The Cytotoxicity of Commercial Available Turmeric Products in Bangkok Against Colon Cancer Cell-Line HT-29

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Abstract

Commercial turmeric products, together with its major metabolites curcuminoid and monoterpenoid, have been claimed to be used as Thai traditional medicine for the treatment of several symptoms such as jaundice, allergy, tuberculosis, and metastasis stage cancer for many centuries. However, no experiment of the effect of commercial turmeric products against the colon cancer cell line exists to support the manufacturer claiming yet. In this work, the researchers aim to clarify that four on-shelf commercial turmeric products can inhibit the growth of colon cancer cell line HT-29. The results from our MTT assays revealed that all of the products could inhibit the growth of HT-29 efficiently with IC_{50} value ranging from 0.15 - 0.4 $mg.mL^{-1}$. Moreover, results from our dissolving experiment also suggested that cooking or preparing turmeric or consuming with oil is better than water. From all data obtained so far, the researchers would suggest that all turmeric products have a potential effect on colon cancer and, thus, may have a benefit to colon cancer patients.

Keywords**:** *Thai traditional medicine, Natural product, Turmeric, Curcumin, Colon cancer, Anti colon cancer*

1. Introduction

Turmeric is one of Thai traditional herb that has been used in Thai traditional medicine, cuisine, cosmetics, and fabric dyeing for many centuries (Kanjanahattakij et al., 2019). Especially, for Thai traditional medicine, both chunk and powder forms of turmeric root were used for the treatment of jaundice, cold, fever, bloating, colic, abnormal menstruation, dizziness, diarrhea, joint pain, fresh wound, scald head, eczema, abscess, allergy, insect bites, tuberculosis, gonorrhea, and metastasis stage cancer (Hewlings & Kalman, 2017). The root itself contains several vitamins, elements, and nutrients including retinol, thiamine, riboflavin, niacin, tocopherol, calcium, phosphorus, iron, carbohydrate, fiber, and proteins, which are important as a source for preparation of health-promoting food recipes (Barchitta et al., 2019). Even though the plant has very high potential benefits for human health, it can hardly dissolve in water. Within this reason, the plant can rarely be applied, unlike the other Thai traditional herbs. Knowing how to improve and choose the right solvent will help us modifying the preparation of both drug and food recipes, which could increase the usage of turmeric in Thai traditional medicine (Gopinath & Karthikeyan, 2018).

There were many studies because of its major metabolites, curcuminoid and monoterpenoid, trying to analyze these compounds for several bioactivities such as anti-oxidation, anti-inflammation, anti-bacterial, anti-parasite, and anticancer. Some works reported that the major metabolite curcumin, one of the curcuminoid, could inhibit the growth of melanoma cell-line (Baena, & Salinas, 2016). Curcumin and its derivatives, dimethoxy curcumin, and bisdemethoxycurcumin, also exhibit very high anti-oxidant, anti-inflammation, anti-Alzheimer, and can lower the level of blood cholesterol (Qin et al., 2017). The easiest way to use turmeric is by mixing it into food or dessert and consuming it directly. Within the traditional consuming method, turmeric will directly interact with the human gut ecosystem. Whether turmeric really has an ability to inhibit the growth of cancer cell-line or not, the bioactivity of turmeric against colon cancer cell-line is not described by any previous works yet. In this work, the researchers aim to compare the solubility of Bangkok's commercial turmeric products in several solvents and determining the cytotoxicity of dissolved turmeric against colon cancer cell-line HT-29 for better understanding of the interaction between the gut and the herb. The results from these studies demonstrated to us the benefits of turmeric for colon cancer patients who take turmeric mixed with Thai traditional foods, supplements, or drugs.

2. Objectives

The researchers focus on analyzing solubility and cytotoxicity of 4 different commercial brands of turmeric products in Bangkok against colon cancer cell-lines HT-29

3. Materials and Methods

3.1 Turmeric samples

We purchased four commercial turmeric products from a general supermarket in Bangkok, Thailand. As the name of the brands cannot be exposed, English letters A, B, C, and D were substituted. Brand A, B, and C were capsules while brand D was a freshly prepared powder from a manufacturer. In each capsule, there are net weights of 500, 365, and 400 mg per capsule for Brand A, B, and C, respectively. However, only Brand A mentioned that the product has at least 7% of total curcuminoids and 6% volatile compounds (confirmed by HPLC). Furthermore, the Thai FDA confirmed that all the tree products are made of 100% *Curcuma longa* linn. For Brand D, the manufacturer prepared the turmeric powder by the traditional method, sold in the countryside, but not branded yet. Roughly, they weighted and ground turmeric into powder form before double-extracting the powder with distilled water using a hot water extractor. The aqueous solutions were then pooled and evaporated to dry by freeze dryer.

3.2 MTT assay of turmeric solution against human cancer cell-line HT-29

In this work, the MTT assay was adapted from the work previously described in 2019 (Jarintanan, Jongrungruangchok, & Uthaisang-Tanechpongtamb, 2019). The human colon cancer cell-line HT-29 obtained from the American Type Culture Collection (ATCC, USA) was routinely maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (10,000 $U.mL^{-1}$ penicillin and 10 $mg.mL^{-1}$ streptomycins) (GIBCO, USA). The cells were then cultured in a CO_2 incubator at 37°C with an atmosphere of humidified air with 5% CO_2. Before the test, the cells were washed with 0.25% w/v trypsin-EDTA (GIBCO, USA) to remove the old medium and were temporarily maintained at 37°C. After removing the washing buffer, the cells were immediately dissolved in complete DMEM medium, counted under an inverted microscope, and approximately 1×10^4 cells in 100 µL suspensions were seeded into each well of 96-well plates. Consequently, dissolved turmeric extracts in 0.5% DMSO (Sigma-Aldrich, Germany) were then added into the wells at the final concentration of 0, 0.125, 0.25, 0.5, and 1 $mg.mL^{-1}$ in triplicates fashion. For the control wells, 0.5% DMSO without turmeric extract was added instead. The mixtures were incubated 24 hours at 37°C in a CO_2 incubator. After the incubation with turmeric extract, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) (USB, USA) dissolved in complete DMEM medium was added into each well at the final concentration of 0.5 $mg.mL^{-1}$ and subsequently incubated for one more hour at room temperature. After carefully aspirate all mediums from the well, the formed formazan crystals were dissolved by the addition of 100 µl of 100% DMSO into each well. The researchers determined the concentration of purple formazan product by a microplate reader with the optical density (OD) measured at 595 nm. The percentage of cell viability was calculated as %viability = (OD of treated cells/OD of control cells) × 100.

3.3 Microscopic analysis

Before adding MTT into each well (after 24 hours incubation) in 96-well plates, the sample from 1 $mg.mL^{-1}$ turmeric product treated HT-29 was taken for microscopic analysis. The effects of the products on cell morphology were observed by an inverted microscope at 20x magnification (Nikon, Japan).

3.4 Data analysis

The results were expressed with mean ± standard deviation (SD, for each group n=3). All data were processed with GraphPad Prism 8 software. Data analysis was performed using Nonlinear Regression to evaluate the Inhibition Concentration at 50% survival (IC_{50}) for MTT assay. SPSS software was used for the statistics of live and dead cells, which were compared by ANOVA test. Linear graphs show linear error for ± standard of the mean.

4. Results and Discussion

To find the best solvent for dissolving turmeric powder in the next step, commercial turmeric product D was dissolved in 5 different solvents at a final concentration of 50 $mg.mL^{-1}$. The solvents used were water, vegetable oil, phosphate-buffered saline (PBS) (GIBCO, USA), 95% ethanol, and 0.1% DMSO. After dissolving product D powder in water, it was found that it yields a light clear yellow aqueous solution with much sedimentation. Similar to water, dissolving the powder in PBS also gave the same kind of solution. When the turmeric powder was dissolved in oil, it gave a more yellowish solution with less sedimentation than that dissolved by water. However, dissolving the powder with DMSO and ethanol gave the different results from the previous three samples; both solvents can dissolve the powder into a concentrated orange solution with just a little bit of sedimentation (Figure 1).

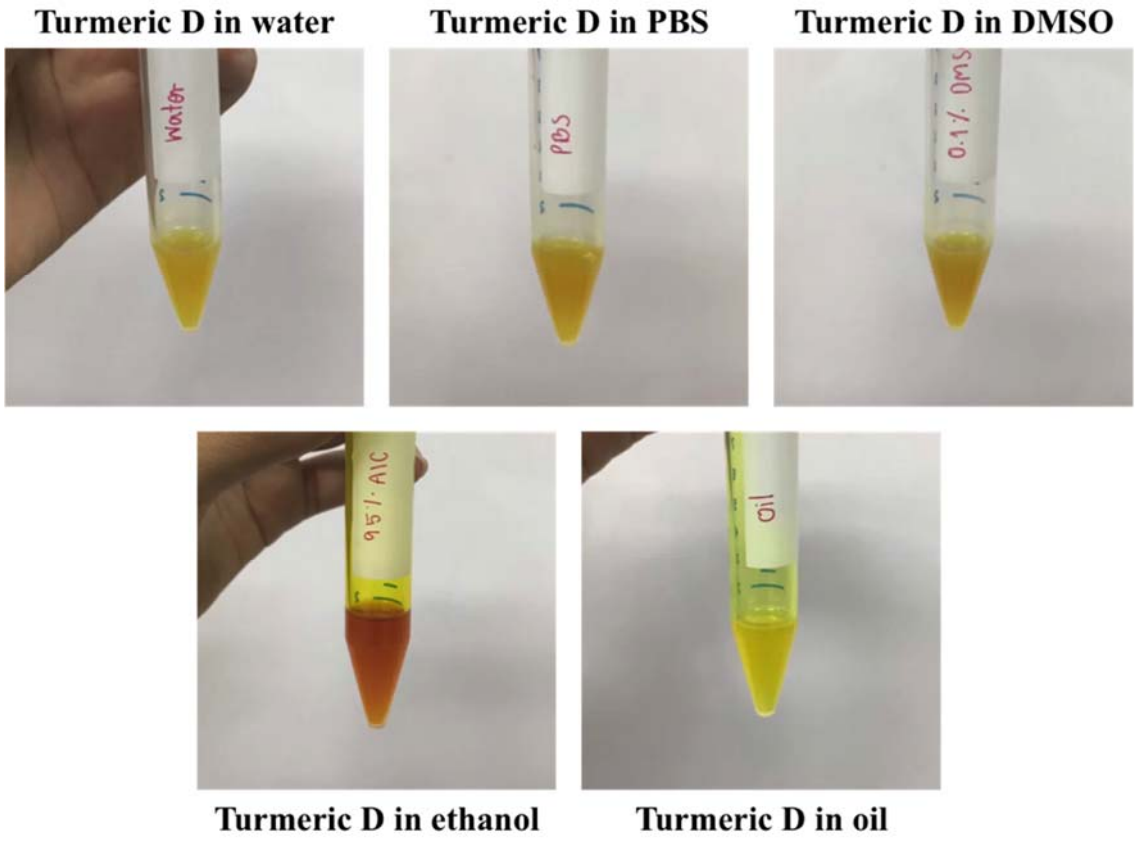

Figure 1 Solubility of turmeric product D in 5 different solvents including water, PBS, DMSO, ethanol, and oil

The dissolving results showed that turmeric powder could be dissolved well by alcohol and DMSO. Nothing surprisingly, because the bioactive compounds found in turmerics such as curcuminoid and monoterpenoid are well extracted from ethanolic fraction and mentioned in several previous works. Curcumin, the curcuminoid major metabolite found in turmeric, is a polyphenol. It can dissolve well in ethanol and DMSO because its molecular structure contains both polar and non-polar functional groups (Hani & Shivakumar, 2014). The results should be different in water and PBS experiments because these solvents have more polarity than ethanol and DMSO. For vegetable oil, as we expected, the solubility of the turmeric powder should be better than the polar solvent, but less than ethanol. In Thai traditional cooking using turmeric as an ingredient, it is better to use it with oily methods such as frying, boiling with coconut milk, or

making curry, rather than the water-based method such as making a soup or steaming (Sun, Ji, & Shen, 2019). The health-promoting compounds will be dissolved out from the herb by oil and give more benefits to the consumers. In this work, DMSO was used as a solvent for the MTT test because it has less toxic effects on cell-line than ethanol (Figure 1).

The results from MTT assay showed that all of the four turmeric products could inhibit the growth of colon cancer cell line HT-29. However, there are differences in IC_{50} value between these samples. The one with the highest IC_{50} value is sample B (0.4 $mg.mL^{-1}$). It is indicated that sample B has the lowest efficiency to inhibit the growth of colon cancer HT-29 cell line. For sample A, it has IC_{50} value at 0.2 $mg.mL^{-1}$, which is the sample that moderately inhibits the growth of colon cancer cell-line HT-29 in this work. Similar to each other, sample C and D have IC_{50} against HT-29 cancer cell-line at 0.15 and 0.16 $mg.mL^{-1}$, respectively (Table 1). According to the IC_{50} values, turmeric C and D are the best turmeric products that can inhibit the growth of the colon cancer cell-line. When we plotted the inhibition concentration results from each assay together, it is clearly supported that samples C and D are the best turmeric products that can inhibit the growth of the HT-29 cell-line (Figure 2). During the assay, the morphology of the cell samples after treatment with four commercial products was also observed under the inverted microscope. The results revealed the shrinkage of the cells after 24 hours treatment, especially by turmeric products C and D. At the highest concentration, 1 $mg.mL^{-1}$, all four turmeric products changed HT-29 cells morphology from epithelial form (untreated control) toward shrinkage form (Figure 3). From our analysis, altogether, we would recommend colon cancer patients to consume turmeric products C and D as supplementation to delay the progression of their pathogenic stage of the disease.

Table 1 IC_{50} value of 4 different commercial turmeric products against HT-29 cell-line

Turmeric products	IC_{50} value ($mg.mL^{-1}$)
Turmeric A	0.20
Turmeric B	0.40
Turmeric C	0.15
Turmeric D	0.16

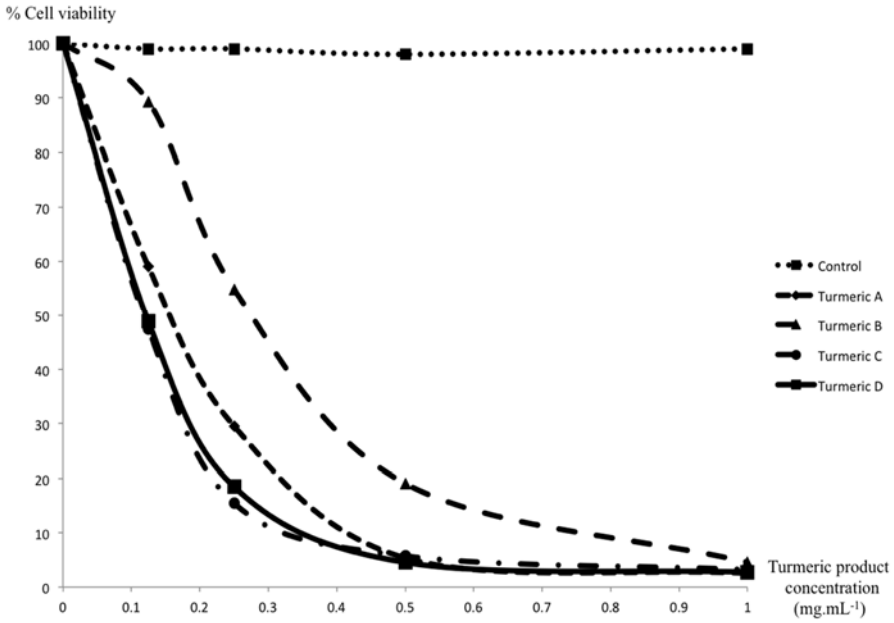

Figure 2 Plot of cell viability against different concentrations of each commercial turmeric product

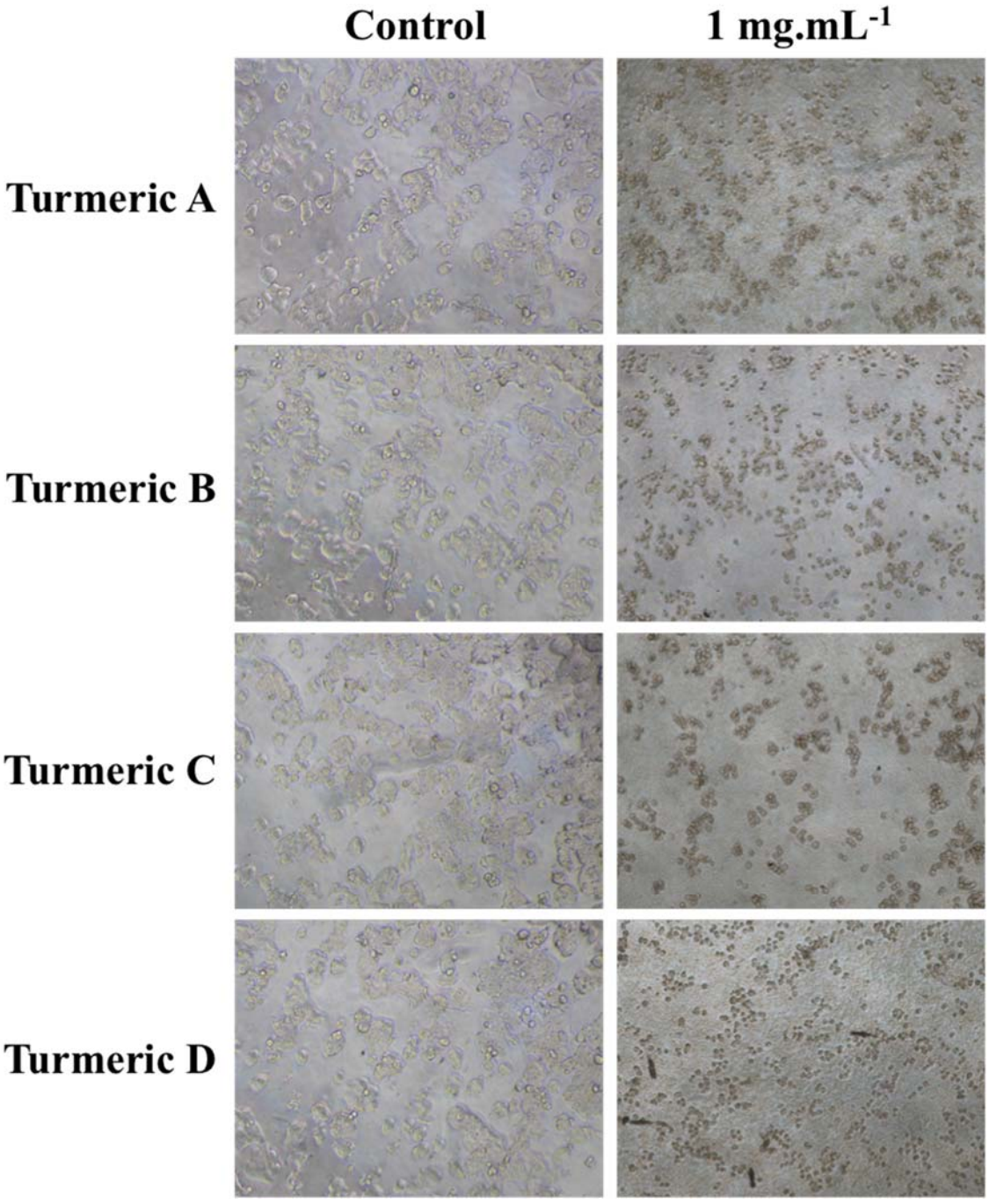

Figure 3 Effect of 4 commercial turmeric products on HT-29 cell morphology at a concentration of 1 mg.mL^{-1} comparing with untreated cell (control)

5. Conclusion

This work strongly showed that commercial turmeric powder could be dissolved well in amphiphilic solvents such as ethanol and DMSO. On the other hand, it does not like to be dissolved by polar solvents and moderately dissolved by non-polar solvents. Owing to the major metabolites existed in turmeric, curcuminoid and monoterpenoid, this is the main reason why we recommend taking or cooking turmeric products with oil rather than water. After we used DMSO as a solvent to dissolve four turmeric commercially available products in the market and analyze for their cytotoxicity, we found that all of them can inhibit the growth of colon cancer cell-line efficiently with IC_{50} value ranging from 0.15 - 0.4 $mg.mL^{-1}$. The results suggested that turmeric products could potentially inhibit colon cancer progression as the manufacturer's claim with different IC_{50} values. Accordingly, it is because each product might contain slightly different active ingredients in turmeric powder, which depend on plantation geographies, harvesting techniques, and manufacturing processes.

6. Acknowledgments

Biochemistry unit Biochemistry Unit, Department of Biomedical Scicence, Faculty of Sciences and Faculty of Medical Technology, Rangsit University, supported the facilities and grant of this work.

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