IN VITRO ANTIOXIDANT STUDIES IN SOME COMMON FRUITS

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ABSTRACT

The present study evaluates the in vitro antioxidant activity of eleven different fruits 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 methanol extract of the fruits was assessed for their antioxidant activity using three different assays like DPPH, reducing power and total antioxidant capacity at different concentrations. In all the three assays, P. emblica has exhibited highest antioxidant activity among the various fruits studied. The results on the antioxidant potentiality of various fruits are discussed.

Keywords: In vitro antioxidant activity, DPPH, Reducing power, Total antioxidant capacity, Fruits.

INTRODUCTION

In recent times, there is an increasing interest in the role of free-radical-mediated damage in the etiology of human diseases. In the status of normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions. Oxidation of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins. Oxidative damage to body cells and molecules has been widely postulated to be involved in the causation and progression of a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts, and several forms of cancer. Human metabolism counts on an antioxidant defensive system involving enzymes and proteins to prevent these effects. However, the defenses can be overwhelmed in certain circumstances so that harmful effects occur. It is accepted that the intake of antioxidant substances reinforces defenses against free radicals. The use of synthetic antioxidants has been limited because of their toxicity. Therefore, it is of great significant and necessity that research focuses on discovering potential natural, effective antioxidants to replace the synthetic ones.

It is widely accepted that fruits and vegetables have many healthful properties. Consumption of fruits is beneficial to health and contributes to decrease the mortality rate of cardiovascular and other diseases. This positive influence is attributed to some natural antioxidant phytonutrients. The majority of the antioxidant capacity of a fruit may be from polyphenols, flavonoids, vitamins, and carotenoids.

In view of huge importance of fruits as antioxidant sources, the present research programme, a comparison of their antioxidant property of commonly consumed fruits was investigated in order to evaluate their antioxidant capacity. Three in vitro antioxidant methods have been used to compare the activity utilizing the methanol extracts of eleven different fruit samples viz. DPPH, reducing power and total antioxidant capacity.

MATERIALS AND METHODS

Plant materials

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

Extraction

After selection, each fresh fruit was washed under running tap water followed by washing with distilled water to remove the surface debris. Exactly 500g of peeled fruit pulps were weighed and were minced using a mixer grinder for fine maceration. After homogenization, it was extracted in 500ml methanol solvent 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. To the marc, 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°C) for further use.

Qualitative phytochemical analysis

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in methanol extracts of eleven different fruit extracts. All chemicals and solvents used in the study were of analytical grade. 2, 2-diphenyl-1-picryl hydrazyl (DPPH), methanol, trichloroacetic acid (TCA) are purchased from Himedia, India. Ascorbic acid, monobasic and dibasic sodium phosphate, potassium ferri cyanide, ferric chloride, sulphuric acid, sodium phosphate, ammonium molybdate is procured from SD Fine chem. Ltd, India.

UV-Vis Spectrophotometer (Elico Sl. 159, India), centrifuge (Remi RM12C, India), low deep freezeer (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.

DPPH (2, 2 - diphenyl-1-picryl hydrazyl) radical scavenging activity

DPPH free radical scavenging assay was measured using the method of Wong et al. 2006. The different concentrations of each extracts prepared in methanol were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30min at room temperature in dark. Changes in absorbance of samples were measured at 517nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard control. All the tests were performed in triplicates.

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Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

\[ \% \text{ Inhibition} = \left( \frac{(A_0 - A_1)}{A_0} \right) \times 100 \]

Where, \( A_0 \) is the absorbance of the control (without test samples) and \( A_1 \) is the absorbance of test samples.

Results are expressed as IC\textsubscript{50}, which is the amount of antioxidant necessary to decrease the initial DPPH\textsuperscript{+} concentration by 50%.

Reducing power assay

The reducing power of the extracts was evaluated according to Oyaizu, 1986. Different amounts of methanol extracts were perched in methanol solvent and dverse with 2.5ml of 0.2M phosphate buffer (pH 6.6), and 2.5ml of 1% K\textsubscript{3}Fe(CN)\textsubscript{6}. This mixture was incubated at 50°C for 20 min. 2.5ml of 10% TCA was added and centrifuged at 3000rpm for 10 min. The upper layer of the solution (2.5ml) was assorted with methanol (2.5ml) and FeCl\textsubscript{3} (0.5ml, 0.1%), and the absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicated increased reducing power. The experiment was conducted in triplicates and the reducing power was expressed as equivalents of ascorbic acid (μg) / mg of extract.

Total antioxidant capacity (Phosphomolybdenum method)

The total antioxidant capacity was measured by spectrophotometric method of Prieto et al. 1999. At different concentration, methanol extracts were prepared in their respective solvents and combined in an eppendorf tube with 1ml of reagent solution (0.6M H\textsubscript{2}SO\textsubscript{4}, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90min at 95°C. The mixture was cooled to room temperature and the absorbance was read at 695nm against blank. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid (μg) / mg of extract.

RESULTS

Qualitative phytochemical analysis

The preliminary qualitative phytochemical analysis revealed that all the eleven methanol fruit extracts showed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins and polyphenols. Phyllanthus emblica, Psidium guajava, Ananas comosus, Citrus sinensis, Malus domestica, Pyrus communis and Artocarpus heterophyllus revealed the presence of alkaloids whereas alkaloids were absent in other fruits viz., Citrus lanatus, Carica papaya, Manilkara zapota, Musa paradisiaca. Analysis also revealed that none of the fruits under study gave positive results for saponins in the methanol extract (Table 1).

Table 1: Results of qualitative phytochemical analysis of eleven methanol fruit extracts

<table>
<thead>
<tr>
<th>Fruit extracts</th>
<th>TESTS</th>
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<tbody>
<tr>
<td></td>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Ananas comosus</td>
<td>+</td>
</tr>
<tr>
<td>Artocarpus heterophyllus</td>
<td>+</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>+</td>
</tr>
<tr>
<td>Citrus lanatus</td>
<td>+</td>
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<tr>
<td>Citrus sinensis</td>
<td>+</td>
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<tr>
<td>Malus domestica</td>
<td>+</td>
</tr>
<tr>
<td>Manilkara zapota</td>
<td>+</td>
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<tr>
<td>Musa paradisiaca</td>
<td>+</td>
</tr>
<tr>
<td>Phyllanthus emblica</td>
<td>+</td>
</tr>
<tr>
<td>Psidium guajava</td>
<td>+</td>
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<tr>
<td>Pyrus communis</td>
<td>+</td>
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</tbody>
</table>

**Reducing power assay**

The ascorbic acid equivalents for reducing power were 595.00, 23.60, 11.90, 11.60, 9.10, 8.50, 8.20, 6.70, 6.20, 3.00 and 2.10 μg per mg of extract in Phyllanthus emblica followed by Psidium guajava, Carica papaya, Citrus sinensis, Malus domestica, Artocarpus heterophyllus, Manilkara zapota, Citrus lanatus, Ananas comosus, Pyrus communis, Musa paradisiaca respectively (Fig.2).

**Total antioxidant capacity**

The total antioxidant capacity was found to be highest in Phyllanthus emblica followed by Citrus lanatus, Psidium guajava, Carica

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**Fig 1:** DPPH radical scavenging activity (IC\textsubscript{50}) of eleven methanol fruit extracts

**Fig 2:** Reducing power assay of eleven methanol fruit extracts (Equivalents of ascorbic acid)
Reducing heterophyllus.

Total ascorbic acid equivalents.

In the present study the methanolic fruit extracts were evaluated for the total antioxidant capacity. Among the eleven methanol fruit extracts, total antioxidant capacity was found to be high in Phyllanthus emblica extract and low in Pyrus communis extract in terms of ascorbic acid equivalents.

A preponderance of epidemiological studies provides convincing evidence of the beneficial role of fruits and vegetables in the diet for the maintenance of health and prevention of disease. The presence of phytochemicals, in addition to vitamins and provitamins, in fruits and vegetables has been recently considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease, and diabetes. Many of these phytochemicals have been found to provide a much stronger antioxidant activity than vitamins C and E and β-carotene within the same food. Synergistically or additively, these dietary antioxidants provide bioactive mechanisms to reduce free radical induced oxidative stress. Prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phytochemical-containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanism to reduce the risk of chronic diseases in humans.

Recent research has also shown that, through overlapping or complementary effects, the complex mixture of phytochemicals in fruits and vegetables provides a better protective effect on health than single phytochemicals. This perspective has been strengthened by the occurrence of inconsistent results in human clinical trials using single antioxidants. Although 5,000 plant phytochemicals have been identified, a large proportion remains unknown. Different plants have different contents of phytochemicals with different structures and thus offer different protective mechanisms to different levels.

Natural antioxidants, particularly in fruits, can be phenolic compounds (tannins, flavonoids, phenolic acids and tocopherols), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and so on), or ascorbic acid as well as carotenoids. These 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 free radicals which are known to be important in cellular prooxidant states. Several researchers have investigated the antioxidative activity of flavonoids and have attempted to define the structural characteristics of flavonoids that contribute to their activity. Phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, and p-coumaric acids, appear to be more active antioxidants than the hydroxy derivatives of benzoic acid such as p-hydroxybenzoic, vanillic, and syringic acids. α-Tocopherol is one of the most active in vitro chain-breaking antioxidants. Vitamin C is a hydrophilic antioxidant, and is considered to be a poor antioxidant within the lipophilic plasma membrane. 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. Carotenoids also have a protective function against oxidative damage, and singlet oxygen is very powerfully quenched by β-carotene.

CONCLUSION

Considering the three assays, it can be concluded that the Phyllanthus emblica is the best fruit followed by Psidium guajava and Carica papaya in terms of antioxidant potential of the fruits tested. It is also interesting to note that the other fruits cannot be ranked due to their varied results in different assays. The present research programme establishes the antioxidant ability of the fruit extracts, even though extent potential varies from case to case. The results and inferences from different methods in other fruits under study differ substantially because each complex chemical reaction generates unique values. Hence, authors are of the opinion that an appropriate index needs to be developed which does not represent a specific antioxidant property but can rank the antioxidant capacity of the fruits.

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The authors wish to thank Prof. Siddaramappa BR for providing laboratory facilities and encouragement. Our sincere thanks also to

Fig. 3: Total antioxidant capacity of eleven methanol fruit extracts

(Equivalents of ascorbic acid)

DISCUSSION

Qualitative phytochemical analysis

The result of present investigation revealed that, the preliminary phytochemical analysis of the plant extracts are bestowed with the presence of several bioactive compounds viz. polyphenols, tannins, steroids, flavonoids and alkaloids in fruit extracts which therefore encourages antioxidant studies.

DPPH radical scavenging activity

DPPH assay is based on the concept that a hydrogen donor is an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals. The antioxidant effect is proportional to the disappearance of DPPH in test samples. A freshly prepared DPPH solution exhibit a deep purple color with absorption maximum at 517nm. The purple color generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals i.e. by providing hydrogen atoms or by electron donation, conceivably via free radical attack on the DPPH molecule and converted them to a colorless stable molecule 2,2-diphenyl-1-hydrazine, or a substituted analogus hydrazine, resulting in a decrease in absorbance at 517nm. Hence absorbance decreases; the more potent the antioxidant more decrease in absorbance is seen. In the present study the methanolic fruit extracts were evaluated for the DPPH radical scavenging activity. Among eleven methanol fruit extracts, P. emblica exhibited the highest radical-scavenging activity whereas A. heterophyllus showed lowest activity.

Reducing power assay

The reducing capacity of extracts Fe3+/ ferriyanyde complex to the ferrous form may serve as a significant indicator of its antioxidant capacity. The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. The reduction of the Fe3+/ ferriyanyde complex to the ferrous form occurs due to the presence of reductants in the solution. Absorbance of Fe5+ can be observed by measuring the O.D. values at 700nm the reduction power of the extract increases with increase in concentration. In the present study the methanolic fruit extracts were evaluated for the reducing power ability. Among eleven methanol fruit extracts, P. emblica exhibited the highest reducing activity whereas M. paradisiaca showed lowest activity in terms of ascorbic acid equivalents.

Total antioxidant capacity

Total Antioxidant Capacity by phosphomolybdenum method assay 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 the present study the methanolic fruit extracts were evaluated for the total antioxidant capacity.
REFERENCES


Ramesh et al.

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