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Effect of kenaf seed oil from different ways of extraction towards ovarian cancer cells

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ABSTRACT

Kenaf (*Hibiscus cannabinus*) from the family of Malvaceae is a valuable fibre plant native to India and Africa. Kenaf is composed of various active components including tannins, saponins, polyphenolics, alkaloids, essential oils and steroids. It has been used to treat bruises, bilious conditions, fever and puerperium. Nevertheless, the anti-cancer properties of kenaf seed oil have not yet been investigated. In this study, kenaf seed oils obtained by Sonication, Soxhlet and supercritical carbon dioxide fluid extraction (SFE) with 9 different combinations of pressure (bars) and temperature (°C) (200/40, 200/60, 200/80, 400/40, 400/60, 400/80, 600/40, 600/60 and 600/80) were investigated for the cytotoxicities. All the oils were cytotoxic towards ovarian cancer (CaOV3) and colon cancer (HT29) cell lines in a dose dependent manner as detected by using the MTT assay and trypan blue dye exclusion method. Oil from Sonication was the most cytotoxic towards CaOV3 cell line. Treated cells exhibited characteristics of apoptosis such as chromatin condensation and nuclear fragmentation. In conclusion, kenaf seed oils from the three extractions were cytotoxic towards CaOV3 cell line in a dose-dependent manner possibly via the induction of apoptosis. In considering the safety of the product, SFE technology is a better alternative extraction method that is suitable in kenaf seed oil extraction.

Keywords: Supercritical carbon dioxide fluid extraction; Kenaf (*H. cannabinus*) seed oil; Cytotoxicity; Apoptosis

1. Introduction

Kenaf (*Hibiscus cannabinus*) from the family of Malvaceae is a valuable fibre plant native to India and Africa (Mohamed et al., 1995). It is now being planted as a potential one to replace tobacco. Kenaf is one of the allied fibres of jute and shows similar characteristics. The plant is composed of various active components including tannins, saponins, polyphenolics, alkaloids, essential oils and steroids, and has long been prescribed in traditional folk medicine in Africa and India (Agbor et al., 2005; Kobaisy et al., 2001).

Kenaf seeds yield vegetable oil that is edible for human consumption (Nyam et al., 2009; Mohamed et al., 1995). The oil contains alpha-linolenic acid (ALA), the essential omega-3 fatty acid that is metabolized to eicosapentaenoic acid, a precursor of eicosanoids with anti-inflammatory and anti-thrombotic activity (Ruiz et al., 2002) and also known as chemopreventive agent (Williams et al., 2007). Additionally, kenaf seed oil contains phytosterol which possesses anti-cancer, anti-oxidant and lipid lowering cholesterol properties (Kritchevsky and Chen, 2005; Berger et al., 2004; Choi et al., 2003).

Kenaf seed oil can be extracted out conventionally by using the organic solvents such as *n*-hexane or petroleum ether. Nevertheless, the oil is always doubted for the safety of consumption due to the residue of the solvents. Hence, supercritical carbon dioxide extraction (SFE) is considered a better way to obtain the oil (Wang and Weller, 2006). To date,

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the anti-cancer properties of kenaf seed oil have not yet been investigated. The objective of this study was to determine the cytotoxicity of kenaf seed oil from Sonication, Soxhlet and SFE towards ovarian cancer (CaOV3) and human colon cancer (HT29) cell lines.

2. Methods and materials

2.1. Extraction of kenaf seeds

Kenaf seeds were purchased from the National Tobacco Board, Pasir Putih, Kelantan, Malaysia. The variety of kenaf seeds was Quiping 3. Kenaf seeds were cleaned, soaked in water at ambient temperature for 24 h and dried at constant temperature (50 °C) overnight in an oven (FD 115, Fisher Scientific, Germany). The final moisture content of the dried seeds was <5%. The dried seeds were stored at 4 °C until further use.

The detailed procedures for kenaf seed oil extraction have been previously reported in the literature (Chan and Ismail, 2009). Briefly, for Sonication or conventional ultrasonic assisted solvent extraction (SONIC), 25 g of kenaf seeds were ground in a stainless steel Waring blender for 1 min and homogenized with 300 ml of *n*-hexane at 13,500 rpm for 3 min (Ultra-turax T25 basic IKA®-WERKE, Germany). Subsequently, the mixture was sonicated for 90 min in an ultrasonic bath (Power sonic 505, Microprocess Controlled Benchtop Ultrasonic Cleaner, Germany). After Sonication, the mixture was filtered through a filter paper (Whatman No. 1) and the filtrates were evaporated using a rotary vacuum evaporator (Buchi, UK).

For Soxhlet extraction, 50 g of kenaf seeds were ground by a stainless steel Waring blender for 1 min and equally divided into two extraction thimbles. Each thimble was then transferred into a Soxhlet extractor (Witeg-Labortechnik GmbH, Wertheim, Germany). Prior to extraction, 300 ml of *n*-hexane was added into the round bottom flask. After extraction was initiated, the solvent flow rate was manually adjusted to 7 min/cycle and the extraction was terminated after 20 cycles (rapid Soxhlet extraction, SOX/S) and 100 cycles (classic Soxhlet extraction, SOX/L), respectively.

For SFE, kenaf seeds were extracted by using the supercritical carbon dioxide fluid extractor (Thar 1000 F, USA) at 9 different combinations of pressure (bars) and temperature (°C). The combinations were 200/40, 200/60, 200/80, 400/40, 400/60, 400/80, 600/40, 600/60 and 600/80. Briefly, kenaf seeds were ground in a stainless steel Waring blender for 1 min and 100 g of kenaf seeds were placed into a 1 l extraction vessel. After the extraction vessel was tightly sealed, the desired temperature and pressure were set. The flow rate of carbon dioxide was set at 25 g/min and regulated by an automated back pressure regulator. The SFE extraction was initiated after the desired temperature and pressure were achieved. The whole extraction lasted for 150 min and the yield was measured.

2.2. Cell culture

The human colon cancer (HT29) and human ovarian cancer (CaOV3) cell lines from the American Type Culture Collection (ATCC, USA) were grown in RPMI 1640, supplemented with 10% foetal bovine serum and maintained in a humidified atmosphere of 5% carbon dioxide at 37 °C.

2.3. Treatment

Cells were treated with different concentrations of kenaf seed oil (100–5000 μg/ml) for 72 h.

2.4. Determination of cytotoxicity

2.4.1. MTT assay

Briefly, 10 μl medium with 0.5 mg/ml MTT in PBS was added into each well. The plate was incubated at 37 °C for 4 h. Next, the medium was totally removed and 200 μl Tris-DMSO solutions were added to each well. The absorbance, which was proportional to cell viability, was measured at 570 nm and a reference wavelength of 630 nm by using an ELISA plate reader (Bio-Rad, Hercules, CA, United States). A graph of percentage of cell viability versus concentration of extracts was plotted and the concentration that gave 50% inhibition of the cell viability (IC_{50}) was determined.

2.4.2. Trypan blue dye exclusion method

Briefly, 15 μl of cell suspension were mixed with 15 μl of trypan blue. Subsequently, the mixture was transferred to a haemocytometer with an overlay of cover slip and viewed under an inverted light microscope. The cell viability was then determined. A graph of percentage of cell viability versus concentration of extracts was plotted and the concentration that gave 50% inhibition of the cell viability (IC_{50}) was determined.

2.5. Morphological changes

The cells were treated for 72 h and viewed under an inverted light microscope.

2.6. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 16.0. Results were analyzed by one-way analysis of variance (ANOVA). Data were expressed as mean ± standard deviation (mean ± SD). A difference was considered to be significant at $p < 0.05$.

3. Results and discussion

There are a lot of efforts to find the suitable extraction method with the highest oil yield, yet taking into consideration the safety of the product. SFE has been documented as an effective method for preparing bioactive products from plant material due to its advantages over the use of liquid solvents (Reverchon and De Marco, 2006). The solvent power of supercritical fluids depends on its density with changes in temperature, pressure, and/or composition. Other important properties of supercritical fluids are their very low surface tensions, low viscosities and moderately high diffusion coefficients, leading to a more favourable mass transfer (Wang and Weller, 2006). Supercritical carbon dioxide solvent was selected in our study as this solvent is easily separated from the extract. This ensures the safe consumption of kenaf seed oil.

Pressure and temperature are the two most important factors that contribute to the yield of kenaf seed oil. Elevation in pressure at certain temperature results in an increase in the carbon dioxide (CO_2) density, thus enhancing solubility of the solutes and increasing the yield. At constant pressure, the density of CO_2 decreases with the increase in tempera-

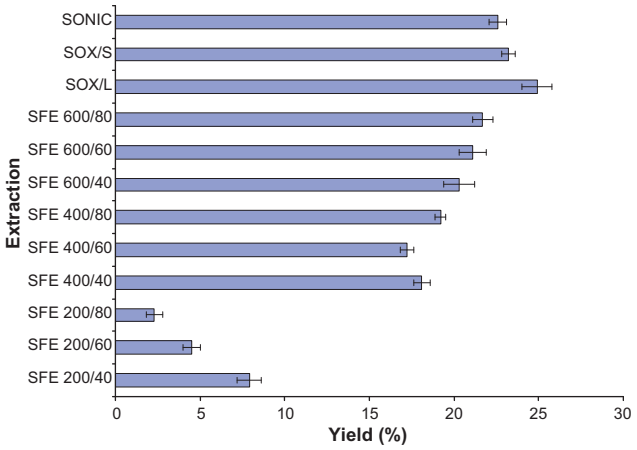

Fig. 1 – Yield of kenaf seed oils from different ways of extraction. Each data point represents the mean of three independent experiments ± SD. *$p < 0.05$ is considered significant.

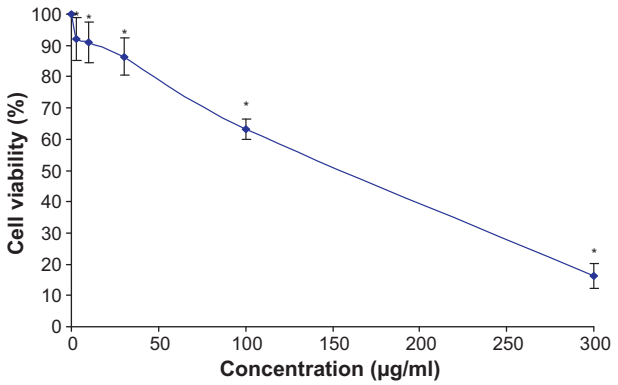

Fig. 2 – Effect of kenaf seed oil extracted by Sonication on the viability of CaOV3 cell line after 72 h as determined by using the trypan blue dye exclusion method. The oil killed the cell line in a dose-dependent manner. Each data point represents the mean of three independent experiments ± SD. *$p < 0.05$ is considered significant.

ture, and becomes more pronounced as the compressibility increases (Pourmortazavi and Hajimirsadeghi, 2007). On the other hand, increase in temperature increases the vapour pressure of analytes. Therefore the tendency of components to be extracted passing through the supercritical fluid will increase (Reverchon and De Marco, 2006). In this study, increase in the oil yield was observed with the increase in temperature at constant pressures of 600 and 400 bar (Fig. 1). Nonetheless, different pattern was noted at constant pressure of 200 bar whereby the yield decreases with increase in temperature. In this experiment, Soxhlet gave the highest oil yield of 25% as compared to other extractions (Fig. 1).

All the kenaf seed oils were more cytotoxic towards CaOV3 than HT29 and the IC_{50} values of all the oils towards CaOV3 and HT29 ranged from 100 to 4000 μg/ml. The lowest IC_{50} of 172.33 μg/ml is shown by the oil from Sonication (Fig. 2). Meanwhile, the oil from SFE (600/40) gave the lowest IC_{50} of 200 μg/ml against HT29. Oil extracted at all constant pressures (600, 400 and 200 bar) at 80 °C was less cytotoxic towards the HT29 cells as compared to the lower temperatures of 60 °C and 40 °C (data not shown). It is speculated that higher temperature may denature some of the heat sensitive compounds exist in kenaf seed oil (Cossuta et al., 2008) or probably they are not being extracted out at that condition (Vaughn et al.,

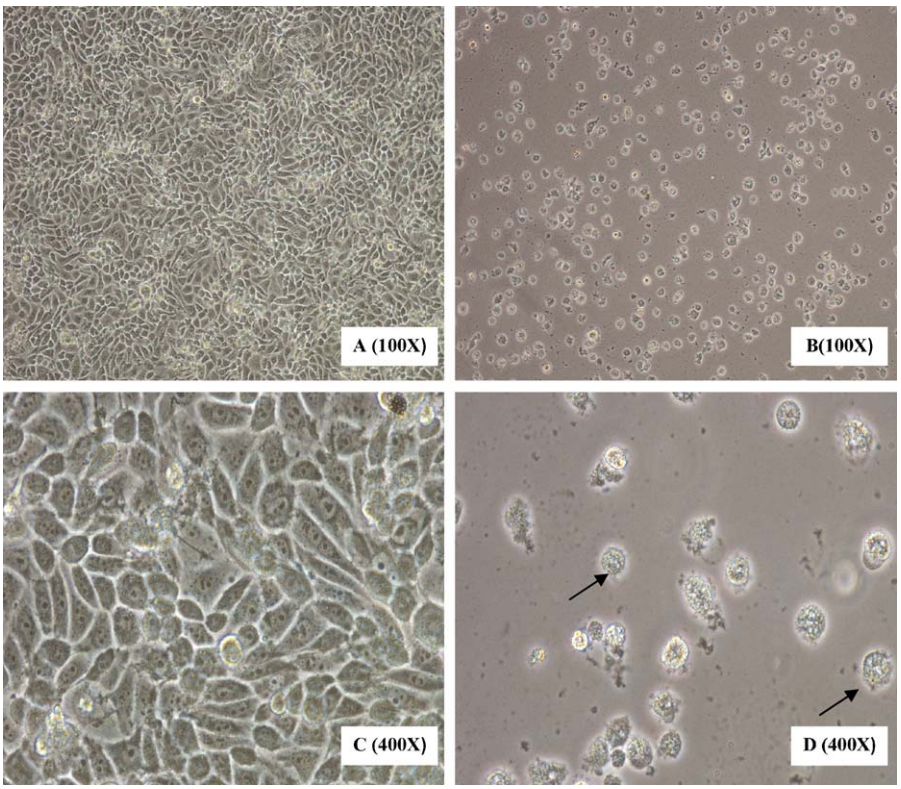

Fig. 3 – Morphological changes of CaOV3 untreated (A and C) and treated with 300 μg/ml of kenaf seed oil extracted by Sonication (B and D) for 72 h. Reduced cell population was observed in the treated sample (B). Affected cells showed some features characteristic of apoptosis such as chromatin condensation and nuclear fragmentation (arrows).

Table 1 – IC_{50} value of kenaf seed oils from different ways of extraction towards CaOV3 cell line after 72 h as determined by using the MTT assay. Each data point represents the mean of three independent experiments ± SD. $p < 0.05$ is considered significant.

Extracts	IC_{50} (μg/ml)
200 bars, 40 °C	184.00 ± 5.00
200 bars, 60 °C	417.00 ± 1.00
200 bars, 80 °C	336.50 ± 1.32
400 bars, 40 °C	206.67 ± 4.04
400 bars, 60 °C	213.00 ± 2.64
400 bars, 80 °C	173.80 ± 7.94
600 bars, 40 °C	211.67 ± 3.79
600 bars, 60 °C	187.00 ± 5.20
600 bars, 80 °C	188.33 ± 10.12
Soxhlet long time (12 h)	201.67 ± 2.31
Soxhlet short time	219.33 ± 2.08
Sonication	172.33 ± 7.23

2008). For CaOV3, increase in temperature did not give significant effects ($p > 0.05$) to the IC50 value (Table 1). This might be due to the bioactive components towards CaOV3 are thermal-stable.

Cell viability decreases with increase in the concentration of the oil (Fig. 2). It shows that the oil killed the cells in a dose-dependent manner. ALA and phytosterols are the two possible candidates responsible for the killing activity (cytotoxicity) of the extract in CaOV3 cell line. Nyam et al. (2009) and Mohamed et al. (1995) reported that kenaf seed oil has high percentage of phytosterols. Phytosterols were found to reduce *in vitro* metastatic ability of MDA-MB-231 human breast cancer cells (Awad et al., 2001) and inhibits growth of HT-29 human colon cancer cells by activating the sphingomyelin cycle (Awad et al., 1998). In addition, phytosterols have been claimed to possess chemopreventive properties against breast, colon and prostate cancers (Awad et al., 2000; Awad and Fink, 2000).

Data obtained from the study can be used as basis for development of a disease-oriented drug-discovery. As oil with IC_{50} value between 125 and 5000 μg/ml is considered to be developed as a cancer therapeutic agent (Manosroi et al., 2006), kenaf seed oil could be a potential one for the management of cancer.

Cell number decreases with the increase in concentration of the oil (Fig. 3B). Affected cells showed some features of apoptosis such as chromatin condensation and nuclear fragmentation (Kerr et al., 1972). Apoptosis is a type of cell death, and agents with the ability to induce apoptosis in tumours have the potential to be used for anti-tumour therapy (Saraste and Pulkki, 2000). Phytosterols, one of the main components of kenaf seed oil, were reported to have effects on the membrane structure and function of tumour and host tissue, and signal transduction pathways that regulate tumour growth and apoptosis (Awad and Fink, 2000).

4. Conclusion

Kenaf seed oils from the three extractions were cytotoxic towards CaOV3 cell line in a dose dependent manner possibly via the induction of apoptosis. In considering the safety of the product, SFE technology is a better alternative extraction method that is suitable in kenaf seed oil extraction.

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