

In vitro antioxidant, antibacterial, and cytotoxicity activities from Karanda (*Carissa carandas* L.) fruit extracts

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Abstract

Background: *Carissa carandas* L. (Apocynaceae), commonly known as Karanda, is a widely used medicinal plant. In Thailand, Karanda fruits are favorite fruits especially at central region due to attractive shape and color with health promoting activities. **Aims:** The aims of the study were to evaluate its antibacterial and antioxidant activities. Furthermore, cytotoxicity of ripped Karanda fruit extracts was also evaluated with normal cells to claim for health safety. **Materials and Methods:** Total phenolic content (TPC) and antioxidant activity of dichloromethane (KD) and methanol (KM) extracts were performed by of Folin–Ciocalteu reagent; 2, 2-diphenyl-1-picrylhydrazyl radical, 2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) cation radical scavenging assays and oxygen radical absorbance capacity. Antibacterial tests against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterococcus faecalis* were performed according to Clinical and Laboratory Standards Institute. Cytotoxicity of KD and KM were performed by resazurin microplate assay and used human dermal fibroblasts-neonatal (HDFn-neonatal) dermal fibroblast and Vero cells as normal cells. **Results:** KD and KM were contained TPC = 5.1 ± 0.5 and 19.4 ± 0.9 mg of gallic acid equivalent of extract, respectively. Antioxidant activities of KD and KM were 90-110 and 130-200 μmol Trolox equivalent antioxidant capacity, respectively. KD and KM were inhibited all tested bacteria at minimum inhibition concentration = 12.5-50 and 25-50 mg/ml, respectively. No any cytotoxic effect of KD and KM extracts to Vero cells and HDFn-neonatal dermal fibroblast. **Conclusions:** Ripped Karanda fruits were preferable antioxidant activities rather than unripe. Ripped Karanda fruits were also possessed antibacterial activity with no cytotoxicity to normal cells.

Key words: Antibacterial, antioxidant, *Carissa carandas*, cytotoxicity, Karanda fruit

INTRODUCTION

Carissa carandas L. (Apocynaceae), commonly known as Karanda (in Thai called Namdaeng, Manaao ho, Naam khee haet), is a widely used medicinal plant. *C. carandas* is large dichotomously branched evergreen shrub with short stem and strong thorn in pairs. This species is a rank-growing, straggly, woody, climbing shrub, usually growing to 10 or 15 feet (3-5 m) high, sometimes ascending to the tops of tall trees. The fruits, leaves, barks, and roots of *C. carandas* have been used for ethnomedicine in the treatment of human diseases, such as diarrhea, stomachic, anorexia, intermittent fever, mouth ulcer and sore throat, syphilitic pain, burning sensation, scabies, and epilepsy.^[1,2] The prominent biological activities reported include antidiabetic,

antimicrobial, cytotoxicity, anticonvulsant, hepatoprotective, antihyperlipidemic, cardiac depressant, analgesic, anti-inflammatory, antipyretic, and antiviral properties.^[3-8] Plant yielded major bioactive compounds, i.e., alkaloids, flavonoids, saponins, large amounts of cardiac glycosides, triterpenoids, phenolic compounds, and tannins. Roots yield volatile principles including 2-acetyl phenol, lignan, carinol, sesquiterpenes (carissone, carindone), lupeol, β -sitosterol,

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16 β -hydroxybetulinic acid, α -amyrin, β -sitosterol glycoside, and des-Nmethylnoracronycine. Leaves yield triterpenoid constitutes as well as tannins. Fruits yield carisol, epimer of α -amyrin, linalool, β -caryophyllene, carissone, carissic acid, carindone, ursolic acid, carinol, ascorbic acid, lupeol, and β -sitosterol.^[9] Fruit is a rich source of iron, with a fair amount of vitamin C. Mature fruit, high in pectin, is useful for making jellies, jams, squash, syrup and chutney.^[1,9]

In Ayurveda, the unripe fruits were used as an anthelmintic, astringent, appetizer, antipyretic, antidiabetic, aphrodisiac, in biliary disorders, stomach disorders, rheumatism, and diseases of the brains.^[13,14] Other folkloric used are including root used as plaster in the Konkan to keep off flies; A concoction pounded with horse wine, lime juice, and camphor used as a remedy for itches; in Cuttack, decoction of leaves used at the commencement of remittent fevers; In Punjab and Cashmere, leaves used in diarrhea, earache, soreness of the mouth and throat, and syphilitic pains; in India, root paste used for diabetic ulcers; used for acidity, flatulence, poor digestion; juice of fresh plant used for wounds that refuse to heal; used for scabies, intestinal worms, pruritus, biliousness; in Ayurveda, stem bark used for obstinate skin diseases and the root for urinary disorders: In Bangladesh, plant parts used for treatment of epilepsy, malaria, fever, dysentery, and diabetes.^[1,12] Fruits have also been studied for its analgesic, anti-inflammatory and lipase 1 activities.^[12] Antimicrobial activities of Karanda fruit were reported that 50% ethanol extract (5 mg/ml) against *Staphylococcus aureus* (ATCC 2593) and *Escherichia coli* (ATCC 8739).^[13] Antioxidant activity of Karanda fruits was relatively high when compared to other tropical fruits.^[14] In Thailand, Karanda fruits are favorite fruits especially at central region due to attractive shape and color with health promoting activities. Many local products were made from Karanda fruits such as juice, jam, and desserts. Health beneficial biological activities of Karanda fruits on ripping state as edible Karanda fruits may need to confirm. Concerning for health promoting information of Karanda fruits are report in Thailand, this study has been designed to evaluate its antibacterial and antioxidant activities. Furthermore, cytotoxicity of Karanda fruit extracts was also evaluated with normal cells to claim for health safety.

MATERIALS AND METHODS

Sample Extraction

In this study, Amphawa Agricultural Office, Samut Songkram, Thailand, provided help to collect the sample Karanda fruits. Fruits were collect during September 2016. Plant characteristic and authenticate was done from experts at Amphawa Agricultural Office, Samut Songkram, Thailand. Fresh ripped fruits (511 g) were selected, cut in small pieces and then air dried. The air dried Karanda fruit (48 g) was grinded in powder and kept for screening biological test. Bring ground Karanda fruit for continuous extraction, then, extract with dichloromethane and methanol using Soxhlet

apparatus. Finally, get the solvent evaporated through rotary evaporation apparatus under vacuum. The extracts could also be dissolved in dimethyl sulfoxide (DMSO) and be test to the anticancer and antimicrobial activities onward.

Total Phenolic Content (TPC)

In this step, 0.1 ml of 1 mg sample extract was input into the test tube, mixing with 4.6 ml distilled water and 1 ml of Folin–Ciocalteu reagent. After that, the extract was left inside the room in room temperature for 3 min. Next, 3 ml of 2% Na_2CO_3 (w/v) was filled into the tube and shaken with the speed of 150 rpm for 2 h. Then, the extract was measured to find out the light absorbance at 760 nm by comparing with the gallic acid at the intensity of 1, 0.875, 0.75, 0.625, 0.5, 0.375, 0.25 and 0.125 mg/ml. The TPC was calculated into mg of gallic acid per g of the extract.^[15]

Assays for Antioxidant Activity

- 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay to measure the decreasing light absorbance of DPPH radical^[16] using negative control by DPPH radical (6×10^{-5} M), promptly measure at nm and positive control using vitamin C.
- 2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) cation radical scavenging assay similar to the 1st method but using ABTS radical instead^[17] and also using Trolox (soluble vitamin E) as standard substance to create standard graph (0.5-5.0 mg/ml concentration). The antioxidant activity of the ripped Karanda would be shown in Trolox equivalent antioxidant capacity (TEAC)/g of the ripped Karanda extracts.
- Oxygen radical absorbance capacity (ORAC) to measure the ability of extract to scavenge oxygen radical^[18] and the florescent signal generated by fluoresceine sodium salt (Sigma-Aldrich, Inc.) was measured by FLUOstar OPTIMA Microplate Reader (BMG) on 1 h. The antioxidant activity of ripped Karanda would be also shown in TEAC/g of the ripped Karanda extracts.

Antibacterial Activity Test

Six bacteria, *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 17978), and *Enterococcus faecalis* (ATCC 29212) were used as tested organisms. The bacterial inoculum was prepared in tryptic soy broth. The number of cfu/ml in the inoculum was determined and standardized. A 0.5 Mc Farland standard is comparable to a bacterial suspension of 10^8 cfu/ml. The minimum inhibition concentration (MIC) was determined by the broth microdilution method using 96-well microtiter plates.^[19] Concentrations range from 1.0 to 50.0 mg/mL for extracts were used in experiment against every microorganism tested. The starting solutions of extracts

were obtained by measuring off a certain quantity of extract and dissolving it in DMSO. The dilutions of extracts were prepared in Müller-Hinton broth for bacterial cultures and potato dextrose agar broth for *Candida albicans* cultures. The MIC was determined by establishing visible growth of microorganisms. The boundary dilution without any visible growth was defined as the MIC for the tested microorganism at the given concentration. As a positive control of growth inhibition, streptomycin was used and 0.5% DMSO was used as negative control.

Cytotoxicity Test

Vero cells (African green monkey) and human dermal fibroblasts, neonatal (HDFn) C-004-5C were used for evaluated cytotoxicity of karanda fruit. The resazurin microplate assay developed by O'Brien *et al.*^[20] was performed for anticancer test. In brief, the cells were cultured in proper condition and diluted by culture medium at $2.2-3.3 \times 10^4$ cells/ml. The next step was to add the 5% DMSO 50 μ l into cell suspension 45 μ l in the 384-well plates. Then, the extract was incubated at 37°C in the incubator which contained 5% of CO₂. After incubation (3-5 days), 12.5 μ l of resazurin (62.5 μ g/ml) was added. The incubation was continued for 4 h, then measured fluorescence signal by SpectraMax M5 multidetection microplate reader (Molecular Devices, USA) at excitation and emission wavelength of 530 and 590 nm, respectively. Dose response curve could be done in the 6th test. Three-fold serial intensity dilution and the intensity of the cell-restraint extract 50% (IC₅₀) could be calculated by SOFTMax Pro software (Molecular Devices, USA). Ellipticine was used as positive control. 0.5% DMSO and water were used as negative control.

Statistical Analyses

TPC and antioxidant values are expressed as mean \pm standard deviation. Statistical analyses were performed with the EXCEL (version 11) and SPSS (version 13) software packages, to determine independent *t*-test was test for different among mean of antioxidant values between BD and BM; and Pearson's bivariate correlation test was carried out to calculate correlation coefficients (*R*²) between the content of total phenolic and the DPPH, ABTS radical scavenging and ORAC activities. Antibacterial and cytotoxicity activity of KD and KM were described as MIC and IC₅₀.

RESULTS

It was found that Karanda fruits extracted with dichloromethane (KD) contained crude extract lower than Karanda fruits extracted with methanol (KM) by concentration = 5.1 ± 0.5 and 19.4 ± 0.9 mg of gallic acid equivalent of extract, respectively, which was contained with the TPC, which was related to potential of antioxidant activities, which were 90-110

and 130-200 μ mol TEAC for KD and KM, respectively) [Table 1]. KD and KM were inhibited all tested bacteria at MIC = 12.5-50 and 25-50 mg/ml, respectively [Table 2]. No any cytotoxic effect of KD and KM extracts to Vero cells and HDFn-neonatal dermal fibroblast [Table 3], which implied that both extracts were not harmed human normal cells.

DISCUSSION

The phytochemical screening of KD and KM were revealed the presence of phenolic compounds. This finding was correspond to previous study^[14] that report high amount of TPC contained in Karanda fruit extract (ethanol extract), and antioxidant activity was also relatively high according to DPPH, ABTS radical scavenging assays and ORAC method when compared to other tropical fruits and concluded that this fruit was contained potent phenolic compounds.^[14] The evaluation of ripening effect on fruit on phytonutrients, such as, soluble proteins, total soluble sugars, antioxidants (phenols, ascorbic acid, lycopene) and antioxidant activity (DPPH, FRAP, and NO scavenging activity) in fruits of *C. carandas* at ripening stages and results showed decrease in soluble proteins, total phenol contents, DPPH radical scavenging and NO scavenging activities, while total soluble sugars, ascorbic acid content and ferric reducing activity increased with maturity of the fruit. There was a significant increase in antioxidant lycopene in the fully ripened red *Carissa* fruit.^[21] In case of antimicrobial activities, this study was showed positive results of KD and KM along with six bacteria at MIC = 12.5-50 mg/ml, which was corresponded to the previous study was reported antibacterial activities of Karanda fruit extracted by ethanol, which revealed antimicrobial activities against *S. aureus* (ATCC 2593) and *E. coli* (ATCC 8739).^[13]

The previous studies were revealed for cytotoxicity of unripe fruits, fully-ripe fruits and leaves of Karanda extracts (40% of ethanol) were inhibited HepG2 cells^[22] and cytotoxicity of leaf extract was inhibit the proliferation of human cervical cancer cells (HeLa), prostate cancer cells (PC-3), and normal mouse fibroblasts (3T3).^[2] Hence, there were concluded that cytotoxicity of leaf extract was highest at 100 μ g/ml, however, fruits (unripe and ripe) were lack of cytotoxicity to inhibit HepG2 cells at same concentration and when increase the dose of assay up to 200 μ g/ml.^[22] There may imply that KD and KM had no cytotoxic effect with normal cells same concentration as previous reports (100 μ g/ml); however, there may be cytotoxic when increase the concentration of extract. Thus, further studies will be conducted to determine the response of normal cells, as well as cell line, with higher concentrations of the extract should be considered. The variation of plant harvest is also important to concern that Karanda fruits were in unripe or ripe stage and result interpretation also needs to consider such as method of biological assay, solubility of extract and assay solution, units of extract, and cutoff concentration of method.

Table 1: TPC and antioxidant activities of ripped KD and KM

Extract**	TPC (mg GAE)	DPPH• (μmol TEAC)	ABTS• (μmol TEAC)	ORAC (μmol TEAC)	R ²
KD	5.1±0.5	90.2±4.6	98.5±5.2	110.0±4.7	1.000*
KM	19.4±0.9	131.0±4.8	157.2±2.5	189.5±10.4	1.000*

KD: Karanda extracted with dichloromethane, KM: Karanda extracted with methanol, TPC: Total phenolic content, GAE: Gallic acid equivalent/g of extract, TEAC: Trolox equivalent antioxidant capacity/g of extract. *Correlation is significant at $P<0.01$ level, **The significant different of antioxidant values (from three methods) between KD and KM at $P<0.05$ level. ABTS: 2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid), DPPH: 2, 2-diphenyl-1-picrylhydrazyl

Table 2: The antibacterial activity of ripped KD and KM extracts against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii* and *E. faecalis*^{a,b}

Antimicrobial activity	MIC (mg/ml)		
	KD	KM	Streptomycin
<i>S. aureus</i>	50	25	13.25
<i>E. coli</i>	25	50	13.25
<i>K. pneumoniae</i>	25	25	1.95
<i>P. aeruginosa</i>	12.5	25	15.62
<i>A. baumannii</i>	12.5	25	15.62
<i>E. faecalis</i>	25	25	15.62

KD: Karanda extracted with dichloromethane, KM: Karanda extracted with methanol, MIC: Minimum inhibition concentration, *Negative control: 0.5% DMSO. *S. aureus*: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *A. baumannii*: *Acinetobacter baumannii*, *E. faecalis*: *Enterococcus faecalis*

Table 3: Cytotoxic effect of ripped KD and KM extracts against HDFn and Vero cells

Extract (100 μg/ml)	% Cytotoxicity of HDFn ^a	% Cytotoxicity of Vero ^b
KD	19.93	-21.76
KM	24.13	-17.53

Interpretation^c

% Cytotoxicity	Activity
<50	Noncytotoxic
≥50	Cytotoxic

^aIC₅₀ of positive control: Ellipticine=2.89 μg/ml, ^bIC₅₀ of positive control: Ellipticine=1.43 μg/ml; ^cNegative control: 0.5% DMSO, KD: Karanda extracted with dichloromethane, KM: Karanda extracted with methanol, HDFn: Human dermal fibroblasts, neonatal, IC50: Inhibitory concentration 50%

CONCLUSION

TPC and antioxidant activities of dichloromethane (KD) and ethanol (KM) extracts of ripped Karanda fruits were preferable rather than unripe as the previous study. Antibacterial of KD and KM against six bacteria including *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *E. faecalis*,

with MIC = 12.5-50 and 25-50 mg/ml, respectively. No cytotoxicity of KD and KM (100 mg/ml) to normal cells (Vero and HDFn cells).

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