Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.2014B433 

# *In-vitro* cancer cell cytotoxicity and alpha amylase inhibition effect of seven tropical fruit residues

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ARTICLE INFO

*Article history:*
Received 21 Dec 2013
Received in revised form 2 Apr 2014
Accepted 26 Jun 2014
Available online 7 Jul 2014

*Keywords:*
Alpha amylase inhibition
Anticancer
Antidiabetic
Flavonoid
Fruit residues

ABSTRACT

**Objective:** To determine quantitative phytochemical, anticancer and antidiabetic effect of seven Indian tropical fruit residues.

**Methods:** *In-vitro* cytotoxic activity ($IC_{50}$) was evaluated against cervical cancer cells (HeLa), breast cancer cells (MCF-7), hepatocellular carcinoma cells (HepG-2) and bone sarcoma cells (MG-63) and alpha amylase inhibition assay was used for antidiabetic activity.

**Results:** Results of phytochemical analysis revealed that all residues contained remarkable amount of alkaloid, saponin, tannin and flavonoid. Notable cancer cell growth inhibition was observed for the extract from *Carissa carandas* pomace and *Litchi sinensis* seeds with $IC_{50}$ values ranged from 56.72 to 89.24 μg/mL. Alpha amylase inhibition assay was measured at six different concentrations (5, 10, 25, 50, 100 and 200 mg/mL) by using different solvent extract. Results showed that *Carissa carandas* possessed best activity with $IC_{50}$ value as 29.66 mg/mL followed by other residues in methanol extract.

**Conclusions:** Study suggests that these fruit residues demonstrate promising antidiabetic and anticancer activity that substantiated its ethno medicinal use and may provide new molecules for the treatment of these diseases.

## 1. Introduction

Non-communicable or chronic diseases have become a burden in all countries and increased in a rapidly growing rate. Globally, the leading chronic disease are: cardiovascular diseases (including strokes), cancer, chronic lung disease (including asthma) and diabetes. These problems are often the result of behavior aspects, which include tobacco smoking, a diet high in saturated fat and low in fruit and vegetables, more alcohol consumption and physical inactivity[1]. Antioxidant polyphenols play an important role as a health protective factor since they neutralize the hazardous effect of free radicals in the cell. Excess amount of free radical in human body can lead to oxidative stress, result in DNA and protein damage and an increased risk of chronic disease. It has been estimated that there were 10 000 oxidative hits to DNA per cell per day in humans[2]. Cancer and diabetes are the major health problems and continue to be one of the foremost causes of death all over the world. Various therapeutic agents to treat these diseases are available in Western medicines; however, they are toxic, expensive and associated with serious side effects. Many pharmacological investigations are carried out to identify new drugs or to find new lead structures for the development of novel therapeutic agents for the treatment of these diseases[3]. Natural products are obtained mainly from medicinal and food plants or their parts which are used as a prominent source in primary health care since long time. The beneficial effects of these products are due to the combinations of high-

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Foundation Project: Supported by the State Grading Laboratory, Directorate of Agricultural Marketing, Delhi, India and the 'Ministry of Land, Transport and Maritime', Republic of Korea.

molecular weight such as dietary fiber and low-molecular weight like secondary metabolites. These components are chemically heterogeneous and comprise different classes of phytoconstitutents (essential oils, alkaloids, acids, steroids, tannins, saponins, polyphenols, *etc.*) and directly associated with a number of health-promoting properties such as anticarcinogenic, anti-inflammatory, antidiabetic, antithrombotic and vasoprotective activities. Generally, these compounds are present in the outer layers of fruits and vegetables in higher amounts[4]. Significant amount of fruits and vegetables wastes/by-products are generated by the food processing industries. Researches show that by-product in general contains a variety of biologically active compounds that are mostly discarded as wastes. This not only wastes a potentially valuable resource but also aggravates a serious disposal problem[5]. The use of these wastes or residues can contribute to lower production costs in the food industry and create new food sources for human consumption. Meanwhile, research also continues for searching ideal candidates to cure these chronic diseases with minimum side effects and effective cost.

This paper provides an overview of the studies of phytochemical analysis, anticancer and antidiabetic activity of seven Indian tropical fruit residues.

## 2. Materials and methods

### 2.1. Plant materials and chemicals

Samples *Carissa carandas* (*C. carandas*) L. (Apocynaceae), (pomace); *Ananas comosus* (*A. comosus*) L. (Bromeliaceae), (skin); *Artocarpus lachoocha (A. lachoocha)* R. (Moraceae), (pomace); *Litchi sinensis* S. (Sapindaceae), (seeds); *Grewia asiatica (G. asiatica)* L. (Malvaceae), (pomace); *Beta vulgaris (B. vulgari)* L. (Amaranthaceae) (pomace) and *Artocarpus heterophyllus (A. heterophyllus)* L. (Moraceae) (skin) were obtained from Market Delhi, India in 2011. Upon arrival in the laboratory, skins and seeds were collected manually by cutting off with a stainless steel knife in small pieces and pomace was collected after extracting the juices. All residues were oven dried at 50 °C, ground to the consistency of powder and stored at 4 °C until analyse. All analyses were performed in triplicate, and samples are dry material (DM).

High performance liquid chromatography (HPLC) and analytical reagent grade solvents were used in the analysis and purchased from Merck chemical, Delhi, India. The reference standards were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). The cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco's Modified Eagle's Medium was purchased from BioWhittaker®, whereas fetal bovine serum and other cell culture materials were purchased from Gibco BRL Life Technologies, USA. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide] was purchased from Biosesang Inc., Korea.

### 2.2 Extraction

Powdered air-dried samples with solvent were extracted by a slight modification of the method of Rehman and Demiray[6,7]. Samples were extracted by using high efficiency homogenizer (IKA® Ultra Turrax T25) and kept for three days in shaker at room temperature. All extracts were centrifuged at 9 000 r/min for 20 min at 4 °C (DuPont, model Sorvall RC-5C) to obtain the supernatant. The residues were re-extracted under the same conditions. Supernatants were pooled, combined and evaporated with a rotary evaporator (Nutronix, Jain Brothers India) at ≤50 °C. All extracts were stored at 4 °C until analyse. The whole procedure was performed in triplicates at three different times.

### 2.3. Phytochemical analysis

#### 2.3.1. Crude alkaloid and saponins

Alkaloid and saponin content was calculated gravimetrically[8,9]. The results were expressed as g/100 g DM.

#### 2.3.2. Tannins

Tannin was calculated spectrophotometrically by mixing 5 mL (1% solution of DM) of the solution with 3 mL of 0.1 mol/L $FeCl_3$ in 0.1 mol/L HCl and 0.008 mol/L potassium ferrocyanide. Absorbance was measured at 605 nm within 10 min and tannic acid was used as the standard[10].

#### 2.3.3. Total flavonoid content

The flavonoid content was determined by colorimetric method at 510 nm[11]. Catechin was used as a positive control and results were expressed as catechin equivalents (CE, mg/g DM).

### 2.4. Cytotoxicity activity

#### 2.4.1. Methyl thiazolyl tetrazolium (MTT) assay

Cells were cultured in T-75 tissue culture flasks (Nunc, Denmark) at 37 °C in a 5% $CO_2$ humidified incubator using appropriate media supplemented with Dulbecco's Modified Eagle's Medium containing 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin. Cells were seeded in a 96 well microtiter plate containing 100 μL medium at a final density of $2\times10^4$ cells/well at identical conditions. After overnight incubation, the cells were treated with different concentrations of test compounds (6.25-500.00 μg/mL) in a final volume of 200 μL. After 24 h, 10 μL of MTT (5 mg/mL) was added to each well and the plate was incubated at 37 °C in the dark for 4 h. Then the media along with MTT was removed and the formazan crystals were solubilised by adding dimethylsulfoxide (100 μL/well).