WALAILAK JOURNAL

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Anticancer Activity of *Lindernia crustacea* (L.) F. Muell. var. Crustacean on Human HCT116 Colon Cancer Cell *via* Cellular Lipid and β-sheet Protein Accumulation[†]

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Received: 22 April 2019, Revised: 5 July 2019, Accepted: 20 August 2019

Abstract

Lindernia crustacea (L.) F. Muell. var. crustacean or "Ya Kap Hoi: YKH", which is an edible vegetable, is commonly seen in Thailand. This study investigated its anticancer properties with high antioxidant activity by reducing power activity, excluding alkylation activity. It explored YKH extract induction of anticancer activity through biomolecular changes in the HCT116 human colon cancer cell line. The ethanolic extract stock solution and water extract stock solution were prepared. NR assay was used for cancer cell cytotoxic testing and Fourier transform infrared (FTIR) microspectroscopy was used for biomolecular changes study on lipid, protein and nucleic acid/DNA. The apoptosis induction by the extracts was detected by using Annexin V-FITC and DAPI staining. The compounds in the YKH ethanolic extract was performed by using GC-MS analysis. As a result, YKH ethanolic extract caused HCT116 colon cancer cell death in the dose dependent manner after 24 h exposure, and the 50 % cell death (IC₅₀ concentration) was $195.4 \pm 12 \,\mu$ g/mL. Cellular biochemical changes observed from FTIR data showed that YKH ethanolic extract treated HCT116 colon cancer cell. There is an increase in lipid content and a reduction in intensity of nucleic acid/DNA, α -helix protein structure at 1,656 cm⁻¹ was reduced and peak position of β -sheet structure (1,637 cm⁻¹) was shifted to lower frequency. From the analysis results. YKH ethanolic extract seems to exert anti-colon cancer effect by changing cellular biomolecular structure of lipids and β -sheet protein accumulation, supporting apoptotic induction. The compounds in the YKH ethanolic extract mainly yielded fatty acids, which may be useful as potential compounds.

Keywords: Lindernia crustacean, Ya Kap Hoi, Anticancer, FTIR

[†]Presented at the International Conferences on Biomedical Sciences and Medical Technology 2019: June 20 - 21, 2019

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Introduction

Lindernia crustacea (L.) F. Muell. var. crustacean or "Ya Kap Hoi: YKH" is commonly found as grass in Thailand and has been used as an edible vegetable. Despite no confirmation of YKH in clinical use, the claimed uses of it for anticancer, antibacterial activity and treatment of joint pain was reported in the newspaper. For example, Thai traditional uses stem for treatment of venereal disease and leaves decoction for postpartum conditions, in India, the dry powder was infused in aqueous for drinking in morning for detoxification purpose, chewing leaves with lemon is used to increasing the biliary flow. The skin application was also reported to be used for the treatment of ringworm, scalding, burning, antifungal activity and antibacterial activity. In Malaysia, it is used for the treatment of scalds and burn, anti-inflammatory specifically from tick and also for postpartum conditions [1]. However, in the literature, empirical evidence about the association of YKH with cancer therapy was not found for L. crustacea (L.) F. Muell. var. crustacean. Our recent analysis of in vitro screening of biological activities of the extract found that both aqueous and ethanolic extracts of YKH showed high antioxidant activity by reducing power activity, but not alkylation activity. YKH ethanolic extract can inhibit immune cells proliferation activity more than the YKH aqueous extract. Only YKH ethanolic extract, but not YKH aqueous extract showed potent anticancer activity on many human cancer cell lines such as colon cancer cell line (HCT116), liver cancer cell (HepG2) and Jurkat leukemic T cell. However, YKH ethanolic extract has less toxicity on human lung cancer cell line (SK-LU1) and non-toxicity on African green monkey kidney cell (Vero normal cell line) [2]. There has been no previous information reported about mechanism of YKH anticancer activity.

Fourier transform infrared (FTIR) microspectroscopy was adapted to detect biochemical changes in cell samples with no need for use of reagents or complicated sample handling [3,4], to differentiate between human brain tumours and normal brain tissue [5] and also can be applied to detect cellular biomolecular components changing and structures of cells be detected before and during cell death [6,7]. It can also applied for studying of cell apoptosis, cell cycle staging, cell differentiation and cell proliferation of a variety of lines and tissues [3,6,7], such as human leukemic U937 cancer cells [8], human lung adenocarcinoma cisplatin-sensitive cell line (SK-LU-1) [9] and human melanoma cell line (SK-MEL2) [10].

Thus, this study aimed to investigate anticancer mechanism of YKH ethanolic extract in the HCT116 human colon cancer cell line using FTIR microspectroscopy for the analysis of changes of major cellular biomolecules such as lipid, protein and nucleic acid/DNA of treated the HCT116 human colon cancer cell line.

Materials and methods

Plant preparation and extraction

Lindernia crustacea (L.) F. Muell. var. crustacean or female "Ya Kap Hoi : YKH" was provided by Kamphaeng Phet Provincial Public Health Office. The YKH was extracted and prepared by using the following method. The crude 50 % ethanol-aqueous extract was obtained as previously described [11,12]. The fresh weight was 200.40 g and dried for 1 - 2 h. The dried YKH was ground and homogenized with 500 mL water, and filtered through gauze cloth. The filtrate was centrifuged at 13,000 rpm for 10 min and freeze-dried and then frozen at - 20 °C. The obtained dried residue after freeze-drying was the YKH aqueous extract (9 g). The remaining residue was subjected to oven drying at 50 °C for 24 h. The obtained dried residue was 29.78 g which was macerated in 100 % ethanol for 7 days with concessionary shaking. The filtration was done using gauze cloth and filter paper, respectively. The filtrate was evaporated under vacuum in 40 °C until the dried YKH ethanolic extract (0.13 g) was obtained. Two parts of YKH crude extracts were dissolved in dimethyl sulfoxide (DMSO) at 20 mg/mL as stock solutions. The stock YKH extracts were freshly diluted in fresh medium at various concentrations before use.

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Anticancer activity by NR assay

Cytotoxic test was performed for detection of anticancer activity. HCT116 human colon cancer cell line was used as cell models and 6×10^5 cells/mL of normal African green monkey kidney cell line (Vero) was used as normal cell control. The stock at 20 mg/mL of YKH in DMSO was freshly diluted before use with fresh culture medium to desired concentrations. The final concentration of DMSO in each concentration did not exceed 1 % v/v to ensure less than 10 % toxicity. The determination of cancer cell cytotoxic activity was performed *in vitro* by using NR assay as previously described [8,12,13]. Briefly, 100 μ L/well of 3×10⁵ cells/mL HCT116 and 6×10⁵ cells/mL of Vero were seeded into 96-wells plate and incubated for 24 h. Cell were treated with the YKH extracts at various desired concentrations ranging from 50 to 500 µg/mL for 24 h and untreated cell was a control cell group. The 96-wells plate was centrifuged at 492 g for 10 min. Supernatant was removed and 100 μ L of 50 μ g/mL of NR solution in media was added into each well. The cells were incubated for 2 h at 37 °C in 5 % CO₂ incubator. The 96wells plate was centrifuged at 492 g for 10 min and supernatant was removed and cells were washed twice with 150 µL of 1x PBS. Cells were solubilized with 100 µL of 0.33 % HCl/isopropanol. The viable cells with NR were measured for absorbance with dual wavelengths at 520 nm and 650 nm (reference wavelength). The % cytotoxicity was calculated and the concentration causing 50 % cell death (IC_{50}) was determined from the plot between the % cytotoxicity and the concentration of YKH for a linear equation calculation.

Cellular biochemical changes study by FTIR microspectroscopy

Based on the property of infrared energy is absorbed by material and causes vibration of chemical bonds in molecules. Functional groups of molecules tend to absorb infrared radiation of the same wavelength range, depending on structure of the molecule [14]. The cellular biochemical changes, such as proteins, lipids and nucleic acids, induced by YKH extract was observed by using Fourier transform infrared (FTIR) microspectroscopy. Treated cells were washed in 0.9 % NaCl and dropped on low-e slides (MirrIR; Kevley Technologies, Chesterland, OH, USA), then rinsed in distilled water [8-10,15]. Fixed cells were dried and kept in desiccators until used. Measurements were performed at the IR End Station, Synchrotron Light Research Institute (Public Organization), Nakhon Ratchasima, Thailand, IR data acquisition was accomplished using a Bruker Hyperion 2000 microscope (Bruker Optics Inc., Ettlingen, Germany), equipped with a nitrogen-cooled MCT (HgCdTe) detector with a 369IR objective lens coupled to a Bruker Vertex 70 spectrophotometer. IR spectra were obtained from the reflection mode by collecting 64 scans, at 4 cm⁻¹ resolution, over a measurement range of 4,000 - 600 cm⁻¹. Spectral acquisition and instrument control were performed using OPUS 7.0 (Bruker Optics Ltd, Ettlingen, Germany) and Unscrambler 9.7 software (CAMO, Norway). Spectra of each condition was used for further analysis including HCT116 treated with 1×IC₅₀, 2×IC₅₀ of YKH ethanolic extract, cisplatin-treated cells, and untreated cells. Infrared spectra of biomolecules provided novel information on the lipids, proteins and nucleic acids investigated. FTIR spectral changes of HCT116 under treatment of YKH ethanolic extract were used for investigation of cellular biochemical changes of cancer cells. To reduce the complexity of FITR spectra and select the high quality spectra, spectral pretreatment with 2nd derivative and extended multiplicative signal correction (EMSC) were performed. Spectral different were determined by principal component analysis (PCA) (Unscrambler 9.7 software - CAMO, Oslo, Norway) across the 1st 6 PCs. The average FTIR absorbance spectra of HCT116 cancer cell lines were compared. Individual FTIR spectra of each study group was analyzed by PCA. Spectra were processed using the 2nd derivative and the vector was normalized using the Savitzky-Golay method (3rd polynomial, 9 smoothing points), and then normalized with EMSC in the spectral regions from 1,800 to 750 cm⁻¹. Results were interpreted by focusing on 2^{nd} derivative spectra with a negative peak. Peaks in raw spectra become negative peaks on either side of the 2^{nd} derivative spectra. The differences between control and treated groups were based on absolute absorbance intensity. PCA provide a visualization of clustering of similar spectra within data sets in score plots and an identification of variables in loading plots. Score plots describe data structure in terms of clustering observed sample patterns and more generally show sample differences or similarities. Loading plots describe data structure in terms of variable contributions and correlations. Results were interpreted by taking 2nd derivative spectra showing the negative peak. Positive and negative loading plots had opposite correlation with negative and positive score plots, respectively.

Apoptosis death mode by Annexin V-FITC/PI staining

The apoptosis induction by the YKH ethanolic extracts was detected by flowing cytometry using Annexin V-FITC apoptosis detection kit (Bender MedSystems GmbH, Austria). Necrotic changes were determined using propidium iodide (PI). In principle, an early event in apoptosis is the flipping of phosphatidylserine (PS) located at the plasma membrane from the inside surface to the outside surface. Annexin V binds specifically to PS and FITC-conjugated Annexin V can be used as a fluorescence probe to label apoptotic cells. Propidium iodide (PI) is used in conjunction with Annexin-V FITC. The cell membrane integrity excludes PI in viable and apoptotic cells, whereas necrosis cells are permeable to propidium iodide. Thus, dual parameter of flow cytometry (FACS) analysis allows for discrimination between viable, apoptotic and necrotic cells. An Annexin-V FITC and propidium iodide double staining method was used. Briefly, harvested HCT116 human colon cancer cell line were resuspended in binding buffer (10 mM HEPES/ NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂). 5 μ L of Annexin-V/FITC reagent and 10 μ L of PI reagent were added to the cell samples. The mixtures was incubated for 15 min in the dark at room temperature and then analyzed by flow cytometry [13,16-19].

Nuclei morphological change in cells undergoing apoptosis by DAPI staining

Apoptosis effect of the YKH ethanolic extract was determined by a fluorescence dye staining employing DAPI to identify the condensation and fragmentation of nuclear DNA. The $1 \times IC_{50}$ and $2 \times IC_{50}$ concentration of the extract obtained from cytotoxic test were treated with HCT116 human colon cancer cell line for 24 h. The $2 \times IC_{50}$ (300 µg/mL) of Cisplatin was used as positive control. After cells were treated, the culture medium was removed and washed by using serum-free medium and cells were fixed by cold methanol. After that the DAPI dye was added to stain nuclear DNA for 1 h. The excess dye was removed and PBS:Glycerin (1:1) was added. The average % apoptotic cells were calculated from 10 eye views under inverted fluorescence microscopy at a 40× magnitude [8,13-16,19-20].

GC-MS analysis

The GC-MS analysis was performed on an Agilent 6890N gas chromatograph (Agilent Technologies, China) coupled to an Agilent 5973N mass selective detector (Agilent Technologies, U.S.A.). Capillary GC analysis was performed on a DB-5ms (30 m × 0.25 mm i.d., 0.25 μ m) capillary column (5 % diphenyl, 95 % dimethylpolysiloxane) with helium as carrier gas. GC conditions were 80 °C and held for 1 min, at the rate of 12 °C min⁻¹ to 189 °C for 0 min, then raised at the rate of 2 °C min⁻¹ to 210 °C for 7 min, at the rate of 15 °C min⁻¹ to 225 °C for 10 min and at the rate of 35 °C min⁻¹ to 275 °C for 7 min. The carrier gas (He) flow rate was 1.7 mL/min. Injection temperature was at 200 °C with splitless mode. The transfer line, ion source and quadrupole analyzer temperatures were maintained at 280, 230 and 150 °C for Electron ionization (EI) mode. A solvent delay of 2.5 min was selected. In the full-scan mode, (EI) mass spectra in the range of 50 - 500 (*m*/*z*) were recorded at 70 eV electron energy with an ionization current of 34.6 mA and a multiplier potential of 1,764.7 V. In the full-scan mode, target ions under study were monitored by database Wiley 7N.1 [13,16,20].

Results and discussion

Cytotoxic effect of YKH extract on HCT116 colon cancer cell

The YKH ethanolic extracts and aqueous extract were tested for the cytotoxicity effect on HCT116 human colon cancer cell lines and African green monkey kidney cell line (Vero). The IC₅₀ represented the concentration of the extract that possessed 50 % cell death after 24 h exposure in only treated HCT116 with YKH ethanolic extracts was 195.4 \pm 12 µg/mL, whereas the IC₅₀ value cannot be calculated even using maximum concentration up to 500 µg/mL of YKH aqueous extract. Similar to Vero, the IC₅₀ value cannot be calculated even using maximum concentration up to 500 µg/mL of 500 µg/mL of YKH aqueous extract or YKH ethanolic extracts which were demonstrated in **Figure 1**. The IC₅₀ value cannot be calculated even

using maximum concentration (500 μ g/mL), considered to be inactive. The results showed that only the YKH ethanolic extract exerted cytotoxicity against HCT116 human colon cancer cell lines but not toxic to Vero normal cell line.



Figure 1 Cytotoxic effect of *Lindernia crustacea* (L.) F. Muell. var. crustacean (YKH) ethanolic extract on HCT116 colon cancer cell after 24 h exposure.

Biochemical changes observed by FTIR microspectroscopy in HCT116 treatment with YKH ethanolic extract

PCA was performed over 500 spectra of all treatments. The best clustering between treatment was achieved by PC1 and PC2 (**Figure 2**). PC1 explained 86 % of the total variance in the data set, separating 2 groups: (i) a group of control and a group of cells treated with $1 \times IC_{50}$ of YKH ethanolic extract and (ii) a group of cells treated with ethanolic extract $2 \times IC_{50}$ and standard chemical cisplatin treatment. The PCA loadings plot (**Figure 2b**) was used to determine the regions of the FTIR spectrum which mostly contributed to the clustering observed in the scores plot (**Figure 2a**). Spectra from $2 \times IC_{50}$ and standard chemical cisplatin were distinguished from control and $1 \times IC_{50}$ by having positive PC1 scores (**Figure 2a**). This was explained by these spectrum having the highest negative values for PC1 loading (**Figure 2b**) with variables between 2,919, 2,850, 1,739, 1,648 cm⁻¹, possibly indicating that the lipid, lipid head group and random coil band of protein are most strongly responsible for discrimination. Moreover, spectra from control and $1 \times IC_{50}$ had the highest positive value for PC1 loading at variable 1662 and 1085 cm⁻¹, indicating the high content of α -helix in secondary structure of protein and nucleic acid.

The results of average 2^{nd} derivative FTIR spectra of HCT116 colon cancer cell lines under treatment of YKH ethanolic extract confirmed PCA analysis (**Figure 3**). The intensity of lipid _{vs}CH₂, _{vas}CH₂ stretching modes (~ 2,852 and 2,921 cm⁻¹, respectively) was observed under 2×IC₅₀ and standard chemical cisplatin treatment. Additionally, the carbonyl band associated with the lipid head-group (_{vs}C=O stretching at 1,740 cm⁻¹) showed correlation result as lipid profile (**Figure 3b**). The α -helix band of protein in control and 1×IC₅₀ treatment presented higher intensity, suggesting that it contained higher amount than that of 2×IC₅₀ and standard chemical cisplatin treatment. Interestingly, 2×IC₅₀ and standard chemical cisplatin treatment seemed to have effects on nucleic acid of colon cancer, which had low intensity at 1,085 cm⁻¹.



Figure 2 PCA score plot a) and loadings plot b) for individual spectra of HCT116 colon cancer cell under treatment of YKH ethanolic extract at $1 \times IC_{50}$, $2 \times IC_{50}$ of YKH ethanolic extract, cisplatin-treated cells, and untreated cells. Biochemical changes from each group classified as per their PC1 versus PC2 score plot. PC1 and PC2 explained 86 and 7 % of the total variance, respectively. Spectra derived using second-derivative processing with the entire biochemical cell fingerprint region between 3,000 - 2,800 cm⁻¹ and 1,800 - 900 cm⁻¹. The lipid, lipid head group and random coil band of protein are most strongly responsible for discrimination. The spectra from control and $1 \times IC_{50}$ have the highest positive for PC1 loading at variable 1,662 and 1,085 cm⁻¹ indicating the accumulation of α -helix structure protein and nucleic acid.



Figure 3 Average second-derivative FTIR spectra of HCT116 colon cancer cell lines under treatment of YKH ethanolic extract at $1 \times IC_{50}$, $2 \times IC_{50}$ of YKH ethanolic extract, cisplatin-treated cells, and untreated cells. FTIR spectra were processed after 9 points of smoothing and normalized with EMSC over the range of 3,000 to 900 cm⁻¹. a) Shows an enlargement of the lipid spectral region from 2,800 to 3,000 cm⁻¹ and b) shows an enlargement of protein, carbohydrate and nucleic region from 1,800 to 850 cm⁻¹.

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IR spectra of macrobiomolecules can provide cellular information such as pattern of lipids, proteins and nucleic acids [21-23]. Therefore, the FTIR spectral changes data can be used to track mode of cell death in many reports by evaluation of cellular biochemical changes at the biomolecular level due to treatment on cells. Our results is in agreement with the erythroleukaemic K562 cell line treated with imatinib mesylate showed the lipid ester band integral area (1,723 and 1,756 cm⁻¹) in apoptotic cells was greater than that of viable cells [24]. The increasing of cellular lipid ester band at 1,740 cm⁻¹ and between 2,800 and 3,000 cm⁻¹ of C-H stretching was showed in the T-lymphoblastic cell line (CEM) treated with etoposide showed [25]. The accumulation of lipid content, raising of β -pleated sheet intensity and α -helix protein shifting confirming induction of apoptosis via pro-apoptotic proteins in human leukaemia cell lines (U937) treated with melphalan, P. kesiya extract and C. formosum extract [8]. Lipid content increasing, nucleic acid/DNA intensity reduction, α -helix protein structure at 1,656 cm⁻¹ reduction and β sheet protein at 1,637 cm⁻¹ shift to lower frequency also confirming alteration in apoptotic proteins in human. Human lung adenocarcinoma cisplatin-sensitive cell line (SK-LU-1) treated with melatonin [9]. FTIR spectra also can assigned to monitor the integrity of the lipid-containing melanosome in human melanoma cells (SK-MEL2) treated with sesamol and kojic acid resulting lipid and nucleic acid bands were significantly depleted with the secondary protein structure shifted to a more β -sheet protein [10].

The lipid, lipid head group and random coil band of protein are most strongly responsible for discrimination in this current study. Under 2×IC₅₀ YKH ethanolic treatment and standard chemical cisplatin treatment, the intensity increasing of lipid vsCH2, vasCH2 stretching modes (~ 2,852 and 2,921 cm⁻¹, respectively) were observed and correlated with the carbonyl band associated with the lipid headgroup (_{vs}C=O stretching at 1,740 cm⁻¹). Many specific markers can be observed during the apoptotic process cell changes such as membrane fluidity, ionic charge, membrane proteins and lipid structure. The presence of phosphatidylserine on the outer leaflet of plasma membranes correlates with the increased of peak FTIR spectra between 3,000 and 2,800 cm⁻¹ [26,27]. The broadened and lower wavelength shifting from 1,655 to 1,649 cm⁻¹ of amide I peak was reported in human melanoma cell line (SK-MEL28) treated with boric acid [15].

The spectra from control and 1×IC₅₀ YKH ethanolic treatment in this study had the highest positive value for PC1 loading at variable 1,662 cm⁻¹ indicating the accumulation of α -helix protein. Average 2nd derivative results of FTIR spectra of HCT116 colon cancer cell lines under treatment of YKH ethanolic extract confirmed by PCA analysis. The α -helix protein were higher presented in control and 1×IC₅₀ YKH ethanolic extract treatment than that of $2 \times IC_{50}$ and standard chemical cisplatin treatment. It is well recognised that caspases play an important role in the apoptotic process [28]. Caspases conformation visualized by X-ray spectography mostly consists of parallel β -sheets, the shifting peak position of β sheets confirms caspases conformation changing [29]. The increasing β -sheet peaks seems to confirm the induction of apoptosis through caspases activity.

The spectra from control and $1 \times IC_{50}$ YKH ethanolic treatment in this study also have the highest positive for PC1 loading at variable 1,085 cm⁻¹ indicating the higher content of nucleic acid. The results of average second-derivative FTIR spectra of HCT116 colon cancer cell lines under treatment of YKH ethanolic extract were confirmed by PCA analysis. The 2×IC₅₀ YKH ethanolic treatment and standard chemical cisplatin treatment tend to effect on nucleic acid of HCT116 cancer cell. Its suggesting nucleic acid content of HCT116 cells was shown to be reduced by YKH ethanolic treatment. These findings support previous reports, indicating nucleic acid content declines after apoptosis, yet induced by standard chemotherapeutic drugs, in human leukaemia cell lines CEM and U937 [8,25,30]. Nucleic acid/protein ratio observed by FTIR data was significantly reduced after SK-MEL28 human skin melanoma cells were treated with boric acid [15].

Apoptosis effect determination by flow cytometry

The apoptosis death mode by using by flow cytometry was attempted but the false positive value was obtained due to the color of the YKH ethanolic extract interfered with the staining dye. So, apoptosis induction effect of the YKH ethanolic extract was, further, performed based on morphological study using DAPI staining assay.

Apoptosis determination by DAPI staining

The YKH ethanolic extract at $2 \times IC_{50}$ concentration could induce the apoptotic cell death in HCT116 colon cancer cells. Moreover, the % apoptotic cancer cells of the YKH ethanolic extract at $2 \times IC_{50}$ was significantly higher than the positive controls-cisplatin standard chemotherapeutic drugs in HCT116 cell lines (**Table 1**). The nuclei morphological changes finding with DNA fragmentation by DAPI staining confirmed FTIR results, which indicated the reduction of nucleic acid content in HCT116 colon cancer cells undergoing apoptosis [9].

 Table 1 % Apoptotic cell induced in human HCT116 colon cancer cells by YKH ethanolic extract compared to cisplatin.

DAPI staining	YKH ethanolic extracts	YKH ethanolic extracts	Cisplatin (positive control)
Concentration	200 µg/mL	400 µg/mL	300 µg/mL
	$(1 \times IC_{50})$	$(2 \times IC_{50})$	$(2 \times IC_{50})$
% Apoptotic cell death	24.2 ± 7.79	$70.8 \pm 8.49 *$	26.2 ± 5.01
(mean±SD)			

*p < 0.05 vs. control

GC analysis

The YKH extract component compounds was summarized according to the GC-MS library as shown in **Table 2**. The GC-MS profile of YKH was set according to the method used to define the polyphenolic compound. It was found that under the condition studied, the main compounds yielded fatty acids such as palmitic acid, its ester and some stearic acid. However, it should be noted that this identification is based on the database and the quality of the detected compound.

Extract solvent	Retention time (min)	Peak height	% of total area	Mass spectra (m/z)	Assigned compounds (% quality)
Ethanol	31.07	33,946	4.13	53, 60, 67 73, 85, 79, 85, 91, 97, 111, 121, 129, 135, 143, 153, 163, 171, 178, 185, 199, 228	Tetradecanoic acid (97 %)
	32.87	63,757	5.02	58, 71, 85, 95, 109, 123, 137, 151, 165, 179, 194, 210, 250	2-Pentadecanone (91 %)
	35.00	386,305	38.95	53, 60, 73, 85, 97, 107, 115, 129, 143, 157, 171, 185, 199, 213, 227, 239, 256	n-Hexadecanoic acid or palmitic acid (99 %)
	35.48	310,896	20.54	55, 73, 88, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 241, 255, 284	Palmitic acid, ethyl aster (99 %)
	37.74	85,838	6.80	55 73 83 97 115 129 143 171 178 185 199 213 227 241 255 263 284	Octadecanoic acid or stearic acid (97 %)

Table 2 GC-MS data of YKH extracts.

Conclusions

This study found that YKH ethanolic extract exerted anticancer activity on human colon cells with specific manner. These were observed through changes in the biomolecular structure of lipids, nucleic acids and proteins *via* FTIR microspectroscopy. Tracking IR signatures of YKH ethanolic extract in HCT116 cancer cells, the differences in intensity of the FTIR bands associated with lipids, proteins and nucleic acids were responsible for determination of the anticancer mode of action. FTIR spectra revealed augmentation of lipid content, reduction in nucleic acid, reduction in α -helix protein structures and β -sheet protein structure accumulation. From the FTIR results, YKH ethanolic extract seems to exert anticolon cancer effect *via* changing cellular biomolecular structure of lipids and β -sheet protein accumulation which supporting apoptotic induction. The compounds in the YKH ethanolic extract mainly yielded fatty acids which may contributed as potential compounds. A further prospective study of the potential compounds for their specificity, and also effective molecular techniques for apoptotic cell death induction still need for more deepen understanding of the mechanism.

Acknowledgements

This work was supported by the Synchrotron Light Research institute (Public organization) for financial support under the 6106/12 project.

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