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Antioxidant, Antidiabetic and Antiproliferative Activities of Juice Extract and Residue Powder from Karanda (*Carissa carandas* L.)

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Abstract

Karanda (*Carissa carandas* L.) fruit has been extensively studied for various applications and used in making a variety of food and nonfood products due to the phytochemical components. In this study, the phenolic content, antioxidant activity, anti-diabetes and antiproliferative activities of the freeze-dried crude karanda juice powder (KJP) and residue powder (KRP) were investigated. The findings revealed a different yield: KJP obtained 7.76 g/100g and KRP obtained 9.89 g/100g ($p \leq 0.05$). In addition, the KRP had less moisture and water activity (a_w) than the KJP, which may be associated with the sugar residue content. The KJP revealed that the total phenolic and anthocyanin content were 58.02 mg GAE/g and 0.17 mg/g, respectively. Furthermore, antioxidation activity using the DPPH and FRAP assays demonstrated 38.86 mg GAE/g and 9.08 µmol $FeSO_4$/g, respectively. In terms of anti-diabetes activity, the crude extract of KJP inhibited α-amylase activity (98.70%) and α-glucosidase (50.86%), whereas KRP inhibited α-amylase activity (50.34%) and α-glucosidase (18.30%) when compared to acarbose. The KRP and KJP were both non-cytotoxic and inhibited the growth of human colorectal adenocarcinoma (Caco-2) cells and human liver hepatocellular carcinoma (HepG2), with IC_{50} values of 97.26 µg/mL and 87.06 µg/mL, respectively. Both the KJP and the KRP freeze-dried showed promise as dietary supplements or functional foods for diabetes and cancer prevention.

Introduction

Carissa carandas L. is a member of the Apocynaceae family that is commonly grown in Malaysia, Thailand, India, Philippines, Cambodia, Vietnam and East Africa. It is known to possess an extensive range of phytochemicals in its fruits that impact enormous medicinal value including vitamin C, anthocyanin, flavonoids, glycosides, alkaloids, carbohydrates, sterols, terpenoids, tannins and saponins

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(Monika et al., 2022; Bhaskar & Balakrishnan, 2009). The unripe fruit has abundant quantities of ferulic acid (80.04 mg/g) and sinapic acid (16.29 mg/g) (Kubola, et al., 2011). These active compounds have medical and pharmacological value as anti-inflammatory, antipyretic, antioxidant, anti-cancer and antidiabetic and antiviral property. (Iyer & Dubash, 2006; Begum et al., 2013; Sudjaroen & Suwannahong, 2017).

Particularly, the fully ripened fruit turns a purple color and contains anthocyanins such as cyanidin-3-o-rhamnoglucoside (Iyer & Dubash, 2006), cyanidin-3-galactoside, cyanidin-3-glucoside (Rohan et al., 2018) and simple phenolics such as hydrocinnamic acid. Anthocyanins, which are red, purple and blue in color, have been classified as powerful functional foods with great potential as food colorants because of their high pigment content and low toxicity, which have increased interest in their development to replace synthetic colorants (Brouillard, 1982). Anthocyanins are used as food coloring in confections, jellies, jams, preserves and frozen desserts, among others (Birks, 1999). Numerous studies have documented the health benefits of anthocyanins (Chen et al., 2005; Wang & Stoner, 2008). Antioxidants are essential substances that have the ability to protect the human body from free radical damage (Madhuri et al., 2019). Antioxidants can help prevent the formation of free radicals by scavenging them or promoting their decomposition and suppression.

Previously, karanda juice was extracted and processed as a functional beverage, jelly and pudding. The remaining fruit, seed and peel after juice extraction were not used. To maximize the value of the source material and reduce industrial waste, these residues might be employed as dietary supplements or functional foods. Freeze-drying is commonly employed for the long-term storage and preservation of foods. This is one of the most exciting and useful procedures for drying food since it protects and retains the characteristics of fresh samples, such as shape, appearance, flavor, nutrients, porosity, color, flavor, texture and biological activity. Consequently, the goals of this study were to evaluate the chemical and biological analysis of fruit extracts and residue powder from *C. carandas* that can be further used as functional foods.

Materials and methods

1. Sample collection and preparation

Fully ripened dark purple to black karanda (*Carissa carandas* L.) fruits were harvested from Tha Maka Kanchanaburi Province, Thailand between July to August 2021. One kilogram of fruits was washed with water and soaked in water for 10 min. The fruits were then placed in a plastic basket and rinsed under running tap water for 5 min. Next, the fruits were separated by juice extractor (Panasonic MJ-DJ01, Japan) into juice extract and residue. The juice extract was concentrated again using a rotary evaporator (BUCHI R300, Japan) at 60°C until 18°Brix solid and then freeze-dried (EPSILON FD 60, Thailand) as juice extract powder (KJP). The residue (containing seed and pulp) after extraction (KRP) was directly pre-chilled at -20°C before freeze drying. The freeze drying process consisted of 1) pre-freezing process at -25°C for 180 min, 2) primary drying at -15°C to 35°C for 480 min and 3) secondary drying at 45°C to 50°C for 30 to 60 min. After that, samples (500 g) were ground into a fine powder using a dry powder herb grinder with high blade rotation at 25000-32000 rpm, 2500 watts and 220 volts. Next, the samples were sifted through a 100 mesh stainless steel sieve and packed into aluminum foil bags for analysis.

2. Determination of yield, physical and chemical characteristics

The karanda powder was calculated by weighing the fruit, freezing-drying fruit extract and residue and then measuring the net weight and percentage of the by-product. After that, the color of the KJP and KRP were measured using a colorimeter (Minolta Colorimeter, CR 400, Japan) in the CIE system (L*a*b*). The pH values were determined by a pH meter (Mettler-Toledo, USA). Finally, the moisture content was determined following the AOAC method (AOAC, 2000). Water activity (a_w) was measured using a water activity meter (Smart Water Activity Meter model AQUALAB 4 TE, USA).

3. Preparation and analysis of crude extract from KJP and KRP

One gram of sample was soaked in 50 mL of 40% ethanol in water and then shaken in an ultrasonic bath (Wiggens UE03SFD, Germany) at 55°C for 60 min. The extract was filtered through No. 4 Whatman filter paper.

3.1 Determination of total phenolic content using by Folin-Ciocalteu reagent

Total phenolic content was determined by the Folin-Ciocalteu reagent following Gorinstein et al., (2001) with slight modifications. First, 0.75 mL of 10-fold diluted Folin-Ciocalteu reagent and 100 mL of the ethanolic extract were placed in a test tube. The mixture

was mixed and allowed to stand at room temperature for 5 min. Then, 0.75 mL of a 6% (w/v) sodium carbonate solution was added. The mixture was homogenized and allowed to stand at room temperature for 90 min.Total phenolic content was determined at 725 nm using a spectrophotometer (Thermo scienctific Evaluation 201, USA). A standard calibration curve was plotted using ascorbic acid at a concentration of 0.02-0.1 mg/mL. Total phenolic content was expressed as gallic acid equivalent (GAE) mg/g (Pewlong et al., 2014).

3.2 Determination of total anthocyanin content using the pH-differential spectrophotometric method

Each sample was diluted with two dilutions including potassium chloride (0.025 M) at pH 1.0 and sodium acetate (0.4 M) at pH 4.5. Samples were allowed to equilibrate for 15 min before detection by a spectrophotometer (Thermo scienctific Evaluation 201, USA), with absorbance measured at 520 nm ($A_{520\,nm}$) and 700 nm ($A_{700\,nm}$). The difference in the absorbance at differing pH values and wavelengths was calculated as:

$$A = (A_{520\,nm} - A_{700\,nm})pH1.0 - (A_{520\,nm} - A_{700\,nm})\ pH4.5$$

The concentration of total anthocyanin pigments was calculated as:

$$\text{Total anthocyanin content (mg/L)} = (A \times MW \times DF \times 1000)/\varepsilon x1$$

Where MW is the molecular weight, DF is the dilution factor, ε is the molar absorptivity and 1 is for 1 cm path length. The molecular weight (MW = 449.2 g mol^{-1}) and molar absorptivity (ε = 26,900 Lcm^{-1} mol^{-1}) of cyanidin-3-glucoside were used (Wrolstad et al., 2005).

3.3 Antioxidant assays; DPPH radical scavenging activity

The DPPH radical scavenging activity was performed as previously described by Khattak et al. (2008). A measurement of antioxidant were 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. One hundred microliter of each extract sample was added to 900 µL of DPPH in method solution (150 µM) in a test tube and shaken vigorously. After incubation at room temperature (25°C) for 15 min in darkness, the absorbance of sample solution was determined at 517 nm. The free radical scavenging activity was expressed as mg ascorbic acid equivalent from 0.01 to 0.1 mg/mL, calculated by standard curve (R^2 = 0.9925).

3.4 Antioxidant assays; Ferric reduction antioxidant potential (FRAP)

Ferric reduction antioxidant potential of KJP and KRP were determined according to the method described by Benzei & Strain. (1996). The reaction reduced the ferric ion (Fe_3^+) to the ferrous ion (Fe_2^+) that changed the color of yellow complex (Fe_3^+/TPTZ) to blue complex (Fe_2^+/TPTZ). For analysis the FRAP reagent was prepared by mixing 16.7 mM $FeCl_3.6H_2O$ and 8.3 mM 2,4,6-tripyridyl-s-striazine (TPTZ) with 250 mM acetate buffer, pH 3.6. A total reaction of 5.25 mL consist of 75 µL sample, 225 µL of distilled water and added to 2.25 mL of freshly prepared FRAP reagent in a test tube. The solution was incubated at room temperature (25°C) throughout the reaction. The absorbance was read at 596 nm using a spectrophotometer (Thermo Scienctific Evaluation 201, USA) immediately and 30 min after mixing. The antioxidant potential of the samples was analyzed based on a standard curve plotted using $FeSO_4.7H_2O$ at different concentrations from 400 to 2,000 µM. The amount of antioxidant potential was expressed in µmol$FeSO_4$/g.

4. Antidiabetic activity assay

Crude extract and residue powder were extracted before determining α-amylase and α-glucosidase inhibition. Karanda extracts were prepared by aqueous extraction of 50 g of powder stirred in 200 mL of distilled water and 95% ethanol solution. The mixture was placed in a rotary shaker for 4 and 24 hr, followed by centrifugation at 8000 rpm for 10 min. The resultant supernatant was filtered using Whatman No.4 filter paper.

4.1 α-Amylase inhibitory assay

The determination of α-amylase inhibition was carried out by quantifying the reducing sugar (glucose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of glucose liberate. A modified dinitrosalicylic acid (DNS) method was adopted to estimate the glucose equivalent (Bhutkar & Bhise, 2012). An aliquot 1 mL of the aqueous extract of the selected karanda extracts was pre-incubated with α-amylase 1 unit/mL for 30 min and then 1 mL (1% w/v) starch solution was added. The mixture was further incubated at 37°C for 10 min. The reaction was stopped by adding 1 mL DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3,5-dinitrosalicylic acid solution) and the contents were heated in a boiling water bath for 5 min. A blank was prepared without plant extracts and another without the amylase enzyme, replaced by equal

quantities of buffer (20 mM sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20°C). The absorbance was measured at 540 nm. Reducing sugar released from the starch was estimated as glucose equivalent from a standard graph. Acarbose was used as the positive control as inhibitor. The extracts were diluted in a buffer to give final concentrations of 5 mg/mL, 7 mg/mL and 9 mg/mL. The anti-diabetic activity was determined by the inhibition of α-amylase, expressed as percentage inhibition and calculated by the following equations:

% Inhibition = (Glucose) test / (Glucose) control x 100

4.2 α-Glucosidase inhibitory assay

Inhibition of α-glucosidase activity was determined using the modified published method (Berna et al., 2012). One milligram of α-glucosidase (Sigma-Aldrich, USA) was dissolved in 100 mL of phosphate buffer (pH 6.8) containing 200 mg of bovine serum albumin (Merck, Germany). The reaction mixture consisting of 10 µL of sample at varying concentrations (0.52 to 33 µg/mL) was premixed with 490 µL phosphate buffer pH 6.8 and 250 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) (Sigma-Aldrich, USA) and preincubated at 37°C for 5 min. Then 250 µL α-glucosidase (0.15 unit/mL) was added and incubated at 37°C for 15 min. The reaction was terminated by addition of 2000 µL Na_2CO_3 200 mM. The α-glucosidase activity was determined at 400 nm on spectrophotometer UV-Vis (Shimadzu 265, Japan) by measuring the quantity of *p*-nitrophenol released from *p*-NPG. Acarbose was used as the positive control of α-glucosidase inhibitor.

5. Cytotoxicity and cell viability assay using MTT assay

The *in vitro* cytotoxicity evaluation was performed using cancerous cell lines and normal cell lines. Human colorectal adenocarcinoma (Caco-2) cells and human liver hepatocellular carcinoma (HepG2) cells, as well as normal human fibroblast (BJ) cells, were used to determine the cytotoxicity of the samples. The cancer cells were seeded in a 96-well plate at a density of 2 x 10^4 cells/well in complete medium. Caco-2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), which contains 10% v/v heat-inactivated fetal bovine serum (FBS) and 1% v/v penicillin-streptomycin. HepG2 cells were cultured in DMEM containing 10% v/v FBS and 1% v/v penicillin-streptomycin. The normal BJ cells were seeded in a 96-well plate at a density of 3 x 10^4 cells/well in minimum essential medium (MEM) supplemented with 10% v/v heat-inactivated fetal bovine serum and 1% v/v penicillin-streptomycin. All seeded cells were incubated at 37°C in a humidified air atmosphere containing 5% (v/v) CO_2. After seeding for 24 hr, the cells were washed with serum free medium and incubated with the samples at concentrations of 1, 5, 10, 20, 50, 100 and 200 µg/mL. The final concentration of dimethyl sulfoxide (DMSO) in the culture medium of each treatment group was 0.5% (v/v). Cells in the serum free medium containing 0.5% (v/v) DMSO were used as the control. After 24 hr, the cells were washed with phosphate buffered saline (PBS) before being incubated for 4 hrs with a solution of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (final concentration 0.5 mg/mL in PBS). Finally, the medium was removed and DMSO was added to each well for dissolving formazan crystals and the absorbance at 540 nm was measured using a microplate reader. The experiment was performed with four replicates (Weerawatanakorn & Pan, 2017). Phytochemical components of *Carissa carandas* and the inhibitory effects of fruit juice on inducible nitric oxide synthase and cyclooxygenase-2.

6. Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD) of three replicates, with data analyzed using the SPSS statistical package (SPSS, version 26.0, Chicago, IL, USA). The data was analyzed using t-test to evaluated the differences in means between KJP and KRP. The *P*-value was used to determine the level of significance at $\alpha \leq 0.05$.

Results and discussion

1. Yield of Karanda powder, moisture content, water activity and color

The karanda juice powder (KJP) and karanda residue powder (KRP) were effectively freeze-dried into a powder. The color characterisation, mechanical characteristics, porosity and water content of the freeze-dried KJP and KRP were assessed through experimentation. Traditional methods, such as sun drying, tray drying and spray drying, resulted in fewer modifications to the active constituents. After freeze-drying, the yields of KRP and KJP were 9.89 and 7.76 g/100 g, respectively. 45% of the residue after extraction yielded fresh fruit, which was less than the amount of fruit extracted (55%). Due to the extract's high water and

acidic content, the freeze-drying procedure is more complex and susceptible to moisture absorption. The KRP had 5.85% moisture, but the KJP contained 12.96% because the acidity of the KJP was a charged material that bound to water and moisture in the environment, as shown in Table 1. The KRP and KJP had water activity (a_w) values of 0.26 and 0.66, respectively.

The CIE (Commission International de L'Eclairage) or L* a* b* values of the two samples were different ($p \leq 0.05$). The KRP had a higher L* value due to its dispersion, flesh, peel and seeds that made the color lighter. The KJP was a combination of karanda flesh and juice after extraction, with further evaporation at 60°C. This process turned the extracted juice brown due to the browning reaction, which darkened the freeze-dried powder. The a* value of the KRP was higher than that of the KJP, at 34.09 and 30.02, respectively. Significantly water-soluble flavonoid compounds, such as anthocyanins in Karanda fruit, gave the KRP purple and red colors that were unstable at high heat. Before being freeze-dried, the two samples were heated in different ways during the preparation process. This caused the anthocyanin in the KJP sample to change color. The b* of the KRP showed a higher yellow value than the KJP at 16.11 and 12.08, respectively. The KJP became dark-purple and darker than the KRP, which was light purple.

2. Determination of total phenolic and anthocyanin content

The total phenolic content analysis revealed that the freeze-dried KJP had a total phenolic content of 69.68 mgGAE/g, which was higher than the KRP total phenolic content of 58.02 mgGAE/g, indicating that the flesh of the karadas fruit had a high total phenolic content. The KJP used in the extraction contained a mixture of flesh and juice. However, the KRP was a combination of the seeds and fruit pulp, which have accumulated various minerals and the amount of some bioactive compounds less than the KJP. According to Table 1, the total anthocyanin content of KJP was 0.13 mg/g, while the KRP contained 0.17 mg/g ($p \leq 0.05$). Pewlong et al. (2014) discovered that the total phenolic content in whole ripe karanda fruit was 4.67 mgGAE/g, which was the highest when compared to immature fruit. The total anthocyanin content was highest when the fruit was ripe, until it turned dark purple to black. Moreover, the KJP contained higher total phenolic content than KRP ($p \leq 0.05$). The KJP had higher karanda juice concentration and flesh than the KRP that included seeds. The KJP and KRP had total phenolic content of 67.68 and 58.02 mgGAE/g, respectively. Results concurred with Pewlong et al. (2014).

The KRP had 0.17 mg/g of total anthocyanin, while the KJP had 0.13 mg/g ($p \leq 0.05$) (Table 1). Purple berries contain glucose-conjugated anthocyanin. Acylated and non-acylated anthocyanins exist. Glucose, lactose, lutinoes and rhamnose are non-acylated anthocyanins, while acylation of glucose and acids such as coumaric, caffeic, malic and acetic generates esterification at the 3rd or 5th positions (Ambika et al., 2015). Acid bonding facilitate anthocyanin stability, which disintegrates at 100°C in mild acid solvents of pH 1– 4. The KRP had more anthocyanin than the KJP after evaporation. The concentration process at 60°C for 1 hr resulted in anthocyanin release due to the long time taken for the thermal process.

Table 1 Physicochemical properties of KRP and KJP

Properties	KRP	KJP
Yield (g/100 g)	9.89±0.15[a]	7.76±0.05[b]
Moisture content (%)	5.58±0.35[b]	12.96±0.23[a]
a_w	0.26±0.01[b]	0.66±0.05[a]
Color		
L*	9.16±0.05[a]	6.23±0.07[b]
a*	34.09±0.18[a]	30.02±0.22[b]
b*	16.11±0.04[a]	12.08±0.44[b]
Total phenolic content (mgGAE/g)	58.02 ±0.95[b]	69.68 ±0.74[a]
Total anthocyanin content (mg/g)	0.17±0.31[a]	0.13±0.85[b]

Remark: Values are expressed as mean ± SD of triplicates. Different letters in the same row indicate statistically significant difference ($p \leq 0.05$)

3. Antioxidant activity of Karanda powder

The antioxidant effects attributed to the polyphenols of plants plays a pivotal role in protecting the body from oxidative stress, diabetes, cardiovascular diseases and cancer (Madhuri et al., 2019; Weerawatanakorn & Pan, 2017). Therefore, the plant-based therapeutics is a potential alternative therapy to explore due to their reported safety and nutraceutical benefits.

To compare the total phenolics and antioxidant capacity of the KJP and KRP. The TPC (Folin Caiocalteu), FRAP and DPPH were performed. Anthocyanins have antioxidant activity and effectively prevent DPPH free radical. The KRP and KJP prevented different DPPH free radicals ($p \leq 0.05$) at 38.86 and 33.65 mg AAE/g, respectively and was also found by Khunchalee & Charoenboon (2019) (Table 2). The FRAP assay of KRP and KJP at 9.08 and 9.84 (μmol$FeSO_4$/g).

Table 2 Antioxidant Activity (DPPH assay) and FRAP of KRP and KJP (1 mg/mL)

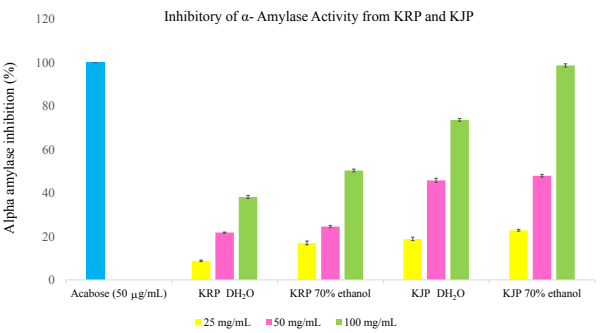
Antioxidant Activity	KRP	KJP
DPPH assay (mg AAE/g)	38.86±0.78[a]	33.65±0.43[b]
FRAP (μmol$FeSO_4$/g)[ns]	9.08±0.72	9.84±0.98

Remark: Values are expressed as mean ± SD of triplicates. Different letters in the same row indicate statistically significant differences ($p \leq 0.05$). ns = not significant ($p > 0.05$)

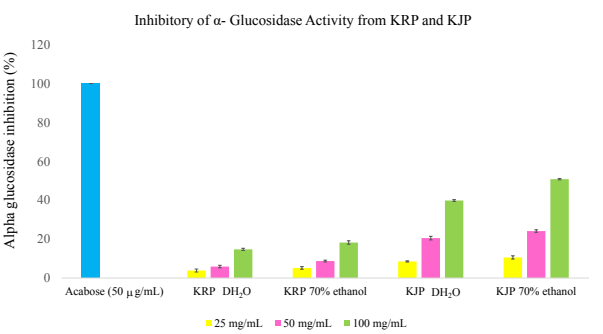
4. Assay for α-amylase and α-glucosidase Inhibition

Aside from antioxidant activity, numerous studies have shown that polyphenols have anti-hyperglycemic effects by binding to glucose transporters (Nistor et al., 2010) and competitively inhibiting digestive enzymes. Carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, digest dietary starch and convert the oligosaccharides to glucose, resulting in an increase in blood glucose levels. As a result, one of the primary approaches to managing patients' hyperglycemic conditions is to inhibit these enzyme activities. Acarbose is the most commonly used α -glucosidase inhibitory drug, but it has side effects.

In this study, α-amylase and α-glucosidase inhibition assays were carried out at three different concentrations (25, 50 and 100 mg/mL) of water and ethanol extracts for 4 hrs and inhibition was observed at all concentrations (Fig.1). Acarbose (50 μg/mL) was used as a positive control. Fig.1 shows the inhibition of α-amylase and α-glucosidase activity by the tested KRP and KJP. The results showed that all the extracts possessed significant activity. The KJP at 100 mg/mL showed the highest activity at 98.70% (ethanol extract) and 73.62% (water extract), while the KRP has less inhibitory activity at 50.34% (ethanol extract) and 38.17% (water extract). Furthermore, the inhibitory values of ethanol and water extract for α-glucosidase inhibition were 50.86% and 39.89%, respectively. The KRP also revealed less inhibitory activity at 18.30% (ethanol extract) and 14.78% (water extract). As previously described, extracts containing 50 to 100 mg/mL of karandas have a higher potential to inhibit α-amylase than α-glucosidase. Ethanol extract exhibited the highest inhibition of both enzymes, which was significantly efficacious compared to acarbose. Several studies have suggested that plant extracts can act as α-glucosidase inhibitors, implying that these extracts may be useful in the treatment of hyperglycemia. The activity of these extracts, such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides and phenolic compounds, can be attributed to their phytoconstituent content. In the present study, among all the ethanol extracts, the KJP and KRP demonstrated a remarkable inhibition potential. The polyphenols are known to interact with the enzyme through non-specific binding, leading to inhibition of enzyme activity. Polyphenols become more effective at inhibiting α-glucosidase as their molecular weight and degree of polymerization increase (Wang et al., 2013).



(A)



(B)

Fig. 1 Inhibitory of α-Amylase Activity from karanda residue powder (KRP) and karanda juice powder (KJP) (A) and Inhibitory of α-Glucosidase Activity from karanda residue powder (KRP) and karanda juice powder (KJP) (B)

5. Cytotoxicity and cell viability assay

Polyphenols can inhibit the growth of a wide range of cancer cells. The sensitivity of cancer cells to polyphenol may differ depending on the tissue from which they are derived, implying a possible link between polyphenol-induced cytotoxicity and specific cancer types (Benavente-Garcia & Castillo, 2008). Here, anticancer activity of aqueous and ethanol extract and residue of *C. carandas* was studied by using MTT assay towards cell line including human fibroblast cells, HepG2 and Caco-2. Dose response curves constructed between 10-200 μg/mL ranges. The results showed that the

incubation of KJP with HepG2 and Caco-2 significantly inhibited cell proliferation with IC_{50} values of about 87.06 and 97.26 µg/mL, respectively. Growth inhibition percentage is illustrated and statistically ($p \leq 0.05$) in Table 3 The proliferation of HepG2 was significantly inhibited by 73.7% suppression while the proliferation of Caco-2 was significantly inhibited by 77.9% using the ethanol extract at 200 µg/mL.

Moreover, the KRP also showed the inhibition with IC_{50} values of about 13.28 mg/mL with HepG2 and 11.79 mg/mL with Caco-2. Our findings demonstrated that ethanol extract and residue may contain a high chemical component compared to previous reports in which a methanolic extract of *C. carandas* fruit was also found to have antiproliferative activity against MCF-7 and HepG2 cells (Pewlong et al., 2014; Priti et al., 2014; Ondee, 2019). Fruit juice containing major phenolic compounds such as ferulic acid, rutin, cyanidin-3-glucoside, flavonoids and anthocyanins has downregulated the induction of inflammatory responses. Polyphenolic compounds such as epicatechin gallate, quercetin, kaempferol and triterpenes, i.e. carissol. The cytotoxicity stopped the cell cycle and caused apoptosis. It also caused a big drop in superoxide anion production, cell adhesion and the movement of tumor cells (Ahamad et al., 2014).

The cytotoxic effect of the extract and residues showed that not only edible parts but also by-products exhibit a good source of anticarcinogenic agents. Therefore, karanda fruit powder showed promise and could be further developed as capsule or tablet supplements or functional foods such as antioxidant tea, antidiabetic tea, or tea for the prevention of cancer.

Table 3 Antiproliferative activities from KRP (mg/mL) and KJP (µg/mL)

Cells	Antiproliferative activities (IC_{50})		Reference
	KRP	KJP	
Fibroblast cell line	85.53±0.91[b]	130.97±0.67[a]	In this study
Caco-2 cell line	11.79±0.20[b]	97.26±0.53[a]	In this study
HepG-2 cell line	13.28±0.68[b]	87.06±0.73[a]	In this study
HepG-2 cell line		56.81±0.97	Priti et al., 2014
Hela cell line		58.62±0.35	Priti et al., 2014
MCF-7 cell line		56.72±0.59	Priti et al., 2014
MG-63 cell line		82.91±0.79	Priti et al., 2014

Remark: Values are expressed as mean ± SD of triplicates. Different letters in the same row indicate significance at $p \leq 0.05$

Conclusion

Our findings demonstrated that freeze-dried karanda powder is particularly beneficial for dietary supplements and functional foods. They have a range of phytochemical, anti-diabetic and anti-proliferative activities. KRP had less total phenolic content than KJP, but it had more total anthocyanin content and showed different antioxidant activity in the DPPH and FRAP assays ($p \leq 0.05$). They also demonstrated anti-diabetic activity by inhibiting α-amylase and α-glucosidase activities. KRP and KJP have less cytotoxicity but could suppress the growth of Caco-2 and HepG2 with IC_{50} values of 11.79 and 13.28 (mg/mL) and 97.26 and 87.06 (µg/mL), respectively. Based on their biological activity, these powders have the potential to be effective antioxidant supplements for diabetes and cancer prevention.

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References

Ahamad, M.S., Siddiqui, S., Jafri, A., Ahmad, S., Afzal, M., & Arshad, M. (2014). Induction of apoptosis and antiproliferative activity of naringenin in human epidermoid carcinoma cell through ROS generation and cell cycle arrest. *PLOS ONE, 9,* e110003.

Ambika, C., Beenu, T., & Intelli, A. (2015). Influence of processing on physiochemical, Nutritional and phytochemical composition of Carissa spinarum (Karonda). Asian Journal of Pharmaceutical and Clinical Research, 8(6), 254-259.

AOAC. (2000). Official Methods of Analysis of AOAC International (17th ed.). Maryland, USA: AOAC International Gaithersburg.

Benavente-Garcia, O., & Castillo, J. (2008). Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity. Journal of Agricultural and Food Chemistry, 56(6), 185–205.

Begum, S., Saqib, A.S., & Bina, S.S. (2013). Carandinol: First isohopane triterpene from the leaves of Carissa carandas L. and its cytotoxicity against cancer cell lines. Phytochemistry Letter, 6(9), 1-5.

Benzie, L.F.F., & Strain, J.J., (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry, 239, 70-76.

Berna, E., Katrin, B., Abdul, M., Wulan, Y., Anastasia, B., & Eva, K.S. (2012). Screening of α glucosidase inhibitory activity from some plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. *Journal of Biomedicine & Biotechnology, 2012,* 1-6.

Birks, S. (1999). The potential of carrots. *Food Manufacture, 47,* 22-23.

Bhaskar, V.H., & Balakrishnan, N. (2009). Analgesic, anti-inflammatory and antipyretic activities of Pergularia demia and Carissa carandas. *DARU Journal of Pharmaceutical Sciences,17*(3), 168-174.

Bhutkar, M.A., & Bhise, S.B. (2012). In Vitro Assay of α amylase inhibitory activity of some indigenous plants. *International Journal of Chemical Sciences, 10*(1), 457-462.

Brouillard, R. (1982). Chemical structure of anthocyanins. In P. Markakis, Ed. *Anthocyanins as food colors* (pp.1-40). New York: Academic Press.

Chen, P.N., Chu, S.C., Chiou, H.L., Chiang, C.L., Yang, S.F., & Hsieh, Y.S. (2005). Cyanidin 3-glucoside and peonidin 3-glucoside inhibit tumor cell growth and induce apoptosis in vitro and suppress tumor growth in vivo. *Nutrition and Cancer, 53,* 232-243.

Itankar, P.R., Lokhande, S.J., Verma, P.R., Arora, S.K., Sahu, R.A., & Patil, A.T. (2011). Antidiabetic potential of unripe *Carissa carandas* Linn. fruit extract. *Journal of Ethnopharmacology, 135*(2), 430-433.

Iyer, C.M., & Dubash, P.J. (2006). Anthocyanin of the Karwand (Carissa carandas) and studies on its stability in model systems. *Journal of Food Science and Technology (Mysore), 30*(4), 246-248.

Khunchalee, J., & Charoenboon, P. (2019). Study of free radical scavenging, total phenolic contents and tyrosinase inhibition activity of crude extract from *Carissa carandas* Linn. *Journal of Science and Technology,11*(1), 26-34.

Khattak, K.F., Simpson, T.J., & Ihasnullah, (2008). Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella sativa seed. Food Chemistry, 110,* 967-972.

Kubola, J., Siriamornpun, S., & Meeso, N. (2011). Phytochemicals, vitamin C and sugar content of Thai wild fruits. *Food Chemistry, 126,* 972-981.

Madhuri, S., Neelagund, Dr., & Neelagund, E. (2019). Anti-oxidant, anti-diabetic activity and DNA damage inhibition activity of *carissa carandas* fruit. *International Journal of Advance Research and Development, 4*(1), 75-82.

Monika, M., Harsimrat, K.B., Gurpreet, K.D., & Poonam, A.S. (2022). Unlocking the impact of drying methods on quality attributes of an unexploited fruit, karonda (*Carissa carandas L.*): A step towards food and nutritional security. *South African Journal of Botany, 145,* 473-480.

Nistor, L.A., Martineau, L.C., Benhaddou-Andaloussi, A., Arnason, J.T., Lévy, É., & Haddad, P.S. (2010). Inhibition of intestinal glucose absorption by anti-diabetic medicinal plants derived from the James Bay Cree traditional pharmacopeia. *Journal of Ethnopharmacology, 132,* 473–82.

Ondee, S. (2019). Antioxidant and antiproliferative activities of *Carissa carandas* Linn. Fruits. *Thai Cancer Journal, 6,* 7–15.

Pewlong, W., Sajjabut, S., Jaruratana, Eamsiri, J., & Chookaew, S. (2014). Evaluation of antioxidant activities, anthocyanins, total phenolics, vitamin C content cytotoxicity of *Carissa carandas* Linn. *Food and Applied Bioscience, 13*(1), 509-517.

Priti, G., Ira, B., Se-Kwon, K., Kumar, V., & Anubhuti, S. (2014). *In-vitro* cancer cell cytotoxicity and α amylase inhibition effect of seven tropical fruit residues. *Asian Pacific Journal of Tropical Biomedicine, 4,* 665-671.

Rohan, S., Aditi, K., Kaushik, B., & Supradip, S. (2018). Anthocyanin composition and potential bioactivity of karanda (*Carissa carandas* L.) fruit: An Indian source of biocolorant. *Food Science and Technology, 93,* 673-678.

Sudjaroen, Y., & Suwannahong, K. (2017). In vitro antioxidant, antibacterial, and cytotoxicity activities from Karanda (*Carissa carandas* L.) fruit extracts. *International Journal of Green Pharmacy, 11*(1), 189-193.

Wang, H., Liu, T., & Huang, D., (2013). Starch hydrolase inhibitors from edible plants. *Advances in Food and Nutrition Research, 70,* 103-36.

Wang, L.S., & Stoner, G.D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters, 269,* 281-290.

Weerawatanakorn, M., & Pan, M.H. (2017). Phytochemical components of *Carissa carandas* and the inhibitory effects of fruit juice on inducible nitric oxide synthase and cyclooxygenase-2. *Journal of Food Biochemistry, 41*(3), e12343.

Wrolstad, R.E., Durst, R.W., & Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends in Food Science and Technology, 16,* 423-428.