Pharmacogn J. 2020; 12(5): 1050-1057
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Cytotoxicity of *Lactobacillus plantarum* KK518 Isolated from Pak-Sian Dong (Thai Fermented *Gynandropsis pentaphylla* DC.) Against HepG2, MCF-7 and HeLa Cancer Cells

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ABSTRACT

Background: Pak-Sian Dong is a fermented vegetable product of Thailand prepared from aerial parts of Pak-Sian (*Gynandropsis pentaphylla* DC.). *Lactobacillus plantarum* KK518 was isolated from Pak-Sian Dong and already assessed for its probiotic attributes. **Objective:** The aim of this work was to determine the untapped cytotoxic effects of *L. plantarum* KK518 extract against HepG2 (liver cancer), MCF-7 (breast cancer) and HeLa (cervical cancer) cells. **Materials and Methods:** The bacterial extracts were prepared from whole cultures; containing cells and broths using ethyl acetate as extracting solvent and the dried extracts were redissolved in ethanol before use. Cytotoxic, antiproliferative and antimigratory effects of the bacterial extracts on three types of cancer cells were determined using 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetra zolium bromide (MTT) assay, clonogenic formation and wound healing assays, respectively. **Results:** *L. plantarum* KK518 extract showed the highest cytotoxicity at 90.88% at 1,000 μg/mL against HeLa cells (IC_{50} of 371.97 μg/mL) over 48 h of exposure. Anti-colony formation test showed that the bacterial extracts at 600, 800 and 1,000 μg/mL over 48 h led to a complete inhibition of colony formation of HeLa cells; however the highest IC_{50} of 418.52 μg/mL was found in HepG2 cells suggesting that HepG2 was least affected by bacterial extract. Likewise, HepG2 cells seemed to be most resistant to antimigratory effects as observed by highest relative area of the wound at most time intervals and most extract concentrations. **Conclusion:** *L. plantarum* KK518 offers a potential use as a bio-therapeutic with chemopreventive effects against cervical, breast and liver cancers.

Key words: HepG2, HeLa, MCF-7, *L. plantarum* KK518, Pak-Sian-Dong.

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History

- Submission Date: 31-04-2020;
- Review completed: 15-06-2020;
- Accepted Date: 22-06-2020.

DOI : 10.5530/pj.2020.12.148

Article Available online

http://www.phcogj.com/v12/i5

INTRODUCTION

Cancer is the most common cause of death in Thailand, with 85,000 fatalities a year from liver and bladder, lung, colon, breast and cervical disease. Over the next 21 years, 24 million Thais are predicted to be diagnosed with cancer.[1] Chemotherapy is one of the most effective treatments for prolonging the patient's life. However, many chemotherapeutic drugs encountered reduction of therapeutic effect due to the problem of drug-resistance[2] and may as well exert toxicity to normal cells leading to unpleasant side effects to the patients. These adverse sides of cancer chemotherapy prompt the continuing discovery of novel anticancer agents or alternative treatments. Microbes have so far established their candidacy as alternative anticancer treatment through the production of several bioactive compounds such as antioxidant enzymes, immune toxins, proteins, and secondary metabolites for therapeutic purposes.[3]

Certain fermented foods have been linked to anticancer benefits due to bioactive natural products from vegetables and also lactic acid bacteria (LAB). For example, kimchi, a fermented cabbage originated from Korean, has been well-recognized for its antioxidant, antiobese, cancer preventive, and other health-promoting benefits.[4] The functionality of LAB mainly from the predominant genus *Weissella* followed by *Lactobacillus plantarum* in the fermentation process of kimchi also contributed to its cancer prevention.[5]

In Thailand, Pak-Sian-Dong is a fermented vegetable product prepared from aerial parts of Pak-Sian (*Gynandropsis pentaphylla* DC.) and it has been commonly consumed in Northeastern Thailand. LAB namely *Pediococcus cerevisiae*, *Lactobacillus brevis* and *Lactobacillus plantarum* have been major species prevalent during fermentation of Pak-Sian-Dong.[6] The previous work showed probiotic attributes of *Lactobacillus plantarum* KK518 isolated from Pak-Sian Dong in Khon Kaen province, Thailand.[7] However, its cytotoxic effect is yet to be evaluated.

L. plantarum has been well-known for prominent probiotic effects documented extensively for rats, poultry and pigs.[8] In spite of the emerging evidence of anticancer attributes of LAB, very limited data is available on cytotoxic and antiproliferative activity of *L. plantarum*. Therefore, the aim of this work was to determine the cytotoxicity of *L. plantarum*

Cite this article: Luang-In V, Saengha W, Buranrat B, Nudmamud-Thanoi S, Narbad A, Pumriw S, *et al.* Cytotoxicity of *Lactobacillus plantarum* KK518 Isolated from Pak-Sian Dong (Thai Fermented *Gynandropsis pentaphylla* DC.) Against HepG2, MCF-7 and HeLa Cancer Cells. Pharmacogn J. 2020;12(5):1050-7.

originated from Thai fermented food on HepG2 (liver cancer), MCF-7 (breast cancer) and HeLa (cervical cancer) cancer cells.

MATERIALS AND METHODS

Source of bacterium and cultivation

L. plantarum KK518 was isolated from Pak-Sian Dong in Khon Kaen province, Thailand and assessed for probiotic attributes already.[7] The bacterial culture was stored in 20% glycerol stocks at -80°C at Natural Antioxidant Innovation Research Unit, Department of Biotechnology, Mahasarakham University, Thailand. *L. plantarum* KK518 was grown in de Man, Rogosa and Sharpe (MRS) broth pH 6.8 (Difco, Detroit, MI, USA) and anaerobically cultured at 37°C for 24 h. Standard cultures were prepared by inoculation of 10 mL corresponding broth with 10 μL of a frozen stock (-80°C) and incubated accordingly as mentioned above. The strain was then subsequently sub-cultured in 10 mL broth for 24 h prior to inoculation into the fermentation tubes.

Crude bacterial extraction

Overnight bacterial culture (1% v/v) was inoculated in corresponding broths (100 mL) in 500 mL flask at 37°C at 200 rpm for 2 days. Negative controls were broths without bacterial inoculations. The crude bacterial extracts were prepared from whole cultures; containing cells and broths. After that, 100 mL ethyl acetate (ETAC) was added to microbial cultures for crude bacterial extraction at 37 °C at 200 rpm for 6 h and the ETAC layer as whole cell metabolite extract was separated and dried using a rotatory evaporator, dissolved in 95% ethanol and stored at -20°C till further analysis.

Cancer cell lines

The human breast adenocarcinoma (MCF-7, ATCC® HTB-22™), human cervical adenocarcinoma (HeLa, ATCC® CCL-2™) and human hepatocellular carcinoma (HepG2, ATCC® HB-8065™) cancer cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% of Fetal bovine serum and 100 U/mL of penicillin, 100 μg/mL of streptomycin, then incubated at 37 °C under 5% CO_2. DMEM media for cell lines cultures were renewed every 2-3 days until 80% confluency was reached. Cultured cell lines were washed with phosphate-buffered saline (PBS), pH 7.2 before trypsinization with 0.25% Trypsin-EDTA. DMEM media were added to cell lines and the cell colonies were counted using inverted microscope (NIB-9000, Xenon, China).

Cytotoxicity assay

Cytotoxicity was measured using MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetra zolium bromide)(Sigma, USA) assay following the method.[9] MCF-7, HeLa and HepG2 cells (5×10^3 cells/mL) were pipetted into 96-well plates and incubated at 37°C under 5% CO_2 for 48 h. Crude microbial extracts (0, 400, 600, 800 and 1,000 μg/mL) were added to wells and incubated for 48 h. MTT (5 mg/mL) dissolved in PBS buffer (pH 7.2) was added to the wells and incubated at 37°C under 5% CO_2 for 4 h. MTT was removed and 200 μL DMSO was added to dissolve the formazan and the purple color appeared if cells were alive. The absorbance was measured at 590 nm using microplate reader (M965+, Mastertech, Taiwan). Cytotoxicity of crude microbial extracts against cancer cells was measured as IC_{50} value. When % cytotoxicity was ≤ 50%, it represented non-cytotoxic effect and when % cytotoxicity was >50%, it represented cytotoxic effect. Cell morphology was also observed using an inverted microscope (NIB-100, Xenon, China).

Clonogenic assay

The colony formation assay was used to evaluate the effect of crude microbial extract on the regrowth of cancer cells as previously described.[9] The viable cancer cells were seeded in 6-well plates at a concentration of 500 cells/well for 24 h. The cells were then treated with various concentrations of crude microbial extracts (0, 400, 600, 800 and 1,000 μg/mL) for 24 h. Subsequently, the cells were washed with PBS buffer and resuspended into fresh DMEM and grown for 24 days. Subsequently, the DMEM medium was discarded, the cells were washed with PBS buffer three times, fixed with 100% methanol at −20°C, stained with 0.5% crystal violet in 100% methanol for 1 h at room temperature, washed with tap water, and the colonies were viewed and captured using a digital camera (Nikon D50).

Wound healing assay

Cell migration was evaluate using a wound healing assay as previously described.[9] Briefly, cancer cells were seeded into 24-well plates for 24 h. Cells were scratched using a sterile 0.2-mL pipette tip, certain cells were untreated and others were treated with different concentrations of crude microbial extracts (0, 400, 600, 800 and 1,000 μg/mL). Images were obtained from 0 to 48 h. The relative area (%) of the scratch = $\frac{\text{area of scratch at T h}}{\text{area of scratch at 0 h}}$ x 100. Cell migration was monitored by phase contrast microscopy (NIB-9000 inverted microscope; magnification, ×10, Xenon, China).

Statistical analysis of data

Data were collected in triplicate and results were reported as means ± standard deviation (SD). Statistical analysis was performed using One-way analysis of variance (ANOVA) and Duncan multiple range's test by the software SPSS (demo version). Statistically significant differences were considered if $p < 0.05$.

RESULTS

Cytotoxicity of *L. plantarum* KK518 extract on cancer cells

It was found that *L. plantarum* KK518 extract had cytotoxic effects on three types of cancer cells; HepG2, MCF-7 and HeLa cells in a dose-dependent manner (Table 1). The greatest antiproliferative effect on all cancer cells was observed at the highest dose (1000 μg/mL) of the bacterial extract. It seems *L. plantarum* KK518 extract was least cytotoxic towards MCF-7 cells based on the lowest cytotoxicity percentage at 68.53% at 1000 μg/mL bacterial extract and most cytotoxic towards HeLa cells at 90.88% at 1000 μg/mL bacterial extract (Table 1). These results were in accordance with the calculated IC_{50} values of *L. plantarum* KK518 extract on cancer cells. The inhibition concentration of 50% growth (IC_{50}) was determined. It was shown that the lowest IC_{50} values was derived from HeLa cells (Table 1) at 371.97

Table 1: Cytotoxicity of *L. plantarum* KK518 extract on cancer cells over 48 h of exposure.

Concentration (μg/mL) of *L. plantarum* KK518	Cytotoxicity (%)		
	HepG2	MCF-7	HeLa
1000	$90.00 \pm 0.31^{a,A}$	$68.53 \pm 1.03^{a,B}$	$90.88 \pm 0.33^{a,A}$
800	$70.00 \pm 4.86^{b,B}$	$63.10 \pm 9.15^{a,C}$	$88.03 \pm 1.05^{a,A}$
600	$27.05 \pm 1.39^{c,C}$	$42.67 \pm 7.37^{b,B}$	$59.95 \pm 1.58^{b,A}$
400	$20.00 \pm 6.02^{c,B}$	$28.21 \pm 1.06^{c,B}$	$54.72 \pm 1.65^{b,A}$
IC_{50} (μg/mL)	661.45 ± 0.80^{B}	682.53 ± 0.67^{C}	371.97 ± 1.23^{A}

Small letter and capital letter superscripts indicate significant differences ($p < 0.05$) in the columns and rows, respectively.

μg/mL and the highest IC_{50} value was from MCF-7 cells at 682.53 μg/mL (Table 1). No cytotoxicity activity was detected for MRS medium as a negative control against three types of cancer cell lines in this study (results not shown).

Apoptosis in cancer cells

Apoptosis is an autonomous process involving the activation, expression, and regulation of a number of genes, which leads to programed cell death to rid of unwanted or abnormal cells in organisms and maintain a stable internal environment.[10] The changes in cell morphology induced by *L. plantarum* KK518 extract treatment at 1000 μg/mL for 48 h under a phase contrast microscope were examined for the preliminary characterization of the cytotoxicity induced by the bacterial extract the cancer cells. In all treated cancer cell lines by bacterial extracts, cell rounding up, cell shrinkage, membrane blebbing and lack of cell adhesion were observed (Figure 1) as opposed to non-treated cancer cells. This indicated that *L. plantarum* KK518 extract in this work was able to induce apoptosis resulting in cancer cell death as observed by apoptotic bodies (Figure 1).

Anti-colony formation effect

In addition to the cytotoxicity effect, the antiproliferative effect of microbial extracts on long-term viability of cancer cells was investigated using a colony formation assay. The results showed that bacterial extracts from *L. plantarum* KK518 led to a dose-dependent decrease in the colony forming capacity of HepG2, MCF-7 and HeLa cells with IC_{50} values (Table 2; Figure 2) lower than those found in cytotoxicity effect to induce cancer cell death (Table 1), except for HeLa cells that its IC_{50} value was not determined since bacterial extract concentrations at 600, 800 and 1,000 μg/mL led to a complete inhibition of colony formation. Thus, HeLa cells are the most sensitive to bacterial extracts whilst HepG2 was most resistant due to the highest IC_{50} value of 418.52 μg/mL. To sum up, lower concentrations of bacterial extracts suffice to exert the antiproliferative effect in a longer time (24 days) in clonogenic assay when compared to the cytotoxic effect in a shorter time (48 h).

Antimigratory effect

Next, antimigratory effects of *L. plantarum* KK518 extracts on cancer cells were also examined. The results demonstrate that the bacterial extract inhibited cancer cell migration by decreasing wound-healing capacity in a dose- dependent manner in all three cancer cells (Table 3; Figure 3). However, HepG2 cells seemed to be most resistant to antimigratory effects caused by *L. plantarum* KK518 extracts as observed by highest relative area of the wound at most time intervals and most concentrations (Table 3). HeLa cells were most sensitive to the bacterial extract at 12 h and 24 h.

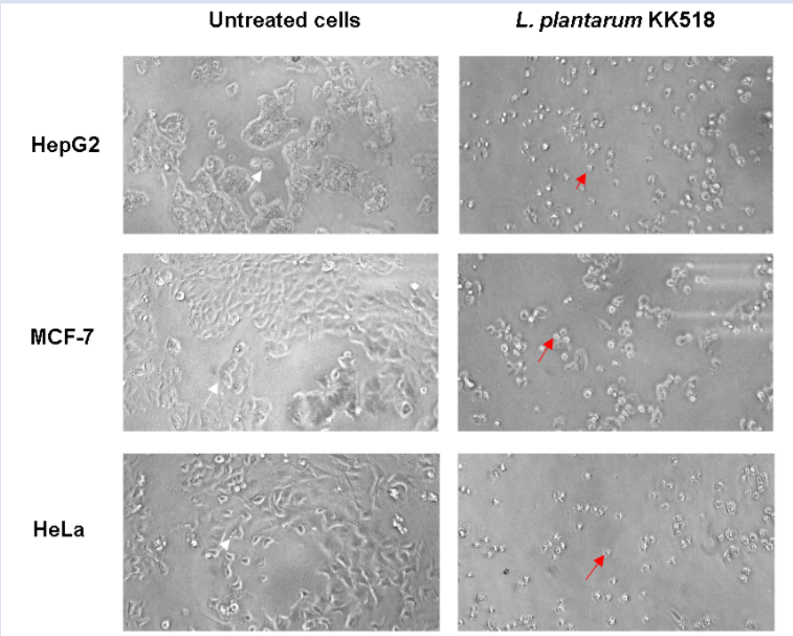

Figure 1: Cell morphology of HepG2, MCF-7 and HeLa cells after treatment with 1000 μg/mL of *L. plantarum* KK518 extract over 48 h of exposure compared to the untreated cells. White arrows point to live cells and red arrows point to dead cells.

Table 2: Anti-colony formation of *L. plantarum* KK518 extract on cancer cells.

Concentration (μg/mL) of *L. plantarum* KK518	Colony formation (% of control)			IC_{50} (μg/mL)		
	HepG2	MCF-7	HeLa	HepG2	MCF-7	HeLa
0	100.00 ± 0.00^{e,A}	100.00 ± 0.00^{e,A}	100.00 ± 0.00^{c,A}			
400	48.14 ± 0.53^{d,B}	14.46 ± 0.21^{d,A}	57.94 ± 0.79^{b,C}			
600	27.99 ± 1.57^{c,C}	12.25 ± 0.33^{c,B}	0.00 ± 0.00^{a,A}	418.52^{B}	246.13^{A}	ND
800	11.94 ± 1.06^{b,C}	5.56 ± 0.32^{b,B}	0.00 ± 0.00^{a,A}			
1000	8.21 ± 1.06^{a,C}	1.30 ± 0.32^{a,B}	0.00 ± 0.00^{a,A}			

Small letter and capital letter superscripts indicate significant differences ($p < 0.05$) in the columns and rows, respectively. ND = Not determined.

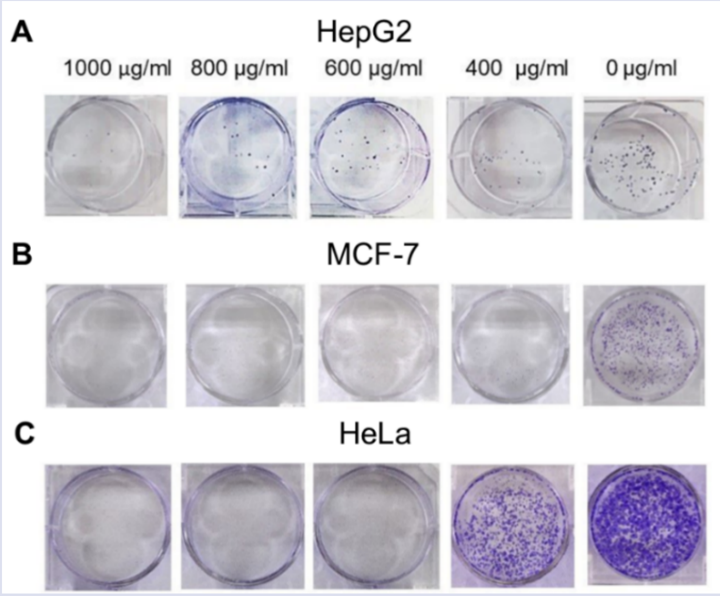

Figure 2: Colony formation assays. **A** HepG2 colonies affected by *L. plantarum* KK518 extracts. **B** MCF-7 colonies affected by *L. plantarum* KK518 extracts. **C** HeLa colonies affected by *L. plantarum* KK518 extracts.

Table 3: Wound healing effect of *L. plantarum* KK518 extract on cancer cells.

Cell line	Concentration (µg/mL) of *L. plantarum* KK518	Relative area of the scratch (%)				
		0 h	12 h	24 h	36 h	48 h
HepG2	0	$100 \pm 0.00^{a,A}$	$98.73 \pm 1.34^{a,B}$	$74.80 \pm 0.28^{c,C}$	$65.70 \pm 0.84^{d,D}$	$0.00 \pm 0.00^{e,E}$
	400	$100 \pm 0.00^{a,A}$	$98.26 \pm 0.81^{a,B}$	$84.50 \pm 0.26^{b,C}$	$79.90 \pm 1.13^{c,D}$	$52.59 \pm 0.98^{d,E}$
	600	$100 \pm 0.00^{a,A}$	$98.76 \pm 1.35^{a,A}$	$84.98 \pm 0.02^{b,A}$	$83.55 \pm 1.48^{bc,A}$	$58.00 \pm 0.78^{c,A}$
	800	$100 \pm 0.00^{a,A}$	$99.53 \pm 0.55^{a,B}$	$97.97 \pm 0.95^{a,C}$	$87.65 \pm 3.60^{ab,C}$	$82.02 \pm 0.42^{b,C}$
	1000	$100 \pm 0.00^{a,A}$	$99.35 \pm 0.54^{a,B}$	$98.89 \pm 0.45^{a,B}$	$90.95 \pm 10.6^{a,B}$	$91.80 \pm 0.85^{a,B}$
MCF-7	0	$100 \pm 0.00^{a,A}$	$80.46 \pm 0.64^{d,B}$	$68.10 \pm 1.83^{e,C}$	$43.39 \pm 1.77^{e,D}$	$0.00 \pm 0.13^{e,E}$
	400	$100 \pm 0.00^{a,A}$	$82.32 \pm 0.00^{c,B}$	$72.93 \pm 0.16^{d,C}$	$56.08 \pm 0.04^{d,D}$	$49.28 \pm 0.61^{d,E}$
	600	$100 \pm 0.00^{a,A}$	$82.58 \pm 0.08^{c,A}$	$77.78 \pm 0.13^{c,A}$	$71.89 \pm 0.09^{c,A}$	$64.46 \pm 0.04^{c,A}$
	800	$100 \pm 0.00^{a,A}$	$95.85 \pm 0.08^{b,B}$	$95.50 \pm 0.07^{b,C}$	$95.58 \pm 0.09^{b,C}$	$95.42 \pm 0.04^{b,C}$
	1000	$100 \pm 0.00^{a,A}$	$99.21 \pm 0.09^{a,B}$	$99.21 \pm 0.09^{a,B}$	$99.25 \pm 0.05^{a,B}$	$99.25 \pm 0.04^{a,B}$
HeLa	0	$100 \pm 0.00^{a,A}$	$59.25 \pm 4.31^{c,B}$	$40.00 \pm 4.10^{d,C}$	$0.00 \pm 0.00^{e,D}$	$0.00 \pm 0.00^{e,D}$
	400	$100 \pm 0.00^{a,A}$	$71.00 \pm 0.84^{b,B}$	$57.30 \pm 0.70^{c,C}$	$44.00 \pm 0.56^{d,D}$	$36.40 \pm 0.42^{d,E}$
	600	$100 \pm 0.00^{a,A}$	$72.45 \pm 0.21^{b,B}$	$61.95 \pm 0.21^{c,C}$	$65.45 \pm 0.21^{c,D}$	$48.40 \pm 0.14^{c,E}$
	800	$100 \pm 0.00^{a,A}$	$87.05 \pm 0.49^{b,B}$	$82.65 \pm 0.50^{a,C}$	$74.60 \pm 0.42^{b,D}$	$62.65 \pm 0.35^{b,E}$
	1000	$100 \pm 0.00^{a,A}$	$95.95 \pm 0.21^{a,B}$	$95.80 \pm 0.14^{b,B}$	$94.90 \pm 0.14^{a,C}$	$94.80 \pm 0.14^{a,C}$

Small letter and capital letter superscripts indicate significant differences ($p < 0.05$) in the columns and rows, respectively.

DISCUSSION

This work aimed to determine the anticancer properties of *L. plantarum* KK518, a probiotic bacterium isolated from Pak-Sian Dong in Thailand. *L. plantarum* is a beneficial microorganism that is extensively used as a starter culture for various Asian fermented food products.[11]

L. plantarum produces large amounts of organic acid during kimchi fermentation and produces natural antibacterial and antifungal products.[11]

L. plantarum's chemopreventive potential has been reported. *L. plantarum*, isolated from kimchi, was able to exhibit a strong antimutagenic effect against *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, 4-Nitroquinoline-1-oxide.[12] Moreover, *L. plantarum*, isolated from kimchi, had more potent antimutagenic effects compared to LAB originated from fermented milk.[13] It has been hypothesized that polysaccharide types on the cell wall of *L. plantarum* rather than glycopeptide play a pivotal role in cancer suppression.[14]

To evaluate cytotoxicity of *L. plantarum* KK518 extract on three different cancer cells including HepG2, HeLa, and MCF-7, MTT colorimetric assay as a routine technique was conducted. This technique relies on the ability of live cancer cells to metabolize the yellow tetrazolium salt MTT to a blue crystalline formazan product while dead cells are unable to do so.[15] HeLa cells (IC_{50} of 371.97 µg/mL and 90.88% cytotoxicity at 1,000 µg/mL) were most cytotoxic to *L. plantarum* KK518 extract over 48 h of exposure whilst MCF-7 cells were least cytotoxic (IC_{50} of 682.53 µg/mL and 68.53% cytotoxicity at 1,000 µg/mL) to the bacterial extract. Likewise, *L. plantarum* KK518 extract was most antiproliferative towards HeLa cells using anti-colony formation test since the bacterial extracts at 600, 800 and 1,000 µg/mL over 48 h led to a complete inhibition of colony formation; however HepG2 was most resistant

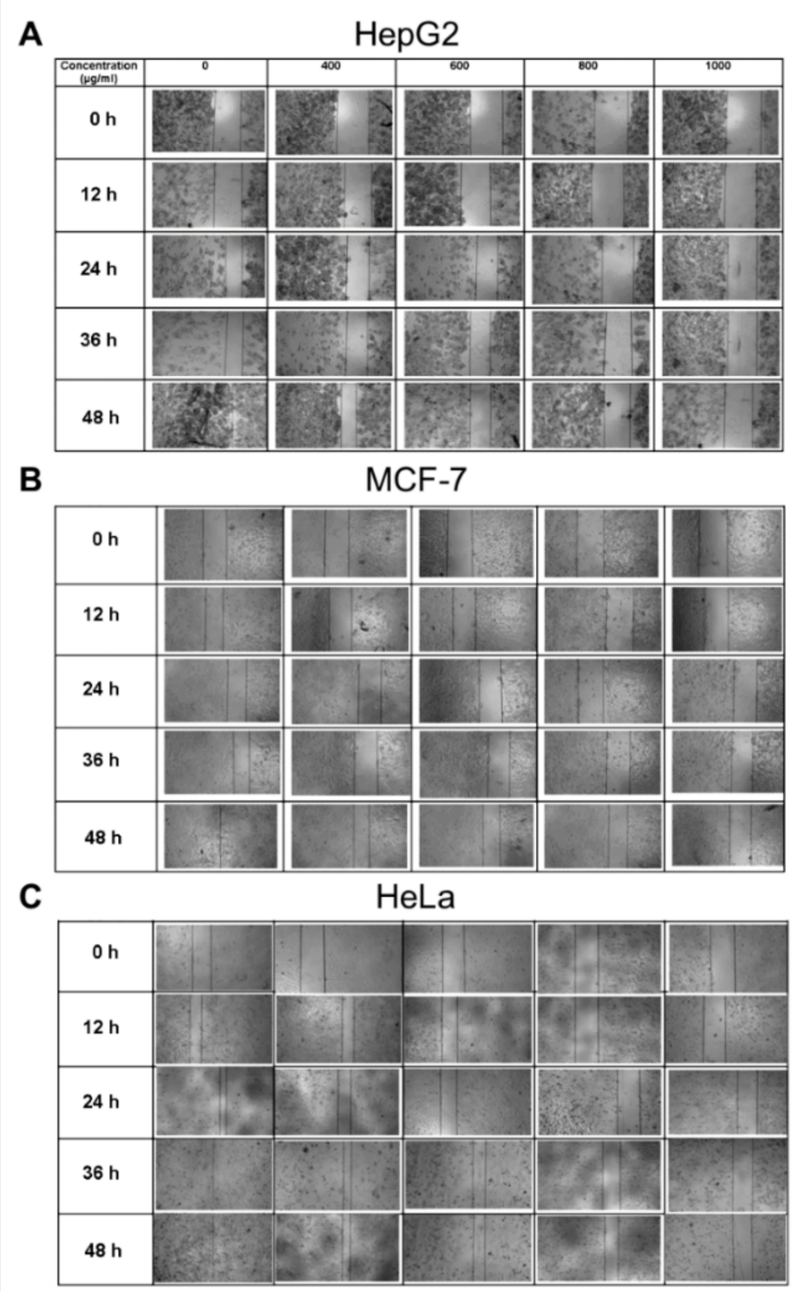

Figure 3: Wound healing assay. **A** HepG2 cell wounds affected by *L. plantarum* KK518 extracts. **B** MCF-7 cell wounds affected by *L. plantarum* KK518 extracts. **C** HeLa cell wounds affected by *L. plantarum* KK518 extracts.

to the bacterial extract (IC_{50} of 418.52 µg/mL). Similarly, HepG2 cells seemed to be most resistant to antimigratory effects. Overall, *L. plantarum* KK518 extract was likely to be effective in treating cancers in the following order: HeLa > MCF-7 > HepG2.

However, components in the bacterial extract responsible for the observed effects are yet to be identified. The possible molecules that play a key role in the cytotoxity of extracted bacterial metabolites include active proteins that binds to procarcinogenic compounds[16,17] or non-protein molecules such as short-chain fatty acids including butyrate and propionate.[16]

Previously, *L. plantarum* 70810 isolated from Chinese Paocai was able to produce exopolysaccharide (EPS) with moderate antitumor activity against HepG2 cells (56.34 % cytotoxicity at 600 µg/mL EPS extract) after a prolonged time (72 h) of treatment.[18] When compared to our finding, *L. plantarum* KK518 extract was less effective in treating HepG2 cells (27.05% cytotoxicity at 600 µg/mL bacterial extract) after 48 h of exposure than *L. plantarum* 70810 EPS. This may be due to the purer form of EPS treatment than our crude bacterial extract treatment and longer time of exposure to HepG2 cells. In accordance with our work, *L. plantarum* 5BL isolated from the vaginal secretion of a healthy and fertile Iranian woman elicited a significant antiproliferative effect on HeLa for all incubation times and doses used. The greatest antiproliferative effect on HeLa was observed at the highest dose (50 µg/mL) of the metabolite and was greater against HeLa cells than against MCF-7 cells.[19]

In contrast to our finding, six bacteriocin-producing *L. plantarum* identified as I-UL4, TL1, RS5, RI11, RG11 and RG14 strains isolated from Malaysian foods[20] produced postbiotic metabolites that were more cytotoxic against MCF-7 cells than HeLa and HepG2 cells. In addition, *L. plantarum* 15HN isolated from traditional dairy products exhibited no significant anticancer effects on MCF-7, and HeLa cells.[21] Moreover, cytotoxicity analysis of plantaricin from *L. plantarum* DM5 isolated from indigenous fermented beverage Marcha from India on HeLa cell lines revealed its non-toxic nature towards HeLa cells.[22]

Compared to these previous reports, our *L. plantarum* KK518 extract seemed to be more efficacious towards HeLa and MCF-7 cells than those of *L. plantarum* 15HN and *plantarum* DM5.

In the previous study, blueberries fermented with *L. plantarum* showed higher antioxidant activities and antiproliferative activities against HeLa cells than did raw blueberries. *L. plantarum* fermentation biotransformed blueberry polyphenols into active phenol metabolites with strong antioxidant and antiproliferative activities.[23] For future practical application, *L. plantarum* KK518 can be used as a starter culture to ferment functional foods with chemopreventive effects.

CONCLUSION

This work revealed that *L. plantarum* KK518 extracts may have potential anticarcinogenic activity in HepG2, MCF-7 and HeLa cancer cells through the dual effect of cell proliferation inhibition, induction of apoptosis and cell migration inhibition. Since literature on cytotoxic and antiproliferative activity of *L. plantarum* is still limited, its mechanisms on cellular levels and gene expressions requires further investigations. Overall, *L. plantarum* KK518 extract appears to have potential as a bio-therapeutic and can be implemented for functional food product development with chemopreventive benefits.

ACKNOWLEDGEMENTS

This research was funded by Thailand Research Fund through the MRG Grant for the Development of New Lecturer (grant no. MRG6180280) and Office of the Higher Education Commission, Thailand awarded to V.L. and Mahasarakham University (Fast track 2021). Authors would like to thank Department of Biotechnology, Mahasarakham University, Thailand for laboratory facilities.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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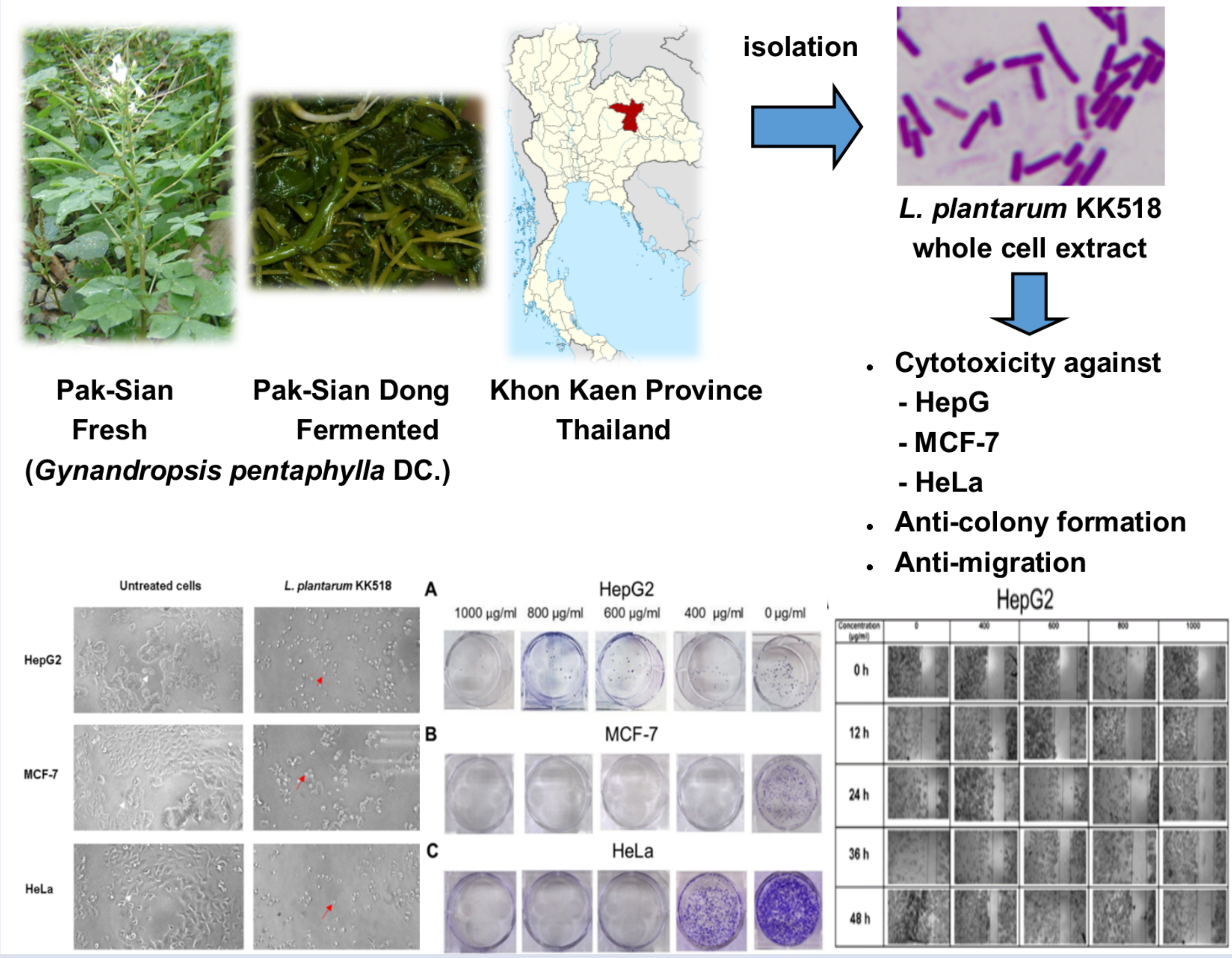
GRAPHICAL ABSTRACT



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Cite this article: Luang-In V, Saengha W, Buranrat B, Nudmamud-Thanoi S, Narbad A, Pumriw S, *et al.* Cytotoxicity of *Lactobacillus plantarum* KK518 Isolated from Pak-Sian Dong (Thai Fermented *Gynandropsis pentaphylla* DC.) Against HepG2, MCF-7 and HeLa Cancer Cells. Pharmacogn J. 2020;12(5):1050-7.