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Biological Activities of Ethanol Extract from Karanda (*Carissa carandas L.*) Fruits

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Carissa carandas L. fruits are is contain high amount of iron, vitamin C and pectin. These fruits have been applied for folk medicine in the reliving of human disorders; and reported for its analgesic, anti-inflammatory and lipase 1 activity. Aims of this study were to determine antioxidant, antimicrobial and anti-inflammation activities of ethanol extract from karanda fruits. Fresh fruits were cleaned, air dried, and extracted with 95% ethanol by maceration. Total phenolic content (TPC) and total flavonoid measurements (TFC) of karanda fruit extract was determined by Folin–Ciocalteu reagent and colorimetric method. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) radical scavenging and lipid peroxidation inhibition activity of extract was evaluated and compared with ascorbic acid and α-tocopherol, respectively. In vitro inflammatory activity was evaluated by monitoring of degraded-albumin reduction and compared with diclofenac diethylammonium. Anti-

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microbial activity of was evaluated against pathogenic bacteria, Streptococcus mutans, and Staphylococcus aureus; and pathogenic yeasts, Candida albicans by disc diffusion method. TPC and TFC of ethanol extract were 11.39±0.18 mg gallic acid equivalent (GAE)/g and 9.15±1.31 mg quercetin equivalent (QE)/g, respectively. DPPH and NO radical scavenging activities of karanda fruit extract were preferable (IC_{50} = 0.56±0.05 and 50.95±14.29 mg/ml). However, this extract was poorly inhibited lipid peroxidation (IC_{50} > 1,000 mg/ml), and lack of anti-inflammation activity. Karanda fruit extract was slightly inhibited S. mutans and S. aureus, therefore there was unable to inhibited C. albicans. The results may support application of karanda fruit for oral anti-septic and sore-throat relieving herb.

Keywords: Anti-bacterial; anti-inflammation; antioxidant; Carissa carandas L.; karanda fruits.

1. INTRODUCTION

Carissa carandas L. is namely as karanda or karonda and its belonging to Apocynaceae family. Berry-shaped fruits are containing high amount of iron, vitamin C and pectin. Even it is used as an ingredient in most of the edible preparations such as jam, jelly, squash and syrup [1]. The fruits, leaves, bark and roots have been applied for folk medicine in the reliving of human diseases, such as diarrhoea, anorexia, intermittent fever, mouth ulcer and sore throat, syphilitic pain, burning sensation, scabies, and epilepsy [2,3]. Fruits have also have been reported for its analgesic, anti-inflammatory and lipase 1 activities [4]. Karanda fruit (50% ethanol extract) were exhibited anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli* [5]. Dichloromethane and methanol extracts of Karanda fruits (25-50 mg/ml) had inhibited *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Enterococcus faecalis* [6]. Phenolic and flavonoid contents in karanda fruits had reported for antioxidant activities and inhibited DNA damage in all solvents [6-8] and especially in ethanol extract [8]. Karanda juice is contain major phenolic compounds, flavonoids and anthocyanins, which had down-regulated the induction of inflammatory inducible nitric oxide synthase as well as cyclooxygenase-2 (COX-2) in lipopolysaccharide-stimulated macrophages [9]. Methanol extract of karanda fruits had anti-inflammation on carrageenan-induced hind paw edema in rats with dose-dependent pattern [10]. Nitric oxide (NO) radical production and lipid peroxidation are play role in progression of inflammation and oxidative stress. Hence, antioxidant assays by inhibition of NO radicals and lipid peroxidation are also need to evaluate as indicators of pathogenesis reduction rather than DPPH or ABTS radicals [11]. Anti-microbial of karanda fruit against oral pathogens is also rarely reported. Aims of this study were 1) to determine antioxidant activities of karanda fruit extract by different assays, such as inhibition of nitric oxide radicals scavenging and lipid peroxidation comparing with DPPH radical scavenging assay; 2) to determine antimicrobial activity of karanda fruit extract against *Streptococcus mutans*, *S. aureus* and *Candida albicans* and 3) to determine *In vitro* anti-inflammation activity of karanda fruit extract. The finding may useful karanda fruit for applying as oral anti-septic and sore-throat relieving herb.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extract Preparation

Applied Thai Traditional Medicine Program, Suan Sunandha Rajbhat University, Samut Songkram, Thailand was purchased karanda fruit samples from different of location in Samut Songkram, Thailand during January to March, 2021. Botanical characterization of ripped karanda fruits was authenticated by from experts and all of samples were pooled them together. Fresh fruits (~1 kg) were cleaned, cut in small flesh pieces and air dried under 40-45 °C. Dried flesh were grinded and grounded powder (100 g) was extracted with 95% ethanol by maceration (72 h) and three-time repeated in same sample; and solvent was removed from extract by rotary evaporator (N-1000, EYELA, Japan).

2.2 Total Phenolic Content (TPC) and Total Flavonoid Measurement

TPC was briefly explained as follow: 0.1 ml of each extract (mg/ml) was added to test tube, and mixed with of distilled water (4.6 ml) and Folin-Ciocalteu reagent (1 ml), respectively. Mixture was left in room temperature for 3 min and 3 ml of 2% Na_2CO_3 (w/v) was added, then shaken for 2 h (150 rpm). Absorbance of sample was

measured at 760 nm against gallic acid standard curve, which had concentrated at 1, 0.875, 0.75, 0.625, 0.5, 0.375, 0.25 and 0.125 mg/ml. TPC of sample was reported as mg of gallic acid equivalent (GAE) per g of dried sample [12]. Total flavonoid content (TFC) of karanda fruit extract was measured by colorimetric method. Dried extract was dissolved in 80 % methanol to obtain a final concentration of 1 mg/ml. The calibration curve was prepared using 0.1–1 ml aliquots of quercetin solution, 500 µL of the acetic acid solution, 2 ml of the pyridine solution and 1 ml of the aluminum chloride solution. The final volume was adjusted to 10 ml with 80 % methanol and the final quercetin concentration was 1-10 µg/ml. Ethanol extract (0.5 ml) was filled to a test tube instead of standard solution as tested sample. Sample tube was kept at room temperature for 30 min and absorbance of test mixture was measured at 420 nm. Test was conducted in triplicate and the flavonoid content was represented as mg of quercetin equivalents (QE) per g of extract (mg QE/g) [13].

2.3 Antioxidant Activity Tests

2.3.1 DPPH radical scavenging activity

Decrement of DPPH radical absorbance was measured in present of sample extract (0.01-1 mg/ml) by 96 well micro-titer plate readers (Tecan, Sunrise, Austria). DPPH radical (6×10^{-5} M) and ascorbic acid were used as negative and positive controls. Results was calculated and represented as 50% inhibitory concentration (IC_{50}) of sample against DPPH radical [14].

2.3.2 Lipid peroxidation inhibition activity

Extract was dissolved with absolute ethanol and diluted to 0.001, 0.01, 0.1, 1 and 10 mg/ml. Each concentration was tested for lipid peroxidation inhibition activity by ferric iron-thiocyanate complex. Absorbance of iron-thiocyanate was monitored by micro-titer plate reader. α-tocopherol was used as positive control. Results was calculated and represented as IC_{50} of sample on reduction of lipid peroxidation [15].

2.3.3 NO radical scavenging activity

Extract was dissolved with absolute ethanol and diluted to 0.001, 0.01, 0.1, 1 and 10 mg/ml. Each concentration was tested for NO radical scavenging in Griess reagent. Absorbance of iron-thiocyanate was monitored by micro-titer plate reader. Ascorbic acid was used as positive control. Results was calculated and represented as IC_{50} of sample on reduction of NO radicals [16].

2.4 In Vitro Anti-inflammatory Activity

Extract was dissolved with Tween 20 (20%) and centrifuged (150 rpm) for 5 min. Supernatant was pipetted and diluted to 0.01, 0.1, 1, 10 and 100 mg/ml. Each concentration of diluent was incubated with albumin solution at 70±2 °C for 5 min. Absorbance of albumin level was monitored by micro-titer plate reader. Diclofenac diethylammonium was used as positive control. Results was calculated and represented as IC_{50} of sample on reduction of albumin degradation [17].

2.5 Antimicrobial Activity

2.5.1 Pathogenic bacteria and yeasts

Pathogenic bacteria including *S. mutans* and *S. aureus*; and Pathogenic yeast, *C. albicans* were provided from Faculty of Medicine, Chiang Mai University, Chiang Mai and Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand, respectively.

2.5.2 Materials and antibiotic discs

Brain heart infusion, BHI (HiMedia Laboratories, India) was used as bacterial culture media. Potato dextrose agar, PDA (HiMedia Laboratories, India) was used as yeast culture media. 6 mm filter paper disc (Macherey-Nagel, Germany), petri dishes (Union Science, Thailand), laminar flow biohazard class II (Renovation Technology, Thailand) and soft incubator SLIO-600ND (EYELA, Japan) were included in this assay. 0.015 mg of erythromycin disc (Oxoid, UK) were positive control antibiotic for *S. mutans* and *S. aureus* and 0.025 mg of fluconazole for *C. albicans*.

2.5.3 Disc diffusion methods

Each bacterium and yeast were inoculated in BHI and PDA plates, respectively. Undiluted ethanolic extract (8 mg) was dissolved with 95% ethanol (sterile with 0.2 µm of membrane filtered) to concentrate at 125 mg/ml for Disc diffusion method. 10 µl of undiluted extract was dropped to filter paper disc on plate. Then applied diluted extract (125 mg/ml) for 1, 2 and 3 times to disc and concentration was 1.25, 2.5 and 3.75 mg,

respectively. Tested disc was compared with standard antibiotic disc and negative control disc (95% ethanol) within same plate. Incubation condition was 37±1 °C for 24-48 h. Interpretation of test was determined by measuring diameter of inhibition zone (mm) surrounded each disc [18-20]. All steps of test were done with aseptic technique.

2.6 Statistical Analyses

All assays were calculated from by triplicate measurements and represented as mean ± standard deviation. Statistical analyses were performed with SPSS program (version 20.0).

3. RESULTS AND DISCUSSION

TPC and TFC contained in karanda fruit extract were 11.39±0.18 mg GAE/g and 9.15±1.31 mg QE/g. DPPH and NO radical scavenging activities of karanda fruit extract were preferable (IC_{50} = 0.56±0.05 and 50.95±14.29 mg/ml) when compared with ascorbic acid. However, this extract was poorly inhibited lipid peroxidation (IC_{50} > 1,000 mg/ml), and lack of anti-inflammation activity by unable to reduce albumin degradation (Table 1). Karanda fruit extract was slightly inhibited *S. mutans* and *S. aureus*, therefore there was unable to inhibited *C. albicans* by disc diffusion method (Table 2).

Table 1. Antioxidant and anti-inflammation activities of ethanol extract from Karanda (*Carissa carandas* L.) fruits [a]

Extract (control) / Assay	TPC [b]	TFC [c]	DPPH	NO	LPI	Anti-inflammation
Ethanol	11.39±0.18	9.15±1.31	0.56±0.05	50.95±14.29	>1000	ND
α-tocopherol	-	-	-	-	0.39±0.10	-
Ascorbic acid	-	-	0.03±0.01	0.25±0.05	-	-
Diclofenac diethyl ammonium	-	-	-	-	-	0.34±0.01

[a] Antioxidant and anti-inflammation activities were represented as 50% of inhibitory concentration, IC_{50} (mg/ml)
[b] Total phenolic content (TPC) was represented as mg of gallic acid equivalent (GAE) per g of extract
[c] Total flavonoid content (TFC) was represented as mg of quercetin equivalent (QE) per g of extract
DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; NO = nitric oxide radical scavenging activity;
LPI = lipid peroxidation inhibition activity; ND = not determined

Table 2. Anti-microbial activity of ethanolic extract from Karanda (*Carissa carandas* L.) fruits

Pathogen /sample	Extract/ Control (mg)*	Diameter of inhibition zone (mm)			
		Plate 1	Plate 2	Plate 3	Average
Streptococcus mutans					
Ethanolic extract	2.5	10.8	11.0	10.5	10.8±0.25
95% Ethanol	-	ND	ND	ND	ND
Erythromycin	0.015	26.9	26.5	26.4	26.6±0.59
Staphylococcus aureus					
Ethanolic extract	2.5	9.8	8.5	9.3	9.2±0.65
95% Ethanol	-	ND	ND	ND	ND
Erythromycin	0.015	26.5	25.7	25.9	26.0±0.51
Candida albicans					
Ethanolic extract	2.5	ND	ND	ND	ND
95% Ethanol	-	ND	ND	ND	ND
Fluconazole	0.025	28.9	29.0	29.5	29.1±0.36

** The concentration of extract was 2.5, 0.25 and 0.025 mg; and determined antimicrobial activity by agar disc diffusion, ND = Not determined*

The results were corresponded with previous study, which had report high amount of TPC contained in ethanol extract [7]. We were showed that flavonoids were major constituents in TPC. DPPH and NO radical scavenging activities were also corresponded with DPPH, ABTS radical scavenging assays and ORAC method from previous studies [6,7]. Poor lipid oxidation of this extract may influence from high polarity of flavonoid contained. Therefore, for lack of anti-inflammation of karanda fruit extract in our finding was controversial with previous studies [9,10]. Because of this extract is inhibit the induction of COX-2 by NO reduction rather than reduction of albumin degradation [9]. In addition, anti-inflammation activity of extract was acted via NO radical inhibition. Antibacterial activity of karanda fruit extract was also corresponded for *S. aureus* inhibition [6,21] and new reporting for S. *mutans* inhibition. However, karanda fruit extract in this study was unable to inhibit *C. albicans* that contrast with previous report, which possessed antifungal activity against *C. albicans*, *A. oryzae* and *T. azoli* fungus strains [21]. It may due to use of different extracts (methanol and petroleum ether) and ripping stage of fruits (unripe). The results may support application of karanda fruit for oral anti-septic and sore-throat relieving herb.

4. CONCLUSION

Ethanol extract of karanda fruits was exhibited antioxidant activity by DPPH and NO radical scavenging; and anti-bacterial activity by inhibition of *S. mutans* and *S. aureus*.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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