Nutritional importance of some dry fruits based on their phenolic acids
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Citation

Abstract
Dry fruits are an important group of agricultural and confectionary commodities being used since human civilizations all over the world because of their nutritional value, taste and nutritional requirements. Healing of various physical, emotional and psychological problems by dry fruits has been reported in ancient traditional medical system (Ayurveda). High performance liquid chromatographic analysis was performed to estimate phenolic acids in eleven dry fruits viz., Date palm (Phoenix reclinata), Cardamom (Ellettaria cardamomum), Almond (Terminalia catappa), Coconut (Cocos nucifera), Groundnut (Arachis hypogea), Kishmish (Vitis vinifera), Cashewnut (Anacardium occidentale), Pista (Pistachia vera), Makhana (Euryale ferox), Chiraunji (Beuchanania latifoli) and resin of higher plants commonly used in India. Among several peaks of phenolic acids, only eight could be identified viz., tannic, gallic, caffeic, vanillic, O-coumaric, ferulic, cinnamic and salicylic acids on the basis of their retention time with standard compounds and co-chromatography. Some phenolic acids were present in rich amount in some of the dry fruits. Tannic, ferulic and salicylic acids were found in high amount in Pista. Gallic and vanillic acids were maximum in Chiraunji, while caffeic and O-coumaric acids were rich in resin and almond, respectively. Cinnamic acid was maximum in groundnut.

INTRODUCTION
The dry fruits are a rich pool of biochemical ingredients that add flavour, taste and nutritional value to food. The curative power of plant-based natural medicines has been relied by nearly two-thirds of the world population for the reason of their knowledge, economics, affordability, availability or their belief in safe traditional system of medicines (Indian medicine-Ayurveda). Dry fruits, condiments and spices have been used by many civilizations as traditional methods to boost energy, improve the nervous system, aid digestion, relieve headache due to stress or cold and against many other diseases (Table 1).

With the development of modern isolation, purification and identification techniques, many bioactive natural products have been isolated from a number of dry fruits. These include alpha-pinene, sabinene, limonene, cineole, para-cymene, linley acetate, alpha-terpinoele acetate and nerol. Besides these, dry-fruits contain enzymes that detoxify carcinogens, inhibit cholesterol synthesis, block estrogen, lower blood pressure and prevent blood clotting. Keeping this in view a detailed analysis of phenolic acids in some dry fruits (Cocos nucifera, Groundnut (Arachis hypogea), Kishmish (Vitis vinifera), Cashewnut (Anacardium occidentale), Pista (Pistachia vera), Makhana (Euryale ferox), Chiraunji (Beuchanania latifoli) and resin of higher plants) has been conducted by High Performance Liquid Chromatography (HPLC) and the results are presented here.

MATERIALS AND METHODS
EXTRACTION OF PHENOLIC ACIDS
Eleven dry-fruits (Chhuara, Cardamum, Chiraunji, Pista, Cashewnut, Kishmish, Almond, Resin, Groundnut, Coconut and Makhana) were purchased from authentic dry-fruit shops. One gram of each dry-fruit was weighed and powdered in a pestle-mortar followed by suspending fine crushed samples in 5 ml ethanol-water (80:20, v/v) in glass tubes. The suspension was subjected to ultra-sonication by Branson sonifiers 450 (Branson Ultrasonic Corps, Danury CT, USA) for 15 min at 4 °C followed by centrifugation at 10,000 rpm for 15 min at room temperature. The supernatant of each sample was collected and the residue was re-extracted twice. Supernatant from each extraction was pooled together and added with charcoal to remove pigments prior to solvent evaporation under vacuum (Buchi type Rotavapor). Dried samples were resuspended in 1.0 ml...
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HPLC grade methanol by vortexing and stored at 4 °C for further analysis by HPLC. The amount of phenolic acids is expressed in terms of (µg/g) dry weight of the dry-fruits.

HPLC ANALYSIS

High performance liquid chromatography (HPLC) of the sample was performed. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable Shimadzu SPD-10 AVP UV-VIS detector and Rheodyne model 7725 in factor with a loop size of 20 µl. Reverse phase chromatographic analysis was carried out using a C-18 reverse phase column (250 x 4.6 mm inner diameter, particle size 5 µm, Luna 5 µ C-18 (2), Phenomenex, Torrance, USA) at 25 °C under isocratic condition included an injection volume of 5 micro liters, mobile-phase methanol: 0.4 % acetic acid (80:20 v/v), flow rate 1 ml / min, and detection at 290 nm. Samples were filtered through membrane filter (pore size 0.45 micron meter, E-Merck, Germany) prior to injection in sample loop. Tannic, gallic, salicylic, ferulic, cinnamic, vanillic, O-coumaric and caffeic acids were used as internal and external standards. Phenolic compound in the samples of dry-fruits were identified by comparing the retention time (Rt) of individual standard. Concentrations were calculated by comparing peak areas of reference compounds with those in sample run under the same elution conditions.

QUANTITATIVE ESTIMATION: PREPARATION OF STANDARD CURVES

Phenolic acid standards were accurately weighted (1 mg/ml) and dissolved in HPLC grade methanol. A range of concentrations form 1 µg/ml to 10 µg/ml was prepared by serial dilution. Quantitative HPLC was conducted using reversed phase C-18 column under similar running conditions as has been described for the analysis of samples. The analysis was carried out in triplicate and the detection was monitored at 290 nm. Calibration curves were plotted showing a linear correlation between concentration and peak areas for phenolic acids separately.

RESULTS

HPLC analysis of phenolic of dry-fruits showed presence of several phenolic acids (Table 2). Maximum amount of tannic acid (43.51 µg/g) was present in pista followed by chhuhara (41.13), almond (7.19), cashew nut (4.36) and resin (4.31) while in the remaining dry fruits the amount of phenolic acids was under detectable level. Gallic acid was maximum in chiranuji (16.03) followed by kishmish (9.88) and coconut (3.96) while in chiranuji it was found in traces. Vanillic acid was maximum in cardamom (3.12) followed by chiranuji (1.83), chhuhara (1.16) while in almond (0.52) it was present in traces. O-coumaric acid was maximum in almond (8.54) followed by cashew nut (1.42), while in other dry fruits such as chhuhara (0.76), cardamom (0.63), unroasted cashewnutt (0.11), makhana (0.14), chiranuji (0.28), resin (0.03) and ash of date (0.01) it was present in traces. Ferulic acid was maximum in pista (11.10) followed by ground nut (1.34) while in other dry fruits such as almond (0.77), cardamom (0.65) makhana (0.41), resin (0.24) and ash of date (0.01) it was found in traces. Cinnamic acid was maximum in ground nut (5.24) followed by almond (1.65) while in cashew nut (0.34), cardamom (0.12), makhana (0.05) and resins of higher plants (0.03) was in traces. Salicylic acid was maximum in pista (284.02) followed by cashew nut (14.36) while in others [almond (0.78), makhana (0.60), chiranuji (0.52), cashew nut (0.47), kishmish (0.30), cardamom (0.27) and cashew nut (0.19 µg/g) was in traces.

Table 1. Dry-fruits used in culinary art and their pharmacological properties
DISCUSSION

Besides adding nutrients and taste to the food, dry-fruits also possess valuable medicinal properties. Most of the dry-fruits under investigations are traditionally known to have pronounced effect on human health. Phenolics constitute an important group of natural products, contributing significantly to the medicinal value of a number of plants including dry-fruits.

Gallic acid and its ethyl ester are the most potent scavengers of super oxide radical. Ellagic acid, a dimer of gallic acid, is a potent antioxidant (molar antioxidant activity in terms of Trolox Equivalent Antioxidant Capacity: TEAR = 3.0), the antioxidant activity of which is three times that of vitamin C or vitamin E. Derivatives of gallic acid with a number of free hydroxyl groups having free radical scavenging property are also powerful antioxidants and possess antibacterial activity against gram-negative and gram positive bacteria. Gallic acid has anti-inflammatory and cytotoxic property against all cancer cell lines studied in vitro. It also possesses hepatoprotective effects at fairly high concentrations corresponding to its level in plasma that might only be achieved by dietary means.

Caffeic acid is the most prominent cinnamate that provides protection against genotoxic agents. It has been demonstrated to possess anti-carcinogenic properties in experimental animals. It has also been reported that caffeic acid in the form of an extract of the artichoke (rich in chlorogenic and caffeic acids) can be used to lower serum cholesterol level in human beings. Cinnamic and hydroxycinnamic acids are rather more abundant and diverse groups of phenolics with higher dietary intake. In terms of...
dietary load, total cinnamic acid intake in different populations ranges up to 1000 mg/day. Cinnamic acid and analogs provide natural protection against infections caused by pathogenic microorganisms. 4- propoxycinnamic acid residue shows antimarial activity . Ferulic acid is present as natural dietary supplements with pronounced anti-inflammatory and antioxidant activity and is a pharmaceutical agent used as photoprotectants in skin lotions . Similarly, ferulic acid is known to possess antifungal and antimicrobial properties .

Very little work has been done on the phenolic acid contents of dry fruits as only anacardic acid, cardanol and cardols have been reported in A. occidentale . The root mucilage of Arachis hypogea contains 4 methoxycinnamic acid and . The total phenolic acids has been estimated in Pistacia vera . The solvent and aqueous extract of Terminalia catappa leaves shown strong antioxidant activity due to presence of several phenolic acids . Therefore the information regarding the presence of several phenolic acids in some dry fruits under study forms the basis of the nutritional value being thought since time immemorial by human beings. This is completely new information to science.

References

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