



# Cytotoxic activities of ethanolic crude extracts from fruiting bodies of bamboo mushrooms (*Dictyophora* spp.) against cholangiocarcinoma cells

Pathanin Chantree<sup>1,2</sup>, Sirilak Chumkiew<sup>3</sup>, Mantana Jamklang<sup>3</sup>, Pongsakorn Martviset<sup>1,2</sup>

1 Thammasat University, Pathumthani, 99/209 Moo 18 Phaholyothin Rd., Klong-Neung, Klong Luang, Pathumthani 12120 Thailand

2 Thammasat University Research Unit in Nutraceuticals and Food Safety, 99 Moo 18 Phaholyothin Rd., Klong-Neung, Klong Luang, Pathumthani 12120 Thailand

3 Suranaree University of Technology, 111 University Ave., Muang, Nakhon-Ratchasima 30000 Thailand

Corresponding author: Pongsakorn Martviset ([pong\\_m@tu.ac.th](mailto:pong_m@tu.ac.th))

Academic editor: Mikhail Korokin ♦ Received 24 July 2021 ♦ Accepted 22 December 2021 ♦ Published 16 March 2022

**Citation:** Chantree P, Chumkiew S, Jamklang M, Martviset P (2022) Cytotoxic activities of ethanolic crude extracts from fruiting bodies of bamboo mushrooms (*Dictyophora* spp.) against cholangiocarcinoma cells. Research Results in Pharmacology 8(1): 33–41. <https://doi.org/10.3897/rrpharmacology.8.72098>

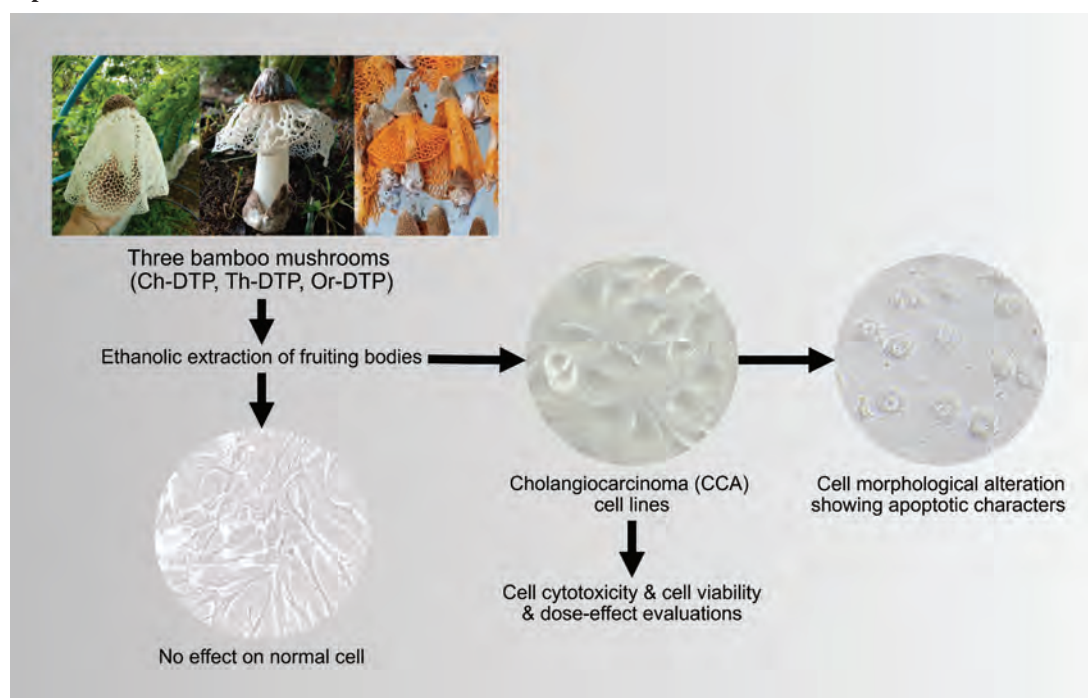
## Abstract

**Introduction:** Cholangiocarcinoma (CCA) is a highly progressive tumor. The standard chemotherapy varies in its effectiveness, with generally low efficacy. So, the discovery of novel chemotherapy is still required. The objective of this preliminary study was to determine the cytotoxic effects induced by three kinds of bamboo mushrooms (*Dictyophora indusiata* or Chinese bamboo mushroom; Ch-DTP, Short skirt bamboo mushroom (Thai isolate); Th-DTP, and orange skirt bamboo mushroom; Or-DTP) on CCA cells.

**Materials and methods:** CCA cell lines, including CL-6, HuCCT1, HuH28, and OUMS normal fibroblast cells, were treated with various concentrations of DTP extracts. The MTT assay was used to determine cytotoxicity, and cell morphology was observed by using phase-contrast microscopy.

**Results and discussion:** The results suggested that Ch-DTP effectively killed all three CCA cell lines in both low (0.3 mg/mL) and high (0.6 mg/mL) doses, but Th-DTP and Or-DTP had significantly reduced cell viability only at high doses ( $p < 0.001$ ). Ch-DTP had the best effect by showing a response of more than 50% at a concentration of 0.3 mg/mL. Th-DTP had moderate effects at a concentration of lower than 0.6 mg/mL but worthwhile at higher concentrations, whereas Or-DTP had limited effects at concentrations of 0.4 mg/mL and downward, although the effects were significantly increased in the higher concentration range. Morphology of the Ch-DTP treated cells was greatly transformed both at low and high doses, but Th-DTP and Or-DTP showed definite alteration only at high doses. The morphological changes revealed apoptotic induction. In OUMS cells, no effects were recognized with any of the three DTPs.

**Conclusion:** This study indicated that DTP extracts could induce cytotoxicity in cholangiocarcinoma, with a high potential of being an effective therapeutic agent.

**Graphical abstract:****Keywords**

bile duct cancer, bamboo mushroom, cytotoxicity.

**Introduction**

Cholangiocarcinoma (CCA) is a highly proliferative and progressive tumor originating from cholangiocytes, the bile duct epithelium cells (Valle et al. 2016). The origin of CCA is not clearly described, but has been related to infection by liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis*, coupled with consumption of carcinogens, e.g. dimethyl nitrosamine (DMN) occurring in fermented meats (Sripa et al. 2007; Kirstein and Vogel 2016). Early diagnostic tools, as well as effective drugs, are not available for controlling CCA, which is the major reason why patients present with severe complications and poor prognosis (Ustundag and Bayraktar 2008; Bertuccio et al. 2019). The current drugs of choice for the treatment of CCA are 5-Fluorouracil (5-FU) and gemcitabine used in combination therapy with varying effectiveness and low efficacy (Valle et al. 2010; Khan et al. 2012; Ramírez-Merino et al. 2013; Park et al. 2015). For these reasons, the discovery of novel chemotherapeutics is still required.

*Dictyophora* spp., or bamboo mushrooms, are a group of fungi in the family Phallaceae. They are distributed mainly in tropical areas of Asia, Africa, America, and Australia. They are characterized by a fruiting body with a conically shaped stalk and a delicate net-like white skirt arranged longitudinally (Elkhateeb et al. 2020). The

most clearly known species is *Dictyophora indusiata*, or Chinese bamboo mushroom. *D. indusiata* has beneficial nutrients like other edible mushrooms. The composition of carbohydrates is about 47% of the dry weight with 38% soluble polysaccharides. Meanwhile, crude fiber and crude protein contents are about 29% and 6%, respectively (Ker et al. 2011). Moreover, it is composed of essential amino acids, beneficial minerals with a high content of vitamin E,  $\beta$ -carotene, ascorbic acid, thiamine, riboflavin, nicotinic acid, phosphate, and calcium (Ouyang et al. 1998; Mau et al. 2001; Ma and Zhang 2004).

There are bioactivity studies of *D. indusiata* extracts, both *in vitro* and *in vivo*. The water-soluble polysaccharide extracts have been shown to have a free radical scavenging effect against hydroxyl radicals (Wang et al. 2019a, b). These effects were also shown with purified *D. indusiata* water-soluble  $\beta$ -d-glucan polysaccharides (Deng et al. 2012). For *in vivo* studies of antioxidant effects, the water-soluble polysaccharides administered to mice could interrupt increased malondialdehyde levels, while increasing levels of antioxidant proteins including superoxide dismutase, glutathione peroxidase, and catalase in the kidney and liver tissues (Wang et al. 2019a, b). Regarding immunomodulatory effects, the *D. indusiata* polysaccharides could induce proliferation of macrophage cell lines with the up regulation of immunostimulant cytokine levels (Deng et al. 2016).

Moreover, the enhancement of NK cell killing ability and macrophage phagocytosis potential were observed in the cells treated with *D. indusiata* polysaccharides (Fu et al. 2015).

Regarding anticancer effects, the crude polysaccharide extract of *D. indusiata* has been shown to exert cytotoxicity in the osteosarcoma S180 cell lines (Zhong et al. 2013). The same trend of cytotoxic effects was also revealed in HeLa and hepatoma HepG2 cell lines when treated with water extract and crude polysaccharides of *D. indusiata* (Li et al. 2012). In breast cancer MCF-7 cell lines, the cytotoxicity of the polysaccharide derivatives of *D. indusiata* was shown via apoptotic induction, with evidence of cell cycle arrest, DNA fragmentation, and activation of caspase cascades (Liao et al. 2015). In an *in vivo* study, administration of the triple-helical polysaccharide has been shown to suppress tumor growth in S180 tumor-bearing mice (Deng et al. 2013). However, there is no determination of the anticancer effect of *Dictyophora* spp. extracts in CCA.

In this study, we evaluated the anticancer effects of three *Dictyophora* spp. ethanolic crude extracts on three cholangiocarcinoma cell lines (CL-6, HuCCT1, and HuH28). Our results suggested anticancer activity of *Dictyophora* spp. extracts that may be favorable for the treatment of cholangiocarcinoma in the future.

## Materials and methods

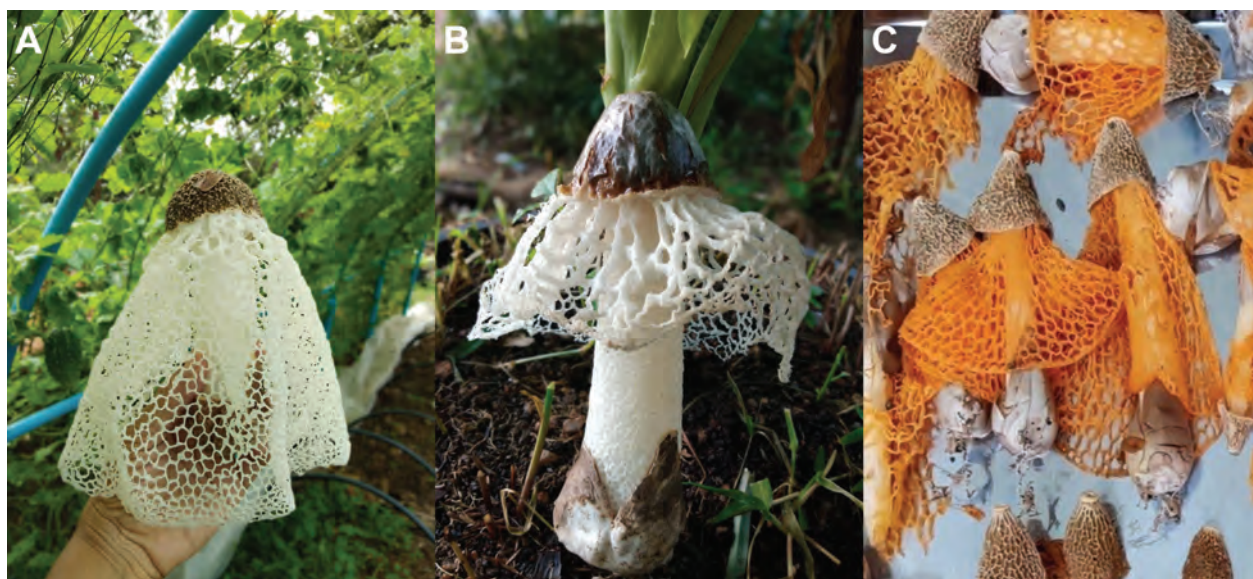
### Preparation of ethanolic crude extracts of bamboo mushrooms fruiting bodies

Three kinds of bamboo mushrooms (*Dictyophora indusiata*, or Chinese bamboo mushroom; Ch-DTP, short skirt bamboo mushroom (Thai isolate); Th-DTP, and orange skirt bamboo mushroom; Or-DTP)

were used in this study as they are the major bamboo mushrooms found in Thailand. The fruiting bodies of bamboo mushrooms were bought from farms located in Prachinburi, Nakhon-Ratchasima, and Khonkaen provinces. The external morphology of the three bamboo mushrooms is illustrated in Fig. 1. The ethanolic crude extract of *Dictyophora* spp. was prepared from the fruiting bodies of the mushrooms. In brief, the mushroom fruiting bodies were cleaned several times by tap water, cut into small pieces of approximately 2–3 mm and dried in a hot air oven (50–55 °C) for 2–3 days. The dried mushrooms were powdered by using an electric grinder, and 100 g of the powder was transferred to a stoppered flask containing 95% ethanol and incubated at room temperature (25–30 °C) for seven days. The ethanolic extract was filtered through Whatman no. 1 filter paper (Sigma-Aldrich, Darmstadt, Germany), evaporated in an evaporator, air dried in a hot air oven at 50 °C, and stored at -20 °C until use.

### Cell culture

Three CCA cell lines, including of intrahepatic origin; CL-6 and HuCCT1, and extrahepatic origin; HuH28, were cultured in RPMI-1640 supplemented with 10% (v/v) fetal bovine serum and 100 U/mL penicillin/streptomycin (Gibco, ThermoFisher Scientific, Rockford, IL, USA) at 37 °C under a 5% CO<sub>2</sub> atmosphere. CL-6 cell was kindly provided by Associate Professor Adisak Wongkajornsilp, Department of Pharmacology, Faculty of Medicine, Sirisaj Hospital, Mahidol University, Thailand. HuCCT1 and Huh28 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank (JCRB Cell Bank, Osaka, Japan). The cells were regularly sub-cultured according to the doubling time of each cell line, by using 0.25% trypsin-EDTA (Gibco, ThermoFisher Scientific, Rockford, IL, USA).



**Figure 1.** External morphology of three bamboo mushrooms used in this study. (A) *Dictyophora indusiata*, or Chinese bamboo mushroom; Ch-DTP, (B) short skirt bamboo mushroom (Thai isolate); Th-DTP, and (C) orange skirt bamboo mushroom; Or-DTP.

OUMS fibroblast cells were used as a normal control of treatment by *Dictyophora* spp. fruiting body ethanolic crude extracts. They were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and 100 U/mL penicillin/streptomycin (Gibco, ThermoScientific, USA) under the same conditions as the CCA cells.

### Cell viability assay

The CL-6, HuCCT1, HuH28, and OUMS cells were plated onto 96-well microtiter plates ( $1 \times 10^4$  cells/well) and cultured at 37 °C under 5% CO<sub>2</sub> atmosphere for 24 h. The quality of the cells was observed under an inverted light microscope before treating with ethanolic crude extract of *Dictyophora* spp. fruiting bodies. For evaluation of cytotoxicity, the cells were treated with various concentrations of ethanolic crude extracts of Ch-DTP, Th-DTP, and Or-DTP fruiting bodies (800, 700, 600, 500, 400, 300, 200, 100, and 50 µg/mL) diluted in 50% ethanol. The vehicle control (final concentration of 5% ethanol) was tested simultaneously. 5-Fluorouracil (5-FU) was used as a reference compound. The cells were incubated at 37 °C under 5% CO<sub>2</sub> for 48 h. After incubation, 20 µL of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma Aldrich, St. Louis, MO, USA) was added to each well and incubated for 3 hours. The culture media containing MTT reagent were removed by careful pipetting, and the precipitates were dissolved by DMSO. The absorbance was measured at a wavelength of 570 nm. The experiment was performed in three independent assays, each in triplicate. The IC<sub>50</sub> (concentration that inhibits cell growth by 50%) was calculated using CalcuSyn™ (Biosoft, Cambridge, UK).

### Concentration-response analysis

The concentration-response of the three CCA cell lines following exposure to various concentrations of ethanolic crude extracts of Ch-DTP, Th-DTP, and Or-DTP fruiting bodies (800, 700, 600, 500, 400, 300, 200, 100, and 50 µg/mL), was determined by MTT assay (Sigma-Aldrich, Mannheim, Germany) as mentioned earlier. The concentration-response curves were calculated using the analysis software CalcuSyn (Biosoft, Cambridge, UK).

### Statistical analysis

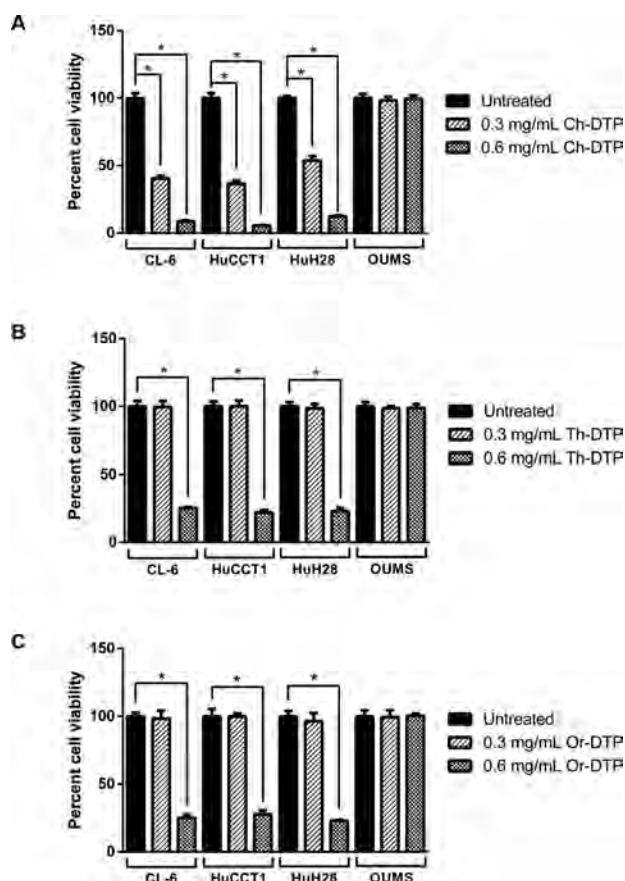
The statistical analysis was performed using Prism GraphPad version 6 (GraphPad Software, San Diego, CA, USA). Data are expressed as mean ± SD values. A comparison of more than two quantitative data sets was performed using ANOVA. A comparison of the two quantitative data sets was performed using an independent *t*-test or paired *t*-test, where appropriate. The statistical significance was set at  $\alpha = 0.05$ .

## Results

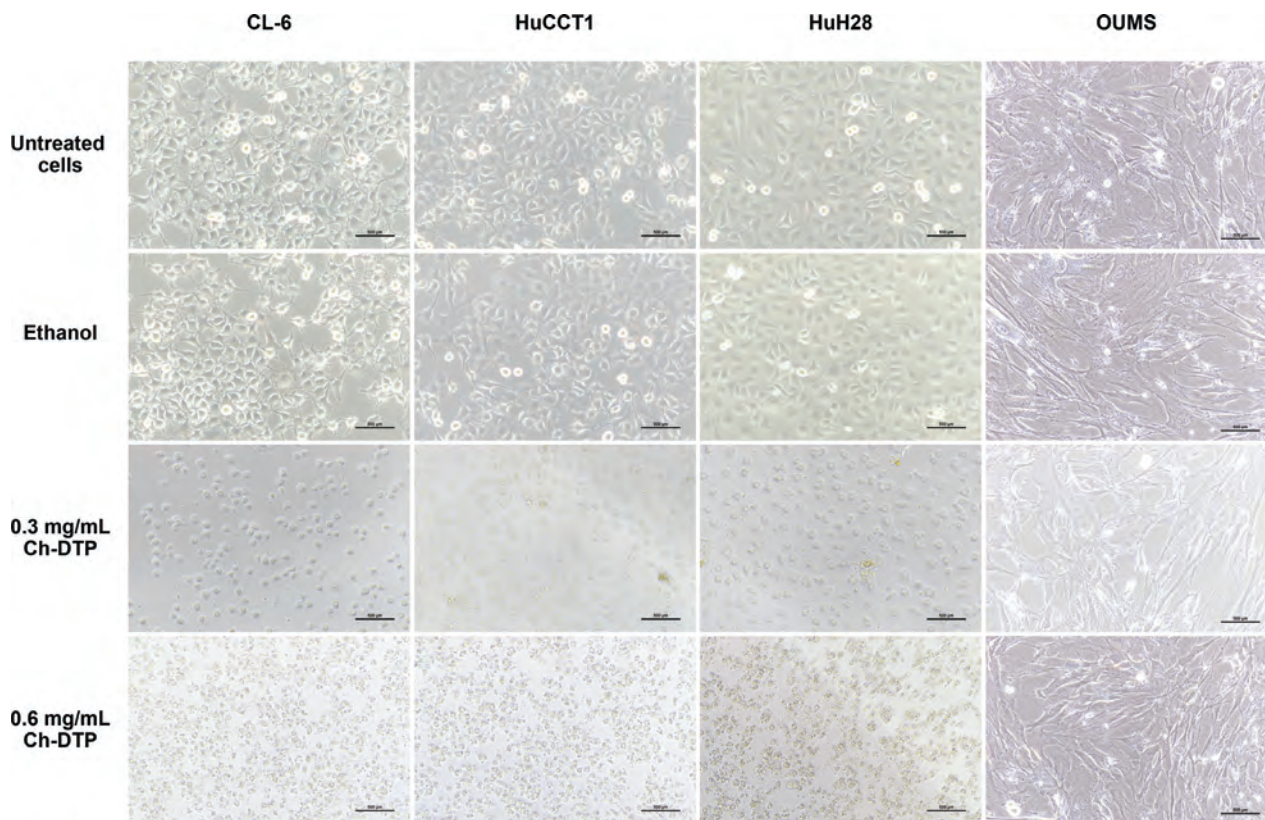
### All ethanolic crude extracts of *Dictyophora* spp. fruiting bodies effectively killed CCA cells

Cell viability results suggest that Ch-DTP effectively killed all three CCA cell lines at both low (0.3 mg/mL) and high (0.6 mg/mL) doses. Th-DTP and Or-DTP had nominal effects at low doses, but significantly reduced cell viability at high doses as shown in Fig. 2, while the vehicle control (ethanol) had no effect on cell viabilities (data not shown).

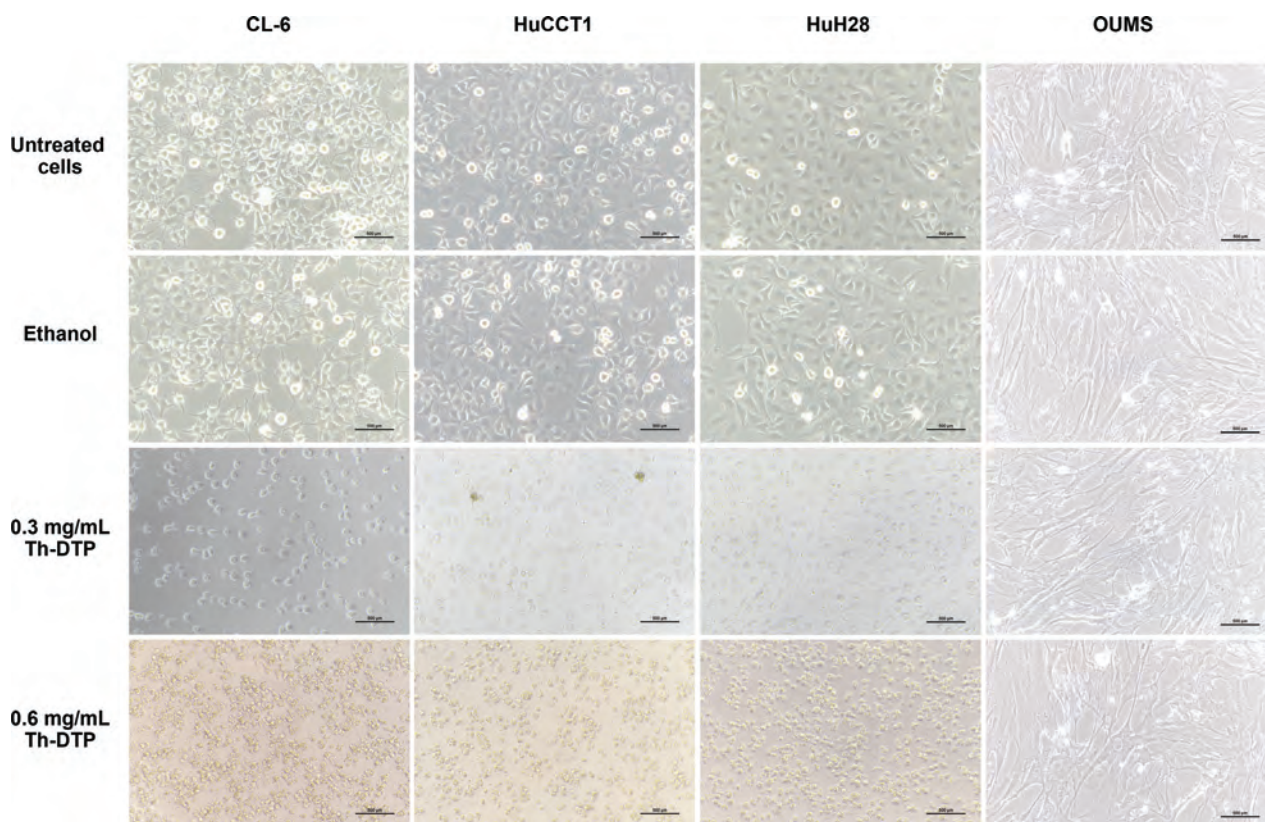
The observation of cell morphological alterations after treatment with ethanolic crude extracts of Ch-DTP, Th-DTP, and Or-DTP fruiting bodies was performed under a phase-contrast microscope in order to verify the efficacy of the extracts. The untreated cells and ethanol-treated cells were not different. In contrast, the Ch-DTP treated cells were greatly transformed at both low and high doses. Correspondingly, in cell viability assays, Th-DTP and Or-DTP showed little alteration at low doses, but a major change at high doses. In OUMS, no effects were recognized for any DTP extracts. Cell appearance is illustrated in Figs 3–6.



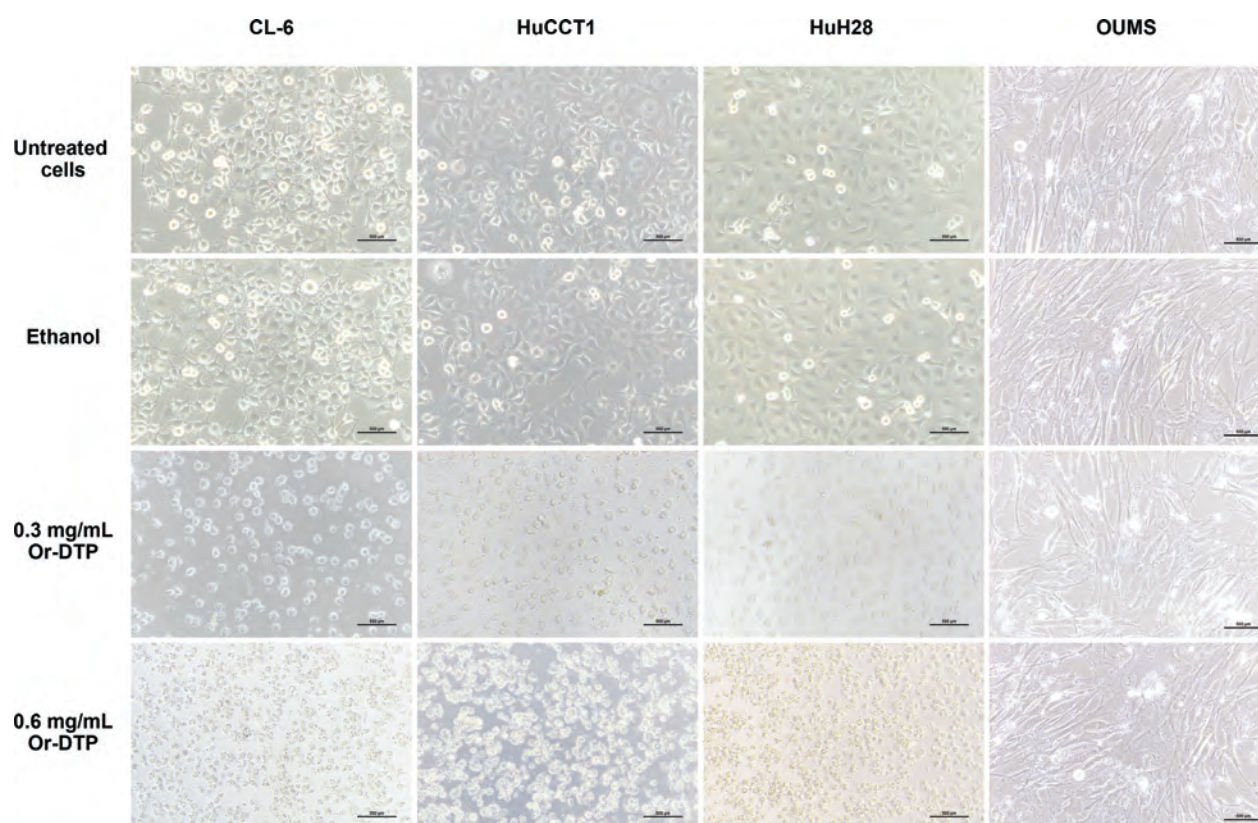
**Figure 2.** Cell viabilities of three CCA cell lines (CL-6, HuCCT1, and HuH28) and OUMS normal fibroblasts treated with ethanolic crude extracts of (A) Ch-DTP, (B) Th-DTP, and (C) Or-DTP fruiting bodies. (\* represents statistically significant differences at  $p < 0.001$ , Chinese bamboo mushroom; Ch-DTP, short skirt bamboo mushroom (Thai isolate); Th-DTP, orange skirt bamboo mushroom; Or-DTP).



**Figure 3.** Cell morphological changes after treating with ethanolic crude extracts of Ch-DTP fruiting bodies in three CCA cell lines (CL-6, HuCCT1, and HuH28) and OUMS normal fibroblasts (Chinese bamboo mushroom; Ch-DTP) (20X amplification).



**Figure 4.** Cell morphological changes after treating with ethanolic crude extracts of Th-DTP fruiting bodies in three CCA cell lines (CL-6, HuCCT1, and HuH28) and OUMS normal fibroblasts (Short skirt bamboo mushroom (Thai isolate); Th-DTP) (20X amplification).



**Figure 5.** Cell morphological changes after treating with ethanolic crude extracts of Or-DTP fruiting bodies in three CCA cell lines (CL-6, HuCCT1, and HuH28) and OUMS normal fibroblasts (Orange skirt bamboo mushroom; Or-DTP) (20X amplification).

#### Ch-DTP had the lowest $IC_{50}$ value among three bamboo mushrooms in each CCA cell line

The  $IC_{50}$  (mean  $\pm$  SD) of Ch-DTP against CL-6, HuCCT1, and HuH28 were  $0.32 \pm 0.06$ ,  $0.29 \pm 0.03$ , and  $0.33 \pm 0.001$  mg/mL, respectively. The  $IC_{50}$  of Th-DTP against CL-6, HuCCT1, and HuH28 were  $0.48 \pm 0.01$ ,  $0.49 \pm 0.02$ , and  $0.47 \pm 0.01$  mg/mL, respectively. The  $IC_{50}$  of Or-DTP against CL-6, HuCCT1, and HuH28 were  $0.53 \pm 0.03$ ,  $0.50 \pm 0.03$ , and  $0.48 \pm 0.02$  mg/mL, respectively. Lastly, the  $IC_{50}$  of the standard drug (5-FU) were  $0.09 \pm 0.02$ ,  $0.09 \pm 0.01$ , and  $0.12 \pm 0.02$  mg/mL, respectively. The  $IC_{50}$  of Ch-DTP-treated cells were significantly different from other DTP-treated cells with  $p < 0.05$  in all three cells. The  $IC_{50}$  of all three cells are summarized in Table 1.

**Table 1.**  $IC_{50}$  of Ethanolic Crude Extracts of Ch-DTP, Th-DTP, and Or-DTP Fruiting Bodies Against Three Cholangiocarcinoma Cell Lines in Units of mg/mL

Bamboo mushrooms	$IC_{50}$ (mean $\pm$ SD) against CCA cell lines (mg/mL)		
	CL-6	HuCCT1	HuH28
Ch-DTP	$0.32 \pm 0.06$	$0.29 \pm 0.03$	$0.33 \pm 0.001$
Th-DTP	$0.48 \pm 0.01$	$0.49 \pm 0.02$	$0.47 \pm 0.01$
Or-DTP	$0.53 \pm 0.03$	$0.50 \pm 0.03$	$0.48 \pm 0.02$
5-FU	$0.09 \pm 0.02$	$0.09 \pm 0.01$	$0.12 \pm 0.02$

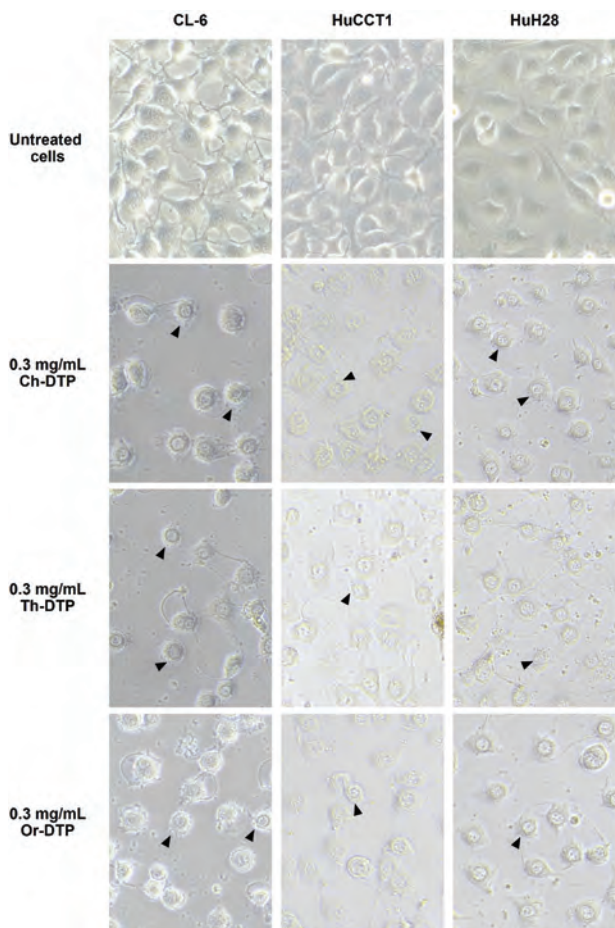
**Note:** Chinese bamboo mushroom; Ch-DTP, Short skirt bamboo mushroom (Thai isolate); Th-DTP, Orange skirt bamboo mushroom; Or-DTP).

#### All three ethanolic crude extracts of *Dictyophora* spp. fruiting bodies showed various effects in three CCA cells with no effect on OUMS cells

The concentration-response analysis showed the efficacy of ethanolic crude extracts of Ch-DTP, Th-DTP, and Or-DTP fruiting bodies against three cholangiocarcinoma cell lines. Ch-DTP had the best effect, showing a response of more than 50% at a concentration of 0.3 mg/mL to nearly 100% at 0.6 to 0.8 mg/mL in all CCA cell lines. The Th-DTP had moderate effects at concentrations below 0.6 mg/mL, but worthwhile ones at higher concentrations. On the other hand, Or-DTP had limited effects below 0.4 mg/mL, but the effects were significantly increased at higher concentrations. In OUMS fibroblasts, ethanolic crude extracts of Ch-DTP, Th-DTP, and Or-DTP fruiting bodies had no effect. The concentration-response curves are shown in Fig. 7.

## Discussion

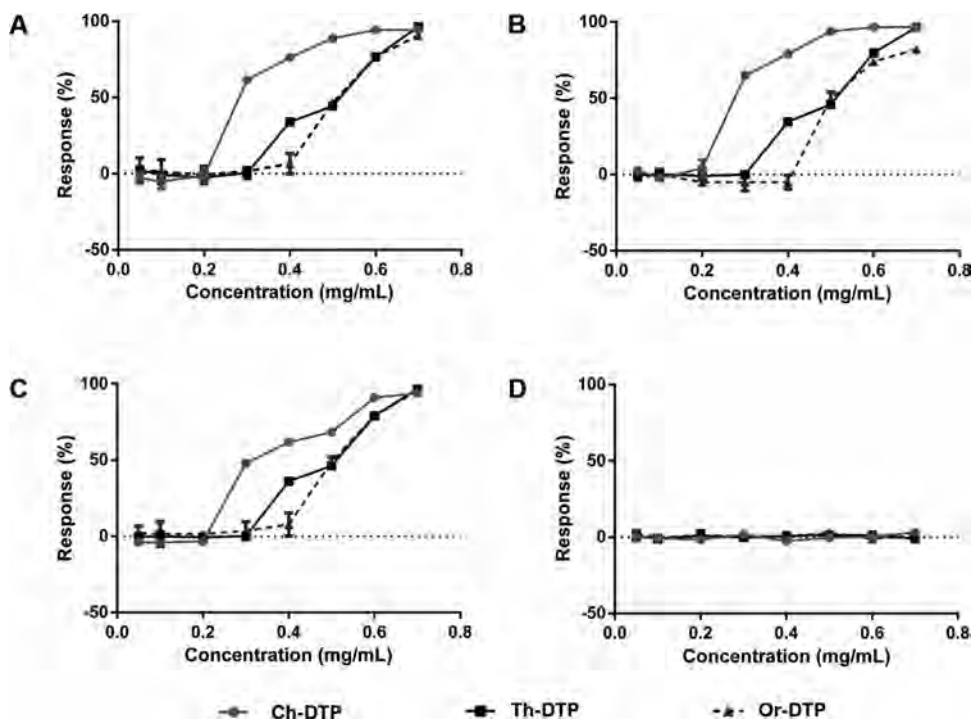
Mushrooms have been valued throughout the world, both as food and medicine because they possess high contents of nutrients (Okhuoya and Okogbo 1990; Elkhateeb et al. 2019). *Dictyophora* spp., or bamboo mushrooms, are a group of fungi in the family Phallaceae, found mainly in tropical areas of Asia, Africa, America, and



**Figure 6.** CCA cell morphological alteration after treatment with Ch-DTP, Th-DTP, and Or-DTP showing cytosol shrinking with nuclear condensation, suggesting that those cells were undergoing apoptosis (arrows)

Australia. *D. indusiata* has nutritional values, and its proteins, carbohydrates, and dietary fiber contents have been extensively studied (Ker et al. 2011; Sitinjak 2017). Modern studies suggest that *D. indusiata* polysaccharides exert many biological effects, such as anticancer, antitumor, anti-proliferative and neuroprotective activities (Yu et al. 2017). Consequently, the present study has focused on examining *Dictyophora* spp. extracts for cytotoxicity against cultured cholangiocarcinoma cells (CCA).

One distinct property of cancer cell is their resistance to apoptotic induction (Hanahan and Weinberg 2011). Hence, newly developed alternative chemotherapeutic agents should have cytotoxicity via apoptotic induction. Apoptosis is a type of programmed cell death resulting in nuclear condensation, cytoplasmic shrinking, and cell membrane blebbing (Burz et al. 2009). In the present study, it was revealed that *Dictyophora* spp. extracts of Chinese bamboo mushrooms (Ch-DTP), Short skirt bamboo mushrooms (Thai isolate, Th-DTP) and Orange skirt bamboo mushrooms (Or-DTP) induced cytotoxicity in cholangiocarcinoma cells. Normal OUMS fibroblast cells were not affected, which indicates that the extracts would probably induce limited adverse effects when administered to patients. The relative cell viability results suggest that Ch-DTP effectively killed all three CCA cell lines at both low and high doses, while Th-DTP and Or-DTP had minimal effects at low doses, but led to significantly reduced cell viabilities at high doses. Furthermore, the concentration-response and  $IC_{50}$  analyses showed that the efficacy of ethanolic crude extracts of Ch-DTP, Th-DTP, and Or-DTP fruiting bodies against three cholangiocarcinoma cell lines corresponded to the cell viability results. Our results suggest that Ch-DTP may



**Figure 7.** The concentration-response curves of ethanolic crude extracts of Ch-DTP, Th-DTP, and Or-DTP fruiting bodies in CCA cells (A) CL-6, (B) HuCCT1, and (C) HuH28 and (D) OUMS fibroblasts.

have the highest potential by showing a response of more than 50% at a concentration of 0.3 mg/mL, and nearly 100% at 0.6 to 0.8 mg/mL in all CCA cell lines. Th-DTP had moderate effects at concentrations below 0.6 mg/mL, but pronounced effects at higher concentrations. Similarly, Or-DTP had limited effects below 0.4 mg/mL, but significantly increased effects at higher concentrations. The IC<sub>50</sub> values of all DTPs from our study were higher than those of the standard drug (5-FU). However, the IC<sub>50</sub> alone does not represent the whole effects of the extracts, especially the alteration of intracellular signaling cascades that need to be proved further. In the morphological study, the Ch-DTP treated cells were greatly transformed at both low and high doses, whereas Th-DTP and Or-DTP showed limited alteration at low doses, but substantial changes at high doses. Nonetheless, DTP-treated CCA cells showed cytosol shrinking with nuclear condensation (Fig. 6), suggesting that those cells were undergoing apoptosis. Taken together, the results suggest that Ch-DTP revealed the best cytotoxicity effect against all CCA cells, followed by Th-DTP, and Or-DTP, respectively.

In the present study, the ethanolic crude extracts of three bamboo mushrooms (*Dictyophora* spp.) fruiting bodies exerted cytotoxicity on cholangiocarcinoma cell lines. We have hypothesized that this effect was exerted through apoptotic induction regarding the characteristics of cellular morphological changes after treatment. Nevertheless, other anticancer effects of those extracts, including autophagy and cell cycle arrest induction, as well as the related signaling pathways, should be further elucidated.

## References

- Bertuccio P, Malvezzi M, Carioli G, Hashim D, Boffetta P, El-Serag HB, Vecchia CL, Negri E (2019) Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma. *Journal of hepatology* 71(1): 104–114. <https://doi.org/10.1016/j.jhep.2019.03.013> [PubMed]
- Burz C, Berindan-Neagoe I, Balacescu O, Irimie A (2009) Apoptosis in cancer: key molecular signaling pathways and therapy targets. *Acta Oncologica* 48(6): 811–821. <https://doi.org/10.1080/02841860902974175> [PubMed]
- Deng C, Fu H, Teng L, Hu Z, Xu X, Chen J, Ren T (2013) Anti-tumor activity of the regenerated triple-helical polysaccharide from *Dictyophora indusiata*. *International Journal of Biological Macromolecules* 61: 453–458. <https://doi.org/10.1016/j.ijbiomac.2013.08.007> [PubMed]
- Deng C, Hu Z, Fu H, Hu M, Xu X, Chen J (2012) Chemical analysis and antioxidant activity in vitro of a β-d-glucan isolated from *Dictyophora indusiata*. *International Journal of Biological Macromolecules* 51(1–2): 70–75. <https://doi.org/10.1016/j.ijbiomac.2012.05.001> [PubMed]
- Deng C, Shang J, Fu H, Chen J, Liu H, Chen J (2016) Mechanism of the immunostimulatory activity by a polysaccharide from *Dictyophora indusiata*. *International Journal of Biological Macromolecules* 91: 752–759. <https://doi.org/10.1016/j.ijbiomac.2016.06.024> [PubMed]
- Elkhateeb W, Daba G, Elnahas M, Thomas P (2020) *Trametes versicolor* and *Dictyophora indusiata* champions of medicinal mushrooms. *Pharmaceutical Research* 4: e000192.
- Elkhateeb W, Daba G, Thomas P, Wen TC (2019) Medicinal mushrooms as a new source of natural therapeutic bioactive compounds. *Egyptian Pharmaceutical Journal* 18: 88–101.
- Fu H, Deng C, Teng L, Yu L, Su T, Xu X, Chen J, Yang C (2015) Immunomodulatory Activities on RAW 264.7 macrophages of a polysaccharide from Veiled Lady Mushroom, *Dictyophora indusiata*. *Higher Basidiomycetes. International Journal of Medicinal Mushrooms* 17(2): 151–160. <https://doi.org/10.1615/intjmedmushrooms.v17.i2.60> [PubMed]
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5): 646–674. <https://doi.org/10.1016/j.cell.2011.02.013> [PubMed]
- Ker YB, Chen KC, Peng CC, Hsieh CL, Peng RY (2011) Structural characteristics and antioxidative capability of the soluble polysaccharides present in *Dictyophora indusiata* (Vent. Ex Pers.) Fish Phallaceae. *Evidence-Based Complementary and Alternative Medicine* 2011: e396013. <https://doi.org/10.1093/ecam/neaq041> [PubMed] [PMC]
- Khan SA, Davidson BR, Goldin RD, Heaton N, Karani J, Pereira SP, Rosenberg WMC, Tait P, Taylor-Robinson SD, Thillainayagam AV, Thomas HC, Wasan H (2012) Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 61(12): 1657–1669. <https://doi.org/10.1136/gutjnl-2011-301748> [PubMed]
- Kirstein MM, Vogel A (2016) Epidemiology and risk factors of cholangiocarcinoma. *Visceral Medicine* 32(6): 395–400. <https://doi.org/10.1159/000453013> [PubMed] [PMC]

## Conclusion

The cytotoxicity of ethanolic crude extracts of three bamboo mushrooms (*Dictyophora* spp.) fruiting bodies, including Chinese bamboo mushroom (Ch-DTP), short skirt bamboo mushroom (Th-DTP), and orange skirt bamboo mushroom (Or-DTP), against cholangiocarcinoma (CAA) were determined by cell viability, IC<sub>50</sub>, concentration-response, and morphological change analyses. The results suggest that Ch-DTP revealed the best cytotoxicity effect against cholangiocarcinoma cells followed by Th-DTP, and Or-DTP, respectively.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

This study was financially supported by the Thammasat University Research Unit in Nutraceuticals and Food Safety, and Center of Excellence in Microbial Technology for Agricultural Industry, Institute of Science, Suranaree University of Technology, Thailand. The CCA cell lines were kindly provided by Professor Kesara Na-Bangchang, Graduate Program in Bioclinical Sciences, Chulabhorn International College of Medicine, Thammasat University, Thailand.



- Li X, Wang Z, Wang L, Walid E, Zhang H (2012) In vitro antioxidant and anti-proliferation activities of polysaccharides from various extracts of different mushrooms. *International Journal of Biological Macromolecules* 13(5): 5801–5817. <https://doi.org/10.3390/ijms13055801> [PubMed] [PMC]
- Liao W, Lu Y, Fu J, Ning Z, Yang J, Ren J (2015) Preparation and characterization of *Dictyophora indusiata* polysaccharide-zinc complex and its augmented antiproliferative activity on human cancer cells. *Journal of Agricultural and Food Chemistry* 63(29): 6525–6534. <https://doi.org/10.1021/acs.jafc.5b00614> [PubMed]
- Ma Y, Zhang F (2004) Determination of nutritive components of mycelia and fruitbody of *Dictyophora indusiata*. *Journal of Xan-Xi Agricultural University* 20: 389–391.
- Mau JL, Lin HC, Ma JT, Song SF (2001) Non-volatile taste components of several specialty mushrooms. *Food Chemistry* 73(4): 461–466. [https://doi.org/10.1016/s0308-8146\(00\)00330-7](https://doi.org/10.1016/s0308-8146(00)00330-7)
- Okhuoya JA, Okogbo FO (1990) Induction of edible sclerotia of *Pleurotus tuber-regium* (FR) Sing, in the laboratory. *Annals of Applied Biology* 117(2): 295–298. <https://doi.org/10.1111/j.1744-7348.1990.tb04215.x>
- Ouyang S, Luo Y, Liu M, Fan J, Guo X, Deng F (1998) Analysis of amino acids, vitamins and inorganic elements in *Dictyophora indusiata*. *Hunan Yi Ke Da Xue Xue Bao* 23(6): 535–536. [PubMed] [in Chinese]
- Park JO, Oh DY, Hsu C, Chen JS, Chen LT, Orlando M, Kim JS, Lim HY (2015) Gemcitabine plus cisplatin for advanced biliary tract cancer: A systematic review. *Cancer Research and Treatment* 47(3): 343–361. <https://doi.org/10.4143/crt.2014.308> [PubMed] [PMC]
- Ramírez-Merino N, Aix SP, Cortés-Funes H (2013) Chemotherapy for cholangiocarcinoma: An update. *World Journal of Gastrointestinal Oncology* 5(7): 171–176. <https://doi.org/10.4251/wjgo.v5.i7.171> [PubMed] [PMC]
- Sitingjak R (2017) The nutritional content of the mushroom *Phallus indusiatus* Vent, which grows in the cocoa plantation, Gaperta-Ujung, Medan. *Der Pharma Chemica* 9(15): 44–47.
- Sripa B, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, Pairojkul C, Bhudhisawasdi V, Tesana S, Thinkamrop B, Bethony JM, Loukas A, Brindley PJ (2007) Liver fluke induces cholangiocarcinoma. *PLoS Medicine* 4(7): e201. <https://doi.org/10.1371/journal.pmed.0040201> [PubMed] [PMC]
- Ustundag Y, Bayraktar Y (2008) Cholangiocarcinoma: a compact review of the literature. *World Journal of Gastroenterology* 14(42): 6458–6466. <https://doi.org/10.3748/wjg.14.6458> [PubMed] [PMC]
- Valle J, Wasan H, Palmer DH, Cunningham D, Anthony A, Maraveyas A, madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J (2010) Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *The New England Journal of Medicine* 362(14): 1273–1281. <https://doi.org/10.1056/NEJMoa0908721> [PubMed]
- Valle JW, Borbath I, Khan SA, Huguet F, Gruenberger T, Arnold D (2016) Biliary cancer: ESMO clinical practice guidelines for diagnosis, treatment, and follow-up. *Annals of Oncology* 27(5): 28–37. <https://doi.org/10.1093/annonc/mdw324> [PubMed]
- Wang W, Song X, Zhang J, Li H, Liu M, Gao Z, Wang X, Jia L (2019a) Antioxidation, hepatic- and renal-protection of water-extractable polysaccharides by *Dictyophora indusiata* on obese mice. *International Journal of Biological Macromolecules* 134: 290–301. <https://doi.org/10.1016/j.ijbiomac.2019.05.028> [PubMed]
- Wang W, Song X, Gao Z, Zhao H, Wang X, Liu M, Jia L (2019b) Anti-hyperlipidemic, antioxidant, and organic protection effects of acidic-extractable polysaccharides from *Dictyophora indusiata*. *International Journal of Biological Macromolecules* 129: 281–292. <https://doi.org/10.1016/j.ijbiomac.2019.01.182> [PubMed]
- Yu WX, Lin CQ, Zhao Q, Lin XJ, Dong XL (2017) Neuroprotection against hydrogen peroxide-induced toxicity by *Dictyophora echinvolvata* polysaccharide via inhibiting the mitochondria-dependent apoptotic pathway. *Biomedicine and Pharmacotherapy* 88: 569–573. <https://doi.org/10.1016/j.biopha.2017.01.103> [PubMed]
- Zhong B, Ma Y, Fu D, Zhang C (2013) Induction of apoptosis in osteosarcoma s180 cells by polysaccharide from *dictyophora indusiata*. *Cell Biochemistry and Function* 31(8): 719–723. <https://doi.org/10.1002/cbf.2961> [PubMed]

## Author contributions

- **Pathanin Chantree**, Ph.D., D.V.M., Assistant Professor of the Division of Anatomy, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathumthani, Thailand; e-mail: [pathaninchan@gmail.com](mailto:pathaninchan@gmail.com), **ORCID ID** <https://orcid.org/0000-0003-4974-9584>. The author defined the idea of research and developed a research plan, took part in conducting experimental work, collecting and analyzing data, writing and editing the text of the article.
- **Sirilak Chumkiew**, Ph.D., Lecturer of the School of Biology, Institute of Science, Suranaree University of Technology, Nakhon-Ratchasima, Thailand; e-mail: [s.chumkiew@gmail.com](mailto:s.chumkiew@gmail.com), **ORCID ID** <https://orcid.org/0000-0003-0871-588X>. The author defined the idea of research and took part in preparing the raw materials and extracts.
- **Mantana Jamklang**, Ph.D., Lecturer of School of Preclinic, Institute of Science, Suranaree University of Technology, Nakhon-Ratchasima, Thailand; e-mail: [mjamklang@sut.ac.th](mailto:mjamklang@sut.ac.th), **ORCID ID** <https://orcid.org/0000-0002-5657-4156>. The author defined the idea of research and took part in preparing the raw materials and extracts.
- **Pongsakorn Martviset**, Ph.D., Assistant Professor of the Division of Parasitology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathumthani, Thailand; e-mail: [psktar@gmail.com](mailto:psktar@gmail.com), [pong\\_m@tu.ac.th](mailto:pong_m@tu.ac.th), **ORCID ID** <https://orcid.org/0000-0003-4168-5950>. The author defined the idea of research and developed a research plan, took part in conducting experimental work, collecting and analyzing data, writing and editing the text of the article.