# In vitro cytotoxic activity study of aqueous crude extracts of an anticancer Thai traditional preparation against COLO 205 cells

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## Abstract

This research aimed to study the cytotoxic activity of an anticancer Thai traditional preparation against COLO 205 cells. The herbal receipt was provided by a local Thai traditional medicine (TTM) practitioner named Luan Noodee. Regarding his experience, it has been effective against several types of cancer such as colon cancer, mouth cancer, leukemia, lymphoma, and nasopharyngeal carcinoma. This anticancer formulation consists of three herbal medicines, namely, the stems of *Cyrtosperma johnstonii* N.E. Br., the leaves of *Shefflera leucantha* R. Vig., and the roots of *Imperata cylindrica* P. Beauv.. The aqueous extracts of the combination of the three herbs along with the formation composing only one single herb were subjected to the test using MTT assay. The results revealed that the crude extracts from the formulation possessed the highest activity with  $IC_{50}$  of 196 µg/ml compared to that of the aqueous extracts from a single herb. The  $IC_{50}$  of the aqueous extract of *S. leucantha*, *C. johnstonii*, and *I. cylindrica* were 226, 375, and 424 µg/ml, respectively. The results support the idea that using mixed herbs in the formulation is better than using a single herb because there is a possibility that some synergistic effect might occur.

Keywords: Cyrtosperma Johnstonii N.E. Br., Shefflera leucantha R. Vig., Imperata cylindrica P. Beauv., Anticancer, Cytotoxic activity

## 1. Introduction

At present, cancer is the number one cause of death in Thailand (Virani et al., 2017). Although chemotherapy is the backbone for cancer treatment, the use of chemotherapeutic is often limited due to undesirable side effects and overly high expenditures. Alternatively, Thai traditional medicine (TTM) has been used for the treatment of cancers for a considerable amount of time (Chokevivat, Chuthaputti, & Khumtrakul, 2005). Many of the anticancer remedies have been found in the TTM scriptures (Lumlerdkij, 2018). It is worth noting that, the concept of cancer in TTM is not the same as cancer in biomedical diagnosis and careful consideration should be undertaken since over nine hundreds of formulations for cancer are found in TTM scripture (The Protection and Promotion of Thai Traditional Medicine Wisdom Act B.E. 2542, 2018).

Moreover, numerous herbal formulations for the treatment of cancer were individually developed by local TTM practitioners, based on the wisdom inherited from their ancestors (Chokevivat & Chuthaputti, 2005). In TTM, the concept in the treatment of cancer is based on observation and interpretation of patients' symptoms, suffering, past history, causes, effects, and responses, mostly without objective information (He, 2015). There is no statistical data on TTM use by cancer patients in Thailand available. In general, most of the TTM preparations consist of 10 - 50 items of herbal medicinal plants or animal products without mentioning the medicinal properties of any single item (Sobhanasiri, 2009). Even though the benefits of TTM against the disease are well known throughout Thailand, scientific evidence to support the use of TTM anticancer is still lacking.

In this research, the anticancer receipt was kindly provided by TTM practitioner from Nakornpathom Province, Luan Noodee. The three herbal medicines in the formulation consist of the stems of *Cyrtosperma johnstonii* N.E. Br., the leaves of *Shefflera leucantha* R. Vig., and the roots of *Imperata cylindrica* P. Beauv.. Based on his TTM practicing experiences, this formulation has been quite effective against many types of cancer such as colon cancer, mouth cancer, leukemia, lymphoma, and nasopharyngeal carcinoma. The herbal decoction is prepared by mixing the equal weight of dried herbs (70 g each), boiling in water for



20 min, and then discarding the solid residue. The supernatant should be taken twice daily before breakfast and at bedtime. The TTM practitioner Luan Noodee indicated that the reduction or disappearance of tumor mass was observed and the cancer patients can further live with an improved quality of life.

*Cyrtosperma johnstonii* N.E. Br. (Araceae), widely known as Singha Mora in Thailand, is used to treat a range of conditions including internal abscess, hemorrhoid, low appetite, and anemia. Furthermore, application of hydroalcoholic extract of *C. johnstonii* helps to reduce toxicity from centipede and scorpion poisoning. It has been used as a component in many ethnomedicinal herbal receipts for the treatment of many diseases including several types of cancer. TTM practitioner Luan Noodee suggested that Singha Mora played a very important role in killing the cancer cells. Details about phytochemical components in *C. johnstonii* were not yet reported. Anyway, antioxidant and cytotoxicity activities of *C. johnstonii* extracts on human cancer cells were established (Okonogi et al., 2013).

For treatment of cancer, some plants in the preparation showed cytotoxic activity against cancer cells while some showed no cytotoxic activity but exhibited another activity such as antioxidant, antiinflammation, antimicrobial and enhanced immunology. It was noted that *S. leucantha* R. Vig. (Araliaceae) and *I. cylindrical* P. Beauv. (Gramineae) are referred to as the assistant medicinal herbs in the anticancer formula because they do not directly affect the cancer cells. The former helps to reduce inflammation caused by the cancer cells while the latter helps to drive the toxins that accumulate within the body. *S. leucantha*, known locally as "Hanuman Prasankai" in Thai, has been used for the treatment of respiratory tract infection, allergy (Matsui et al., 2010), chronic cough, and asthma (Potduang et al., 2017). It was reported that the active bronchodilator principle is a mixture of saponins found in *S. leucantha*. The very low cytotoxicity activity to brine-shrimp was reported. *Imperata cylindrica* P. Beauv., with Thai name of Yakha, is widely distributed in the tropics and subtropical regions. The roots of *I. cylindrical* have been described as a diuretic (Sripanidkulchai et al., 2001), anti-inflammatory or antipyretic agent in TTM. The water extract of *I. cylindrica* has been reported to possess immunostimulant property (Pinilla & Luu, 1999).

In accordance with the traditional method for the preparation of the herbal decoction, the cytotoxic properties of water extracts of the three herbs combined together and of a single herb were subjected to an investigation.

#### 2. Objectives

This research aimed to study the cytotoxic activity of an anticancer Thai traditional preparation against COLO 205 cells as compared to an individual herb.

#### 3. Material and method

## 3.1 Preparation of herbal extracts

The plant materials, including the stems of *C. johnstonii*, the leaves of *S. leucantha* and the roots of *I. cylindrica*, were provided by a TTM practitioner Luan Noodee. The thorns around the stem of *C. johnstonii* were thoroughly removed before being used. All fresh herbs were washed and dried in hot air oven at 60°C for 24 h. Then, the dried herbs were cut and ground into powder. Aqueous extracts of a single herb (30 g) and a combination of the three herbs (10 g each) were prepared separately by boiling the herb powder in 250 ml distilled water for 4 hr. and occasionally stirring. Afterward, the filtrates were collected by filtering through gauze cloth and Whatman paper No.1, respectively. Then, the filtrates were placed on the water bath for solvent evaporation and the residues were transferred to the hot air oven at 60°C until dried.

The stock solutions of the crude extracts in distilled water were made and serially diluted to give concentrations of 500, 50, 5, 0.5, and 0.005  $\mu$ g/ml, respectively.

3.2 COLO 205 cell line

The cells of COLO 205 were suspended in culture medium at a density of 20,000 cells and seed into 96 well plates at 100  $\mu$ l per well. Culture media was RPMI 1640 supplemented with 10% fetal bovine serum, 10 mM HEPES, 4.5 g/l Glucose, and 1 mM sodium pyruvate. The cells were incubated for 24 h at 37 °C, in 5% CO<sub>2</sub> 24 h prior to the assay.



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# 3.3 MTT assay

The cytotoxicity of the herbal extracts on COLO 205 was tested by MTT assay. The COLO 205 cells were treated with the herbal extracts (dissolved in DMSO) and doxorubicin (positive control) at concentrations ranging from  $0.005 - 500 \mu g/ml$  in triplicates. An untreated group was added with 0.5% DMSO and used as a negative control. The cells were incubated with MTT dye (Thiazolyl Blue Tetrazolium bromide: (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) at 0.5 mg/ml and incubated for 2 h. The formazan crystals product formed were dissolved by adding 100  $\mu$ l of DMSO. After 15 min incubation at the room temperature, the amount of purple formazan product was determined using ELISA microplate reader which the optical density (OD) was measured at 595 nm. The percentage of cell viability was calculated as % viability = (OD of treated cells/OD of control cells) x 100.

## 4. Results and discussion

Figure 1 shows the results of the MTT assay from the herbal crude extracts. It was found that the aqueous extracts from the combination of three herbs had the highest cytotoxicity to COLO 205 cells with  $IC_{50}$  of 196 µg/ml compared to that of the aqueous extracts from a single herb. The  $IC_{50}$  of the aqueous extract of *S. leucantha*, *C. johnstonii* and *I. cylindrica* were 226, 375, and 424 µg/ml, respectively. The result indicated that the traditional formulation had a greater effect in the treatment of cancer than a single herb. This support the idea of using mixed herbs in the formulation is better than using single herbs because there is a possibility that some synergistic effect might occur. Nevertheless, when comparing to the anticancer agent such as doxorubicin, the result reveals the far greater activity with the  $IC_{50}$  value of 0.4 µg/ml (Figure 2) which was approximately 500 times higher than that of any herbal extracts.

It is widely recognized that the efficacy of modern drugs is inevitably higher than that of traditional medicine due to the chemical purity of the drug which also unavoidably lead to many undesirable side effects. On the contrary, the medicinal plants naturally contain large varieties of a small number of chemical compounds which resulting in slower retreat of the disease. But in the end, it can heal most of the illness without suffering from the severed counter effects. In this formulation, aside from the increase of cytotoxic activity, the other medicinal herbs also help strengthen the body to fight against the disease by other mechanisms such as blood nourishment, detoxification, reduction of internal inflammation, and so on.



**Figure 1** Cytotoxic activity of aqueous extracts of combination of three herbs (F), *S. leucantha* (H), *C. johnstonii* (S) and *I. cylindrica* (K)





Figure 2 Cytotoxic activity of doxorubicin

## 5. Conclusion

This study showed that the cytotoxicity of aqueous extracts of the herbal formulation as a whole was higher than the extracts from an individual herb. The results support the use of herbal medicines combination based on the cumulative experiences of the TTM practitioners and prescriptions recorded in ancient scriptures. Nevertheless, a modification of the traditional preparation into the modern and easy-to-use dosage form to increase patient's compliance is still necessary. To develop semi-purified herbal medicinal products, further studies of bioactive compounds in the herbs should be carried out for assessment of the stability and quality of the crude herbal extracts as well as the finished products.

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