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Effect of *in vitro* simulated gastrointestinal digestion of *Phoenix loureirii* on polyphenolics, antioxidant and acetylcholinesterase inhibitory activities

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ABSTRACT

Phoenix loureirii is a dwarf date palm belongs to the family Arecaceae. Regular consumption of peduncle and fruit of this plant helps to improve our health system. The present study aims to investigate the stability of polyphenolics, antioxidant and acetylcholinesterase inhibition activities of peduncle and fruit by *in vitro* simulated gastrointestinal digestion method. The fruit methanol extract showed higher total phenolic (46.17 g GAE/100 g extract), flavonoid (79.25 g RE/100 g extract) and pro-anthocyanidin (0.24 g CE/100 g extract) contents than peduncle. *In vitro* digestion method proved that most of the polyphenolic compounds (gallic acid, catechin, rutin and quercetin) are easily bioaccessible in the fruit than peduncle. The stability of antioxidants (DPPH•, $ABTS^{\bullet+}$, phosphomolybdenum and FRAP) and acetylcholinesterase inhibition activities were found maximum after gastric digestion than pancreatic digestion. In conclusion, polyphenolic compounds are not completely degraded and made easily accessible to exert their biological effects in the gastro-intestinal tract.

1. Introduction

Date palm (*Phoenix* species) fruits are widely consumed as fresh fruit and also used as ingredients in the processed food products, which is a rich source of dietary compounds, possesses pharmacological properties. Among such, *Phoenix loureirii* (Syn. *Phoenix humilis*) peduncle and fruits are eaten in the diet. The fruits are used to treat astringent in the intestinal troubles (Anonymous, 1969; Rasingam, 2012; Pavani, Sankara, Rao Nath, & Appa, 2012). Peduncle exudates are taken orally to arrest diarrhea (Maruthupandian & Mohan, 2010). A dietary intake of polyphenolics from the edible plants coupled with bioaccessibility throughout the gastrointestinal tract is key factors to assess their biological significance upon human health. The nature of extractability and stability of polyphenolics/antioxidant activity may vary during gastric and intestinal digestion, it depends on food matrix, pH, temperature, presence of inhibitors or enhancers of absorption, presence of enzymes and other related factors (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Therefore, there is a need to study the bioaccessibility of polyphenolic contents and antioxidant activity in the intestinal tract.

In vitro simulated gastrointestinal digestion is a conventional method to determine the antioxidant potential of polyphenolics (Bhatt & Patel, 2013). Dietary polyphenolic compounds in the food exposed to *in vitro* digestion, various enzymes interfered and transformed them into different structural forms and possess different chemical properties (Ryan & Prescott, 2010). The amount of nutrients and phytochemicals absorbed during digestion is governed by the physical properties of the food matrix, which affects the efficiency of physical, enzymatic and chemical digestion (Hsu, Hurang, Chen, Wenig, & Tseng, 2004). These findings were proved by previous reports, the polyphenolics composition and antioxidant activity may increase or it may degrade even after gastric and pancreatic digestion (Bouayed, Hoffmann, & Bohn, 2011). Recent research focus on bioaccessibility of dietary compounds from food matrix and in condition exerts their beneficial effects on the gastro-intestinal tract (Tagliazucchi et al., 2010). Therefore, the bioaccessibility of polyphenolics from peduncle and fruit were studied by *in vitro* simulated biological gastrointestinal digestion method. In addition, changes in the antioxidant and acetylcholinesterase inhibition activities were investigated after the digestion treatments.

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2. Materials and methods

2.1. Chemicals

All the chemicals used in this study were obtained from Sigma chemicals Co (St. Louis, MO, USA) and Himedia laboratories (Mumbai, Maharashtra, India). All reagents used were of analytical grade. HPLC grade solvents were used for HPLC analysis.

2.2. Collection and identification of plant materials

The fresh parts of peduncle and fruit of *Phoenix loureirii* were collected from Kotagiri hills, Tamil Nadu, India during the month of May 2014. The taxonomic identity of the plant was confirmed by Botanical Survey of India, Southern circle, Coimbatore and voucher specimen (No. 6238) was deposited at Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India.

2.3. Preparation of crude extracts from P. loureirii

The plant material were washed, air dried under room temperature and ground into a fine powder using mechanical mixer (MG 172; Preethi Kitchen 82 Appliances Pvt., Ltd., Chennai, India). The powdered raw plant materials (5 mg) was dissolved in MilliQ water (10 mL) and particles size was analyzed by Zetasizer (Malvern Instruments, model ZEN 3600, Worcestershire, United Kingdom). The particles size of peduncle and fruit was found to be 794.90 nm and 507.30 nm respectively (Fig. S1). The powdered parts were packed in small thimbles separately and extracted successively with solvents such as petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus. The different solvent extracts were concentrated by rotary vacuum evaporator (RE300; Yamato Scientific America Inc., Santa Clara, California, United States) and then air dried. The dried extracts were weighed and used for further *in vitro* studies.

2.4. Quantification of total phenolic, flavonoid, total proanthocyanidin and saponin contents

The total phenolic contents of plant extracts were quantified based on the method described by Siddhuraju & Becker (2003). The results were expressed as gallic acid equivalents (GAE). The flavonoid content of extracts was determined based on the method of Zhishen, Mengcheng, & Jianming (1999). The presence of flavonoid contents were measured at 510 nm and the results were expressed in rutin equivalents (RE). Total proanthocyanidin contents of the extracts were estimated Spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan) at 500 nm and the results were expressed as catechin equivalents (Butler, Price, & Brotherton, 1982). Saponins content of all the extracts were estimated by the method of Sadasivam & Manikam (2008) and the results were expressed as disogenin equivalents (DE). Saponin contents of plant extracts were measured at 544 nm.

2.5. In vitro digestion by gastric and pancreatic juices

The assay was adapted from Yamamoto et al. (1999) and Porfirio et al. (2010). The gastric juice was prepared by mixing 160 mg of porcine-pepsin and 100 mg of NaCl in 50 mL distilled water and the pH was adjusted to 1.2 using HCl. The peduncle (150 mg/10 mL) and fruit (250 mg/10 mL) methanol extract was added to gastric juice respectively and the samples were incubated at 37 °C, and the reaction was stopped after 2 h by liquid nitrogen. 5 mL of this peduncle and fruit gastric juice samples was spared for pancreatic digestion. To prepare the pancreatic juice, 1250 mg of pancreatin were added to 50 mL of phosphate buffer (50 mM, pH 8.0). Then, 5 mL pancreatic juice was added to each peduncle and fruit methanol extracts (1:1, v/v). The reaction mixture was left to incubate at 37 °C for 2 h. Reaction was stopped by liquid nitrogen. The gastric and pancreatic digested samples were filtered through a 0.45 μm PVDF membrane and analyzed for high performance liquid chromatography (HPLC) analysis, antioxidant and acetylcholinesterase inhibition activities.

2.6. HPLC analysis

The chromatographic separation was performed on a LC-6AD Shimadzu LC (UFLC) chromatography system equipped with LC-6AD-Shimazhu LC pump system and PDA Detector-SPD-20 A- (Shimadzu, Tokyo, Japan). The HPLC analysis was performed on a Luna 5u C18 (2)-100A (250 mm × 4.60 mm) 5μ coloumn. The mobile phases included 0.5% acetic acid (solvent A) and 99.5% of acetonitrile in acetic acid (solvent B). A gradient elution programme was used for separation as follows: 10% B (5 min), 20% B (10 min), 30% B (15 min), 40% B (20 min), 50% B (25 min), 60% B (30 min), 70% B (35 min), 80% B (40 min), 90% B (45 min), 100% B (50 min), 100% B (60 min) and 100% B (70 min). The flow rate was 1 mL/min. The injection volume was 20 μL while the column temperature was maintained at 40 °C. The analytes were monitored at 280 nm with photo diode array detector (PDA). The HPLC equipment was controlled using 'LC' Solution Software (version 1.24 SP2). The standard compounds of phenolics (gallic acid, chlorogenic acid, p-coumaic acid and ferulic acid) and flavonoids (rutin, catechin and quercetin) were used and the standards are prepared by mixture of DMSO and water (1:9 v/v). Various concentrations of standard phenolic and flavonoid compounds were analyzed by HPLC analysis (Fig. S2) and linear calibration curve was plotted concentration versus peak area to quantify the phenolic and flavonoid contents of samples (Fig. S3).

2.7. In vitro antioxidant assays

The DPPH• was used to measure the free radical scavenging activity of peduncle and fruit extracts by the method of Blois (1958). The total antioxidant activity of *P. loureirii* extracts was determined by ABTS radical cation scavenging assay (Re et al., 1999). The results were expressed as trolox equivalents antioxidant capacity (TEAC). The total antioxidant activity of peduncle and fruit extracts was determined by the phosphomolybdenum assay (Prieto, Pineda, & Aguilar, 1999). The results reported are mean values and expressed as ascorbic acid equivalents (AAE). The reducing ability of plant extracts was estimated by the method of Pulido, Bravo, & Sauro-Calixto (2000). The ferric reducing ability of extracts was measured at 595 nm and the results were expressed as Fe (II) equivalents. The binding of ferrous ions with the *P. loureirii* extracts was determined by the method of Dinis, Madeira, & Almeida (1994). The metal ion binding capacities of the extracts were expressed as ethylenediaminetetraacetic acid (EDTA) equivalents. Biological radicals such as nitric oxide (Sreejayan & Rao, 1997), super oxide (Zhishen et al., 1999) and hydroxyl radical (Hagerman et al., 1998) scavenging assays were determined and percentage of inhibition was calculated.

2.8. Acetylcholinesterase (AChE) enzyme inhibition assay

The AChE activity was measured according to the method developed by Ellman, Courtney, Andres, & Featherstone (1961). Galanthamine served as the positive control. The percentage of inhibition was calculated using the following formula; % Inhibition = [(Cc − Cs)/Cc] × 100 Where, Cc is the initial velocity of