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Phenolics-saponins rich fraction of defatted kenaf seed meal exhibits cytotoxicity towards cancer cell lines

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ABSTRACT

Objectives: To determine the cytotoxicity of crude ethanolic extract, *n*-butanol fraction and aqueous fraction on selected cancer cell lines, and to observe the morphological changes of the cancer cells treated with *n*-butanol fraction.

Methods: The cytotoxic effect of *n*-butanol fraction, crude ethanolic extract and aqueous fraction on breast cancer (MCF-7 and MDA-MB-231), colon cancer (HT29), lung cancer (A549), cervical cancer (HeLa) and normal mouse fibroblast (3T3) cell lines was determined using MTT assay. The morphological changes of the treated cells were observed under an inverted light microscope.

Results: *n*-Butanol fraction was the most cytotoxic towards HT29 and MCF-7 cells in a dose-dependent manner compared to crude ethanolic extract and aqueous fraction ($P < 0.05$). The IC_{50} of *n*-butanol fraction for HT29 and MCF-7 was (780.00 ± 28.28) and (895.00 ± 7.07) μg/mL, respectively. Cell shrinkage, membrane blebbing and formation of apoptotic bodies were noted following treatment of HT29 cells with *n*-butanol fraction.

Conclusions: In conclusion, *n*-butanol fraction was more cytotoxic than crude ethanolic extract and aqueous fraction towards the selected cancerous cell lines and induced apoptosis in HT29 cells.

1. Introduction

Kenaf (*Hibiscus cannabinus* L.) belongs to the Malvaceae (Mallow) family, which is locally known as "Ambadhi" in Marathi or "Ambashta" in Sanskrit [1]. Kenaf is an annual plant that grows to 3–4 m in height within 4–5 months and can be harvested 2–3 times a year [2]. Traditionally, the kenaf flower is used to treat biliousness with acidity, the seed to promote weight gain, the leaf to relieve cough and the stem to treat anemia [1]. Malaysia has taken the initiative to cultivate kenaf plant in order to replace tobacco plantation. The kenaf fiber is commercially used in industrial products such as bio-composite materials, absorbents and fiber board. In general practice, kenaf seeds are usually disposed as waste material after the plants have been harvested [3]. Although kenaf is mainly used for fiber, the seed (by-product) also has the potency to be turned into value-added products [4]. Previous study has shown that kenaf seed oil possessed substantially high antioxidant activity [5]. In addition, the kenaf seed oil extracted by supercritical carbon dioxide fluid extraction exhibited anticancer properties towards colon cancer in rats [6], and ovarian cancer cell line [7]. Large scale extraction of oil from kenaf seeds produces huge amount (80% of the seed) of

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defatted kenaf seed meal (DKSM), which contains considerable amounts of health beneficial bioactives such as phenolic compounds and saponins [8]. DKSM has been compared with some edible flours (rice flour, sweet potato flour and wheat flour), and it showed the highest content of phenolic compound and antioxidant properties [3]. The fractionation of DKSM crude ethanolic extract by *n*-butanol successfully elevated the level of phenolics and saponins as well as antioxidant activity of the extract. Most of the active compounds are extractable in solvent system of intermediate polarity, which justifies higher recovery in *n*-butanol fraction [8]. Based on the strong antioxidant activities, it is postulated that *n*-butanol fraction might also possess better anticancer properties in comparison to crude ethanolic extract and aqueous fraction. This study aimed to investigate the cytotoxic effects of DKSM crude ethanolic extract as well as its derived *n*-butanol and aqueous fraction against the breast cancer-hormone dependent (MCF-7), breast cancer-hormone independent (MDA-MB-231), colon cancer (HT29), cervical cancer (HeLa), lung cancer (A549) and normal mouse fibroblast (3T3) cell lines.

2. Materials and methods

2.1. Materials

2.1.1. Cell lines

Breast cancer-hormone dependent (MCF-7), breast cancer-hormone independent (MDA-MB-231), colon cancer (HT29), cervical cancer (HeLa), lung cancer (A549) and normal mouse fibroblast (3T3) cell lines were purchased from the American Type and Culture Collection, USA.

2.1.2. Reagents

The tissue culture medium (RPMI-1640) was purchased from Nacalai TesqueInc (Kyoto, Japan). Penicillin/streptomycin antibiotic and trypsin-ethylene diamine tetraacetic acid were purchased from PAA Laboratories (Pasching, Austria). Mycoplex™ fetal bovine serum was purchased from Commerce Ave (California, USA). Trypan blue solution, MTT powder and propidium iodide were purchased from Sigma Chemicals (St. Louis, USA).

2.2. Methods

2.2.1. Preparation of crude ethanolic extract, aqueous fraction and n-butanol fraction from DKSM

The crude ethanolic extract, aqueous fraction and *n*-butanol fraction of DKSM extracted from kenaf seeds were kindly provided by Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia [8]. Kenaf seeds were purchased from Malaysian Kenaf and Tobacco Board, Kelantan, Malaysia. Firstly, the seeds were pulverized and homogenized with *n*-hexane to extract out the oil. The residue which is DKSM was collected. Next, DKSM was refluxed in 50% of aqueous ethanol to obtain the crude ethanolic extract. For fractionation, crude ethanolic extract was dispersed in distilled water and partitioned with *n*-hexane to remove the residual lipids. Then, the *n*-hexane layer was removed and the aqueous layer was partitioned with *n*-butanol. *n*-Butanol fraction was then pooled and concentrated under the reduced pressure. Finally, aqueous fraction left from this partitioned layer was subjected to lyophilization [8].

2.2.2. Cell culture

MCF-7, MDA-MB-231, 3T3, HT29, HeLa and A549 cell lines were cultured in RPMI-1640 culture medium supplemented with 10% fetal bovine serum and 1% antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin). The cultured cells were maintained in an incubator at 37 °C, and in humidified atmosphere of 5% CO_2.

2.2.3. Determination of cytotoxicity

Cytotoxicity of the tested materials was determined using the MTT assay. The assay measures the metabolic activity of cells based on the reduction of mitochondrial succinate dehydrogenase from yellow (tetrazolium bromide) to dark purple (formazan) [9]. Briefly, the cells were seeded in 96-well plates at cell density of 1.0×10^5 cells/mL in 100 μL complete growth medium. After 24 h, the cells were treated with crude ethanolic extract, *n*-butanol fraction and aqueous fraction at various concentrations (15.625, 31.250, 62.500, 125.000, 250.000, 500.000 and 1 000.000 μg/mL) in triplicate. Untreated cells served as control. The cells were incubated at 37 °C in an incubator with humidified atmosphere of 5% CO_2 for 72 h. Subsequently, 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline) was added. Three hours later, the medium was discarded and 100 μL of dimethylsulfoxide was added to dissolve the purple formazan product. The absorbance was measured at wavelength of 570 nm and 630 nm (as background) by using a microplate ELISA reader (BioTek EL 800, United States).

The percentage of cell viability was obtained by using the following formula:

$$\text{Percentage of cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

The dose response curve of percentage of cell viability versus the extract concentration was plotted to determine the IC_{50}. The cytotoxic effects of the extracts were expressed as IC_{50} value (the extract concentration reducing the absorbance of treated cells by 50% with respect to untreated cells) [10].

2.2.4. Morphological analysis

Based on the IC_{50}, *n*-butanol fraction was found to be the most potent towards colon cancer cell line (HT29). Further experiment was then conducted by using *n*-butanol fraction only. HT29 cells were seeded at cell density of 0.5×10^5 cells/mL in 6-well plates. After 24 h incubation, the cells were treated with *n*-butanol fraction at concentration of 200, 300, 400, 500 and 600 μg/mL for 24, 48 and 72 h. Untreated cells were included as a control. The cell morphology was examined under magnification of 10× and 40× by using an inverted light microscope (Olympus, Tokyo, Japan). The same spot of the cell was captured at different time interval by drawing the cross under the plate as reference.

2.2.5. Statistical analyses

All data were expressed as mean ± SD. The data were analyzed with One-way ANOVA and Dunnett *post-hoc* test