Original Article

Phytochemical constituents, anti–microbial, anti–inflammatory and cytotoxic activities of *Carissa carandas* L. fruit and seed extracts

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Abstract

Introduction: Karandas or Namdaeng (*Carissa carandas* L.) is a fruit traditionally used to treat skin and abdominal diseases. This research was to evaluate its antibacterial, anti-inflammatory and cytotoxic properties and its chemical composition to further understand its efficacy.

Methods: Different extracts of *C. carandas* L. fruit and seed were tested for their total phenolic (TPC) and total flavonoid (TFC) with Folin–Ciocalteu reagent and aluminium chloride colorimetric method, respectively. Their antibacterial activity was tested against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Bacillus subtilis*. Their anti-inflammatory activity was determined using nitric oxide (NO) inhibitory assay in LPS-stimulated RAW 264.7 macrophage cells. Cytotoxicity of the extracts was performed by SRB assay against human amelanotic melanoma cells (C32) skin cancer cell line and the normal human keratinocyte cell line (HaCaT).

Results: All extracts were found to contain TPC in the range of 8.60 - 60.05 mg GAE/g DW and TFC in the range of 15.18 - 42.27 mg QE/g DW. The 95% ethanolic extract of dried seed showed the highest TFC at 186.62 mg QE/g DW. At the concentration of 100 µg/mL, the 95% ethanolic extract of dried unripe fruit showed the highest nitric oxide inhibition activity with the percent inhibition of 46.66±2.93%. The ethanolic extract of fresh unripe fruit showed highest antibacterial activity ($p<0.05$). The seed extracts were cytotoxic to C32 and HaCaT cells, especially the 50% ethanolic extract which showed cytotoxicity with IC_{50} <10 µg/mL.

Conclusion: The 95% ethanolic extract of dried *C. carandas* L. seed appeared to be a good source of antioxidants. All extracts showed low anti-inflammatory and antimicrobial activities but the seed extracts showed good cytotoxic activity. The seed extract should be studied further for its role in the traditional medicine.

Keywords: *Carissa carandas* L., antibacterial activity, anti-inflammatory, human amelanotic melanoma cells (C32), cytotoxicity

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Introduction

C. carandas L. belongs in the family Apocynaceae and is commonly known as Karandas in India, Namdaeng or Manao ho in Thai. It is a large evergreen shrub with strong thorns. The bark is dark brown and the stem is rich in white latex. Its oval leaves are green, and young leaves are red. Its white flower is fragrant. The unripe fruit is light-pink and becomes reddish-purple when ripes. Seeds are flat and light in color.[1] Its bark, roots, leaves and fruits have been widely used in Thai traditional medicine for treatment of human disorders such as stomachic, skin diseases, burning sensation and for its anti-microbial and antifungal properties.[2] Its use for treatments based on its anti-inflammatory, cytotoxic, antimicrobial and antiviral properties has been reported.[3, 4] In India, the unripe fruits are used as an antihelmintic, astringent snack, and appetizer.[5, 6] Using the disc diffusion method,[7] the methanol, ethyl acetate and hexane extracts and juice from the fruit (500 mg/mL) have been shown to have antimicrobial activity against *S. aureus, E. coli, K. pneumoniae, S. typhimurium* and *V. cholerae*. The methanol and dichloromethane extracts of the fruits were also shown to be effective against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 70063), *P. aeruginosa* (ATCC 27853), *A. baumanii* (ATCC 17978), and *E. faecalis* (ATCC 29212), using the broth dilution method.[2] Karandas fruits exhibited antioxidant activity at different values depending on the extraction method.[7, 8] Cytotoxicity of Karandas fruits against HeLa cells (human cervical cancer cells), MCF-7 (Human breast cancer cells), Hep G2 (Hepatocellular carcinoma cells) and MG-63 (Bone sarcoma cells) have also been reported.[9] The objective of this study was to confirm these health benefits of *C. carandas* fruits and seeds.

Materials and Methods

1. Plant materials

We collected three parts of Manao ho (*C. carandas* L.), i.e. ripe fruits, unripe fruits and seeds in May and June, 2017 from Phon Sawan district, Nakhon Phanom province, Thailand. A specimen voucher was deposited at the Faculty of Pharmaceutical Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand, with collection number SKP 072 03 03 01.

2. Preparation of plant extracts

Fresh ripe fruit, unripe fruit and seed of Manao ho were washed and dried in hot air oven at 50°C and ground into powder. Each dried powder was macerated with 95% ethanol, 50% ethanol and hot water. In addition, the flesh of ripe and unripe fruits and seeds were washed and macerated with 95% ethanol, 50% ethanol, and hot water and squeezed. The extraction methods were taken from previous studies.[10] After filtration through Whatman No. 1 filter paper, ethanol filtrates were concentrated in a rotary evaporator under vacuum at 45°C. The decocted extracts were dried in a lyophilizer. The crude extracts were kept at -20°C before use. The extraction yields are summarized in (Table 1).

3. Preparation of sample solution

Concentrated samples of 95% and 50% ethanol extracts were dissolved in sterile dimethyl sulfoxide (DMSO). Concentrated decocted extracts were dissolved in sterile distilled water.

4. Phytochemical evaluation

Total phenolic assay

Total phenolic content of the extracts was determined using the Folin–Ciocalteu method.[11] A 20 µL of each extract (1 mg/mL) and standard gallic acid (2.5 to 100 µg /mL) were added to 96-well microplate and mixed thoroughly with Folin-Ciocalteu reagent and sodium carbonate solution. The plate was allowed to stand at room temperature and the absorbance was measured at 765 nm with a UV-visible spectrophotometer. The phenolic content was expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total flavonoid assay

Total flavonoid content was measured using the aluminium chloride colorimetric method.[12] A 500 µL each of the extracts (1 mg/mL) or a quercetin standard (20 to 480 µg/mL) was added to a centrifuge tube. Then, 5% w/v sodium nitrite and 10% w/v aluminium chloride were added into the tube. Lastly, 1M sodium hydroxide was added and the final volume was made up to 1.5 mL with distilled water. During the incubation at room temperature, 20 µL of the solution was pipetted into 96-well plates. The solution absorbance was determined at 510 nm. Total flavonoid content is expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW).

5. Determination of nitric oxide (NO) production from RAW 264.7 cells

The inhibition of NO production from RAW 264.7 cells was evaluated using the following modified method.[13] RAW 264.7 cells were cultured in RPMI 1640 medium supplemented with 10% heat inactivated FBS, 10,000 units/mL penicillin, and 10,000 mg/mL streptomycin. Each of the 96 well plates was seeded with $1x10^5$ of the cultured cells/well. The plate was incubated for 24 h at 37°C in a humidified 5% CO_2. Then, the cultured cells in each well were replaced with fresh medium containing 10 ng/mL of LPS and was treated with different concentrations of the extract samples and incubated for 24 h at 37°C in 5% CO_2. The NO production was analyzed using Griess reagent. The absorbance was measured at 570 nm in a microplate reader. The cell viability was determined by the MTT assay in which MTT solution (5 mg/mL) was added to each well and incubated for 2 h at 37°C in 5% CO_2. The medium was then aspirated and added with isopropanol containing 0.04 M HCl to dissolve the formazan dye in the cells. The absorbance was measured at 570 nm. The samples were deemed cytotoxic when their % cytotoxicity was more than 30% of the control group. The % inhibition of the samples was calculated using the following equation:

$$\text{Inhibition (\%)} = \left[\frac{\text{ODcontrol - ODsample}}{\text{ODcontrol}}\right] \times 100$$

6. Cytotoxic activity

The SRB assay was performed to estimate growth inhibition by the extracts by staining total cellular protein with SRB dye using the following modified method.[14] The HaCaT cell line (normal human keratinocyte) and cancer cells and C32 cell line (human amelanotic melanoma) were used in the tests. Briefly, the cells were seeded in 96-well plate and incubated for 24 h at 37°C. Then, the cells were treated with various concentrations of the samples and incubated for 72 h in 5% CO_2 incubator. After 72 h, 100 µL of ice-cold 40% trichloroacetic acid (TCA, Aldrich Chemical) was added to fix the cells and the plate was washed with tap water and stained with SRB after which the protein-bound dye was dissolved with 10 mM Tris base solution. The optical density of the solution was measured at 492 nm using a 96-well microtiter plate reader (SLT Labinstrument, Australia). A 0.2% DMSO and medium were used as negative control. The IC_{50} values were calculated using GraphPad Prism Software (San Diego, USA). The percentage growth inhibition was calculated using the following formula:

$$\text{\% inhibition} = \left[\frac{\text{ODcontrol - ODsample}}{\text{ODcontrol}}\right] \times 100$$

7. Antimicrobial activity

Microorganisms used to determine antibacterial activities were as follows: (1) gram-positive bacteria, *B. subtilis* ATCC 6633 and *S. aureus* ATCC 25923; (2) gram-negative bacteria, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 9027 and *E. coli* ATCC 25922; (3) Yeast, *Candida albicans* ATCC 9028. The microorganisms were cultured on Nutrient Agar for bacteria and Sabouraud Dextrose Agar (Difco, USA) for yeast, and were incubated for 18 to 24 h (bacteria) or 48 h

(yeasts) at 37°C. A single colony was transferred to a tube and adjusted with sterile Mueller Hinton Broth to 0.5 McFarland standard.

A: Disc diffusion. Disc diffusion was conducted to test antimicrobial susceptibility of the extracts.[15] A 10 µL sample of each plant extract (concentration 500 mg/mL) was dropped on a sterile disc and air-dried. The bacterial culture was swabbed on Muller Hinton agar plates. The dry disc was placed on the plate surface. The standard antibiotic discs were Ampicillin 10 µg/disc and Norfloxacin 10 µg/disc. The plate was then incubated at 37°C for 18 to 24 hours depending on the species of bacteria. Then, the inhibition zone was measured using a caliper. The test was repeated in triplicate.

B: Broth microdilution method. The method for the assessment of minimum inhibitory concentration (MIC) using 96-well microplate was modified.[16] The extract was serially diluted two-fold to obtain 5 mg/mL, which served as the first concentration in the wells. An equal volume of 50 µL of bacterial culture was added to the well and incubated (24 h for bacteria and 48 h for yeast). After that, resazurin (10 µL/well) was added into each well and further incubated for 2 hours. The lowest concentration that inhibits the growth of bacteria (no change in resazurin color) was considered the MIC for the extract.

C: Minimal bactericidal concentration. The MBC is defined as the lowest concentration of an antimicrobial agent that kills > 99.9% of bacteria.[17] After testing for MIC values, the MBC was taken from the wells that showed no visible growth when streaked on agar plates and incubated 24 h for bacteria and 48 h for yeast at 37°C. The MBC was the lowest concentration of the extract where there was no visible growth on the plate.

Statistical analysis

All data are expressed as mean ± standard error of the means (SEM) from triplicate experiments. Statistical significance was determined with one-way ANOVA and the level of significance was $P < 0.05$.

Results

Total phenolic and flavonoid contents

Maximum total phenolic content (TPC) was found in the ethanolic extract FSE50 (75.35 ± 1.20 mg GAE/g DW), followed by FSW (60.05 ± 1.20 mg GAE/g DW) and DSE50 (57.83 ± 3.45 mg GAE/g DW), while minimum value was in FUSQ (8.60 ± 0.93 mg GAE/g DW). Total flavonoid content (TFC) was very high in DSE95 extract (186.62 ± 3.54 mg QE/g DW), followed by FSW (51.23 ± 1.81 mg QE/g DW) and FSE50 (42.27 ± 3.49 mg QE/g DW) and the least value was in FUSQ (5.22 ± 0.30 mg QE/g DW) (Table 2) (Figure 1).

Anti-inflammation

Anti-inflammatory activity of extracts was tested against LPS-induced NO production in RAW 264.7 cells and compared with prednisolone as a standard anti-inflammatory drug. The percentage of inhibition of all extracts at concentration 100 µg/mL are shown in (Table 2) and (Figure 2). All extracts showed inhibition of not more than 50%. The 95% ethanolic extract of dried unripe fruits (DUE95) exhibited the most inhibition followed by 95% ethanolic extract of dried ripe fruits (DRE95) with values of 46.66 ± 2.93% and 33.08 ± 1.78%, respectively. Other extracts showed less than 30% inhibition. Thus, all extracts were inactive against inflammation (IC_{50} >100 µg/mL). Cytotoxicity was assessed by MTT assay to confirm that the inhibition of NO production was not caused by cell death. Some of the extracts were slightly toxic to cells at 100 µg/mL. However, a cell viability of more than 85% was observed in every extract (data not shown).

Anti-microbial activity

The zones of inhibition diameters produced by the extracts against 5 bacteria are tabulated (Table 3). Fresh unripe *C. carandas* fruit macerated in 95% and 50% ethanol (FUE95 and FUE50) showed activity against five bacteria, i.e. *S. aureus, B. subtilis, E. coli, K. pneumoniae* and *P. aeruginosa* at the concentration of 500 mg/mL, while DUE50 and FRE95 were active against all bacteria except *K. pneumoniae*. DUE95

and FRE50 were active against 3 bacteria followed by DRE95 against *B. subtilis* and *E. coli*. The inhibition zones of the above extracts were significantly different when compared with the positive controls (Ampicillin 10 µg/disc and Norfloxacin 10 µg/disc) ($P < 0.05$). Other extracts did not show inhibition zones. The MICs of FUE95 and FUE50 were in agreement with their zones of inhibition against *B. subtilis* and *P. aeruginosa* but not the other bacteria. The MICs and MBCs were in agreement for FUE95 (*B. subtilis* and *P. aeruginosa*) and FUE50 (*P. aeruginosa*), with the concentration of 5 mg/mL for both assays. The MIC and MBC values of FUE50 for *B. subtilis* was 5 mg/mL and >5 mg/mL, respectively. No extract could inhibit *C. albicans* but Amphotericin B could at the concentration of 100 µg/mL (MIC and MBC values 0.195 and 0.0975 mg/mL, respectively).

Cytotoxicity

DSE50 showed the highest cytotoxic activity against human amelanotic melanoma cell line (C32) with IC_{50} of 5.87 ± 0.08 µg/mL and DSE50 had cytotoxicity against normal human keratinocyte cell line (HaCaT). FSE50, FSE95 and DSE95 showed cytotoxicity against C32 with IC_{50} = 6.44 ± 0.161, 7.38 ± 0.34, and 14.07 ± 2.27 µg/mL, respectively, and were also toxic to HaCaT. Aqueous seed extracts were less toxic against C32 but were toxic against normal cells (Table 2).

Discussion and Conclusion

C. carandas is a plant in family Apocynaceae found commonly in Thailand. Its fruits and seeds are widely used for traditional medicine. This study is a new report on the extracts of the fruits and seeds of this plant as different extraction methods were used. Total phenolic contents of all extracts were found to be in the range of 8.60 - 75.35 mg GAE/g DW, and total flavonoid in the range of 9.79 - 186.6 mg QE/g DW. Similar results were noted in a previous study for 40% ethanolic extract of fresh *C. carandas* fruit where total phenolic contents in the extracts of ripe and unripe fruits were reported as 4.67 and 1.25 mg GAE/g, respectively.[8] Our study showed higher TPC of 50% ethanolic extract of ripe and unripe fruit at 42.69 and 25.94 mg GAE/g DW, respectively. Prakash found flavonoid content in a methanolic extract of the fruit as 2.92 mg rutin equivalent/g extract, considerably lower than our results. This may also be due to different geographical areas where the plants were taken from.[18] Moreover, using different solvents for the extraction may affect total phenolic and flavonoid contents. Generally, phenolic compounds have redox properties, which act as antioxidants.[19] Total phenolic concentration could be used as a basis for the screening of antioxidant activity. Flavonoids are a subgroup of phenolic compounds that act as antioxidants. Antioxidant capacities of flavonoids depend on their free OH groups.[20] Therefore, it appeared that, by using our extraction methods, the extracts had higher phenolic and flavonoid contents than those previously reported.

In the present study, DUE95 at the concentration of 100 µg/mL displayed potent inhibitory activities of NO product, released in LPS-activated macrophages, at 46.66 ± 2.93%. Other reports have shown that ripe fruit juice of *C. carandas* at 500 µg/mL exhibited the inhibition of nitrite production at 29.5% as compared to our result at 8.90%.[21]

The ethanolic extracts of dried fruit appeared to be the best preparation among *C. carandas* fruit extracts. In an *in vivo* study, the methanol extract of dried fruit was reported to reduce the hind paw carrageenan -induced edema in rats when it was compared with the control group.[22] Using the disc diffusion method, antibacterial activities of the fruit extracts showed the inhibition zone diameters of 7.33 ± 0.33 to 10.00 ± 0.00 mm. Other studies have revealed that methanol, ethyl acetate and hexane extracts and juice of *C. carandas* plant at the concentration of 500 mg/mL showed inhibition zones against *S. aureus, E. coli, K. pneumoniae, S. typhimurium* and *V. cholerae* ranging from 10.57 to 21.33 mm[7], which are considerably better than our results.

In our study, the MIC and MBC of the the ethanolic extracts of the unripe fresh fruit were 5 mg/mL. A previous report has showed the MICs of methanol and chloromethane extracts of 12.5 - 50 mg/mL[2]. The MIC range in our study was higher than those in previous report where the MIC values were below 1 - 50 mg/mL, may be because the solvents used were different.[23] Thus, it appeared that not all extracts have antimicrobial properties.

An earlier report of *C. carandas* fruit extracts evaluated against human cervical cancer cells (HeLa), human breast cancer cells (MCF-7), hepatocellular carcinoma cells (Hep G2) and bone sarcoma cells (MG-63) showed IC_{50} values of 58.62 ± 0.35, 56.72 ± 0.59, 56.81 ± 0.97, and 82.91 ± 79.00 µg/mL, respectively at the exposure time of 72 h.[9] In our cytotoxicity study, the ethanolic extracts of the *C. carandas* seed were toxic against cancer and normal cells with IC_{50} < 17 µg/mL. According to the criterion of the National Cancer Institute guide-lines for extracts, an active extract has to show IC_{50} value of less than 30 µg/mL.[24] Thus, our study has otherwise shown that the extracts of *C. carandas* seed exhibited cytotoxicity against malignant melanoma cell lines.

In conclusion, our results showed that the *C. carandas* extracts exhibited low in *vitro* anti-inflammatory and antimicrobial activities. The 95% ethanolic extract of dried seed showed the highest TFC, thus it could be used as a source of antioxidants. In addition, the ethanolic extracts of the seed showed cytotoxic activity against cancer and normal skin cells (human amelanotic melanoma cells and human keratinocyte cells). Therefore, ethanolic extracts of *C. carandas* seed could be isolated for cytotoxic compounds for the treatment of skin cancer. However, these results are still preliminary and more research is needed to confirm the efficacy of this plant as a traditional medicine.

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Table 1 List of different extractions and yields of *Carissa carandas* L. extracts

Type	Part used	Extracts	Code	Yield of crude extract
Dry	Unripe fruit	95%EtOH	DUE95	11.02%
		50%EtOH	DUE50	11.84%
		Water	DUW	18.50%
	Ripe fruit	95%EtOH	DRE95	13.91%
		50%EtOH	DRE50	21.88%
		Water	DRW	22.50%
	Seed	95%EtOH	DSE95	1.73%
		50%EtOH	DSE50	6.67%
		Water	DSW	8.84%
Fresh	Unripe fruit	95%EtOH	FUE95	3.96%
		50%EtOH	FUE50	4.63%
		Water	FUW	4.46%
		Fruit juice	FUSQ	6.79%
	Ripe fruit	95%EtOH	FRE95	3.94%
		50%EtOH	FRE50	5.78%
		Water	FRW	5.63%
		Fruit juice	FRSQ	3.79%
	Seed	95%EtOH	FSE95	2.46%
		50%EtOH	FSE50	2.19%
		Water	FSW	2.62%

Table 2 Total phenolic and flavonoid contents, anti-inflammatory and cytotoxicity of *C. carandas* extracts (Mean ± SEM.) (n=3)

Code	Phenolic content (mg GAE/g DW)	Flavonoid content (mg QE/g DW)	Inhibition of NO product (%) at concentration 100 µg/mL	IC_{50} of cytotoxicity (µg/mL) C32	HaCaT
DUE95	53.16 ± 0.46	33.54 ± 4.82	42.66 ± 2.93*	>100	NT
DUE50	52.44 ± 2.28	35.98 ± 1.63	4.38 ± 0.41	>100	NT
DUW	41.44 ± 1.38	28.42 ± 3.92	15.07 ± 1.24*	>100	NT
DRE95	45.44 ± 1.42	15.18 ± 0.62	33.08 ± 1.78*	>100	NT
DRE50	33.81 ± 2.67	24.33 ± 0.15	14.47 ± 2.41*	>100	NT
DRW	28.07 ± 0.27	34.16 ± 2.27	13.60 ±2.36*	>100	NT
DSE95	26.67 ± 2.12	186.62 ± 3.54	25.59 ± 4.16*	14.07 ± 2.27	16.89 ± 1.43
DSE50	57.83 ± 3.45	42.27 ± 3.49	18.93 ± 0.71*	5.87 ± 0.08	5.81 ± 0.50
DSW	33.94 ± 4.26	22.42 ± 3.80	16.77 ± 0.42*	33.96 ± 1.36	24.36 ± 3.28
FUE95	20.17 ± 2.09	17.38 ± 2.85	6.61 ± 0.01	>100	NT
FUE50	25.94 ± 1.42	19.28 ± 1.85	12.82 ± 0.08*	>100	NT
FUW	26.25 ± 1.03	26.03 ± 2.28	6.88 ± 1.93	>100	NT
FUSQ	8.60 ± 0.93	5.22 ± 0.30	14.94 ± 3.34*	>100	NT
FRE95	40.05 ± 0.54	16.27± 0.70	18.90 ± 4.33*	>100	NT
FRE50	42.69 ± 2.07	35.86 ± 3.67	13.02 ± 2.17*	>100	NT
FRW	37.37 ± 0.50	36.67 ± 1.43	8.44 ± 1.72	>100	NT
FRSQ	14.70 ± 1.67	9.79 ± 2.30	8.90 ± 0.26	>100	NT
FSE95	35.99 ± 3.48	22.76 ± 0.97	19.50 ± 3.09*	7.38 ± 0.34	6.13 ± 0.31
FSE50	75.35 ± 1.02	42.27 ± 3.49	9.45 ± 1.11	6.44 ± 0.16	8.39 ± 1.60
FSW	60.05 ± 2.76	51.23± 1.81	18.80 ± 2.86*	33.47 ± 0.18	17.24 ± 2.75

NT= not tested. Positive control of NO production was Prednisolone with IC_{50} value of 0.15 ± 0.03 µg/mL. * significant difference $P < 0.05$ compared with negative control (0.2% DMSO).

Table 3 Antimicrobial activity of *C. carandas* extracts against five microorganisms using disc diffusion method

Code	Inhibition zone (mm.) (Conc. 500 mg /mL)				
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
DUE95	NI	8.00 ± 0.00*	8.00 ± 0.00*	NI	7.00 ± 0.00*
DUE50	9.33 ± 0.67*	9.00 ± 0.00*	7.00 ± 0.00*	NI	8.67 ± 0.33*
DUW	NI	NI	NI	NI	NI
DRE95	NI	7.33 ± 0.33*	7.67 ± 0.33*	NI	NI
DRE50	NI	NI	7.33 ± 0.33*	NI	7.33 ± 0.33*
DRW	NI	NI	NI	NI	NI
DSE95	NI	NI	NI	NI	NI
DSE50	NI	NI	NI	NI	NI
DSW	NI	NI	NI	NI	NI
FUE95	8.50 ± 0.50*	10.33 ± 0.33*	8.33 ± 0.33*	7.33 ± 0.33*	9.33 ± 0.33*
FUE50	8.33 ± 0.88*	10.00 ± 0.00*	7.33 ± 0.33*	7.67 ± 0.33*	8.67 ± 0.33*
FUW	NI	NI	NI	NI	NI
FUSQ	NI	NI	NI	NI	NI
FRE95	8.33 ± 0.33*	8.67 ± 0.33*	7.00 ± 0.00*	NI	7.33 ± 0.33*
FRE50	NI	7.00 ± 0.00*	8.00 ± 0.00*	NI	7.67 ± 0.33*
FRW	NI	NI	NI	NI	NI
FRSQ	NI	NI	NI	NI	NI
FSE95	NI	NI	NI	NI	NI
FSE50	NI	NI	NI	NI	NI
FSW	NI	NI	NI	NI	NI
Ampicillin	40.33 ± 1.45	27.67 ± 0.88	21.00 ± 0.58	NI	NI
Norfloxacin	25.00 ± 0.58	34.33 ± 1.86	35.67 ± 0.67	21.17 ± 0.73	35.33 ± 0.33

NI= no inhibition zone. Values are mean ± SEM. * P value < 0.05 when compared with positive control (Ampicillin and Norfloxacin).

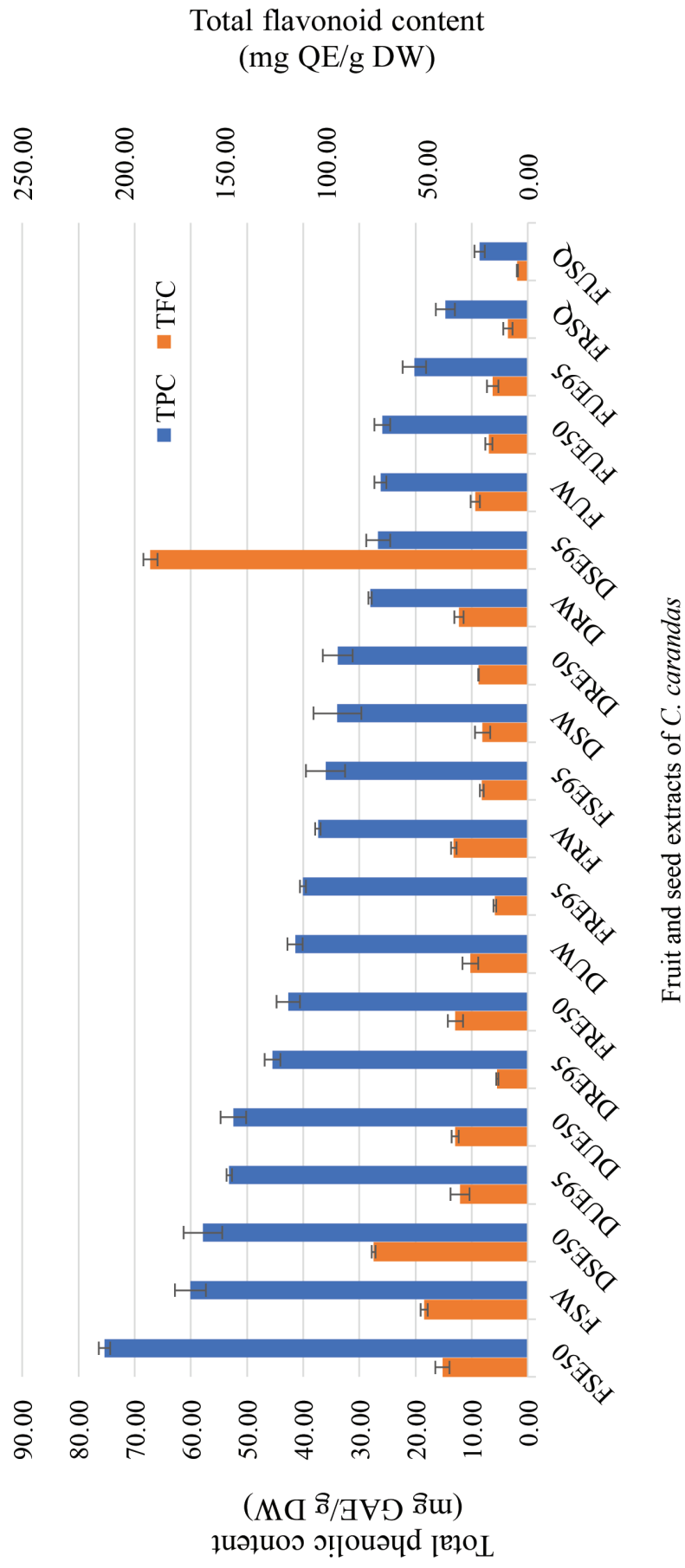

Figure 1 Total phenolic content (TPC) and total flavonoid content (TFC) of *C. carandas* extracts using different extraction (mean ± SEM).

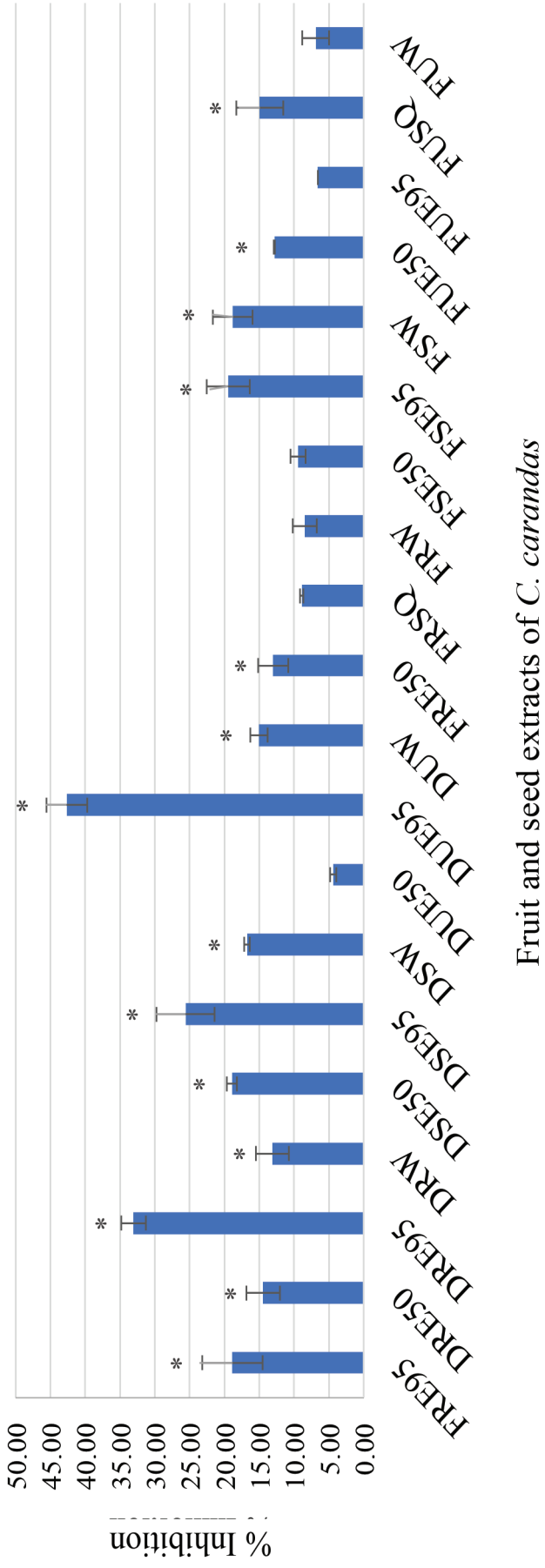

Figure 2 Percent inhibition at 100 µg/mL of *C. carandas* extracts against LPS-induced NO production in RAW 264.7 cells using Griess roagent (mean ± SEM)* significant diffevence P < 0.05 compared with negative control (0.2/DMSO).

บทคัดย่อ

องค์ประกอบทางพฤกษเคมี ฤทธิ์ต้านเชื้อแบคทีเรีย ฤทธิ์ต้านการอักเสบและความเป็นพิษต่อเซลล์ของสารสกัดผลและเมล็ด *Carissa carandas* L.

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** ศูนย์ความเป็นเลิศทางการแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์

บทนำ: มะม่วงหาวมะนาวโห่หรือหนามแดง (*Carissa carandas* L.) ทางการแพทย์แผนโบราณใช้ผลของมะม่วงหาวมะนาวโห่ในรักษาโรคผิวหนังและโรคบริเวณช่องท้อง ในการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาฤทธิ์ต้านเชื้อแบคทีเรีย ฤทธิ์ต้านการอักเสบ ความเป็นพิษต่อเซลล์และสารประกอบเคมีของสารสกัดมะม่วงหาวมะนาวโห่เพื่อนำสนับสนุนการใช้แบบดั้งเดิม

วิธีการศึกษา: การวิจัยครั้งนี้เป็นการนำผลและเมล็ดมะม่วงหาวมะนาวโห่มาทำการสกัดด้วยวิธีที่ต่างกันและนำไปวิเคราะห์ปริมาณสารประกอบฟีนอลรวมและฟลาโวนอยด์รวมด้วยวิธี Folin-Ciocalteu reagent และ aluminium chloride colorimetric method ตามลำดับ ศึกษาฤทธิ์ต้านเชื้อแบคทีเรีย *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* และ *Bacillus subtilis* ศึกษาฤทธิ์ต้านการอักเสบของสารสกัดด้วยวิธีการยับยั้งการหลั่ง Nitric oxide ในเซลล์แมคโครฟาจ RAW 264.7 ที่ถูกกระตุ้นด้วย LPS ศึกษาความเป็นพิษต่อเซลล์ด้วยวิธี SRB assay โดยใช้เซลล์มะเร็งผิวหนัง (C32) และเซลล์ผิวหนังปรกติชนิดเคราติโนไซท์ (HaCaT)

ผลการศึกษา: สารสกัดทั้งหมดมีปริมาณสารประกอบฟีนอลรวมอยู่ในช่วง 8.60 - 60.05 mg GAE/g DW และปริมาณสารฟลาโวนอยด์รวมอยู่ในช่วง 15.18 - 42.27 mg QE/g DW ยกเว้นสารสกัดชั้นเอทานอล 95% ของเมล็ดแบบแห้งแสดงปริมารสารฟลาโวนอยด์สูงสุดเท่ากับ 186.62 mg QE/g DW สารสกัดทั้งหมดแสดงค่าการยับยั้งไนตริกออกไซด์น้อยกว่า 50% นอกจากนี้สารสกัดชั้นเอทานอลของผลดิบแบบสดมีฤทธิ์ต้านเชื้อแบคทีเรียสูงที่สุดอย่างมีนัยสำคัญทางสถิติ สารสกัดจากเมล็ดมีความเป็นพิษต่อเซลล์มะเร็งผิวหนังและเซลล์ปรกติ โดยพบว่าสารสกัดชั้นเอทานอล 50% ของเมล็ดมีความเป็นพิษต่อเซลล์โดยค่า IC_{50} น้อยกว่า 10 µg/mL

สรุปผลการทดลอง: งานวิจัยนี้ได้แสดงให้เห็นว่าสารสกัดเอทานอล 95% ของเมล็ดแบบแห้งเป็นแหล่งของสารต้านอนุมูลอิสระ โดยสารสกัดทั้งหมดเมื่อนำไปศึกษาฤทธิ์พบว่า มีฤทธิ์ต้านการอักเสบและฤทธิ์ต้านเชื้อแบคทีเรียในระดับต่ำแต่สารสกัดของเมล็ดมีความเป็นพิษต่อเซลล์ทั้งสองชนิด ควรมีการศึกษาเพิ่มเติมเกี่ยวกับสารประกอบที่มีความเป็นพิษต่อเซลล์จากเมล็ดต่อไป

คำสำคัญ: มะม่วงหาวมะนาวโห่, หนามแดง, ฤทธิ์ต้านเชื้อแบคทีเรีย, ฤทธิ์ต้านการอักเสบ, human amelanotic melanoma cells (C32), ความเป็นพิษต่อเซลล์