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Antioxidant, cytotoxic and antineoplastic effects of *Carissa carandas* Linn. leaves

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ABSTRACT

For scientific clarification of some traditional uses, this study was designed to explore the antioxidant, cytotoxic and antineoplastic properties of leaf extract of *Carissa carandas* Linn., a traditional medicinal plant of Bangladesh. The methanol extract of *Carissa carandas* leaves (MELC) was applied on DPPH and ABTS experiments to determine its antioxidant activity. *In vitro* the cytotoxic effect of MELC was evaluated against colonic adenocarcinoma cell lines (SW-480 and SW-48) whereas *in vivo* its antineoplastic property was tested against Ehrlich ascites carcinoma (EAC). The DPPH and ABTS assays revealed the antioxidant activity of MELC with IC_{50} 10.5 ± 1.2 and 1.75 ± 0.3 μg/ml that was comparable to L-ascorbic acid. *In vitro* cytotoxic study, MELC reduced the viability of adenocarcinoma cells in dose dependent manner and *in vivo*, administration of MELC (25 mg/kg) resulted in a significant ($p < 0.05$) decrease in viable EAC cell count thereby increasing the life span of the EAC cell bearing mice. Restoration of hematological parameters such as red blood cells (RBC), hemoglobin and white blood cells (WBC) to normal levels in MELC-treated mice was also observed. Moreover, treatment with MELC induced apoptosis of EAC cells as observed in fluorescence microscopic view of DAPI (4,6-diamidino-2-phenylindole) stained cells and also increased p53 gene expression MELC-treated cells in respect to untreated EAC control. In addition, the MELC was rich in polyphenol content and its GC–MS chromatogram confirmed the presence of some compounds all of which showed anticancer and cytotoxic activities in previous studies. In a word, this study supports the use of *Carissa carandas* in traditional medicine as well as highlights the need to further explore the potentials of MELC as an antineoplastic agent.

1. Introduction

Cancer, a group of diseases characterized by uncontrolled growth and spread (invasion and metastasis) of abnormal cells, is considered as the second most common cause of death in the developed world (Tanaka, 1994). A similar picture has also arisen in the developing countries like Bangladesh. Both several external environmental factors (tobacco, chemicals, radiation, and infectious organisms) and/or internal factors (mutations, hormones, and altered immunity) are responsible for neoplastic transformation (Tanaka, 1997). As cancer chemoprevention, the use of natural or synthetic chemical agents is an important strategy to reduce the risk of cancer. Many previous studies have suggested the beneficial effects of various phytochemicals in reducing the risk of cancer (Kuno et al., 2012). So research on the medicinal plants for their antineoplastic effect bears a significant value.

Carissa carandas Linn. commonly known as Karamcha, is a common herb of Apocynaceae family and it is found throughout all over the Bangladesh. It has a long history of use in traditional system of medicine. The fruits, leaves, barks and roots of *Carissa 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 (Chanchal et al., 2013). Earlier studies have shown that the extracts of different parts of this plant possesses cardiotonic, antipyretic, anticonvulsant, antidiabetic and antiviral activities (Ya'u et al., 2008; Itankar et al., 2011; Chanchal et al., 2013). Chemical constituents of *Carissa carandas* include steroids, terpenes, tannins, flavonoid, benzenoids, phenylpropanoid, lignans, sesquiterpenes, and coumarins (Begum et al., 2013). Moreover, this plant is used by traditional practitioner of some districts in Bangladesh for the treatment of cancer. But, no scientific data is available to confirm this folkloric use. Here, the methanol extract of *Carissa carandas* dried leaves (MELC) was evaluated

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for antioxidant, cytotoxic and antineoplastic activities and its various phytochemical compounds were analyzed by GC–MS.

2. Materials and method

2.1. Plant materials

Leaves of *Carissa carandas* (Linn) plant (Family: Apocynaceae) were used for this study. The plant samples were collected during the month of June-July 2015 from the relevant area of Rajshahi district. This medicinal plant was identified and authenticated by plant taxonomist of Department of Botany, University of Rajshahi, Rajshahi, Bangladesh. Voucher specimen (voucher specimen No. 1150) was deposited at Department of Botany, University of Rajshahi, for future reference.

2.2. Extraction

The leaves of *Carissa carandas* was shade dried and ground into powder. The powder (0.3 kg) was extracted with methanol (Sigma Aldrich, Germany) at room temperature for 7 days. The solvent was completely removed by rotary vacuum evaporator and the crude methanol extract of *Carissa carandas* leaves (4.4 g) (designated as MELC) was stored in a vacuum desiccator for further use.

2.3. Determination of total phenolic and total flavonoid content

The Folin–Ciocalteu method modified by Ranilla et al. (2010) was applied to determine the total polyphenolic content of MELC. One milliliter of sample solution was transferred into a 10 ml volumetric flask and mixed with 6 ml of distilled water. 0.5 ml of Folin–Ciocalteu reagent (50%, v/v) (Labscan, Thailand) was added to sample and mixed. After 5 min, 1 ml of Na_2CO_3 (5%, m/v) (Sigma Aldrich, Germany) was added to the mixture and adjusted to 10 ml with distilled water. After standing for 60 min at room temperature, the absorbance was measured at 760 nm. Gallic acid (Sigma Aldrich, Germany) was used for constructing the standard curve. The total phenolic content was expressed as gallic acid equivalents (mg/g of dry weight of extract).

Total flavonoids content of MELC were estimated using the method described by Ordonez et al. (2006). Here, catechin (Sigma Aldrich, Germany) was used as standard. 1.5 ml of methanol, 100 µl of 10% aluminum chloride (Sigma Aldrich, Germany), 100 µl of 1 M potassium acetate (Labscan, Thailand) solution and 2.8 ml of distilled water was added to 0.5 ml of sample/standard. After one hour 30 min of incubation at room temperature (RT), the absorbance was measured at 420 nm. The samples/standard was evaluated at a final concentration of 0.1 mg/ml. Total flavonoid contents were expressed in terms of catechin equivalent, (mg/g of dry weight of extract).

2.4. DPPH· scavenging activity

Free radical scavenging activity was determined by DPPH (Sigma Aldrich, Germany) radical scavenging assay as previously described method (Mohsen and Ammar, 2009) with some modification. A solution of 0.1 mM DPPH in methanol was prepared and 3 ml of this solution was mixed with 1 ml of extractives in methanol at different concentrations (6.25–37.5 µg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference standard. Percentage of DPPH radical scavenging was calculated as DPPH· scavenging effect (%) = $[1 - (A_{sample}/A_{cont})] \times 100$

EC_{50} values (µg/ml), the effective amount of the sample needed to scavenge DPPH· by 50%, were determined from the plotted graphs of scavenging activity against the concentration of the extracts.

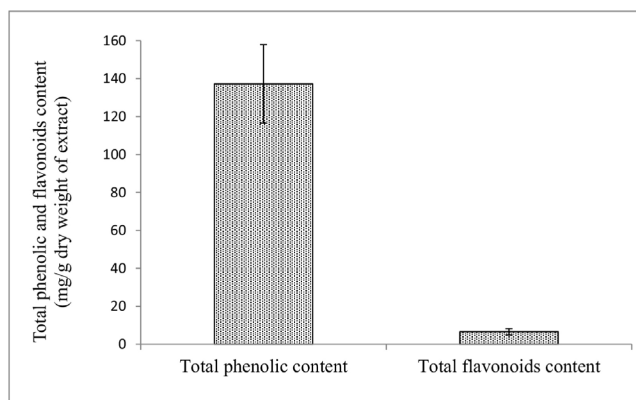

Fig. 1. Phenolic and flavonoid content of MELC.

Table 1
Chemical composition of MELC analyzed by GC–MS.

Sl	Retention time (min)	Compounds	Area (%)
1	3.34	Isobenzofuran	3.80
2	3.41	Ethylbenzene	0.32
3	3.60	o-Xylene	1.02
4	4.45	1,2,4-trimethylbenzene,	0.68
5	10.80	Card-20(22)-enolide	2.72
6	10.90	Methyl 4-*O*-methyl-D-arabinopyranoside	1.86
7	11.17	n-Decanoic acid	0.25
8	11.64	4-*O*-Methylmannose	10.6
9	12.81	Hexadecanal	1.20
10	13.93	Hexadecanoic acid	12.06
11	15.65	9,12-*O*ctadecadienoic acid	1.53
12	15.71	8,11,14-Docosatrienoic acid	14.20
13	26.57	Stigmasterol	1.04
14	27.72	β-Asarone	0.79
15	28.31	24-Noroleana-3,12-diene	4.91
16	29.12	Lupeol	17.10
17	28.84	Urs-12-en-3-ol	0.58
18	30.77	Lup-20(29)-en-3-ol	4.89
		Total area =	79.25

2.5. ABTS·+ scavenging capacity

ABTS·+ assay was carried out according to the method described previously (Cai et al., 2004). The ABTS·+ solution was prepared by mixing 7 mM ABTS (Sigma Aldrich, Germany) and 2.45 mM potassium persulfate (Carl Roth, Germany) and incubating in the dark at room temperature for 12 h. The ABTS·+ solution was then diluted with water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. ABTS·+ solution (3 ml) was added to 0.1 ml of the test sample with various concentrations (0.625–3.75 µg/ml) and mixed vigorously. The absorbance was measured at 734 nm after standing for 6 min. The ABTS·+ radical scavenging activity of the samples was expressed as

ABTS·+ scavenging effect (%) = $[1 - (A_{sample}/A_{cont})] \times 100$

where A_{cont} is the absorbance of the blank control (ABTS·+ solution without test sample) and A_{sample} is the absorbance of the test sample.

2.6. GC-analysis

The chemical composition of MELC was established by gas chromatography mass spectrometry/Quadropole detector analyses using GCMS-QP2010S (Shimadzu Kyoto, Japan) spectrometer. They equipped with a flame ionization detector and capillary column with HP-5MS (30 m × 0.25 mm × 0.25 µm). The temperature program for the column was from 120 °C (1 min) to 230 °C at a rate of 6 °C/min and then held at 200 °C for 35 min. Helium was used as a carrier gas at a flow of 14 psi (Split 1:10), and the injection volume of each sample was 1 µl.

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