavour. The phenolic compounds act as antioxidant agents. As a whole the antioxidants are vital substances which possess the ability to protect body from damage by free radical induced oxidative stress.

The ability of fruit extracts to scavenge the DPPH and HRSA measured as IC_{50} varied significantly. Papaya and Guava showed high antioxidant activity. *Manilkara zapota* (Sapota) and *Musa acuminate* (Banana) fruits with moderate antioxidant activity when compared to standard L-ascorbic acid. Our data suggests an inverse correlation between the amount of polyphenolic and the value of IC_{50}. This implies that polyphenolic compounds in fruits might contribute to their radical scavenging activity.

CONCLUSION

Our observation demonstrates that *Psidium guajava* (Guava) is better than *Carica papaya* (Papaya), *Manilkara zapota* (Sapota), and *Musa acuminate* (Banana), as a good source of dietary antioxidants determined by the chemical DPPH and HRSA methods. However, the ability of these fruits to protect cell components from oxidative damage remains to be investigated.

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Competing interests

The authors have declared that no competing interests exist.

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