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

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## ABSTRACT

**Objective:** To determine quantitative phytochemical, anticancer and antidiabetic effect of seven Indian tropical fruit residues.**Methods:** *In-vitro* cytotoxic activity (IC<sub>50</sub>) 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<sub>50</sub> 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<sub>50</sub> 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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molecular weight such as dietary fiber and low-molecular weight like secondary metabolites. These components are chemically heterogeneous and comprise different classes of phytoconstituents (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. vulgaris*) 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-diphenyltetrazolium 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 9000 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<sub>3</sub> 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<sub>2</sub> 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 2×10<sup>4</sup> 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).

Finally, the reduction of MTT was quantified by reading the absorbance at 570 nm by GENios® microplate reader (Tecan Austria GmbH). Effects of the test compounds on cell viability were calculated using untreated cells as control. Only methanol solvent was used for cytotoxicity activity. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight–line fit.

#### 2.4.2. Altered morphology study

The altered morphology of exposed cells ( $1 \times 10^5$  cells/well) at  $IC_{50}$  concentration was studied after 24 h using phase contrast microscope (DMI6000B, Leica Microsystems, Wetzlar, Germany). Subsequently, the cells were Hoechst stained to observe the nuclear/chromosomal condensation that occurred after treatment with the test compound. For staining the cells, 96 well cell culture plates were used to culture the cells ( $1 \times 10^5$  cells/well) in three replicates to treat with the ideal *C. carandas* pomace crude extract. Then the cells were incubated at 37 °C overnight and the media was removed to wash the cells twice with phosphate buffered saline and fixed with 4% paraformaldehyde in phosphate buffered saline for one day at –4 °C. Further, the cells were stained with nuclear binding dye Hoechst 33342 (1 µg/mL of the fluorescent DNA–binding dye, bis–benzimidazole Hoechst 33342 stain was added to the fixed cells and incubated for 20 min at room temperature) to expose the nuclear condensation/aggregation due to the effect of the *C. carandas* pomace extract. The Hoechst stained cells were visualized and photographed under fluorescence microscope (CTR 6000; Leica, Wetzlar, Germany).

#### 2.5. Amylase inhibition assay

Different solvent systems were used to evaluate the effectiveness of solvent type for alpha amylase inhibition assay. The samples were extracted with aqueous acetone (acetone: water, 80:20 v/v; AAE), aqueous methanol (methanol: water, 80:20 v/v; AME) and mix solvent (ethanol: hexane: water, 80:10:10; MSE). The modified method of McCue and Shetty was followed; briefly 500 µL of extract and 500 µL of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L sodium chloride) containing amylase solution (0.5 mg/mL) were incubated at 25 °C for 10 min[12]. After pre–incubation, 500 µL of 1% starch solution in 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L sodium chloride) was added

to each tube at timed intervals. The reaction mixtures were then incubated for another 10 min at 25 °C. The reaction was stopped with 1.0 mL of dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature and absorbance was measured at 540 nm after being diluted to 10 mL with distilled water. Two of these inhibitors: catechin (0.2 mg/mL to 10 mg/mL) and acarbose (0.2 µg/mL to 10 µg/mL) were used as positive controls in this experiment, where acarbose is an anti–diabetic drug and catechin is a flavonoid found in plants. Results were calculated as % inhibition=[(absorbance of control–absorbance of sample)/absorbance of control]×100 and reported as  $IC_{50}$  in mg/mL.

#### 2.6. Statistical analysis

All the data are reported as mean±SD. The mean values were calculated based on the data taken from at least three independent experiments conducted on separate days. Correlation analysis was done with SPSS (version 10) statistical package. Differences at  $P < 0.05$  were considered significant. All samples were prepared and analyzed in triplicates.

### 3. Results

#### 3.1. Phytochemical analysis

Quantitative results of phytochemical analysis estimated by gravimetric and colorimetric methods showed that all powdered residues possessed remarkable amount of alkaloid, saponin, tannin and flavonoid content (Table 1). The level of alkaloid content was assessed in the range of (3.05±0.49) g/100 g DM (*A. heterophyllum*) to (1.25±0.90) g/100 g DM (*B. vulgaris*), whereas, saponin was ranged between (1.03±1.30) g/100 g DM (*C. carandas*) to (2.67±0.17) g/100 g DM (*A. heterophyllum*). Among the all tested extracts *B. vulgaris* was identified as the weakest residues for the tannin content. However, *A. heterophyllum* skin powder presented more tannin content as compare to other residues in this study. The result of flavonoid content analysis showed that all residues contained significant amount of flavonoid. Flavonoid content was more in *A. comosus* skin powder (59.77±1.07) mg/g as CE on dry weight basis) while low in *G. asiatica*.

**Table 1**  
Phytochemical analysis of the fruits extracts (mean±SD, n=3).

Fruit residue	Flavonoid [mg/g (DM) as CE]	Alkaloid (g/100 g DM)	Saponin (g/100 g DM)	Tannins (g/100 g DM)
<i>C. carandas</i>	24.40±0.60	1.96 ±0.12	1.03±1.30	1.02±0.75
<i>A. comosus</i>	59.77±1.07	2.34±1.10	1.03±0.50	0.96±0.24
<i>A. lachoocha</i>	28.09±0.88	2.61±0.50	2.10±1.30	0.88±1.20
<i>L. sinensis</i>	26.22±0.58	1.69±1.50	1.10±1.20	1.01±1.10
<i>G. asiatica</i>	12.42±0.56	1.56±1.20	1.05±0.96	0.52±1.25
<i>B. vulgaris</i>	14.65±0.87	1.25±0.90	1.08±0.25	0.15±0.70
<i>A. heterophyllum</i>	20.01±0.52	3.05±0.49	2.67±0.17	1.45±0.29

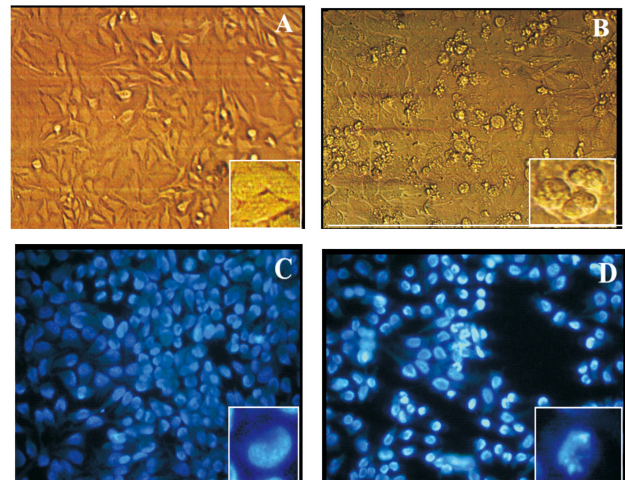
### 3.2. Cytotoxicity assays

The *in vitro* cytotoxic activity ( $IC_{50}$ ) of extracts was evaluated against cervical cells (HeLa), breast cancer cells (MCF-7), hepatocellular carcinoma cells (Hep G-2), bone sarcoma cells (MG-63) using the MTT assay. The  $IC_{50}$  values of extracts on the viability of cancer cells after 72 h of incubation are listed in Table 2. Among all *C. carandas* pomace and *L. sinensis* seed extracts exhibited significant activity against all tested cell lines with  $IC_{50}$  value ranged from 56.72 to 89.24  $\mu\text{g/mL}$ , *A. comosus* skin extract diminished the growth of all tested cell lines but did not affect bone sarcoma viability at a concentration of 100  $\mu\text{g/mL}$ . Interestingly, the cytotoxicity of this extract was most effective among all the tested extracts. *C. carandas* and *L. sinensis* seeds were effective against all tested cell lines and to some extent, also affected bone sarcoma cancer cell growth. However, the extract of *G. asiatica* pomace did not show significant effect against the tested cell lines except breast cancer cell. The skin extract of *A. heterophyllus* also diminished the viability of HeLa, MCF-7, HepG-2 cell lines besides MG-63 at the tested concentrations of extracts. *B. vulgaris* pomace did not show any significant activity against the used cell lines in this experiment.

### 3.3. Altered morphology study

Morphological alterations in the HeLa cell line were observed under the phase contrast microscope. It was found that the untreated cells exhibited normal shapes with clear outline and intact nuclei (Figure 1a). However, the growth of the extract-treated cells was obviously inhibited. Early signs of apoptosis were visible and characterized by slight blebbing followed by cell membrane destruction (Figure 1b). The extract-treated cells were observed to be rounded with loose inter-cellular connection, proliferation was inhibited and the granular content of cytoplasm increased. In order to evaluate nuclear morphological alterations and chromatin fragmentation, treated and control cells were stained with nuclear staining dye Hoechst 33342 and visualized under fluorescent microscope at 360 nm/470 nm excitation/emission. Fluorescence microscopic images of normal control cells and the cells treated with 58.6  $\mu\text{g/mL}$  of *C. carandas* pomace crude extract are shown in Figure 1c and Figure 1d. Control cells (untreated) were observed to be integrated, healthy with intact nuclei and a well defined cell

membrane that was smooth (Figure 1c), whereas the treated cells contained condensed chromatin (pyknotic nuclei) marginated into a horseshoe-shaped structure (Figure 1d). Although significant blebbing was not observed in the treated cells, however, nuclear disintegration is clear as observed in Hoechst staining (Figure 1d).



**Figure 1.** Light and fluorescent micrographs of normal and treated HeLa cells of *C. carandas* pomace crude extract.

Phase contrast microscope images: (a) Normal cells; (b) Cells treated with *C. carandas* (58.6  $\mu\text{g/mL}$ ) pomace crude extract shows early signs of apoptosis characterized by the slight blebbing followed by cell membrane destruction. Fluorescence microscope images: (c) Normal cells; (d) Cells treated with *C. carandas* (58.6  $\mu\text{g/mL}$ ) pomace crude extract, stained with Hoechst 33342. Healthy cells (c) and extremely condensed chromatin (pyknotic nuclei) marginated into a horseshoe-shaped structure (d). Magnification: 20 $\times$ .

### 3.4. Alpha-amylase inhibition assay

In this study, alpha amylase inhibition assay was measured at six different concentrations (5, 10, 25, 50, 100 and 200  $\text{mg/mL}$ ) of three different solvent extracts and inhibition was observed at all concentrations (Figure 2). Acarbose and catechin were used as positive control. The observed  $IC_{50}$  value *i.e.*, the concentration of the extracts, containing the alpha-amylase inhibitor that inhibited 50% of the enzyme activity is reported in Table 3. The results showed that all the extracts possessed significant activity excluding *A. lachoocha* ( $IC_{50}>250.00$   $\text{mg/mL}$ ) and *A. heterophyllus* ( $IC_{50}>250.00$   $\text{mg/mL}$ ). Among all, *C. carandas* showed the highest activity ( $IC_{50}=29.66$   $\text{mg/mL}$ ) followed by *L. sinensis* ( $IC_{50}=32.1$   $\text{mg/mL}$ ), *G. asiatica* ( $IC_{50}=45.70$   $\text{mg/mL}$ ), *A. comosus* ( $IC_{50}=58.40$   $\text{mg/mL}$ ) and *B. vulgaris* ( $IC_{50}=62.40$   $\text{mg/mL}$ ) in methanol extract, respectively. Similarly, the  $IC_{50}$  values of acarbose and catechin were found to be 0.39  $\mu\text{g/mL}$  and 707.80  $\mu\text{g/mL}$ , respectively.

**Table 2**

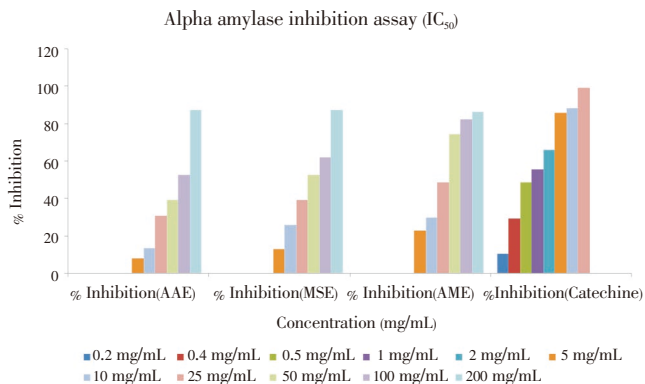
Cytotoxicity of the fruit extracts [ $IC_{50}$  ( $\mu\text{g/mL}$ )] (mean $\pm$ SD,  $n=3$ ).

Fruit residue	Cervical (Hela) cell line	Breast cancer (MCF-7) cell line	Hepatocellular carcinoma (HepG-2) cell line	Bone sarcoma (MG-63) cell line
<i>C. carandas</i>	58.62 $\pm$ 0.35	56.72 $\pm$ 0.59	56.81 $\pm$ 0.97	82.91 $\pm$ 79.00
<i>A. comosus</i>	53.41 $\pm$ 0.32	50.82 $\pm$ 0.36	51.87 $\pm$ 0.29	>100.00
<i>A. lachoocha</i>	84.64 $\pm$ 0.41	82.23 $\pm$ 0.51	83.73 $\pm$ 0.16	>250.00
<i>L. sinensis</i>	64.31 $\pm$ 0.25	62.96 $\pm$ 0.82	63.75 $\pm$ 0.12	89.24 $\pm$ 0.35
<i>G. asiatica</i>	>100.00	68.91 $\pm$ 0.53	>250.00	–
<i>B. vulgaris</i>	>100.00	–	>100.00	–
<i>A. heterophyllus</i>	67.42 $\pm$ 0.57	64.51 $\pm$ 0.23	72.81 $\pm$ 0.21	>100.00

**Table 3**  
Antidiabetic activity of the fruit extracts [IC<sub>50</sub> (mg/mL)].

Fruit residue	AME	MSE	AAE
<i>C. carandas</i>	29.66	39.65	79.31
<i>A. comosus</i>	58.40	92.70	151.60
<i>A. lachoocha</i>	>250.00	>250.00	>250.00
<i>L. sinensis</i>	32.10	53.50	84.60
<i>G. asiatica</i>	45.70	85.20	138.10
<i>B. vulgaris</i>	62.40	94.80	161.00
<i>A. heterophyllus</i>	>250.00	>250.00	>250.00

AME: Aqueous methanol solvent (methanol: water, 80:20 v/v); MSE: Mix solvent (ethanol: hexane: water, 80:10:10); AAE: Aqueous acetone solvent (acetone: water, 80:20 v/v); Values are mean±SD; n=3.



**Figure 2.** Alpha amylase inhibition of *C. carandas* in AME extract.

#### 4. Discussion

Crude phytochemicals provides a tremendous reservoir of various potential therapeutic properties[13]. In this study, phytochemical analysis showed that these plant residues contained abundant amount of phytoconstituents e.g., alkaloids, tannins, saponins and flavonoid, which are alleged to have antioxidant activity, and various biological functions may originate from this property[1,14]. A good positive correlation was found between tannin content and cytotoxicity assay ( $r \geq 0.80$ ,  $P \leq 0.05$ ). Research shows that saponins and tannins are known for a wide range of pharmacological effects like anti-inflammatory, antimicrobial, antispasmodic, antidiabetic, anticancer, anticonvulsant and cytotoxic activities[15]. Significant correlation ( $r \geq 0.91$ ,  $P \leq 0.05$ ) was also found between saponins content and cytotoxicity assay. This is in close conformity with the findings that saponins have been found to possess significant anti-cancer properties[16]. Niaz *et al.* reported that the presence of saponins can support its anti-nutritional activities including the reduction of cholesterol and glucose in the gut through intra luminal physicochemical interaction[17]. Similarly, alkaloids are abundant secondary metabolites found in plants and represent one of the most widespread classes of compound endowed with multiple, varied pharmacological properties including cancer[18]. Alkaloid plants have been used

in a long time with a wide range of medical activities including antihypertensive, anti-inflammatory, antioxidant, antidepressant, anticancer, antidiarrhoeal, hepatoprotective and antimicrobial. Recent study has thrown light on antidiabetic and hypolipidemic activities of alkaloids[19]. In this study, a moderate correlation was found between total alkaloids and cytotoxicity assay ( $r \geq 0.65$ ,  $P \leq 0.05$ ). These phytoconstituents may be responsible for antidiabetic and anticancer activity of these plant residues. In the previous literature, Shailendra carried out the preliminary phytochemical study of methanol extract of *A. lachoocha* fruit pericarp and reported for the presence of tannins and alkaloids[20]. In another study, alkaloid, saponin and tannins were screened in the leaf and stem extract of *C. carandas*[21]. All the prior phytochemical literature is available only on the qualitative screening based analysis. A few studies exist for the quantitative phytochemical analysis. These phytochemicals of various plant extracts have been of great interest in both research and industry, because the possible use as natural additives emerged from a growing tendency to replace synthetic therapeutic agent with natural ones[22]. Clinical studies have provided evidence of a potential role of flavonoid in multiple health benefits. Significantly good amount of flavonoid suggests that these plant residues may be used for management of chronological diseases and oxidative stress. Flavonoid reduces the risk of estrogen-induced cancers by interfering with the enzymes that produce estrogen. For example, flavonoid inhibits estrogen synthetase, an enzyme that binds estrogen to receptors in several organs. Flavonoid significantly inhibits lysosomal enzyme secretion and arachidonic acid releases from membranes by inhibiting lipoxygenase, cyclooxygenase and phospholipase A2[23]. In the earlier study by Vaghasiya, flavonoid content was recorded as 27.5 and 28.65 mg/g in *C. carandas* leaf and stem extract, respectively[21]. Additionally, Prakash found flavonoid content in methanol fruit extract of *C. carandas* as (2.92±0.03) rutin equivalent mg/g extract[24]. Flavonoid content was as (20.7±1.21) mg/g as CE in *A. heterophyllus* skin powder in this experiment, which was higher than the earlier report in the result of the edible part (1.20 mg of rutin equivalent/g)[25].

In the earlier study, anticancer activity of aqueous fruit extract of *G. asiatica* was studied by using MTT assay towards cell line NCLH522, HEK 293, HeLa, MCF 7 and HEP-2. The IC<sub>50</sub> value (mg/mL) was reported as 59.03, 53.88, >100, 58.65 and 50.31, respectively[26]. Results found in our study were significantly higher than the previous report. In another study of *A. comosus* (fruit peel) extract was tested by Adoum using brine shrimp test assay and reported that LC<sub>50</sub> was to be >1000 µg/mL towards ethanol, chloroform, water and ethyl acetone fractions[27]. Fairly good cytotoxic effect of these plant residues showed that not only edible part but also by-products exhibit a good source of anticarcinogenic agent.

A good positive correlation between alpha amylase inhibition assay and flavonoid content ( $r \geq 0.87$ ,  $P \leq 0.05$ ) showed that flavonoid compound might be responsible for the alpha amylase inhibition property of these residues. This agreed with the findings of Elena that flavonoids are promising candidate for the controlling of the digestion of starch and postprandial glycemia<sup>[28]</sup>. It has been evident that phenolic and other phytochemicals play a role in mediating amylase inhibition and therefore have potential to contribute to the management of type 2 diabetes<sup>[12]</sup>. Alpha amylase enzyme is known as one of the key enzyme in human digestive system to degrade starch to monosaccharide and cause the rise of blood glucose<sup>[29]</sup>. Amylase acts upon large polysaccharides (starch) at internal bonds. Natural amylase inhibitors offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia by ultimately decreasing glucose released from starch. Different researches for antidiabetic potential were previously carried out on different parts of the plants, which were selected in this study. Literature shows that *C. carandas* is an herbal plant and is used for the treatment of epilepsy, malaria, fever, dysentery and diabetes *etc.* Mizanur determined antihyperglycemic activity of *C. carandas* methanol leaves extract through oral glucose tolerance tests. It showed dose-dependent reductions in serum glucose levels when administered to glucose-loaded mice at doses of 50, 100, 200 and 400 mg/kg body weight<sup>[30]</sup>. In addition, it was also found that unripe *C. carandas* fruit methanol extract and its ethyl acetate soluble fraction had significantly lowered the blood glucose levels by 48% ( $P < 0.001$ ) and 64.5% ( $P < 0.001$ ) respectively at a dose level of 400 mg/kg per oral after 24 h as compared to diabetic control<sup>[24]</sup>. In another study of *A. heterophyllum* leaves extracts, it was significantly improved that glucose tolerance was observed by Fernando in the normal subjects and the diabetic patients when investigated at oral doses equivalent to 20 g/kg of starting material<sup>[31]</sup>. Hypoglycemic action was also carried out by Chandrika and it was found that flavonoid fraction of *A. heterophyllum* leaf effect (49%) was higher than that of tolbutamide (27%), (a sulphonyl urea drug) commonly used for the treatment of hyperglycemia<sup>[32]</sup>. But in this study, no significant alpha amylase inhibition was found at the concentration of 250 mg/mL in *A. heterophyllum* skin extract. Litchi seeds have been found as a great potential application in the treatment of diabetes and used in Chinese medicines. The aqueous extract of litchi seeds had lowered the blood glucose levels in normal and alloxan-reduced diabetic mice and the hypoglycemic effect was nearly equals to glibenclamide and phenformin<sup>[33]</sup>. Nowadays, litchi seeds have been developed into medicinal tablets to treat diabetes, especially diabetes in clinic in China.

The inhibition of alpha amylase has been suggested as a strategy for diabetes and obesity management by reducing

sugar levels in blood. Though, modern medicines have resulted in the initiation of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications. In relation to plants, excluding a few studies<sup>[34]</sup>, most of the studies have not assessed the impact of different plants on the course of diabetic complications. From the earlier studied results, it can be seen that there are no reports on antidiabetic properties by alpha amylase inhibition assay on the selected plants. This is potentially an important observation to reduce the side effects of excessive high alpha amylase inhibition of drug treatments. The results revealed that these plant residues might possibly reduce the blood glucose level in diabetes patients as well as possess significant cytotoxic effect.

In conclusion, the result of this study shows that these fruit residues offer a safe method or supplement treatment strategy to control diabetes and cancer through its alpha amylase inhibition and anticancer effect in addition to beneficial nutritional effects. Therefore, their derived products may be an important source of nutrition and therapy. Furthermore, the utilization of these residues would also add some economic value.

### Conflict of interest statement

We declare that we have no conflict of interest.

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