Chapter 1

Screening of selected fruits and vegetables for antioxidant activity
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Introduction

Free radicals are implicated in several degenerative diseases such as atherosclerosis, diabetes, arthritis, cancer, and aging. The harmful effects of free radicals on living systems could be attenuated by antioxidants that scavenge the free radicals. Plants are rich sources of natural antioxidants, which play a vital role in the prevention or progression of the degenerative diseases. The consumption of fruits, vegetables, and herbs rich in antioxidants is associated with a decline in the incidence of degenerative diseases and cancer (Halliwell and Gutteridge, 1992; Ames et al., 1993). Therefore, there is a great deal of interest in edible plants that contain antioxidants and health promoting phytochemicals, in view of their health implications.

Fruits and vegetables are ‘treasure houses’ for a repertoire of nutritional compounds and also nutraceutical molecules, which are mostly produced as secondary metabolites. Most of these bioactive phytochemicals are reported to be polyphenols which has multiple functions. Recently the antioxidant activity has much explored due to increasing oxidative related degenerative diseases.

High consumption of fruits and vegetables is associated with low risk for these diseases, which is attributed to the antioxidant vitamins and other phytochemicals (Ames, Shigenaga, & Hagen, 1993; Prior, 2003; Weisburger, 1999).

Fruits and vegetables have a regular place in traditional Indian cuisine and also in traditional Indian system of medicine viz., Ayurveda (2500 B.C. - 900 B.C.), Sushrutha samitha (c. 1000 B.C.), Charaka samitha (c. 800 B.C.) and Vaghabhatta’s Shakavarga (c. 600 B.C.) which are the oldest available compendium of nutritional and nutraceutical properties of fruits and vegetables in India. They provide descriptions and therapeutic properties of fruits and vegetables that are still a source
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of medicine in Ayurveda. Most of the formulations have taken their face-lift into the modern medicines through many pharmaceuticals and made attractive in their final form. The more popular being as major ingredient of nutraceutical, specialty foods like geriatric foods, diabetic foods, pediatric foods etc. Still there is an untapped potential source of nutraceuticals.

In this context, natural, multifunctional, stable, non-toxic and natural bioactive compounds from fruits and vegetables may prefer panacea for disease. India is the second largest producer of fruits and vegetables. It produces a variety of fruits and vegetables due its varied agro-climatic conditions. India is endowed with variety of fruits and vegetables, which plays a vital role in nutritional, nutraceutical and/or economical role in rural population. The following selected fruits and vegetables are one belong to such group. These selected fruit/vegetables are very important for their nutraceutical value and they are widely consumed in rural India. Further various pharmaceutical properties were attributed in Ayurvedic medicine for these fruits and vegetables. Though they were used from time immemorial, the antioxidant properties and associated bioactive molecules in them have not been reported. It is believed that fruits and vegetables with potential antioxidant activity exhibit various biofunctional properties which prevent chronic diseases. Hence, the present work has been initiated to screen the selected fruits/vegetables for their antioxidant property and elucidation of bioactive properties from the most promising fruit/vegetable. The work presented in this chapter describes the antioxidant activity of various extracts of the following fruits and vegetables.

Ber (Ziziphus jujube), wood apple (Feronia limonia), pseudo stem of banana (Musa paradisiaca), brinjal (Solanum melangina), chow-chow (Sechium edule),
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cluster bean (*Cyamopsis tetragonoloba*), elephant foot yam (*Amorphophallus paeoniifolius*) and kenaf (*Hibiscus cannabinus*)

Materials and Methods

Plant material

Fresh and healthy selected fruits (Ber (*Ziziphus jujube*) and woodapple (*Feronia limonia*) and vegetables (pseudo stem of banana (*Musa paradisiaca*), brinjal (*Solanum melangina*), chow-chow (*Sechium edule*), cluster bean (*Cyamopsis tetragonoloba*), elephant foot yam (*Amorphophallus paeoniifolius*) and kenaf (*Hibiscus cannabinus*)) were procured from the local market, Mysore, India. Shell of wood apple was removed before drying and ret of the plant material was washed, sliced and dried in a hot air oven at 50°C for 36 hrs and powdered to 60 mesh in an apex grinder (Apex Constructions, London).

Chemicals

All the organic solvents used for extraction were of AR grade from E. Merck (Mumbai, India). Tris HCl was from Sisco Research Laboratories (Mumbai, India). DPPH (1, 1-diphenyl-2-picrylhydrazyl) and butylated hydroxyl anisole (BHA) were from Sigma chemicals, U.S.A.

Preparation of extracts

Sequential extraction was carried out using solvents of increasing polarity (from non-polar to polar). Sequential extraction was employed to resolve the compounds of different polarity effectively and completely. About 100 g of different fruit/vegetable powder was sequentially extracted using n-hexane, followed by
chloroform, ethyl acetate, acetone, methanol at room temperature (25±2°C), at normal atmospheric pressure, by shaking at 100 rpm for 48 hrs. Each extract was filtered and concentrated by using rotary evaporator (Buchi Rotavapor R-124, Switzerland). The concentrated extracts were freeze-dried and stored in refrigerator (-18°C) until further use.

**Preparation of test sample**

1mg of extract was dissolved in 1ml of ethanol/water. 100µl of the sample was used for the DPPH assay. BHA was used as the standard.

**Antioxidant activity**

**DPPH assay**

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined according to the method described earlier (Blois, 1958; Bondet et al., 1997; Moon and Terao, 1998). The test samples (100 µl) were mixed with 0.9 ml of Tris-HCl buffer (pH 7.4) to which 1 ml of DPPH (500 µM in ethanol) was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm in a UV-Visible Spectrophotometer (UV-160A, Shimadzu co. Japan). The radical scavenging activity was measured as a decrease in the absorbance of DPPH. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

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\text{Antioxidant activity (\\%)} = \left(1 - \frac{A_{\text{sample} \ (517\text{nm})}}{A_{\text{control} \ (517\text{nm})}}\right) \times 100
\]
Statistical analysis

All the samples were tested in triplicate. Values are expressed as mean ± standard error of three replicates.

Results and discussion

Non polar and polar organic solvents viz. hexane, chloroform, ethyl acetate, acetone and methanol were used to extract entire range of components from selected fruits and vegetables. Solvent extracts were screened for their potential antioxidant activity by DPPH assay.

Antioxidant activity of selected fruits and vegetables extracts

Ber (*Ziziphus jujube*)

DPPH radical scavenging activity of ber fruit extracts ranged from 3 to 19% (Fig 1.1) Methanol extract of ber fruit showed highest activity (19%) followed by ethyl acetate extract (6%).

Wood apple (*Feronia limonia*)

Acetone extract of wood apple exhibited an activity of 2%. Rest of the extracts showed no activity (Fig 1.2)

Chow-chow (*Sechium edule*)

Antioxidant activity of chow-chow extracts ranged from 2 to 18% (Fig 1.3). Methanol extract showed highest activity (18%) followed by ethyl acetate extract (15%).
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Fig 1.1: DPPH radical scavenging activity of different solvent extracts of ber fruit.

Fig 1.2: DPPH radical scavenging activity of different solvent extracts of wood apple.
Fig 1.3: DPPH radical scavenging activity of different solvent extracts of chow-chow.

Elephant-foot yam (*Amorphophallus paeoniifolius*)

Antioxidant activity of elephant-foot yam extracts ranged from 12 to 75% (Fig 1.4). Acetone extract showed 75% activity followed by methanol extract (73.5%).

Brinjal (*Solanum melangina*)

Acetone extract of brinjal showed 65% activity followed by methanol extract with 34% (Fig 1.5).

Kenaf (*Hibiscus cannabinus*)

Methanol extract of kenaf showed 34% activity followed by its acetone extract with 29% (Fig 1.6).
Fig 1.4: DPPH radical scavenging activity of different solvent extracts of elephant-foot yam.

Fig 1.5: DPPH radical scavenging activity of different solvent extracts of brinjal.
**Pseudo stem of banana (Musa paradisiaca)**

DPPH radical scavenging activity of pseudo stem of banana extracts ranged from 20 to 63% (Fig 1.7). Acetone extract exhibited 63% activity followed by ethyl acetate extract with 35%.

**Fig 1.6: DPPH radical scavenging activity of different solvent extracts of kenaf.**

Cluster bean (Cyamopsis tetragonoloba)

Methanol extract of cluster bean showed 18% activity (Fig 1.7). Rest of the extracts didn’t show any activity.

**Standard BHA**

DPPH radical scavenging activity of standard butylated hydroxyl anisole was found to be 84% at 1mg/ml concentration.
Fig 1.7: DPPH radical scavenging activity of different solvent extracts of banana pseudo stem.

![Graph showing DPPH radical scavenging activity for various solvent extracts of banana pseudo stem.]

Fig 1.8: DPPH radical scavenging activity of different solvent extracts of clusterbean.

![Graph showing DPPH radical scavenging activity for various solvent extracts of clusterbean.]

DPPH scavenging activity has been largely used as a quick and reliable parameter to assess the *in vitro* antioxidant activity of plant extracts (Goncalves et al., 2005). It possess a proton free radical with characteristic absorption, which decreases significantly on exposure to proton radical scavengers.

In the present study, serial extraction with solvents was carried out to separate the bioactive components into respective solvents. Hexane and chloroform extracts showed lesser antioxidant activity compared to other polar extracts because of absence of polyphenols in non-polar extracts. Acetone and methanol extracts exhibited good antioxidant potential due to presence of high molecular weight antioxidants which get extracted in polar solvents.

DPPH radical scavenging activity of the extracts may be attributed to the presence of hydrogen-donating ability of -OH and -CH₃ groups in extracts/compounds (Chen and Ho, 1995; Nikolaos, *et al.*, 2003).

**Conclusion**

In the current study, out of 40 extracts from 8 selected fruits and vegetables the acetone extract of elephant-foot yam exhibited highest antioxidant activity by DPPH assay. Hence elephant-foot yam was selected for further investigation of its functional properties both *in vitro* and *in vivo*. 