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Fruits: A source of natural antioxidants (Polyphenols)

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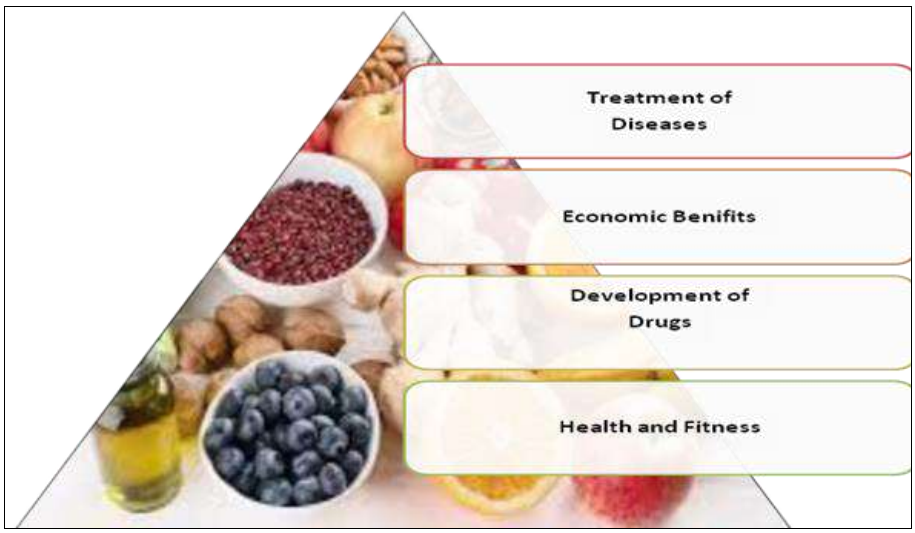
Abstract
Fruits and vegetables are important components of a healthy diet and it is widely accepted all over the world that their consumption helps in prevention of chronic diseases. Phenolic compounds or Polyphenols constitute one of the most numerous and widely distributed groups of substances in the plants. These compounds have at least one aromatic ring with one or more hydroxyl groups and may be classified as flavonoids and non-flavonoids. Fruits contain a variety of compounds with antioxidant activity, including ascorbic acid, carotenoids and polyphenols such as flavonoids and phenolic acid. The study was conducted to determine total phenolic content in various fruits. Total phenolic content estimated in fruits were compared. Total phenols are estimated by Folin Denis reagent that produces blue color with polyphenols. Tannic acid was used as standard and results were expressed as tannic acid equivalent (Singh and Jambunathan, 1981) [11]. Total polyphenol content in the present study showed variation within fruits estimated. Sapota was found to be the richest (1.082 g/100 g) source of polyphenols followed by guava (872 mg/100 g), banana (544.43 mg/100 g), apple (372.2 mg/100 g), pomegranate (188.86 mg/100 g) and grapes green (179.46 mg/100 g). Fruits must be included in our daily diet for good health.

Keywords: Polyphenols, antioxidants, fruits, total polyphenol content, ageing, folin denis reagent, spectrophotometer, optical density

Introduction
Long years ago Hippocrates said, *"*Let food be thy medicine and medicine be thy food" The health benefits of natural foods have been considered for different goals since ancient eras. Oxidative stress plays an important role in the occurrence of most of the chronic lifestyle diseases such as cancer, cardiovascular disease, obesity, type II diabetes and hypertension. It is caused by an excessive production of reactive oxygen species (ROS), which affect the intensity of the body's antioxidant defense system. An excessive production of free radicals is responsible for the cellular injury and cell death (Halliwell, 1997) [3]. Phenolic compounds are major coloring components of fruits as well as vegetables. These compounds cannot be synthesized in human body, but are an integral part of human diet and play many different roles in healthy life. Natural antioxidants present in a diet represent protective qualities against production of free radicals. The total antioxidant activity of food is due to the integrated action of different compounds (Strazzullo, *et al*. 2007) [13]. Polyphenols are major source of natural antioxidants in the diet. The average daily intake of polyphenols is estimated to be approximately 1 g, which is more than the intake of known dietary antioxidants, such as vitamins and minerals, taken altogether. (Scalbert. and Williamson, 2000) [8]. Foods specially fruits and vegetables are reported to contain antioxidants such as, including vitamin C, β-carotene and polyphenols {Sies and Stahl, 1995.) [9].

Polyphenols are the most common and widely distributed class of plant secondary metabolites and several thousand different compounds have been identified till date. They are polyhydroxylated phytochemicals, which contain the two main classes, i.e., flavonoids and phenolic acids. Flavonoids comprise a large group of polyphenols divided into several subclasses, such as flavones (apigenin, luteolin), flavonones (naringenina, hesperidin), Flavanols (quercetin, myricetin, kaempferol), Flavanols (catechin, epicatechin), and anthocyanidins (cyanidin, Malvidin) (Manach, 2004) [5]. Frequent intake of fruits and vegetables rich in polyphenols may reduce risk of cancer, cardiovascular diseases and stroke. (Duthie, and Kyle, 2000.) [2].

Benefits of Polyphenols



The consumption of antioxidant-rich foods is the simplest and most effective possible way against prevention of many diseases related to oxidative stress. Keeping in view, beneficial health effects of polyphenols, this study was carried out to estimate the total polyphenolic content of selected fruits most commonly consumed in India.

Review of Literature

Park (2011) [6] studied major phenolic compounds from basil, lemon thyme, mint, oregano, rosemary, sage and thyme using high performance liquid chromatography (HPLC) profiling technique. Fifteen phenolic compounds derived from plants were selected and their DPPH-scavenging activities were first determined. Then, a standard HPLC profiling of these phenolics was constructed using the HPLC method. Methyl Alcohol extracts of plants were used to isolate antioxidant and anti-inflammatory phenolic compounds.

The study dealt with phenolic compounds in plants and reports on recent studies. Moreover, the present work includes information on the relationships between the consumption of these compounds, via feeding, and risk of disease occurrence, i.e. the effect on human health. Results obtained on herbs, essential oils, from plants grown in tropical, subtropical and temperate regions, were also reported. Temperate regions were also reported (Ruiz. *et al.* 2018) [16].

Scalbert and Williamson (2000) [8] reviewed main dietary sources of polyphenols and daily intake was calculated from a diet containing fruits, vegetables and beverages. Main polyphenols dietary sources were fruits and beverages and to a lesser extent vegetables. Total intake was reported about 1g/day.

Methodology

Estimation of polyphenols in Fruits

Principle

Folin Denis reagent produces blue color with polyphenols. Tannic acid was used as standard and results were expressed as tannic acid equivalent (Singh and Jambunathan, 1981) [11].

Reagents

Folin Denis reagent

100 g sodium tungstate, 20 g phosphomolybdic acid, 50 ml phosphoric acid were added to 750 ml distilled water and refluxed for 2 h, cooled and diluted to 1 liter.

Saturated sodium carbonate

45 g anhydrous sodium carbonate was dissolved in 100 ml distilled water at 70º-80 ºC and cooled. Supersaturated solution with sodium carbonate crystals was filtered through glass wool.

Tannic acid

100 mg tannic acid was dissolved in distilled water and volume was made to one liter with water. Solution was prepared fresh for each determination.

Methanol-HCl

10 ml conc. HCl was mixed in 100 ml methanol and volume was made to one liter with methanol.

Sample selection and preparation

Sample of each fruit was purchased from the local market of Rohtak city of Haryana**.** All the fruits were bought fresh and no canned, frozen and tinned fruits were used for estimation. Samples were cleaned; edible parts were removed in fruits analyzed immediately. Each sample was taken in triplicate and mean was reported. Polyphenolic compounds were extracted from fruits on a dry/fresh weight basis using Folin Denis reagent.

Extraction

Different samples were weighed and then homogenized. A 200 mg defatted homogenized sample was taken in a 250 ml round bottom flask and refluxed with 100 ml methanol-HCl for 2 hours, was allowed to cool. Extract was filtered through what man no. 40 filter paper in 100 ml volumetric flask. Volume was made with methanol-HCl after a few washing. 0.2 ml extract was taken for estimation of polyphenols. The amount of polyphenolic compounds were estimated as tannic acid equivalent according to the Folin-Denis procedure (Swain and Hills, 1959) [14].

Estimation

0.2 ml of the extract was diluted with 7.5 ml water in 10 ml volumetric flask. Standard tannic acid of different

concentrations ranging between 0.2 to 1 ml was taken. 0.5 ml Folin Denis reagent was added to blank, standard and extract and shacked. One ml of saturated sodium carbonate solution was added to all (blank, standards and sample). Volume was made to 10 ml with distilled water. Vortexed thoroughly and allowed to stand for 30 minutes. Color was stable for up to 40 minutes. Absorbance was read in the spectrophotometer at 760 nm using a suitable blank. A standard curve was plotted by taking different concentrations of tannic acid, 0.300 O.D. corresponds to 60µg tannic acid. Suitable volumes of sample were taken to fit into standard concentrations in the standard curve.

Result and Discussion

The mean polyphenol content in fruits tested in present study ranged between 179.46-1082 mg/100 g on fresh weight basis (Table 1).

Table 1: Mean polyphenol content in Fruits

Food group	Name of food stuff	Total Polyphenol content (mg /100 g)
Fruits FW	Apple	372.20± 53.56
	Banana	544.43±41.92
	Guava	872.20±38.45
	Grapes green	179.46±8.89
	Pomegranate	188.86±9.64
	Sapota	1082.00±19.05

Values are mean ± SD
FW fresh weight basis

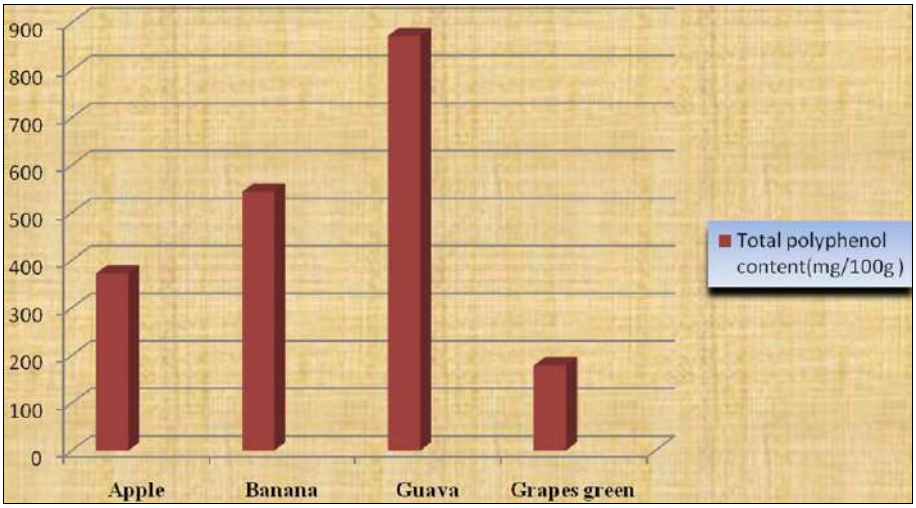


Fig 1: Mean polyphenol content in Fruits

Sapota was found to be the richest (1082±19.05 mg/100 g) source of polyphenols followed by guava (872.20±38.45 mg /100 g), banana (544.43±41.92 mg/100 g), apple (372. 20±53.56 mg/100 g), pomegranate (188.86±9.64 mg /100 g) and grapes green (179.46±8.89 mg/100 g).

Sreeramulu *et al.* (2013) [12] estimated phenolic content in fresh fruits which ranged from 26 to 374 mg/100 g, with the highest in guava (374 mg/100 g) and the least in watermelon (26 mg/100 g). Results were found to be almost similar in case of some fruits as studied by Zujko and Witkowska (2011) [15] and Brat *et al.* (2006) [1]. Variations in the results could be due to various factors such as soil composition, crop variety, plant genetics, storage, harvest conditions, postharvest conditions and climatic conditions. Polyphenol used as a standard could affect results. Among all the studies Gallic was used as standard, in the present study tannic acid was used as standard.

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